UNIVERSITY OF MINNESOTA

This is to certify that I have examined this copy of a doctoral thesis by

Marvin Jon Ross

and have found that it is complete and satisfactory in all respects, and that any and all revisions required by the final examining committee have been made.

William Schmid Advisor

William D. Schmid

16 May 1996
Date

GRADUATE SCHOOL

DISTRIBUTION, PREDATION, PHYSIOLOGY AND BEHAVIOR OF CLONES OF DAPHNIA PULICARIA IN A SINGLE BASIN FRESHWATER LAKE

A THESIS
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY

MARVIN JON ROSS

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DR. WILLIAM SCHMID, ADVISOR

MAY, 1996

ACKNOWLEDGMENTS

I am indebted to many people who through their generosity and expertise made this project possible. Dr. James Curtsinger introduced me to the field of ecological genetics, provided technical assistance with electrophoresis early on and was instrumental in the formative stages of this research. Dr. Robert Megard provided the hydro-acoustic system so critical as the underwater 'eyes' for this research as well as training on its use. He also provided many challenging and stimulating ideas over the course of the project and his diligent editorial efforts significantly improved the prose. Drs. Curtsinger and Megard co-authored chapter one. I am especially indebted to Dr. William Schmid who guided and strongly encouraged all phases of my Ph. D. program and tirelessly reviewed many versions of the manuscript. Drs. Donald Siniff, John Tester, David Czarnecki and Michael Auerbach, stimulated and supported my program from its inception providing both intellectual and moral encouragement. Dr. Czarnecki also assisted with field work, reviewed an early version of chapter one and provided phytoplankton identification. Dr. Richard Meyer also provided phytoplankton identification and helped me work through techniques with the spectrophotometer. Dr. Kinley Larntz introduced me to the fascinating and extremely useful discipline of applied statistics, especially log-linear modeling, that was so important in tracking the numerous interacting variables in this field study that was conducted under uncontrolled conditions. Dr. Marc Boileau provided many helpful suggestions for enzyme screening and assisted with the screening.

I would like to thank the staff and the many undergraduate and graduate students in the Ecology and Ecological Genetics classes at the Lake Itasca Biological Station for their assistance. In particular I wish to acknowledge Dr. Hank Fukui, Dr. Ming Ruan, Oksana Piterman, Lynn Berquist, Jenny Anderson, Clare Gibson and Pam Nelson for their assistance with field work, electrophoresis and zooplankton counts.

I am grateful to the residents of Long Lake, including Jim Garrison, Gwen and Scott Stahnke, Norman and Anita Chase, and the John Hanson family who generously provided year-round access, equipment storage, docking facilities and logistic support. Judy Haroldson assisted with much of the field work. I also want to thank Dan Traun, David Traun and Bob Abraham for providing the rainbow trout stomachs.

Finally I need to express my sincere gratitude to my family who not only gave so generously of their time in direct assistance, but who also by their patience and understanding, indirectly supported this work more than they realize, over the past few years. Often postponing, altering and sacrificing personal plans, Cindy and Adriane helped with lab work; Andy drove the boat and helped with field work.

This research was supported by grants from the Sigerfoos fund, Dayton and Wilke Funds, Sigma Xi and the Lake Itasca Biological Program.

SUMMARY

I investigated the summer distribution of clones of *Daphnia pulicaria* in Long Lake in north central Minnesota from August 1990 to August 1994. During the first three summers the zooplankton were aggregated in two distinct layers during daylight hours. One was in the metalimnion and the other deeper in the hypolimnion. By early August each year, the *D. pulicaria* population was genetically differentiated. One clone, homozygous slow at the *PGI* locus (PGI_{SS}), was more frequent in the hypolimnetic layer than the metalimnetic layer. More detailed observations in 1991 indicated that this increase of PGI_{SS} coincided with a progressive decline in dissolved oxygen in the hypolimnion throughout the summer.

Avoidance of visual predation in darker waters and physiological adaptations to low oxygen conditions were invoked to explain the genetic structure. Large adult *D. pulicaria* and pigmented individuals were found to be much more vulnerable to predation by rainbow trout. Although the sample was small (n = 14), *D. pulicaria* that were homozygous slow at the *PGI* locus were 6 times more likely to be found in trout stomachs than in the plankton. However, no significant association was found between *D. pulicaria* genotypes and size or pigmentation. When the predation observations were made the dominant pigment was not hemoglobin, which normally dominated colored morphs of *D. pulicaria* in Long Lake.

Concentrations of dissolved oxygen, genotypes and annual variation affected the proportion of *D. pulicaria* that contained elevated levels of hemoglobin. Individuals collected in the hypolimnion, where dissolved oxygen levels were at or below 2.8 mg/liter, were 9 times as likely to have more hemoglobin than individuals collected in the metalimnion, where dissolved oxygen levels ranged from 8.8 to 13.4 mg/liter. The PGI_{SS} clone was three times as likely to have elevated hemoglobin than the PGI_{FS} clone. Variation between two years (odds ratio = 1.6 : 1) while statistically significant, was less than the effects ascribed to oxygen level and clone type.

When transplanted from the deep layer to the shallow layer, the PGI_{SS} clone was more likely to survive than the heterozygous (PGI_{FS}) clone. Reciprocal transplant experiments from the shallow layer to the deep layer were inconclusive.

A brief examination of clone-specific vertical migration indicated that the PGI_{SS} clone was more likely than other genotypes to migrate upward from the deep layer after sunset.

Large increases in the numbers of rainbow trout fingerlings and increased dissolved oxygen in the hypolimnion characterized the final two summers of the study. The density of the shallow layer of *D. Pulicaria* declined in 1993 and it failed to develop in 1994. The deep layer failed to differentiate genetically. The frequency of hemoglobin pigmentation in *D. pulicaria* declined in 1993 and disappeared in 1994. Vertical migration from the deep layer was no longer dominated by the PGI_{SS} clone. Because the changes in predator density and dissolved oxygen occurred simultaneously it was not possible to assign a hierarchy of responses to these two parameters that were proposed to account for the perpetuation of genetic diversity in parthenogenetically reproducing *D. pulicaria.*

Three techniques were instrumental to this project. Acoustic methods permitted the detection and sampling of discrete layers of zooplankton. Protein electrophoresis allowed subdivision of samples of *D. pulicaria* into clonal groups. Log-linear modeling afforded the power to the analyze effects, relative magnitudes and interactions of categorical data.

TABLE OF CONTENTS

PREFACE

The development of genetic structure in a population of *D. pulicaria* during summer is described in chapter 1 where most methods also are described. The next 4 chapters detail the mechanisms for the development of this structure. Chapter 2 investigates the role of predation and details some additional techniques. The selective advantage of increased hemoglobin levels on clones residing in a reduced oxygen environment is discussed in chapter 3. Transplant experiments conducted to compare clone-specific survival are described in chapter 4. Differences in the migratory behavior of clones are described in chapter 5.

In addition to the planned experiments and observations described in this dissertation, two unexpected perturbations, one natural and one man-made occurred at Long Lake. These changes offered an opportunity to further investigate hypotheses concerning the effects of predators and the importance of hemoglobin production in genetically structuring the *D. pulicaria* population. The three 'typical' summers from 1990 to 1992, addressed in chapter one, were followed by two summers when the depletion of dissolved oxygen in the hypolimnion was much less severe. The man-made perturbations began in the fall of 1992, when the Minnesota Department of Natural Resources, Section of Fisheries increased the stocking rate of rainbow trout by 100% each year for two years. Chapter 6 discusses the combined effects of these two perturbations on the *Daphnia* population in view of the results from earlier chapters.

CHAPTER 1

Development Of Population Structure

Introduction

Spatially and temporally fluctuating selection have been invoked to explain genetic diversity and clonal coexistence in cladoceran populations that reproduce parthenogenetically for protracted periods (Hebert, 1978; Lynch, 1984; Weider, 1985). Because Cladocera reproduce by ameiotic parthenogenesis during most of the year, selection operates on specific genotypes for repeated generations (Lynch 1983). As Young (1979a) noted, all the members of a clone from the first ex-ephippial female to her last surviving clonal descendent constitute the extended soma of a single genetic individual. A clone may persist through several seasonal or even annual cycles in permanent populations. Because the average lifetime of a clone is much longer than a single individual, subtle environmental effects may be more readily observed.

The spatial distribution of clones in the genus *Daphnia* has been studied extensively. Substantial differences in clone composition have been observed both among ponds in the same geographic area (Hebert and Crease, 1980; Weider and Hebert, 1987a; Weider, 1989) and within individual lakes and ponds (Weider, 1984, 1985, 1989). Spatial patterns of clone composition have been observed (Weider and Hebert, 1987a) and ascribed to environmental variation such as salinity/conductivity gradients (Weider and Hebert, 1987b), and dissolved oxygen (Weider and Lampert, 1985).

Beginning with Hebert's (1974) observation of drastic temporal shifts in genotypic frequencies of *Daphnia magna*, many authors have observed significant temporal changes within ponds and lakes in the clonal composition of permanent populations of *Daphnia* (Hebert and Ward, 1976; Young, 1979b; Weider, 1984, 1985; Mort and Wolf, 1985; Jacobs, 1990; Muller and Seitz, 1993). However, an underlying pattern to these temporal shifts remains elusive. With the exception of Hebert and Ward (1976) and Young (1979b), the aforementioned studies were conducted in relatively

deep bodies of water ranging from six to sixty meters in maximum depth. Carvahlo and Crisp (1987) and Carvahlo (1987), working in a much shallower environment (1.2m max. depth, 0.6m mean depth), were the first to provide an example of clear seasonal patterns of clone frequencies in *Daphnia magna* correlated with seasonal changes in water temperature.

Weider (1985) pointed out that the planktonic habitat in deeper lakes and ponds is much more structured than previously thought, especially in the vertical dimension. Results from Weider (1984, 1985) with *Daphnia pulex* in a seasonally stratified pond only 6 m deep suggest that the genetic structure of planktonic cladoceran populations also may be more complex spatially (both vertically and horizontally) and temporally than previously thought within individual ponds and lakes. Temporal patterns of clonal succession could be complicated by significant spatial differences within lakes, especially in deeper basins.

Here, we examine spatial and temporal distributions of clones of pelagic *Daphnia pulicaria* in a vertically stratified, single-basin, freshwater lake. *D. pulicaria* dominates the *D. pulex* complex in North American lakes (Cerny and Hebert, 1993).

Carvahlo and Crisp (1987) and Weider (1989) demonstrated the need to use relatively sophisticated analytical techniques to describe the effects of interacting variables on the frequency of *Daphnia* clones. Many previous studies have limited analyses to pairwise observations of clone frequency and either spatial or temporal explanatory variables. Here we used a four dimensional log-linear approach to analyze how clone frequency, the response variable, was related to horizontal location, depth, and time as well as more complex second and third order interactions with this group of explanatory variables.

Study Area

Long Lake (Fig. 1), located in Clearwater County in northwestern Minnesota (latitude 47 17' N., longitude 95 17' W.), is 2.3 km long from northwest to southeast, with an average width of 0.4 km., a surface area of 66.5 hectares and a maximum depth of 24 m. The lake is very clear compared to other lakes in the region, with Secchi transparency up to 10.5 m (Moyle, 1969). The lake basin is characterized by extremely steep slopes. The basin shape combined with rocky substrate in the littoral zone results in virtually no emergent aquatic vegetation. Submergent vegetation comprised principally of *Chara contraria* is relatively abundant especially on the southwest facing slope (Schmid, 1965). Limnologically, the lake

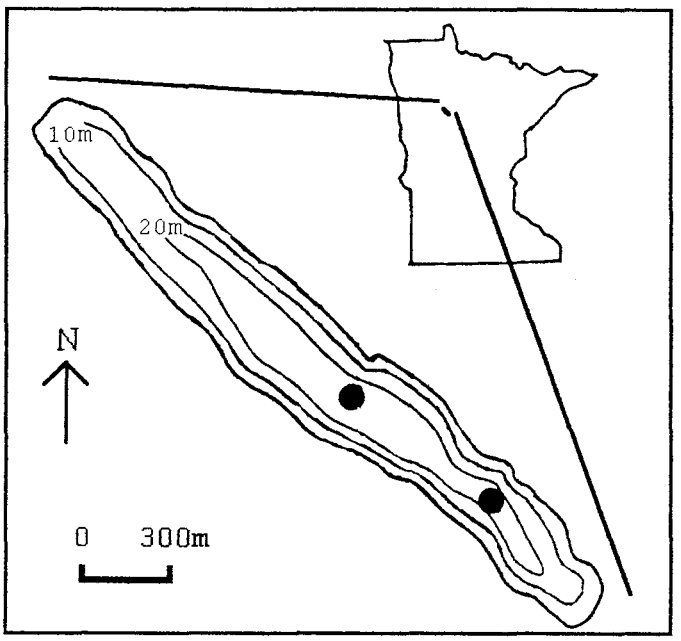


Figure 1. Long lake: dark circles indicate the mid-lake and southeast sampling areas.

is dimictic (mixing throughout the water column in spring and autumn). It is high in carbonates and in the mesotrophic to oligotrophic range of freshwater productivity (Moyle, 1969; Megard, 1967, 1968). Thermal stratification results in depleted levels of

dissolved oxygen below 15 m, especially during summer and early autumn.

Methods

The spatial distribution of zooplankton was discerned with a high frequency (192 kHz) sonar system that consisted of a Lowrance XI6 graph-type echolocator coupled to a modified microcomputer (Megard et al., 1989; Megard at al., In prep.). Backscattering of high frequency sound was measured along transects on the lake. Specific sampling sites were selected based on an initial lengthwise acoustic transect each day that collections were made. Two sites were selected and acoustic cross sections (short axis of the lake) were obtained. Site selection was based upon a combination of two criteria: the first was the appearance of discrete depth layers of zooplankton on the computer monitor and the second was the need to maintain a relatively large distance between sampling sites. Sampling areas are indicated in figure 1. Previous experience had indicated that zooplankton could form mobile aggregations, therefore, we used acoustic information to select specific sampling sites and depths each day within the confines of the general sampling areas. Sampling sites were positioned with LORAN C, or with a sextant using three known locations on shore.

D. pulicaria were collected with a closing plankton net (20 cm x 1 m, 63 *u* mesh, and a removable bucket with a 200 u filter) towed vertically 2-5 m through the backscattering layers. All collections and field observations were made between 1000 and 1600 CDT. Field work was conducted from August 7, 1990 to August 2, 1992.

Temperature and dissolved oxygen profiles were obtained with a YSI model 57 oxygen meter. Water transparency profiles were obtained with a Montedoro-Whitney model TMU-IB transmissometer. These limnological parameters were observed at 1 m intervals on each sampling trip at the northwesterly (mid-lake) of the two sampling locations.

D. pulicaria samples were returned to the laboratory and either processed within 72 hours or frozen up to six months for later electrophoresis. Freezing involved placing individuals in 96-well

micro culture plates with a drop of distilled water. Each loaded micro culture plate was initially placed directly on the cooling coils of a standard upright freezer for rapid freezing and stored at -19 C.

Either fresh or thawed *D. pulicaria* were prepared for electrophoresis by placing individuals directly in Super Z applicator wells (Helena Scientific) and grinding with a spatula in distilled water. Individual *D. pulicaria* homogenates were applied to cellulose acetate gels and exposed to a 200 v electric field for 20 minutes with a Gelman Sciences model PS500 regulated power supply. Electrophoresis chemicals, buffers and stains followed the recipes of Hebert and Beaton (1989).

The phosphoglucose isomerase *(PGI)* locus demonstrated sufficient activity and variability to permit subdivision of the *D. pulicaria* population into three single-locus genotypes: FF = homozygous fast, SS = homozygous slow and FS = heterozygous. These single-locus genotypes will be referred to as clones because we did not observe males in our samples and because the large deviations from Castle-Hardy-Weinberg equilibrium were consistent with a parthenogenetically reproducing population. Each of these single-locus clones are more appropriately viewed as aggregations of genotypes that share the same characteristics at the *PGI* locus. I have screened an additional 28 loci and found additional variation only at the phosphoglucomutase *(PGM)* locus.

Identification of individuals as *D. pulicaria* was made by comparing both rostrum reticulation pattern (Brandlova et. al., 1972) and lactate dehydrogenase *(LDH)* electromorphs (Cerney and Hebert, 1993) with those of *D. pulex* collected at Ice House Pond located at the Lake Itasca Biological Station 10 Km southeast of Long Lake. *D. pulicaria* exhibited an elongate reticulation pattern on the rostrum and a homozygous fast electromorph at the *LDH* locus compared to the more symmetric reticulation and a homozygous slow electromorph at the *LDH* locus of *D. pulex.* Identification to species based on the *LDH* electromorph that has gone to fixation in *D. pulicaria* was confirmed independently by M. Boileau (Pers. Com.).

Data were analyzed using log-linear modeling procedures with the statistical package 'S' on the University of Minnesota's Academic Computing Services UNIX computer. Log-linear techniques were

invoked because the response variable (clone frequency) and the cluster of explanatory variables (water depths, locations on the lake and time) were all categorical. Log-linear modeling permitted the examination of main effects as well as higher order interactions of the explanatory variables, which was not possible with more conventional chi-square analysis. Other data were processed with EXCEL software on a Macintosh SE computer and with STATISTIX, SYGRAPH, DESCSCAN and PLOT-IT software on an IBM computer.

Results

Limnological data (Fig. 2) collected in 1991 indicated that the lake was thermally stratified during the summer months. The epilimnion (warm layer of surface water) thickened from 4 m in May to 7 m in August. A thermocline in the metalimnion extended from 5 m to 12 m in May and moved deeper over the course of the summer. The hypolimnion (deep cool water) occurred below 12 m in May and was reduced in size to water deeper than 15 m in August.

Water transparency measurements (Fig. 2) indicated relatively high phytoplankton concentrations in the metalimnion, especially mid- to late summer 1991. This resulted in a positive heterograde dissolved oxygen profile (Fig. 2) due to excess photosynthesis relative to community respiration at these depths. A qualitative examination of the metalimnetic phytoplankton indicated that a cryptophyte in the genus *Chroomonas* dominated the algal populations and was presumably largely responsible for the increased levels of dissolved oxygen observed in the metalimnion. Dissolved oxygen levels declined sharply in the hypolimnion late in the summer, resulting in near anoxia in deep water by late August. Limnological conditions were not monitored in 1990, however, similar conditions were observed in August 1992.

Preliminary lengthwise acoustic transects indicated that major mid-day concentrations of zooplankton were usually found in the southeast two-thirds of the lake and that the vertical distribution was typically concentrated in three layers; an upper layer located in the epilimnion, middle layer in the metalimnion and the upper hypolimnion (this layer will be referred to as the metalimnetic layer) and the lowest layer located deeper in the

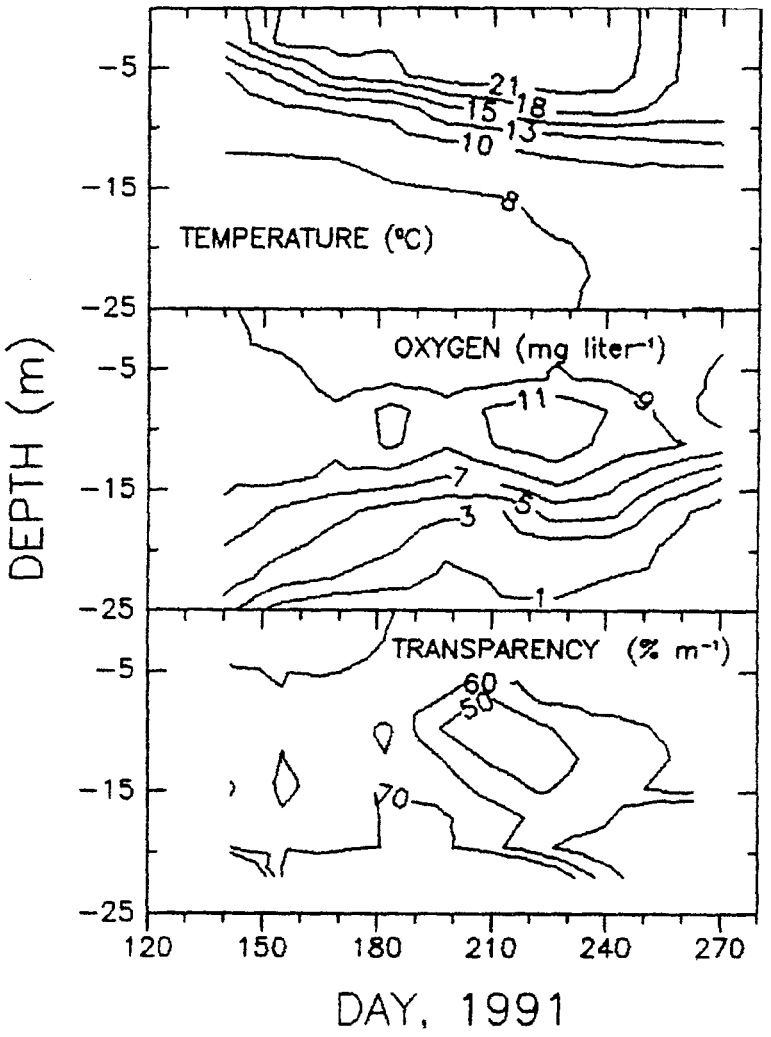
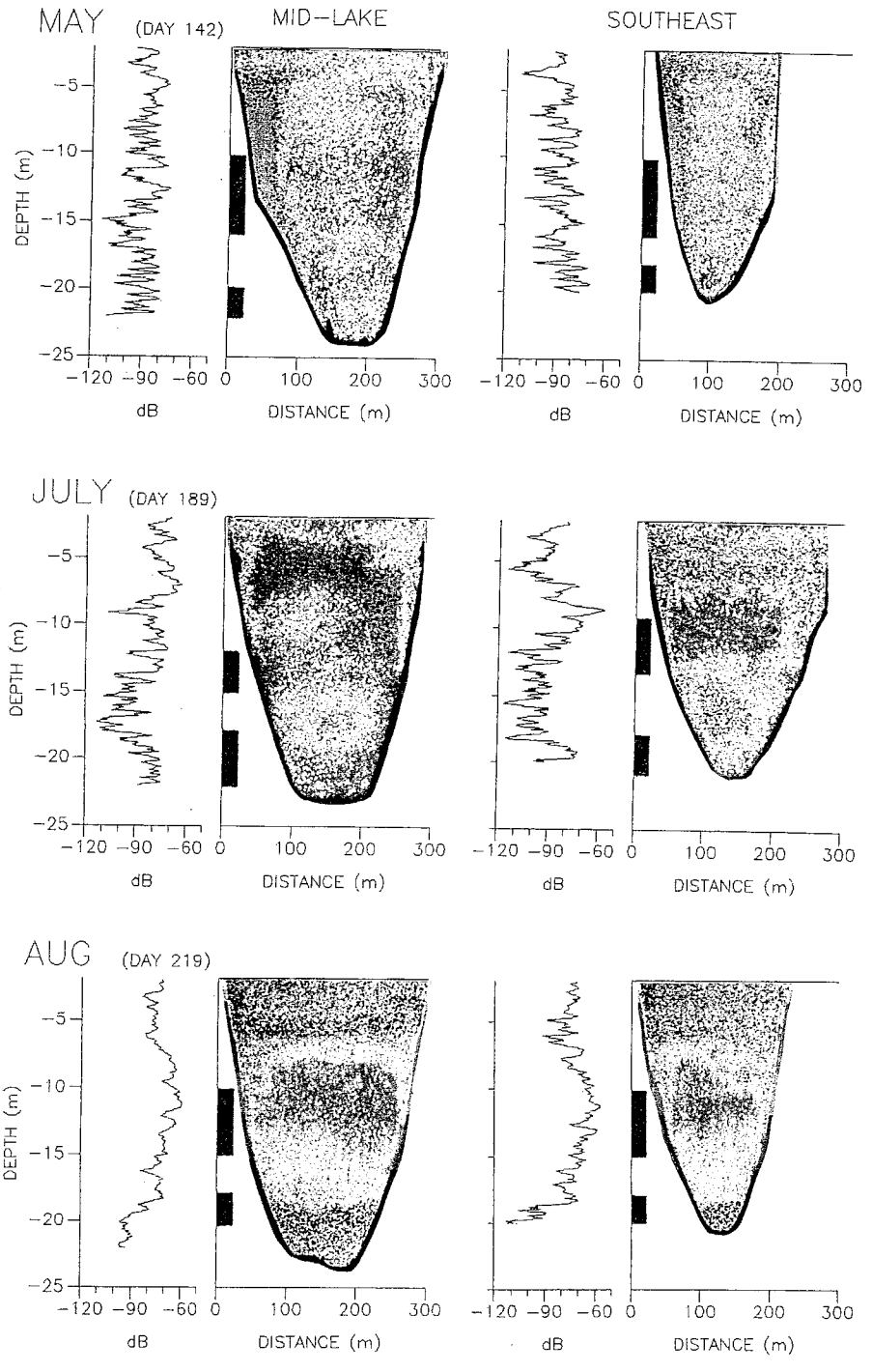


Figure 2. Water temperature, dissolved oxygen and transparency at the mid-lake sampling site in Long Lake during the summer 1991.

hypolimnion. Acoustic cross sections made on three occasions across the mid-lake and southeast sampling points (Fig. 3) illustrate the vertical distribution of zooplankton. The graphs accompanying each cross section show a more quantitative depth profile; constructed from averages of five echo returns from the center of each cross section at the site where *D. pulicaria* were collected. While the layers indicating concentrations of zooplankton were not

Figure 3. Acoustic cross section transects (color photos) and averaged sample site echo profiles (line graphs) of mid-lake and southeast sampling areas on 22 May, 8 July and 7 August, 1991. High zooplankton densities are red, moderate densities are yellow and low densities are blue. Acoustic backscattering was measured as volume scattering strength (corrected for depth) and expressed in terms of decibels (dB).


MAY
(DAY 142)
MID–LAKE
SOUTHEAST
DEPTH (m)
-5
-10
-15
-20
-25
-120 -90 -60
dB
0 100 200 300
DISTANCE (m)
JULY
(DAY 189)
AUG
(DAY 219)

necessarily composed exclusively of *D. pulicaria*, the acoustic profiles indicated separations in the vertical distribution so that each layer could be sampled separately. The rectangles in figure 3 show the depth strata where *D. pulicaria* were collected for genetic analysis. We did not find *D. pulicaria* in the epilimnion during mid-day; epilimnetic layer(s) were composed principally of copepods.

The acoustic cross sections (Fig. 3) indicated that within the general 3-layer framework there was both temporal and spatial variation. For example, on 22 May the zooplankton layers were more clearly defined in the mid-lake sampling area compared to the south east area. On 8 July, the separation between the layers had become more apparent. By 7 August, zooplankton densities had become much lower in the epilimnion and had virtually disappeared from the deepest portions of the hypolimnion where dissolved oxygen was depleted, resulting in a more compressed distribution between 7m and 18m.

Table 1 presents sample size data organized by calendar and Julian dates, sample locations and sample depths from the summer of 1991. The number of observations decreased in the August hypolimnetic samples as the population densities declined. *D. pulicaria* were virtually absent from the metalimnion at the southeast location in September.

Four *PGI* alleles were found in *D. pulicaria* from Long Lake. Alleles were designated F = fast, MF = medium fast, M = medium and S = slow. The rare MF and M alleles were omitted from the analyses.

Early summer samples appeared homogeneous regardless of depth or location. However, by 8 July the proportion of homozygous slow (SS) individuals increased significantly in the hypolimnion at both the mid-lake and southeast locations, while proportions of heterozygous (FS) individuals decreased in the hypolimnion (Fig. 4). Much less variability appeared in the proportions of clones in samples from the metalimnion. By late August there appeared to be differences in clone proportions associated with each location. At this time SS individuals made up a larger proportion of the clones in both the metalimnion and hypolimnion at the southeast location. The 104 homozygous fast (FF) individuals were omitted from figure 4

because they constituted less than 4% of the sample and because no trend was apparent.

Table 1. Sample sizes for the single locus *(PGI)* genotype (= clone) proportions.

		DAY 1991:							
DEPTH	LOC.	5/22 (142	6/3 154	6/16 167	7/8 189	7/23 204	8/7 219	8/19 231	9/20 263)*
Meta-limnion	S. East	94	96	93	92	96	94	90	0
	Mid-lake	93	90	89	92	91	93	89	94
Hypo-limnion	S. East	44	92	88	88	92	59	18	92
	Mid-lake	46	92	93	76	96	32	41	95

Total: 2530

* Julian dates in parentheses

Log-linear modeling of the clone proportions in figure 4 (including FF individuals) indicated that all three main effects (date, location and depth) as well as the date-location and date-depth interactions were significant in explaining the proportions of clones observed over the summer months during 1991. Log-linear results were summarized in table 2 using Pearson Chi-squared statistics and deviance values (G square) for all applicable models, i.e. models with interactions among the explanatory variables included. The only log-linear model that fit the data (Chi-square $P \geq 0.05$) was the second model: [123] [124] [234]. This model states that the response

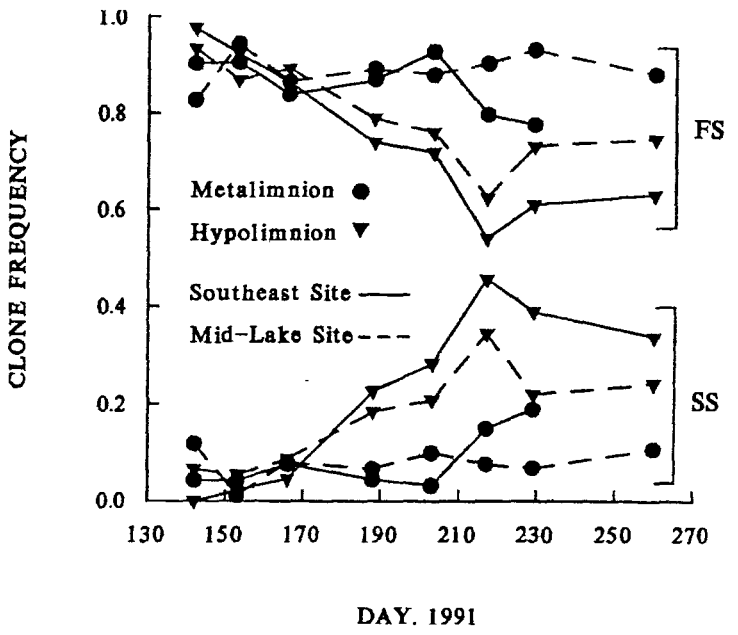


Figure 4. Relative frequencies of heterozygous (FS) and homozygous slow (SS) *D. pulicaria* clones in metalimnetic and hypolimnetic samples from mid-lake and southeast sites in Long Lake during the summer 1991. Sample size data are in table 1.

variable [1] (clone frequency) was related to the explanatory variable cluster [234] (date, location and depth) by date, location and the date-location interaction [123]; and independently by date, depth and the date-depth interaction [124]. Additional explanation of the modeling notation is provided at the bottom of table 2. Examination of the model components (reduction in the G square statistic) revealed that date and depth main effects were greatest; location was less important, although significant, in explaining clone proportions. Further, the date-depth interaction was greater than the date-location interaction in explaining clone proportions during a single summer season.

The frequency of slow (S) alleles at the *PGI* locus increased over the course of the summer in 1991 in populations from both the metalimnion and the hypolimnion, but, the increase was considerably

Table 2. Summary of log-linear models applied to the data in figure 4.

Model	Pearson Chi-sq.	*df*	G sq.	Change in G sq. from base	Change in *df* from base
[123] [124] [134] [234]	21.29	12	25.28	257.12	48
[123] [124] [234]	**22.39**	**14**	**26.47**	**255.93**	**46**
[123] [134] [234]	58.11	26	62.17	220.23	34
[124] [134] [234]	42.67	26	44.04	238.36	34
[124] [13] [234]	44.00	28	49.07	233.33	32
[123] [14] [234]	60.04	28	64.80	217.60	32
[134] [12] [234]	80.00	40	86.68	175.72	20
[124] [234]	49.38	30	55.14	227.26	30
[123] [234]	118.01	30	126.45	155.95	30
[134] [234]	186.30	54	210.59	71.81	6
[12] [13] [14] [234]	82.86	42	88.08	194.32	18
[12] [14] [234]	88.75	44	95.07	187.33	16
[13] [12] [234]	138.85	44	152.98	129.42	16
[13] [14] [234]	187.83	56	212.74	69.66	4
[12] [234]	149.48	46	162.38	120.02	14
[14] [234]	199.62	58	220.02	62.38	2
[13] [234]	274.36	58	274.54	7.86	2
[1] [234]	291.00	60	282.40	Base Model	

[1] -> Clone = Response Variable
[2] -> Date = Explanatory Variable
[3] -> Location = Explanatory Variable
[4] -> Depth = Explanatory Variable

Model Selected: [123] [124] [234]

Intrepretation of the model notation is provided in the following examples:

[1] [234] -> Clone proportions were independent of date, location and depth.
[12] [234] -> Clone proportions were related only to date.
[12] [14] [234] -> Clone proportions were related to date and independently to depth but not location.
[124] [234] -> Clone proportions were related to date and depth and the date - depth interaction but not location.
[123] [124] [234] -> Clone proportions were related to date, location and the date - location interaction; and independentaly to date, depth and the date - depth interaction.

greater in the hypolimnetic population. During the first three sampling dates in May and June the S allele occurred at a frequency of 0.50 in samples of *D. pulicaria* from both the metalimnion and hypolimnion. After a transition period in early July, the proportion of S alleles had changed in late summer to 0.54 in the metalimnion and 0.65 in the hypolimnion.

Examination of clone proportions in early August over a three year period (table 3) revealed that the proportions of SS individuals were consistently greater in the hypolimnion at this time of the year. However, variability was observed from year to year and between locations. The proportion of FF individuals decreased from 1990 to 1992 while there was an increasing trend in the proportion of FS and SS individuals. Additionally, there appeared to be a slightly higher proportion of FS individuals and slightly lower proportions of FF and SS individuals at the southeast location.

Log-linear modeling of the multi-year data in table 3 indicated that all three main effects: depth, location, and year; as well as the location-year interaction were significant in explaining the clone proportions. Log-linear results were summarized in table 4 for all models that fit the data, i.e. that fulfilled the initial criteria of having an acceptable Pearson Chi-squared value ($P \geq 0.05$). While several models met this criteria, model selection procedures including information criteria, information criteria plotted against the number of model parameters, stepwise selection and the principal of parsimony, all converged on the model: [12] [134] [234]. This model indicated that the response variable: [1] (clone frequency) was related to the explanatory variable cluster [234] (depth, location and year) by depth [12] and independently by location, year and the location year interaction [134]. Examination of the model components revealed that depth and year effects were greatest in explaining clone frequencies followed to a lesser, although significant, degree by location and a relatively high location-year interaction.

Table 3. Proportions of single locus *(PGI)* genotypes (= clones) from 825 *D. pulicaria* at two locations and two depths in Long Lake during early August 1990 - 1992. The sample size from each depth at each location is in parentheses.

DATE	LOCATION	CLONES	DEPTH META-LIMNION	DEPTH HYPO-LIMNION
		FF	.33	.12
	Mid-lake	FS	.65	.60
		SS	.02	.28
August 7 1990			(60)	(60)
		FF	.38	.17
	S. East	FS	.60	.80
		SS	.02	.03
			(60)	(60)
		FF	.02	.03
	Mid-lake	FS	.90	.63
		SS	.08	.34
August 7 1991			(93)	(32)
		FF	.05	0
	S. East	FS	.80	.54
		SS	.15	.46
			(94)	(59)
		FF	.03	.07
	Mid-lake	FS	.81	.56
		SS	.16	.37
August 2 1992			(68)	(84)
		FF	.02	0
	S. East	FS	.92	.77
		SS	.06	.23
			(95)	(60)

Table 4. Summary of log-linear models applied to the data in table 3.

Model	Pearson Chi-sq.	*df*	G sq.	Change in G sq. from base	Change in *df* from base
[123] [124] [134] [23‹	7.66	4	7.62	209.47	18
[124] [134] [234]	9.20	6	8.96	208.13	16
[123] [134] [234]	12.83	8	13.79	203.30	14
[124] [234]	16.07	9	16.47	200.62	13
[12] [134] [234]	**16.53**	**10**	**16.64**	**200.45**	**12**
[1] [234]	234.59	22	217.09	Base Model	

[1] -> Clone = Response Variable
[2] -> Depth = Explanatory Variable
[3] -> Location = Explanatory Variable
[4] -> Year = Explanatory Variable

Model Selected: [12] [134] [234]

Discussion

Results from this study indicate that *D. pulicaria* in Long Lake were distributed in two relatively discrete layers during daylight hours, one in the metalimnion and very upper hypolimnion, and the other deeper in the hypolimnion. The change in *D. pulicaria* clonal composition was associated with changing pelagic environmental conditions deep in the hypolimnion. Specifically, an increase in the frequency of homozygous slow (SS) individuals at the PGI locus was associated with oxygen depletion deep in the hypolimnion. The clone frequency remained more constant in the more stable metalimnetic and upper hypolimnetic environment, where phytoplankton photosynthesis produced enough oxygen to more than balance demands by respiration. Further, the increased frequencies of the SS clone in the deep hypolimnion by early August appeared to be a repeatable pattern over the three year study.

In many circumstances the most conspicuous environmental changes in small to medium sized freshwater lakes correlate with

depth, especially during the period of summer thermal stratification. Progressive oxygen depletion during the summer months in the hypolimnion of stratified lakes has been well documented and has been attributed principally to oxidation of organic matter (Hutchinson, 1957). Middle to late summer hypolimnetic oxygen deficits occur annually in the pelagic environment at Long Lake (Megard, 1968; Ross, In prep.).

The hypolimnion at Long Lake was characterized by lower values of both dissolved oxygen and temperature compared to the metalimnion. Both parameters have been shown to affect *Daphnia* clone composition in laboratory experiments. Weider and Lampert (1985) reported that certain clones of *D. pulex* produced less hemoglobin in response to a reduced oxygen environment and were therefore less tolerant of low oxygen conditions than clones that produced relatively higher amounts of hemoglobin. Weider (1985) reported that competitive interactions between clones of *D. pulex* were influenced by oxygen concentration. LaBerge and Hann (1990) also found significant differences in *D. pulex* clonal tolerance to low oxygen concentrations and that this difference was related to clones differing at the hemoglobin locus. Their results also suggested clone specific responses to temperature. Carvalho (1987) found marked thermal differentiation among clones of *D. magna* and reported that seasonal changes in clone frequency were related to differences in thermal tolerance of specific genotypes.

We believe the *D. pulicaria* response observed deep in the hypolimnion of Long Lake was due primarily to oxygen depletion. Clonal proportions did not vary with depth in early summer samples when oxygen concentrations were more uniformly distributed even though the lake was thermally stratified. Most of the above cited literature documented clone specific responses to temperature and/or oxygen in 30 days or less. It was not until early to mid July that proportions of the SS clone increased deep in the hypolimnion, corresponding to the onset of oxygen depletion in the physiologically significant range of 1-3 mg/l (Kring and O'Brien, 1976). Further, the pattern of a progressive increase in SS clone frequency during July and August corresponded to a simultaneous progressive decline in oxygen concentration deep in the hypolimnion, while the thermal environment remained relatively stable.

While depth-related changes in summer clone composition clearly appeared to dominate the data, horizontal location was found to have a significant, albeit lesser, effect on clone frequency. This secondary effect was observed both within a single summer season (1991) and among years in early August. Additionally, there was a significant reduction in FF clone frequency after 1990. However, given the horizontal and annual differences, depth, time and the time-depth interaction were found to explain the largest proportion of the changes in clone frequencies.

Our observations that most changes of clone frequency occurred in the hypolimnion during a period of oxygen depletion provide further support for the concept that spatial and temporal gradients can obviate competitive exclusion and promote the high degree of genetic variation and clonal coexistence that Hebert and Crease (1980) found in parthenogenetically reproducing cladocerans. As LaBerge and Hann (1990) noted, patterns of clone frequency may develop as a consequence of genotypically distinct clones preferentially inhabiting those regions of a pond or lake according to genetically based physiological tolerances. Our observation that clone frequency changes were limited to a changing hypolimnetic environment but relatively constant in a more stable metalimnetic environment also supports the concept of Carvahlo and Crisp (1987) that a population may consist of a mix of ecological generalists and seasonal specialists.

While many studies have observed non-random temporal distribution of *Daphnia* clones, it has been difficult to delineate patterns and to identify environmental conditions that may have produced them. The elusive nature of temporal patterns in *Daphnia* clone structure can be attributed to the complexity of the habitats. Only Carvahlo and Crisp (1987) have been able to demonstrate repeatable temporal patterns. However, they were working in a small (0.1 sq. Km), shallow (1.2 m max. depth) environment. More complex environments such Big Smith Pond (6 m max. depth) that undergo seasonal vertical stratification (Weider, 1984, 1985) provide confounding vertical and horizontal gradients that can obscure subtle temporal patterns of clone succession which may be restricted to particular areas of aquatic habitats.

As Wieder (1985) noted, both spatial and temporal components need to be examined simultaneously in a complex environment in order to provide a more thorough understanding of mechanisms that influence genetic patterns. We attempted to meet these criteria in a deep, but relatively simple, single-basin lake system. To the extent that we found a temporal pattern in clone composition, we believe two elements were critical. The ability to precisely locate and sample persistent vertical bands of zooplankton with acoustic methods in a heterogeneous and dynamic environment was fundamental to this study. Sampling at one meter intervals Muller and Seitz (1993) reported that clonal groups were not homogeneously distributed with depth. Earlier studies such as those reported by Mort and Wolf (1985) and Jacobs (1990) used vertical tows, thus, combining *Daphnia* samples from all depths. The integration of *Daphnia* from more than one vertical habitat could have obscured subtle temporal patterns in these studies. We were able to detect aggregations of zooplankton with sonar before we selected sampling depths. Second the power afforded by log-linear modeling to analyze the effects, relative magnitudes and interactions of the temporal and spatial explanatory variables on clone frequencies was instrumental in discriminating between temporal variation and spatial variation and elucidating the temporal pattern in the hypolimnion.

Two mechanisms not mutually exclusive, could account for the increase in the S allele proportions in *D. pulicaria* populations in the hypolimnion. An increase in fitness of individuals with the S allele in the hypolimnion could explain the increase. An alternative is that the behavior of SS individuals was different from that of other clones. These individuals may select different habitats. Conceivably these individuals could have moved from the dense aggregations in the metalimnion to the deeper layer where densities were lower. Our only estimates of population density are from acoustic profiles that cannot discriminate between *D. pulicaria* and other zooplankton species. Therefore, we cannot decide with any certainty the relative contributions of the micro-evolution hypothesis versus the behavior hypothesis. However, because the proportions of the S allele increased in both layers during summer, we believe that the increase in the proportions of the S allele in *D. pulicaria* in the hypolimnion was more likely a result of a change in gene frequency than a differential preference in habitat.

References

BRANDLOVA, J., BRANDL, Z. AND FERNANDO, C. H. 1972. The Cladocera of Ontario with remarks on some species and distribution. *Can. J. Zool.*, 50, 1373-1403.

CARVALHO, G. R. 1987. The clonal ecology of *Daphnia magna* (Crustacae : Cladocera). II. Thermal differentiation amoung seasonal clones. *J. Anim. Ecol.*, 56, 469-478.

CARVALHO, G. R. AND CRISP, D. J. 1987. The clonal ecology of *Daphnia magna* (Crustacae : Cladocera). I. Temporal changes in the clonal structure of a natural population. *J. Anim. Ecol.*, 56, 453-468.

CERNY, M. AND HEBERT, P. D. N. 1993. Genetic diversity and breeding system variation in *Daphnia pulicaria* from North American lakes. *Heredity,* 71, 497-507.

HEBERT, P. D. N. 1974. Enzyme variability in natural populations of *Daphnia magna* II. Genotypic frequencies in permanent populations. *Genetics,* 77, 323-334.

HEBERT, P. D. N. 1978. The population biology of *Daphnia* (Crustacea, Daphnidae). *Biol. Rev.*, 53, 387-426.

HEBERT, P. D. N. AND BEATON, M. J. 1989. *Methodologies for allozyme analysis using cellulose acetate electrophoroesis.* Helena Laboratories, Beaumont, Texas.

HEBERT, P. D. N. AND CREASE, T. J. 1980. Clonal coexistance in *Daphnia pulex* (Leydig): Another planktonic paradox. *Science*, 207, 1363-1365.

HEBERT, P. D. N. AND WARD, R. D. 1976. Enzyme variability in natural populations of *Daphnia magna*. Ecological differention and frequency changes of genotypes at Audley End. *Hederity,* 36, 331-341.

JACOBS, J. 1990. Microevolution in predominantly clonal populations of pelagic *Daphnia* (Crustacae: Phyllopoda): Selection, exchange, and sex. *J. Evol. Biol.*, 3, 257-282.

KRING, R. L. AND O'BRIEN, W. J. 1976. Effect of varying oxygen concentrations on the filtering rate of *Daphnia pulex*. *Ecology* 57, 808-814.

LABERGE, S. AND HANN, B. J. 1990. Acute temperature and oxygen stress amoung genotypes of *Daphnia pulex* and *Simocephalus vetulus* (Cladocera, Daphiniidae) in relation to environmental conditions. *Can. J. Zool.*, 68, 2257-2263.

LYNCH, M. 1983. Ecological genetics of *Daphnia pulex*. *Evolution,* 37 358-374.

LYNCH, M. 1984. The genetic structure of a cyclical parthenogen. *Evolution*, 38, 186-203.

MEGARD, R. O. 1967. *Limnology, primary productivity, and carbonate sedimentation of Minnesota lakes.* Limnological Research Center, U. of Minnesota. Interim Report No. 1.

MEGARD, R. O. 1968. *Planktonic photosynthesis and the environment of calcium carbonate deposition in lakes.* Limnological Research Center, U. of Minnesota. Interim Report No. 2.

MEGARD, R.O., KUNS, M. M., WHITESIDE, M. C. AND J. A. DOWNING. (In prep.) Spatial distributions of zooplankton during coastal upwelling in western Lake Superior.

MEGARD, R.O., PEARSON, K. A. AND D. A. LARSEN. 1989. A microprocessor based sonar data acquisition system for fish and plankton research. *Proc. Inst. Acoustics Symposium* (Edinborgh), Vol. 11.

MORT, M. A. AND WOLF, H. G. 1985. Enzyme variability in large-lake *Daphnia* populations. *Heredity*, 55, 27-36.

MOYLE, P. B. 1969. *Ecology of the Fishes of a Minnesota Lake with Special Reference to the Cyprinidae.* Ph. D. Thesis, University of Minnesota.

MULLER, J. AND SEITZ, A. 1993. Habitat partitioning and differantial vertical migration of some *Daphnia* genotypes in a lake. *Arch.Hydrobiol. Beith.,* 39, 167-174.

HUTCHINSON, G. E. 1957. *A Treatise on Limnololy.* Vol.1. John Wiley and Sons, New York.

SCHMID, W. D. 1965. Distribution of aquatic vegetation as measured by line-intercept with SCUBA, *Ecology* 46, 816-823.

WEIDER, L. J. 1984. Spatial heterogeneity of *Daphnia* genotypes: Vertical migration and habitat partitioning. *Limnol. Oceanogr.* 29, 225-235.

WEIDER, L, J. 1985. Spatial and temporal genetic heterogeneity in a natural *Daphnia* population. *J. Plankton Res.* 7, 101-123.

WEIDER, L. J. 1989. Spatial heterogeneity and clonal structure in Arctic populations of apomictic *Daphnia. Ecology*, 70, 1405-1413.

WEIDER, L. J. AND HEBERT, P. D. N. 1987a. Microgeographic genetic heterogeneity of melanic *Daphnia pulex* at a low-Arctic site. *Heredity* 58, 391-399.

WEIDER, L. J. AND HEBERT, P. D. N. 1987b. Ecological and physiological differentiation amoung low-Arctic clones of *Daphnia pulex. Ecology* 68, 188-198.

WEIDER, L. J. AND LAMPERT, W. 1985. Differential response of *Daphnia* genotypes to oxygen stress: respiration rates, hemoglobin content and low-oxygen tolerance. *Oecologia* 65, 487-491.

YOUNG, J. P. W. 1979a. Enzyme polymorphism and cyclic parthenogenesis in *Daphnia magna.* II. Heterosis following sexual reproduction. *Genetics* 92, 971-982.

YOUNG, J. P. W. 1979b. Enzyme polymorphism and cyclic parthenogenesis in *Daphnia magna*. I. Selection and clonal diversity. *Genetics* 92, 953-970.

CHAPTER 2

Predation

Introduction

During the past 30 years the effects of predation on lacustrine communities has received a great deal of attention. Beginning with seminal papers by Hrbacek et al. (1962) and Brooks and Dodson (1965) and continuing with the development of the trophic cascade paradigm by Carpenter et al. (1985), predation has been invoked as major force structuring the plankton assemblage in aquatic communities. As Kerfoot et al. (1980) noted, "The biased foraging of dominant predators, such as visually feeding fishes and grasping copepods constitutes the force, in conjunction with competition, which drives prey toward certain adaptive modes". In the preface of their book on predation in aquatic communities, Kerfoot and Sih (1987) distinguish between the direct effects of predation on organismal traits, population dynamics and community structure, and the indirect effects of predation upon trophic cascades. It is the former, more restrictive, view of predation that I will be concerned with in this chapter, perhaps more closely aligned with the ideas on predation in Zaret (1980). However, I examined the effect of predation on clones of the same species, whereas, he addressed differential predation among species differing in size and among developmental stages within a species.

Predators have been shown to affect the structure of freshwater communities at several levels of organization. At the interspecific level, larger species are usually replaced with smaller species in the presence of vertebrate predators that preferentially select large prey (Brooks and Dodson, 1965; Wells, 1970). Hebert and Loaring (1980) reported that the presence or absence of copepod predator explained the occurrence of two species of *Daphnia* as well as the distribution of a vulnerable and a less vulnerable morph of a third *Daphnia* species in 135 Arctic ponds.

At the intraspecific level, cladocerans possess considerable phenotypic plasticity in life history, morphology and behavior due to predation. Changes in life history such as delayed maturity and rapid

juvenile growth in *D. pulex* were reported recently by Black (1993). Predator-induced changes, including helmet and crest formation, and changes in body length, have been widely documented in the Cladocera, as reviewed by Havel (1987). Both experimental evidence (Leibold, 1990) and distributional data (Leibold and Tessier, 1991), indicated that the vertical distribution of *D. pulicaria* was associated with predation intensity by planktivorous fishes. The phenomena of vertical migration in zooplankton as a predator avoidance behavior has been addressed extensively in the literature (see Chapter 5).

Recently some of these phenotypic changes induced by predators have been linked to changes in the distribution of genotypes. Spitz (1992) found that, predator-induced formation of neck teeth and life history changes in *D. pulex,* varied by genotype but were not closely associated with each other. Wilson and Hebert (1993) reported both distributional and experimental evidence of differential vulnerability to predation of conspecific genotypes of *D. pulex*. As they noted, tradeoffs between clones within a species parallel those observed between species because, "The clonal system acts as a simplified representation of interactions within and among zooplankton species". Further they noted, "As cladoceran populations reproduce parthenogenetically, small differences in fitness can become amplified over several generations, as long the directionality of the fitness remains unchanged".

Zaret (1980) dispelled the notion that indiscriminate filter feeding by fish is an important component of predation in most freshwater systems. His review argued persuasively that indiscriminate filter feeding is rare; most vertebrate predation in fresh water consists of highly selective particle feeding. He discussed the three major phenotypic characteristics, (size, pigmentation and motion) that increased the probability of predation on zooplankton by vertebrate particle feeders. Galbraith (1967) documented size-selective predation by rainbow trout *(Salmo gairdneri)* on *D. pulex*, a species very closely related to *D. pulicaria*. His data indicated that *D. pulex* larger than 1.3 mm were especially vulnerable to predation.

The objectives of my observations on predation were threefold. First I wanted to confirm an earlier casual observation that *D.*

pulicaria made up a large proportion of the rainbow trout diet and that the trout seemed to be selectively feeding on the largest *D. pulicaria* from the zooplankton community in Long Lake. Second, I hypothesized that the trout predation may have disproportionately targeted a specific clone. Finally, I formed a series of three hypotheses that related clone specific predation to the phenotypic characters of size and pigmentation that Zaret (1980) had identified as likely to make zooplankton more vulnerable. I hypothesized that the body size of a clone made its adults more likely to occur in the stomachs of trout than the general population of *D. pulicaria* in the lake. The other two hypotheses dealt with clone specific pigmentation and the possibility that either general body pigmentation, or the presence of the relatively highly pigmented eggs and embryos, or both of these types of pigmentation in concert could have made certain clones more vulnerable to predation.

Two distinct color morphs of *D. pulicaria* have been observed in Long Lake. During earlier sampling (Chapter 1), a transparent morph was observed to be most abundant at most times, locations and depths. However, occasionally larger proportions of pigmented individuals were observed. Further this whole body pigmentation appeared to be of two distinct types. The first, most commonly found in deep samples, was identified to be hemoglobin (Chapter 3). The second type of pigmentation, occurring only in summer samples in June and early July, was more orange and appeared like an iron hydroxide. It is this second, as yet unidentified, type of pigmentation that I will be referring to in this chapter. Additionally eggs and embryos in the brood chamber consistently exhibited an intense green color that was clearly visible through the carapace of both transparent and pigmented adults.

Prior to and concurrent with the predation observations, data were collected on three additional topics related to methods. Twenty eight additional enzymes were screened in an attempt to resolve the *PGI* clones into subgroups. It was anticipated that additional resolution of clone structure could enhance the detection of changes in clone distribution relative to environmental changes and predation, as well as with the competition experiments and physiological observations. Additionally, because of time constraints, it was often necessary to freeze samples of *D. pulicaria* for up to six months prior to making phenotypic observations and

carrying out electrophoretic techniques. Therefore, it was necessary to evaluate the effects of the freezing on comparisons with fresh *D. pulicaria.* Also, specifically for the predation observations reported in this chapter, it was necessary to evaluate the variation in stomach contents among the eight trout selected for observation.

The observations reported in chapter one were conducted during summer, 1990-1992. In October 1992 the Minnesota Department of Natural Resources increased the number of rainbow trout fingerlings that were stocked in Long Lake. From 1981 to 1991 the stocking rate had been stable, between 7,500 and 8,000 fingerlings most years. The rate was increased to 14,500 in October 1992 and to 22,500 in October 1993 (see Chapter 6 for additional details).

Methods

Trout were caught by angling with hook and line at depths ranging from 13 m to 16 m, approximately 100 m west of the southeast sampling area (Fig. 1, Chapter 1). Water depths ranged from 16 m to 18 m in the area where fish were captured. Twelve fish were collected between 1000 and 1500 CDT on 23 June 1993. The stomachs from 8 trout were randomly selected and the contents examined. Twenty four *D. pulicaria* from the anterior portion of each stomach were removed for detailed analysis. Half of each sample was examined within 24 h, the remaining 12 *D. pulicaria* from each stomach were frozen at -19 C for analysis up to 6 months later.

Zooplankton from Long Lake were collected on 2 occasions in order to make comparisons with the trout stomach contents. A collection was made between 13 m and 15 m at 1700 CDT on 24 June, 1993, at the same location where the trout were caught the previous day. *D. pulicaria* from this sample were used to compare the size, color and clone distributions in free living *D. pulicaria* with those in trout stomachs. Observations from this collection were made on 96 adult *D. pulicaria* within 24 hours while the sample was fresh, another 96 adults and 96 juveniles were frozen for later analyses. Collections were also made on 28 June, 1993 at 2 m intervals between 8 m and 22 m at the mid-lake sampling site (Fig. 1, Chapter 1) to more-generally characterize the zooplankton

community and contrast it with the trout stomach contents. These samples were preserved in formaldehyde and examined within 3 days. Zooplankton were not analyzed from the upper 8 m of the water column because the water temperatures exceeded 13.6 C, the upper temperature limit preferred by rainbow trout (Ferguson, 1958); therefore, this region was not considered available to trout. All zooplankton were collected with the closing plankton net described in chapter one except that a smaller mesh (130 *u*) filter was used so that smaller sized species as well as smaller *D. pulicaria* could be retained for comparisons.

Temperature and dissolved oxygen were measured as in Chapter 1. Field techniques used for acoustic sampling of zooplankton distribution also remained as described in chapter 1. However, a simple black and white echogram, copied directly from the sonar display, depicted the vertical distribution of zooplankton adequately, and involved considerable savings in time and cost compared to the color images used to display zooplankton distributions in Chapter 1.

Laboratory and electrophoresis methods were the same as those detailed in chapter 1 with several additions. Twenty eight additional loci were screened for enzyme activity and variability using the methods of Hebert and Beaton (1989). Shadowing, which appeared on the cellulose acetate gels with *D. pulicaria* homogenates from the trout stomach contents, was eliminated by rinsing each individual in a drop of distilled water before grinding. The size, color and reproductive condition were recorded as individual *D. pulicaria* were placed in applicator wells (Helena Laboratories), before grinding. Size was measured to the nearest 0.3 mm with the aid of a clear plastic ruler glued to the applicator wells. Color was scored as either transparent or pigmented depending on the presence or absence of pigment. Reproductive condition was categorized by the presence of eggs or embryos in the brood chamber and nonreproductive if the brood chamber was empty. The 'embryo' stage was separated from the 'egg' stage by the differentiation of the head from the thorax and the presence of a compound eye. Observations on size and reproductive condition were made with a dissecting microscope.

Pigment extraction and quantitative analyses followed the methods of Landon and Stasiak (1983) using a Bausch and Lomb Spectronic 70 spectrophotometer. Adult *D. pulicaria* separated into groups of 20 individuals, categorized as either transparent or pigmented, were used for these analyses because of the small amount of pigment from each individual.

Results

Of the additional 28 loci screened, activity and variation was found only at the phosophglucomutase *(PGM)* locus (Table 1). Variation at the *PGM* locus was limited to two alleles. Two additional, but very rare, alleles were discovered at the *PGI* locus. These were designated MS = medium slow and US = ultra slow and brought to 6, the total number of alleles at the PGI locus (Chapter 1). The variation at the *PGM* locus permitted the resolution of *PGI* clones into 23 clonal groups, but, sample sizes in most groups were very small. No trends were apparent that associated *PGM* allele frequencies with the other variables of interest such as predation, nor the phenotypic characters of size, reproduction and pigmentation, therefore, the data were recategorized using only the *PGI* locus prior to the analyses.

Before I examined hypotheses concerning the relationships between predation and clone frequency, and the relationships among clone type and phenotypic characters such as pigmentation, size and reproductive condition, I examined other sources of variation. These preliminary analyses indicated that there was significant (t-test $P = 0.0006$) variation among *D. pulicaria* mean sizes in the trout stomach samples. Mean size ranged from 2.0 mm to 2.6 mm, most of the variability was due to just one individual trout. We did not have the fish to measure, but the fishermen who provided the stomachs said that all the trout were of the same size class, approximately 0.5 Kg. Neither pigmentation nor clone frequency in *D. pulicaria* were found to vary significantly in the stomach contents among trout.

Table 1. Enzyme screening with *D. pulicaria* from Long Lake.

ENZYME	Abbr.*	Activity	Variation
Aconitase	ACON	X	
Adenylate Kinase	AK	X	
Alcohol Dehydrogenase	ADH	X	
Aldehyde Oxidase	AO	X	
Alkaline Phosphotase	ALP	X	
Amylase	AMY (3)	X	
Arginine Phosphokinase	APK	X	
Esterase	EST (2)	X	
Fumarate Hydratase	FUM	X	
Glucose-6-Dehydrogenase	G6PDH	X	
Glutamate-Oxaloacetate Transf.	GOT (2)	X#	
Glyceraldehyde-3-Phosphate Deh.	G3PDH	X	
Glycerol-3-Phosphate Deh.	GPDH	X	
Hemoglobin	HEM	X	
Hexokinase	HEX	X	
Isocitrate Dehydrogenase	IDH	X	
Lactate Dehydrogenase	LDH	X	
Leucine Aminopeptidase	LAP		
Malate Dehydrogenase	MDH	X	
Malate Dehydrogenase	ME	X	
Mannose Phosphate Isomerase	MPI	X#	
Peptidase	PEP (2)	X	
Phosphoglucomutase	PGM	X	X
6-Phosphogluconate Deh.	6PGDH	X	
Phosphoglocose Isomerase	PGI	X	X
Superoxide Dismutase	SOD		
Trehalase			
Triose Phosphate Isomerase	TPI	X	
Xanthine Dehydrogenase	XDH	X	

Numbers in parentheses indicate the number of loci if more than one.

* Hebert and Beaton, 1989.

From M. Boileau (Pers. Com.).

Results with AO, APK, FUM, LDH, PGM and PGI confirmed by M. Boileau (Pers. Com.).

The process of freezing had significant effects on three of the four parameters of interest. *D. pulicaria* frozen for later observation were significantly (t-test $P = 0.003$), smaller (2.3 mm) than fresh individuals (2.4 mm). Frozen samples had significantly (Chi-square $P = 0.02$) fewer individuals with eggs. Eighteen of 96 (19%) of the fresh *D. pulicaria* contained eggs but only to 5 of 87 (6%) observed after freezing. Likewise, frozen samples had significantly smaller (Chi-squared $P = 0.05$) proportions of pigmented individuals. One hundred thirty nine of 192 (72%) of the fresh were pigmented compared to 113 of 180 (63%) after freezing. No significant difference (Chi-square $P = 0.70$) was detected in the distribution of *PGI* clones in fresh versus frozen samples. Because freezing was observed to cause significant reductions in the estimates of size, reproduction and pigmentation, analyses of these parameters were restricted to fresh individuals.

Long Lake was thermally stratified when predation observations were made in late June, 1993. Epilimnetic temperatures ranged from 18.3 C at the surface to 16.3 C at 7 m. A thermocline existed from 8 m (14.9 C) to 12 m (7.2 C). Temperatures in the hypolimnion dropped gradually from 6.7 C at 13 m to 4.9 C at 23 m. An acoustic profile (Fig. 1) indicates that the zooplankton distribution closely mirrored the thermal regime. A relatively dense layer existed in the epilimnion down to 7 m. The lighter colored region from 8 m to 14 m indicated a depth increment in the metalimnion with considerably lower zooplankton biomass. The dark vertical lines were electronic interference. A dark layer between 14 and 22 m corresponded to increased zooplankton biomass in the hypolimnion. The narrow, light colored layer between 22 m and 23 m indicated fewer zooplankton, likely do to depletion of dissolved oxygen near the lake bottom at 23 m. I did not collect reliable oxygen data at this time do to a malfunctioning meter, but limnological data from late June in other years indicated that dissolved oxygen levels were near saturation in the epilimnion and metalimnion and were greater than 3 mg/l in the hypolimnion except within 1 m of the bottom.

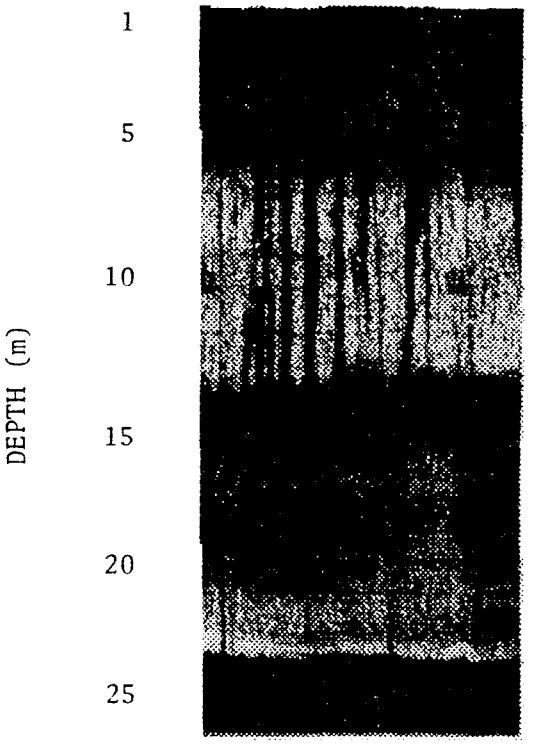


Figure 1. Echogram of zooplankton distribution in Long Lake on 28 June, 1993.

A dramatic shift in the composition of the zooplankton occurred at 14 m. corresponding to the change in backscattering in figure 1. The zooplankton community between 8 m and 14 m was dominated by copepods (Fig. 2); *Bosmina sp.* and juvenile *D. pulicaria* were present at lower densities. No adult *D. pulicaria* were observed in samples taken above 14 m. Below 14 m the zooplankton was made up entirely of *D. pulicaria*, where adults outnumbered juveniles, and transparent adults outnumbered pigmented adults (Fig. 3). The cumulative zooplankton density of 2.90 individuals/L between 8 m and 14 m (Fig. 2) was considerably greater than that of 1.76 individuals/L between 14 m and 22 m (Fig 3) but the animals at 14-22 m were much larger. This result may seem to conflict with the acoustic distribution (Fig. 1). However, the acoustic record depends on both the number and size of the sound backscatterors and it is a function of the sixth power of the length of zooplankton in this size range, (Clay and Medwin, 1977). Thus, fewer large animals appear as proportionately more biomass compared to more small zooplankton. I did not measure the copepods nor the *Bosmina sp.*, but the adult *D. pulicaria* were almost twice as long as the juvenile *D. pulicaria* (see below) and the juveniles were at least as large or larger than the other zooplankton. Thus, while concentrations were greater between 8 m and 14 m, acoustic backscatter from the large adult *D. pulicaria* was greater in the deeper layer between 14 m and 22 m.

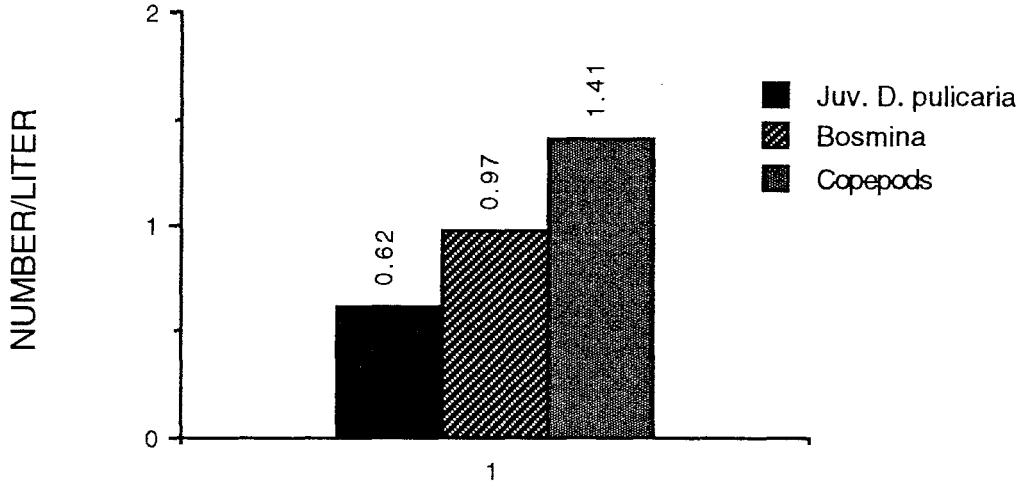


Figure 2. Zooplankton densities between 8m and 14m on 28 June, 1993.

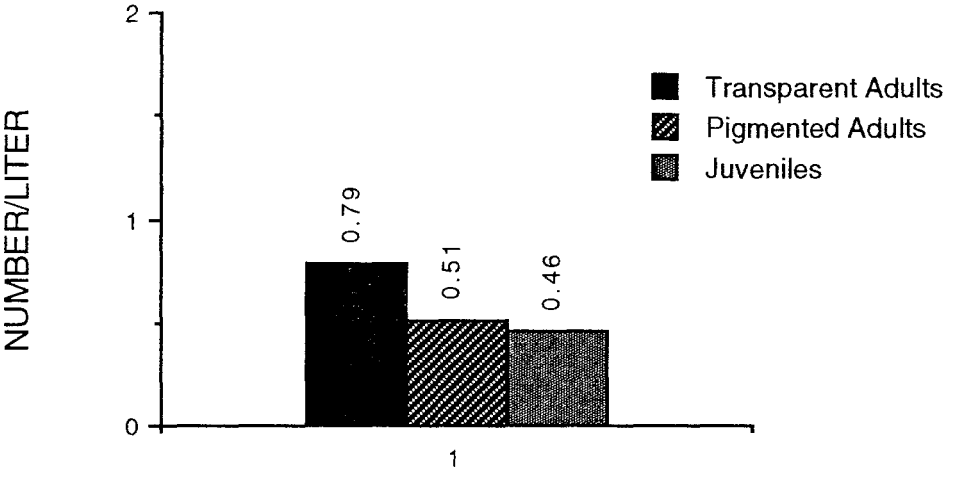


Figure 3. D. pulicaria densities between 14m and 22m on 28 June, 1993.

Many of the adult *D. pulicaria* exhibited a distinct translucent orange color that appeared much less red than the color of hemoglobin (Chapter 3). A hemoglobin extraction and a scan of light absorbance from 350 nm to 710 nm comparing transparent and pigmented adults was inconclusive. The only distinct peak, at 413 nm, corresponded to hemoglobin. Throughout the spectrum the sample from transparent individuals consistently absorbed more light than the pigmented sample. A secondary peak occurred at 550 nm in the transparent but not the pigmented extract.

Rainbow trout were highly selective, feeding almost exclusively on adult *D. pulicaria*. Except for the worms used for bait, the stomachs of all eight trout contained only pigmented *D. pulicaria*, with a mean length of 2.4 mm. *D. pulicaria* in Long Lake exhibited a bimodal size distribution (Fig. 4); mean adult length was 2.4 mm and mean juvenile length was 1.6 mm. The mean length of *D. pulicaria* from trout stomachs was identical that of adults from the lake (Fig. 4). Trout clearly selected individuals from the upper lobe of the bimodal distribution of sizes of *D. pulicaria* available in Long Lake. Selection for pigmented adults was also clearly evident. Only 39% of adult *D. pulicaria* were pigmented in the free-living population in Long Lake (Fig. 3), but all of the individuals from the trout stomachs were pigmented.

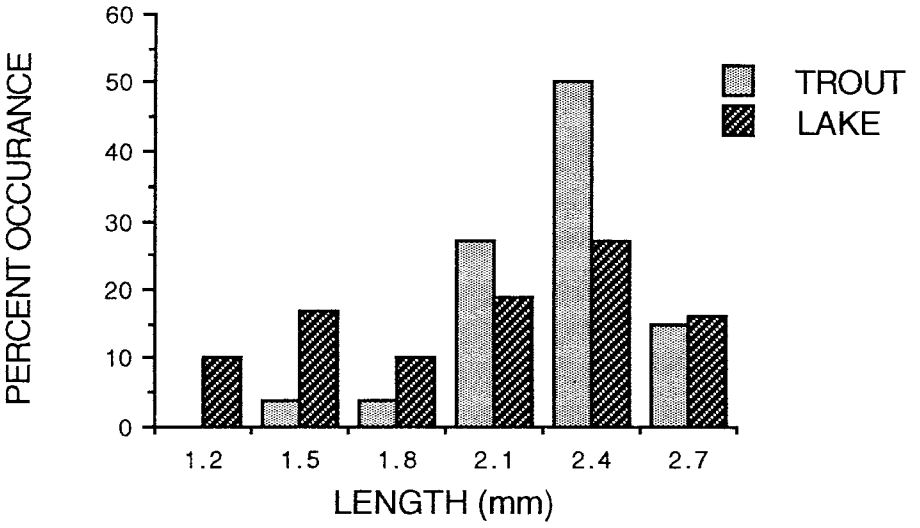


Figure 4. Comparison of the size distributions of *D. pulicaria* from rainbow trout stomachs and free-living in Long Lake.

The frequency of clones in trout stomachs was significantly (Chi-square $P = 0.002$) different from the frequency in the lake (Table 2). The fast/slow (FS) heterozygote was most frequent in both samples. While the slow/slow (SS) homozygote represented a relatively small proportion of the individuals in either sample, it was 6 times more likely to occur in the sample from the trout stomachs. Conversely, the medium-fast/slow (MFS) clone was 3.5 times less likely to be preyed upon by rainbow trout.

Table 2. Distribution of clones in *D. pulicaria* from trout stomachs and free-living in Long Lake.

PGI CLONE	TROUT STOMACH	LONG LAKE	RATIO T/L
FF	2	2	1.0
MFS	6	21	0.3
FS	74	69	1.1
SS	12	2	6.0
Other	0	1	0.0
Total	94	95	

A pairwise examination of the distribution of pigmentation, reproduction and size with clone type failed to indicate any significant (Chi-square $P \leq 0.05$) associations between genotype and these phenotypic characters. Analyses of the distribution of pigmentation in *PGI* clones (Table 3) was limited to *D. pulicaria* from the lake because all of the *Daphnia* from the trout stomachs were pigmented. The MFS clone was slightly more likely to be transparent than the FS clone, sample sizes of the other clones were very small. The analyses of the distribution of reproduction (Table 4) was also limited to clones from the Lake because there were large numbers of free eggs and embryos in the trout stomachs. Therefore, I could not determine reproductive condition of the *D. pulicaria* in the trout stomach samples with any certainty. No trends were apparent in the reproduction data (Table 4). Neither pigmentation (Chi-square $P = 0.18$) nor reproductive condition (Chi-square $P = 0.50$) were found to

Table 3. Distribution of clones in transparent and pigmented *D. pulicaria* from Long Lake.

PGI CLONE	TRANSPARENT	PIGMENTED
FF	0	2
MFS	14	7
FS	36	33
SS	2	0
Other	1	0
Total	53	42

vary significantly by clone type. A three-way log-linear analysis combining pigmentation and reproduction distributions by clone also indicated that the three parameters were distributed independently in *D. pulicaria* collected from the lake. Likewise a single factor ANOVA indicated that I could not reject the null hypothesis (P = 0.08) of equality of mean lengths among *PGI* clones from Long Lake.

Table 4. Distribution of clones in *D. pulicaria* from Long Lake that contained either eggs or embryos, or had empty brood chambers.

PGI CLONE	EGGS	EMBRYOS	NONREPRODUCTIVE
FF	0	0	2
MFS	3	5	13
FS	14	14	41
SS	0	0	2
Other	1	0	0
Total	18	19	58

Discussion

The relatively low genetic diversity observed in *D. pulicaria* from Long Lake is consistent with the previous observations on this species. I found variability at only two of 33 loci. In a survey of genetic variation in *D. pulicaria* from 55 North American lakes, Cerney and Hebert (1993), found variation limited to the same two loci *(PGI* and *PGM)* in most lake populations. They further reported that four of the six loci commonly polymorphic in members of the *D. pulex* complex were invariant in *D. pulicaria.* Compared to the variation within-loci reported by Cerney and Hebert (1993) I found slightly more variation at the *PGI* locus (6 vs. 4 alleles) and less variation at the *PGM* locus (2 vs. 4 alleles). Three of the *PGI* loci (M, MS and US) were very rare and contributed little to my analyses.

Compared to *PGI,* the *PGM* locus is evidently not as closely linked to other loci that control characters of ecological significance. Variation at the *PGM* locus permitted resolution of the *PGI* clones into subgroups. However, this additional information did not help to clarify associations among phenotypic characters and clone types; nor did it alter the interpretation of the observations on clone specific predation. Cerney and Hebert (1993) found regional shifts in the frequency of *PGI* alleles, but, no obvious geographical patterning of *PGM* alleles in *D. pulicaria.*

Other preliminary analyses indicated, that due to significant changes in the distributions of size, pigmentation and reproductive condition associated with the freezing process, it would be desirable to make the observations on these phenotypic characteristics prior to freezing. However, the freezing and thawing process did not appear to result in any change to the enzymes that would cause misinterpretation of the electrophoresis results.

The thermal regime in Long Lake would have allowed rainbow trout to forage on denser concentrations of small zooplankton in the metalimnion; however, the stomach contents indicated that the trout were feeding exclusively on larger adult, pigmented *D. pulicaria* confined to the hypolimnion (14-22m). Preference for the largest-sized zooplankton has been thoroughly documented for planktivorous fish in general (Brooks and Dodson, 1965; Wells, 1970; Werner and

Hall, 1974; O'Brien et al., 1976; Zaret, 1980) and specifically for rainbow trout (Galbraith, 1967).

While rainbow trout clearly preferred pigmented adult *D. pulicaria*, and while the central hypothesis of clone-specific predation within adult *D. pulicaria* was accepted, a mechanism relating phenotypic characters to genotypes that could explain this preference was not observed. Zaret (1980) demonstrated the importance of size, pigmentation and motion as the parameters that could make zooplankton more vulnerable to predation. My analysis of adult *D. pulicaria* found no significant associations among genotype and size, or genotype and either of two types of pigmentation. I could not evaluate the relationship of motion to genotype with these field studies. O'Brien et al. (1976) reported that bluegill sunfish would always choose moving *Daphnia* over motionless *Daphnia*, but they noted that relatively little work has been done evaluating prey motion as a factor in predation. This is especially evident for prey in the size range of large Cladocera like *D. pulicaria*. Kerfoot et al. (1980) reported that small cladocerans such as *Bosmina* and *Chydorus* invoke a defense of playing dead (akenesis) when attacked by invertebrate predators. Reviewing the literature, Zaret (1980) concluded prey motion has not been as well defined as other parameters such as size and pigmentation that contribute to prey visibility.

The relative contribution of rainbow trout feeding to the overall predation pressure on *D. pulicaria* in Long Lake cannot be assessed directly with the data that I collected. In addition to rainbow trout several other fish species occur in Long Lake (Moyle, 1969) that have been reported to graze on large Daphnids. These include yellow perch *(Perca flavescens)*, (Galbraith, 1967; Mills and Forney, 1983), bluegill sunfish *(Lepomis macrochirus)*, (Werner and Hall, 1974; O'Brien et al., 1976), and mimic shiners *(Notropis volucellus)* (Moyle, 1969). The larval stages of many other freshwater fish are planktivorous and likely also contribute to the predation pressure on *Daphnia* in Long Lake. While, none of these other fish species are necessarily restricted from deeper, cooler waters, rainbow trout is the only cold water species in this assemblage that is limited to the cooler waters where the large *D. pulicaria* are normally found during daylight hours.

Invertebrates very likely did not play a significant role in predation on *D. pulicaria. Chaoborus* has been shown to have significant effects on *Daphnia* populations (Spitz, 1991; Black 1993); however, we have never observed *Chaoborus* in Long Lake. The only potential invertebrate predator found in Long Lake was *Leptodora sp.*, but it was exceedingly rare.

The introduction of more rainbow trout beginning in the fall of 1992 provided indirect evidence that predation by trout was a substantial proportion of the predation on *D. pulicaria*. These effects are detailed in Chapter 6. Briefly summarized, however, increases in trout stocking were associated with the 1993 disappearance of the shallow layer of *D. pulicaria,* that was so conspicuous during the summer from 1990 to 1992, and the virtual collapse of the entire *D. pulicaria* population in early 1994.

Concurrent with the increase in trout stocking and the disappearance of the metalimnetic layer of *D. pulicaria* was a conspicuous increase in the frequency of the of the MFS clone. From 1990 to 1992 the MFS clone made up only a small percentage of the population (Chapter 1). However, by June 1993 this clone represented 22% of the adults in the Lake. This observation coupled with the observation that this clone was significantly under-represented in the trout stomachs, is strong evidence that clone-specific predation by rainbow trout can had substantial effect on the genetic composition of the *D. pulicaria* population. Additionally, even though the SS clone made up only 2% percent of the population in the lake, it was significantly over-represented in the trout stomach contents. The results from chapter 1 suggested that the proportion of this clone was largest in the deep layer, where light levels were lowest. Predation pressure from rainbow trout, a visual particle feeder, was probably less effective lower in the water column.

During the past 35 years the limnological literature has thoroughly documented the effect of vertebrate predators on zooplankton, especially the disproportionate grazing on large species such as *D. pulicaria.* My observation that the shallow layer of *D. pulicaria* disappeared following an increase in stocking of rainbow trout closely paralleled those of Leibold (1990) and Leibold and Tessier (1991), suggesting that predation was a significant force on *D. pulicaria.* Additionally the observation that *D. pulicaria*

virtually disappeared from Long Lake with further increases of rainbow trout indicated that predation, specifically by rainbow trout, was a significant force on *D. pulicaria*. The SS clone was instrumental in structuring the *D. pulicaria* population the previous three summers (Chapter 1). It made up only a small proportion of the population when the predation observations were made, but, it was over-represented in the stomach contents, indicating that this clone may be especially vulnerable to predation. Likewise, even though I could not establish associations linking phenotypic vulnerability to the disproportionate distribution of genotypes in trout stomachs, the under-representation of the MFS clone in stomach contents indicated that grazing by rainbow trout favored a specific clone. Furthermore, the observation that the proportion of this same clone increased substantially in the population following the initial increase in stocking of trout, argues persuasively that predation by rainbow trout can genetically structure the population of *D. pulicaria*.

References

BLACK, A. R. 1993. Predator-induced phenotypic plasticity in *Daphnia pulex:* life history and morphological responses to *Notonecta* and *Chaborus. Limnol. Oceanogr.*, 38, 986-996.

BROOKS, J. L. AND DODSON, S. I. 1965. Predation, body size and composition of plankton. *Science,* 150, 28-35.

CARPENTER, S. R., KITCHELL, J. F. AND J. R. HODGSON. 1985. Cascading trophic interactions and lake productivity. *BioScience,* 35, 634-639.

CERNY, M. AND HEBERT, P. D. N. 1993. Genetic diversity and breeding system variation in *Daphnia pulicaria* from North American lakes. *Heredity,* 71, 497-507.

CLAY, C. S. AND MEDWIN H. 1977. *Acoustic Oceanography: Principles and Applications.* John Wiley and Sons, New York.

FERGESON, R. G. 1958. The preferred temperature of fish and their midsummer distribution in temperate lakes and streams. *J. Fish. Res. Bd. Canada,* 15, 607-624.

GALBRAITH, M. G. 1967. Size-selective predation on *Daphnia* by rainbow trout and yellow perch. *Trans. Am. Fish. Soc.*, 96, 1-10.

HAVEL, J. E. 1987. Predator-Induced Defenses: A Review. In: Kerfoot, W. C. and Sih, A. (eds) *PREDATION: Direct and Indirect Impacts on Aquatic Communities.* pp. 263-278. University Press of New England, Hanover and London.

HEBERT, P. D. N. AND BEATON, M. J. 1989. *Methodologies for allozyme analysis using cellulose acetate electrophoroesis.* Helena Laboratories, Beaumont, Texas.

HEBERT, P. D. N. AND LOARING, J. M. 1980. Selective predation and the species composition of Arctic ponds. *Can. J. Zool.,* 58, 422-426.

HRBACEK, J., DVORAKOVA, M., KORINEK, V. AND PROCHAZKOVA. L. 1961. Demonstration of the effect of the fish stock on the species composition of zooplankton and the intensity of metabolism of the whole plankton assemblage. *Verh. Int. Ver. Theoret. Angew. Limnol.,* 14, 192-195.

KERFOOT, W. C., KELLOGG, D. L. AND J. R. STRICKLER. 1980. Visual observations of live zooplankters: evasion, escape and chemical defenses. In: Kerfoot, W. C. (ed) *Evolution and Ecology of Zooplankton Communities.* pp. 10-27. University Press of New England, Hanover and London.

KERFOOT, W. C. AND SIH. A. 1987. *PREDATION: Direct and Indirect Impacts on Aquatic Communities.* University Press of New England, Hanover and London.

LANDON, M. S. AND STASIAK, R. H. 1983. *Daphnia* hemoglobin concentration as a function of depth and oxygen availability in Arco Lake, Minnesota. *Limnol. Oceanogr.,* 28, 731-737.

LEIBOLD, M. 1990. Resources and predators can affect the vertical distributions of zooplankton. *Limnol. Oceanogr.,* 35, 938-944.

LEIBOLD, M. AND TESSIER, A. J. 1991. Contrasting patterns of body size for *Daphnia* species that segregate by habitat. *Oecologia*, 86, 342-348.

MILLS, E. L. AND J. L. FORNEY. 1983. Impact on *Daphnia pulex* of predation by young yellow perch in Oneida Lake, New York. *Trans. Am. Fish Soc.,* 112, 154-161.

MOYLE, P. B. 1969. *Ecology of the Fishes of a Minnesota Lake with Special Reference to the Cyprinidae.* Ph. D. Thesis, University of Minnesota.

O'BRIEN W. J., SLADE, N. A. And VINYARD, G. L. 1976. Apparent size as the determinant of prey selection by bluegill sunfish *(Lepomis macrochirus). Ecology*, 57, 1304-1310.

SPITZ, K. 1991. *Chaoborus* predation and life-history evolution in *Daphnia pulex:* temporal pattern of population diversity, fitness, and mean life history. *Evolution,* 45, 82-92.

SPITZ, K. 1992. Predator-mediated plasticity of prey life history and morphology: *Chaoborus americanus* predation on *Daphnia pulex. Am. Nat.* 139, 229-247.

WELLS, L. 1970. Effects of alewife predation on zooplankton populations in Lake Michigan. *Limnol. Oceanogr.,* 15, 556-565.

WERNER, E. E. AND HALL, D. J. 1974. Optimal foraging and the size selection of prey by the bluegill sunfish *(Lepomis macrochirus). Ecology,* 55, 1042-1052.

WILSON, C. C. AND HEBERT, P. D. N. 1993. Impact of copepod predation on distribution patterns of *Daphnia pulex* clones. *Limnol. Oceanogr.*, 38,1 304-1310.

ZARET, T. M. 1980. *Predation and Freshwater Communities.* Yale University Press, New Haven and London.

CHAPTER 3

Hemoglobin Pigmentation

Introduction

Pigmentation in the Cladocera is due to a variety of chemical compounds associated with several adaptations. Goodwin (1960) divided crustacean pigments into two major groups: 1) those whose major function is to provide the external color pattern of the animals and 2) those which have little value as color but which have important metabolic functions. Melanin and carotiniod pigments are characteristic members of the first group; hemoglobin is an important member of the second group. Melanin in the carapace is thought to provide protection from photodamage (Dodson and Frey, 1991). Hobaek and Wolf (1991) observed that melanic morphs of *D. longispina* inhabited clear water lakes and ponds, while transparent morphs inhabited more humic waters. They reported that the difference in distribution of color morphs was in response to short-wave radiation and, furthermore, that it was clone specific. Hebert and McWalter (1983) established the polyphyletic origin of pigmentation in *D. middendorffana* with the observation that distantly related clones often share cuticular pigmentation, while closely related clones often do not. Carotenoids can cause cladocerans to appear light yellow, blue or green. These pigments are derived from algae in the diet because cladocerans cannot synthesize carotenoids *de novo* (Ringelberg, 1980). Epibionts including diatoms, protozoans and rotifers (Green, 1955) attached to the carapace can give a brownish cast to *Daphnia* especially to older individuals that have not molted for a long time. *D. pulicaria* from Long Lake often take on a translucent orange cast in late spring and early summer (Chapter 2). While this pigment has not been identified, it was clearly not hemoglobin. This orange cast was associated with an ephemeral rust colored, presumably an iron, flock present in the water column.

Hemoglobin has been the most apparent and widely investigated pigment in the Cladocera. Writing about *Daphnia magna* Green (1955) stated, "The animals were bright red with haemoglobin in the blood and were present in such numbers that large patches of the water surface were streaked red as if with blood". Fox's (1948)

observation that the hemoglobin content in *Daphnia* was inversely related to dissolved oxygen concentration in the environment has been confirmed by several authors (Carvalho, 1984; Engle, 1985). Carvalho (1984) discussed the ecological differentiation of populations of *D. magna* based upon environmental oxygen differences in two neighboring ponds. The relationship between dissolved oxygen concentration and hemoglobin appeared to be quantitative rather than qualitative. His review indicated that the amount of pigment rather than the type was altered in response to the environment. He further speculated that the differences in ability of *D. magna* from differing environments to synthesize hemoglobin was probably related to variability in either the oxygen threshold for induction of synthesis or in the kinetics of synthetic enzymes involved.

Several benchmarks have been reported in the literature for the response of *Daphnia* and closely related groups to low levels of dissolved oxygen. Dodson and Frey (1991) reported that many entomostracans produce hemoglobin at oxygen concentrations of 1-2 mg/liter. *Daphnia* oxyhemoglobin deoxygenated at ambient oxygen pressures of 1.18 ml/liter (= 1.65 mg/liter)* (Fox, 1948). *D. magna* with small amounts of hemoglobin could remove oxygen from environmental water with dissolved oxygen concentrations as low as 0.3 ml/liter (= 0.4 mg/liter) (Kobayashi, 1982). *D. pulex* died at oxygen levels of 0.5-1.0 mg/liter (Weider and Lampert 1985).

* Dissolved oxygen concentrations have been reported with two different units of measurement in the literature. Early physiological literature originating in England and oceanographic work often report results in ml/liter. Most limnologists report results in the now conventionally accepted units of mg/liter. Assuming standard temperature and pressure (STP), I converted from ml/liter to mg/liter for clarification of comparison. Mortimer (1967) reported that at STP a mole of gas occupied 22.4 liters and that a mole of diatomic oxygen weighed 32 grams. Therefore, the conversion from ml to mg was calculated to be: 32.0 g/mole / 24.4 liter/mole = 1.4 g/liter = 1.4 mg/ml.

Hemoglobin formation in *D. pulex* was experimentally induced by reducing oxygen concentrations to 3 mg/liter (Engle, 1985). Fox and Phear (1953) reported that *Daphnia* responded to low oxygen levels of 1.2 ml/liter (= 1.7 mg/liter) by producing hemoglobin at increasing levels over a two week period. Further, hemoglobin levels returned to pre-treatment levels at about the same rate when the *Daphnia* were returned to aerated water. Both Kring and O'Brien (1976) working with *D. pulex* and Heisey and Porter (1977) working with *D. magna*, observed that filtering (feeding) rates dropped significantly when dissolved oxygen concentrations dropped below 3 mg/liter.

While Fox et al. (1949) found that no factor other than oxygen depletion stimulated hemoglobin production, the concentration of hemoglobin in daphnids has been shown to be a function of a wide variety of other environmental, phylogenetic and life history variables. Increased water temperature in the 17 - 28 ^{o} C range and iron concentration, have both been shown to result in increased hemoglobin levels in *D. obtusa* (Fox and Phear, 1953). Green (1956) has shown that hemoglobin levels in *Daphnia* can be attributed to differences in species, age, size, sex, and developmental stage. He further concluded that at any one concentration of dissolved oxygen, individual variation in hemoglobin concentration could be explained by individual differences in metabolic rate associated with age, size and sex of the individual. Chandler (1954) reported *Daphnia* hemoglobin content differed from pond to pond and month to month. Additionally she reported that *D. pulex* synthesized more hemoglobin than *D. obtusa* in 9 out of 10 trials. Engle (1985) found that reproductive state influenced hemoglobin level in *D. pulex*, but contrary to earlier work, she found that oxygen levels in nature were not necessarily a good predictor of hemoglobin levels, perhaps because visual predators in the pond with lower levels of oxygen selected against individuals with high levels of hemoglobin. Additionally, she alluded to the possibility of a genetic component regulating hemoglobin production:

"The results of this experiment indicate that when races of the same species of *Daphnia* differ in the ability to produce haemoglobin in low oxygen conditions, the differences will not necessarily correspond to patterns of oxygen availability in nature. Habitat variables (such as oxygen supply, temperature, and visual predation) may interact in complex ways with physiological variables (such as metabolic rate) in determining the extent to which *Daphnia* will synthesize haemoglobin. Additionally, it is not clear what role genetic differences, perhaps involving the mechanism for haemoglobin induction, may play in regulating this biosynthetic trait."

Intraspecific differences in hemoglobin concentration attributed to genetic differences were first reported by Green (1956). He found that a British race of *D. magna* produced more hemoglobin than a race of the same species from France when the groups were held at 0.9 ml/liter (= 1.3 mg/liter) in the laboratory. LaBerge and Hann (1990) found clones of *D. pulex*, differing at the hemoglobin locus, to vary in response to tolerance of oxygen depletion. However, their results were equivocal because results obtained in pond studies were the reverse of laboratory findings. Weider and Lampert (1985) found clone specific differences in tolerance to low oxygen concentrations and hemoglobin production. The differential vertical distribution of clones of *D. pulex* documented by Weider (1984) provided evidence of clone specific responses to dissolved oxygen in a stratified 6 m deep pond. Weider (1985) found that one clone of *D. pulex* replaced another at high, but not at low oxygen levels.

The production of hemoglobin in *Daphnia* has been associated with behavioral, physiological and life history advantages. Fox et al. (1951) reported that increased egg production, longer life, increased food gathering, accelerated embryonic development and more energetic swimming were all associated with increased hemoglobin production. However, as Engle (1985) suggested, the physiological and life history advantages associated with hemoglobin production need to be considered in light of the increased exposure to predation resulting from increases in pigmentation. Zaret (1980) clearly presented a very persuasive argument for the increased vulnerability of pigmented zooplankton to visual predators.

During the summer months, when Long Lake was thermally stratified, increasing depth was associated with decreases of both

oxygen and light. I hypothesized that lower concentrations of dissolved oxygen were associated with the increased frequency of pigmented *D. pulicaria* in the deep layer. This adaptation would carry a lower risk of predation in deeper waters of an extremely clear lake. Hemoglobin production by the SS clone may have explained the increased proportions of this clone found under low oxygen conditions in the deep zooplankton layer (Chapter 1).

Methods

D. pulicaria were collected on 10 August, 1991 and 2 August, 1992 with vertical tows of a closing plankton net. Collections were made from discrete layers of zooplankton detected by sonar at the northwest sampling location (Chapter 1). Limnological, acoustic and electrophoretic methodology remained as described in Chapter 1. Light intensity was measured with a LI-COR model LI-185A light meter and a model LI-192S under-water sensor.

D. pulicaria were scored visually as either transparent or pigmented with the aid of a dissecting microscope before electrophoresis. The small size of each individual daphnid and the need to use most of the homogenate from each individual for electrophoresis precluded analyzing for hemoglobin levels from individual *Daphnia.* Forty additional *D. pulicaria* categorized as either transparent or pigmented were homogenized in groups of 20 and analyzed for hemoglobin concentrations. Pigment extraction and quantitative analyses followed the methods of Landon and Stasiak (1983) using a Bausch and Lomb Spectronic 70 spectrophotometer with rabbit hemoglobin as a standard.

Results

A scan of light absorbance from 350 nm to 610 nm in the hemoglobin extracts revealed a single peak between 410 and 415 nm in samples from both pigmented and transparent groups. Quantitative comparisons made with standards of rabbit hemoglobin (peak absorbance 413 nm) indicated that, samples scored qualitatively as pigmented, consistently contained more hemoglobin than transparent samples (Table 1).

Table 1. Hemoglobin levels (mg/ml) for *D. pulicaria* categorized as transparent and pigmented in groups of 20 individuals.

DATE 1993	TRANSPARENT	PIGMENTED
19 May	0.063	0.099
18 Aug.	0.035	0.089
30 Aug.	0.074	0.120
Mean	0.057	0.103

While, the number of samples was small, each sample was a composite of 20 individuals and the difference between the means was significant (t-test, $P = 0.01$). It should be noted that the quantitative measurements on hemoglobin level, that verified the routine visual qualitative scoring, were made retrospectively. I did not have a routine procedure for quantifying hemoglobin concentrations in 1991 and 1992 when the following samples were collected.

Three hundred forty seven *D. pulicaria* collected in early August, 1991 and 1992 were categorized by color, oxygen concentration at the point of collection and *PGI* clone type (Table 2). Individuals in the low oxygen category were collected from the deep layer of zooplankton, between 17 and 23 m in 1991 and between 15 and 19 m in 1992, where thermal stratification had resulted in oxygen depletion. At these depths dissolved oxygen concentrations averaged 2.8 mg/liter in 1991 and 2.0 mg/liter in 1992. Individuals categorized from a high oxygen environment were collected between 8 m and 14 m in 1991 and between 9 m and 13 m in 1992. At these depths, dissolved oxygen concentrations averaged 13.4 mg/liter in 1991 and 8.8 mg/liter in 1992. The FS clone (65%) dominated the samples especially in the transparent morphs from high oxygen environments. The SS clone made up 29% of the individuals; the remaining 6% of the individuals were from all of the other *PGI* clones combined.

Table 2. Pigmented and transparent *D. pulicaria* collected at on Aug. 10, 1991 and Aug. 2, 1992 categorized by oxygen concentration and clone type.

COLOR	OXYGEN CONC.	YEAR	*PGI* CLONES FS	SS	OTHER
Pigmented	Low	1991	37	37	3
		1992	31	22	7
	High	1991	16	6	1
		1992	3	1	1
Transparent	Low	1991	13	3	0
		1992	16	9	1
	High	1991	59	11	2
		1992	52	10	6

The level of dissolved oxygen had the largest effect on the relative proportions of pigmented versus transparent *D. pulicaria* followed by clone type. Comparisons of the pairwise effects of the explanatory variables are presented in figure 1 in descending order of the magnitude of the effect. The odds ratio of an elevated hemoglobin level in a low oxygen environment was 9.5 to 1. Ignoring the 'other' clone category in the second graph, the odds ratio of an elevated hemoglobin level given that the individual was S/S at the *PGI* locus was 3.2 to 1. The least significant effect was the year or sampling time. The odds of an increased hemoglobin level given that the sample was from 1991 was 1.6 to 1.

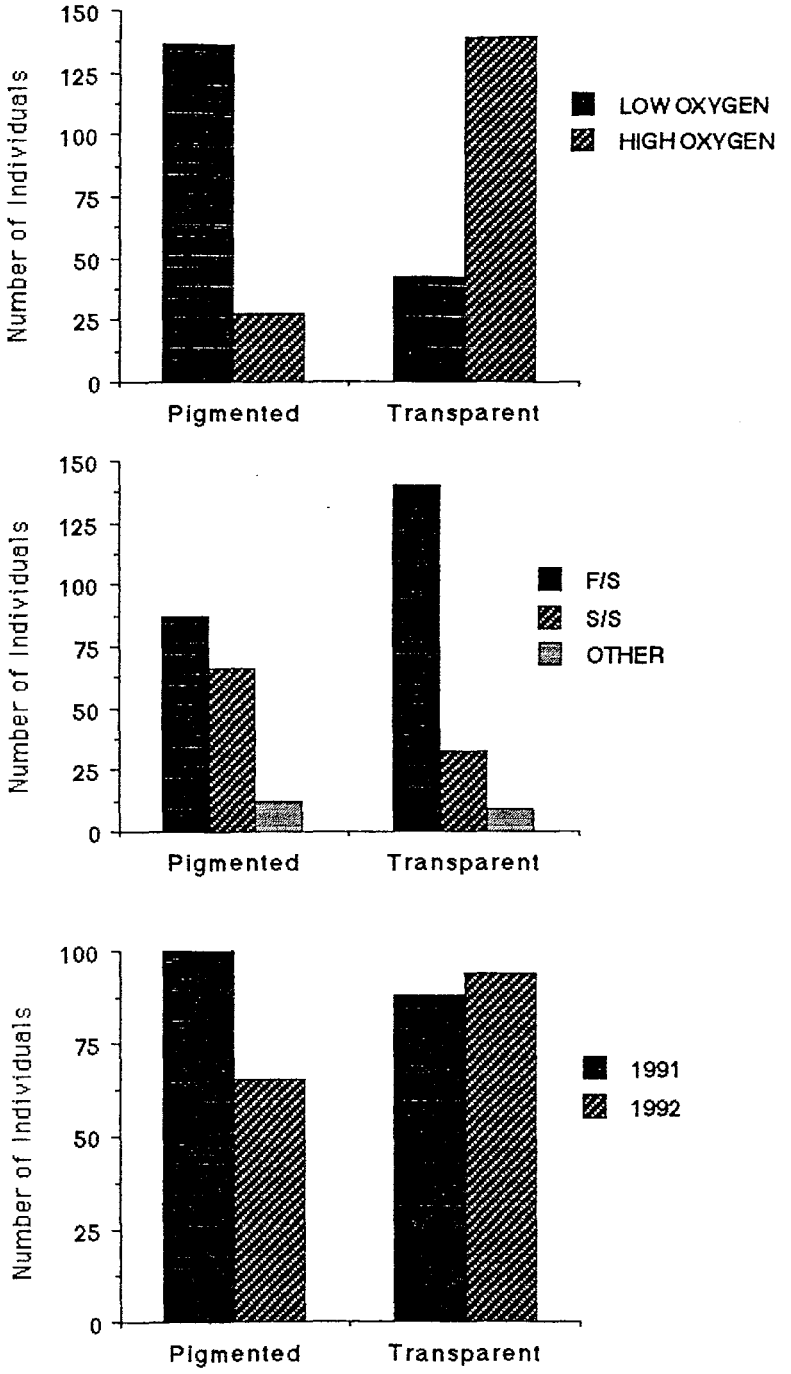


Figure 1. Pairwise comparisons of the effect of dissolved oxygen levels, clone type and year on the distribution of hemoglobin pigmentation in *D. pulicaria.*

The effects of oxygen concentration, sample time and clone type on the distribution of pigmentation (= hemoglobin level) in table 2 were examined with log-linear modeling. All of the models that fit the data, i.e. that fulfilled the initial criteria of an acceptable Pearson Chi-square value ($P \geq 0.05$) and the base model of independence are presented in table 3. Ten models fit the data, most were closely grouped well below the critical Pearson Chi-square values for the respective degrees of freedom. The G square statistic was substantially reduced when the clone effect, [24], was included. Reading up from the bottom of table 3, note the reduction in the G sq. column from 10.77 to 4.74 or less, when the clone effect, [24], is included. Model selection criteria of parsimony, and, information criteria plotted against the number of model parameters converged on the [123] [14] [24] [34] model. The interpretation of this model was that each of the explanatory variables had a significant effect on the distribution of color in *D. pulicaria* and that the effects were independent of each other, and further, that higher order interactions among the explanatory variable cluster, [123], did not add significantly to the interpretation of these data.

Table 3. Summary of log-linear models applied to the data in table 2.

Model	Pearson Chi-sq.	*df*	G sq.	Change in G sq. from base	Change in *df* from base
[123] [124] [134] [234]	0.77	2	0.90	157.66	9
[123] [124] [134]	1.27	4	1.56	157.00	7
[123] [124] [234]	2.36	3	2.57	155.99	8
[123] [134] [234]	2.49	4	2.70	155.86	7
[123] [134] [24]	2.76	6	3.07	154.99	5
[123] [124] [34]	3.29	5	3.53	155.03	6
[123] [234] [14]	3.94	5	4.10	154.46	6
[123] [14] [24] [34]	**4.52**	**7**	**4.74**	**153.82**	**4**
[123] [134]	9.99	8	10.77	147.79	3
[123] [14] [34]	11.83	9	12.17	146.42	2
[123] [4]	138.71	11	158.56	Base Model	

Model Selected: [123] [14] [24] [34]

[1] -> Year = Explanatory Variable
[2] -> Clone = Explanatory Variable
[3] -> Oxygen Concentration = Explanatory Variable
[4] -> Color = Response Variable

Light intensity between 8 m and 14 m, the depth range where the shallow daytime layer of *D. pulicaria* was normally found, averaged 24.5 *u*E/m^2/sec. or 5.5% of incident light (Fig. 2). The corresponding values between 15 m and 20 m, the depth range where the deep layer of *D. pulicaria* was normally found, were 2.9 *u*E/m^2/sec. and 0.6% of incident light. The data were an average of five vertical profiles obtained between 8 July, 1991 and 28 June, 1993. The reduction in light intensity was nearly logarithmic. The average value of ambient sunlight immediately above the lake surface was 692 *u*E/m^2/sec. Incident sunlight at the surface of the lake averaged 446 *u*E/m^2/sec. A light intensity equal to 10% of the mean incident light intensity was observed at a depth of 8.5 m. The 1% light level was at 16 m.

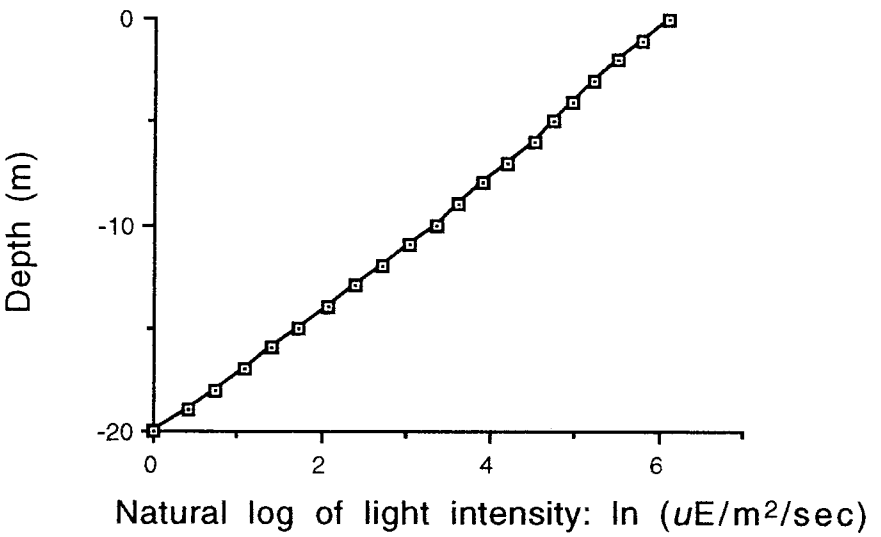


Figure 2. Average light extinction in Long Lake.

Discussion

The hypothesis central to these observations was substantiated. A clone specific response to lower oxygen concentrations in the hypolimnion could, at least in part, account for the genetic structuring observed during summer months in Long Lake as oxygen becomes progressively depleted with depth (Chapter 1, Fig 2). While both the numerically dominant FS clone and the SS clone responded with increased proportions of pigmented individuals to lower dissolved oxygen, the response by the SS clone was 3 times greater. This disproportionate response of increased frequency of higher hemoglobin levels was undoubtedly an adaptation to lower concentrations of dissolved oxygen in the hypolimnion.

The routine visual categorization of *D. pulicaria* into groups labeled transparent and pigmented was somewhat subjective; contemporaneous quantitative verifications were not conducted. However, I was confident that if the number of categories was held to a minimum I could be consistent in my methodology of assigning individuals to color categories. Several pieces of evidence suggest that this assumption was warranted. Retrospective quantification of

hemoglobin concentrations in groups of *D. pulicaria* indicated that there was no overlap between transparent and pigmented groups. A gap occurred between the highest hemoglobin concentrations of 0.074 mg/ml in the transparent group and the lowest hemoglobin concentrations of 0.089 mg/ml in the pigmented group. Additionally the means of three separate samples of groups of 20 individuals in each category were significantly different. A visual index of hemoglobin concentration using human blood as a reference was developed by Fox (1948) has been used extensively in the past (Fox and Phear, 1953; Chandler, 1954; Green, 1956). Carvalho (1984) evaluated this method and reported an experimental error of less than 5%. I did not use a reference, but, my level of visual discrimination into two categories was much simpler than Fox's visual index that used a scale of 0-160 units.

My observation that low oxygen concentrations were associated with increased frequencies of visually observable hemoglobin pigmentation in *D. pulicaria* is consistent with an extensive literature on this phenomena in other daphnids (Carvalho, 1984). The clone specific nature of this response to low oxygen concentrations is consistent with results reported by Weider and Lampert (1985) and LaBerge and Hann (1990) in the closely related species, *D. pulex.*

Predation also likely contributed to the increased proportion of pigmented individuals in deeper, less illuminated waters. Engle (1985) showed that the presence of visual predators may select against hemoglobin pigmentation in *D. pulex* even at low levels of dissolved oxygen in shallow (0.6 m and 2 m maximum depths), and presumably well lit, ponds. In Long Lake, *D. pulicaria* adapting to low oxygen levels via increased hemoglobin pigmentation would be less vulnerable to visual predators by remaining in darker deep water during daylight hours.

Long Lake is extremely transparent. Moyle (1969) reported Secchi transparency up to 10.5 m My observation that an average of 1% of incident surface light was still available at 16 m in Long Lake compared to a 1% level of 3-6 m in Lake Itasca (Ross unpubl.). Longer wavelengths (red light ca. 720 nm) of the visible spectrum are attenuated disproportionately quickly in water with increasing depth (Ruttner, 1969), therefore, color discrimination involving the

red hemoglobin pigment was likely not a factor for predators. While most teleosts have color vision (Munz, 1971), the eyes of freshwater fish are maximally sensitive at 540 nm (Brett, 1957), a wavelength that readily penetrates freshwater. Due to the extreme clarity of the water in Long Lake, pigmented *D. pulicaria* might have stood out as more opaque particles compared to transparent morphs. Zaret and Suffern (1976) pointed out that planktivores, being light dependent predators, rely on the contrast between their prey item and the background to see and capture moving prey. One hundred percent of the *D. pulicaria* found in the stomachs of rainbow trout caught between 13 m and 16 m were pigmented, albeit not with hemoglobin as the dominant pigment (Chapter 2).

Information concerning the quantitative effects of light intensity and the foraging efficiency of zooplanktivorous fishes would be most helpful in assessing the relative vulnerabilities of color morphs of *Daphnia.* In a qualitative sense, predation has been thoroughly documented to be associated with the vertical distribution of Zooplankton during daylight hours (Chapter 5). However, I have been able to find only one reference in the literature that remotely approaches the situation at Long Lake in a quantitative sense. Zaret and Suffern (1976) reported that the feeding rate of golden shiners *(Notemigonus chrysoleucas),* eating *Daphnia galeata mendotae,* increased dramatically when light intensities reached 2200 ergs/cm^2/sec in the laboratory. The comparison to Long Lake is worth examining even though confounded on several levels. *D. galeata* are normally only about one half the size of *D. pulicaria*, golden shiners are not part of the zooplankton predator assemblage in Long Lake and Zaret and Suffern's (1976) units of light measurement (ergs/cm^2/sec) are not directly comparable to mine. The conversion from ergs/cm^2/sec to *u*E/m^2/sec is wavelength dependent (Wetzel, 1975); the spectral composition of light in Long Lake at a given depth was unknown. However, it is interesting to note that 2200 ergs/cm^2/sec was 0.5% of the mid-day surface light value reported at Fuller Pond, Zaret and Suffern's (1976) field study site. The 0.5% light level in Long Lake occurred at 19 m and the average center of the deep layer of *D. pulicaria* was 18.5 m. By extrapolation it appears that light intensities in the vicinity of 0.5% of mid-day surface intensity may be a significant threshold for visual predators. While, rigorous

quantitative examination of specific effects of light intensity on selective predation awaits further work, most authors have assumed that more light is disproportionately deleterious to large and pigmented zooplankton exposed to visual predators (Zaret, 1980).

Wright and Shapiro (1990) pointed out that refugia can be formed in either of two ways. In addition to a gradient in light intensity that could reduce the rate of predation where spatial overlap occurs, a physical or chemical gradient (e.g. temperature, dissolved oxygen) might act to spatially separate predator and prey. The low oxygen concentrations associated with deeper waters in Long Lake could have provided a refuge for *D. pulicaria* from predation. Davis (1975) reported that rainbow trout required a minimal dissolved oxygen concentration of 4.59 mg/liter. *Daphnia*, especially clones that produced elevated levels of hemoglobin, survived at oxygen concentrations down to a threshold of 0.5-1.0 mg/liter (Weider and Lampert, 1985). While brief feeding forays by rainbow trout into water with oxygen concentrations below 4.59 mg/liter were certainly possible, the combination of reduced visibility and reduced oxygen levels with depth, argued for a relatively lower predation pressure in deep water by rainbow trout, a visual, particle feeding predator. Wright and Shapiro (1990) observed the importance of deep mid-summer refuges for large bodied *Daphnia* in several lakes. They also experimentally demonstrated that refuge size was important in modifying zooplanktivore-induced mortality. In conclusion hemoglobin production in PGI_{SS} clones is an adaptation that allows them to exploit deeper, less illuminated waters with lower oxygen concentrations. This physiological adaptation, which was significantly over-represented in PGI_{SS} clones, in concert with disproportionate predation on pigmented individuals in shallower, brighter waters with more dissolved oxygen could have explained the genetic structuring of this population that I observed each summer in Long Lake which was described in chapter one.

References

BRETT, J. R. 1957. The sense organs: the eye. In: Brown, M. E. (ed) *The Physiology Of Fishes.* pp. 121- 154. Academic Press, New York.

CARVALHO, G. R. 1984. Haemoglobin synthesis in *Daphnia magna* Straus (Crustacae: Cladocera): ecological differentiation between neighbouring populations. *Freshwater Biology.* 14, 501-506.

CHANDLER, A. 1954. Causes of variation in the haemoglobin content of *Daphnia* (Crustacae: Cladocera) in nature. *Proc. Roy. Soc. Lond.* B. 124, 625-630.

DAVIS, J. C. 1975. Minimal dissolved oxygen requirements of aquatic life with emphasis on Canadian species: a review. *J. Fish. Res. Board Can.,* 32, 2295-2332.

DODSON, S. I. AND FREY, D. G. 1991. Cladocera and other Branchiopoda. In: Thorp, J. H. and Covich, A. P. (eds) *Ecology and Classification of North American Freshwater Invertebrates.* Chapter 20, pp 723-786. Academic Press, San Diego.

ENGLE. D. L. 1985. The production of haemoglobin by small pond *Daphnia pulex* : intraspecific variation and its relation to habitat. *Freshwater Biology.* 15, 631-638.

FOX, H. M. 1948. The haemoglobin of *Daphnia. Proc. Roy. Soc. Lond.* B. 135, 195-212.

FOX, H. M., GILCHRIST, B. M. AND PHEAR, E. A. 1951. Functions of haemoglobin in *Daphnia. Proc. Roy. Soc. Lond.* B. 138, 514-528.

FOX, H. M., HARDCASTLE, S. M. AND DRESEL, E. I. B. 1949. Fluctuations in the heamoglobin content of *Daphnia. Proc. Roy. Soc. Lond.* B. 136, 388-399.

FOX, H. M. AND PHEAR, E. A. 1953. Factors influencing haemoglobin synthesis by *Daphnia. Proc. Roy. Soc. Lond.* B. 141, 179-189.

GREEN, J. 1955. Studies on a population of *Daphnia magna*. *J. Animal Ecol.* 24, 84-97.

GREEN, J. 1956. Variation in the hemoglobin content of *Daphnia*. *Proc. Roy. Soc. Lond.* B. 145, 214-232.

GOODWIN, T. W. 1960. Biochemistry of Pigments. In: Waterman, T. H. (ed) *The Physiology of Crustacae, Vol I: Metabolism And Growth*. pp. 101-140. Academic Press, New York.

HEBERT, P. D. N. AND MCWALTER, D. B. 1983. Cuticular pigmentation in Arctic *Daphnia* : adaptive diversification of asexual lineages. *Am. Nat.*, 122, 286-291.

HEISEY, D. AND PORTER, K. G. 1977. The effect of ambient oxygen concentration on filtering and respiration rates of *Daphnia galeata mendotae* and *Daphnia magna*. *Limnol. Oceanogr.* 22, 839-845.

HOBAEK, A. AND WOLF, G. W. 1991. Ecological genetics of Norwegian *Daphnia longispina* genotypes in relation to short-wave radiation and water colour. *Hydrobiologia* 225: 229-243.

KOBAYASHI, M. 1892. Relationship between oxygen consumption and resistance to oxygen deficiency of pale *Daphnia magna*. Comp. Biochem. Physiol. 73A, 339-241.

KRING, R. L. AND O'BRIEN, W. J. 1976. Effect of varying oxygen concentrations on the filtering rate of *Daphnia pulex*. *Ecology* 57, 808-814.

LABERGE, S. AND HANN, B. J. 1990. Acute temperature and oxygen stress among genotypes of *Daphnia pulex* and *Simocephalus vetulus* (Cladocera, Daphiniidae) in relation to environmental conditions. *Can. J. Zool.*, 68, 2257-2263.

LANDON, M. S. AND STASIAK, R. H. 1983. *Daphnia* hemoglobin concentration as a function of depth and oxygen availability in Arco Lake, Minnesota. *Limnol. Oceanogr.*, 28, 731-737.

MORTIMER, C. E. 1967. *Chemistry: A Conceptual Approach.* Reinhold, New York.

MOYLE, P. B. 1969. *Ecology of the Fishes of a Minnesota Lake with Special Reference to the Cyprinidae.* Ph. D. Thesis, University of Minnesota.

MUNZ, F. W. 1971. Vision: visual pigments. In: Hoar, W. S. and Randall, D. J. (eds). *Fish physiology.* pp. 1-32. Academic Press, New York.

RINGELBERG, J. 1980. Aspects of red pigmentation in zooplankton, especially copepods. In: Kerfoot, C. W. (ed) *Evolution and Ecology of Zooplankton Communities.* pp. 91-97. University Press of New England, Hanover and London.

RUTTNER, F. 1969. *Fundamentals of Limnology.* University of Toronto Press, Canada.

WEIDER, L. J. 1984. Spatial heterogeneity of *Daphnia* genotypes: Vertical migration and habitat partitioning. *Limnol. Oceanogr.* 29, 225-235.

WEIDER, L. J. 1985. Spatial and temporal genetic heterogeneity in a natural *Daphnia* population. *J. Plankton Res.* 7, 101-123.

WEIDER, L. J. AND LAMPERT, W. 1985. Differential response of *Daphnia* genotypes to oxygen stress: respiration rates, hemoglobin content and low-oxygen tolerance. *Oecologia* 65, 487-491.

WETZEL, R. G. 1975. *Limnology.* W. B. Sanders, Philadelphia.

WRIGHT, D. AND SHAPIRO, J. 1990. Refuge availability: a key to understanding the summer disappearance of *Daphnia. Freshwater Biol.,* 24, 43-62.

ZARET, T. M. 1980. *Predation and Freshwater Communities.* Yale University Press, New Haven and London.

ZARET, T. M. AND SUFFERN, J. S. 1976. Vertical migration in zooplankton as a predator avoidance mechanism. *Limnol. Oceanog.* 21, 804-813.

CHAPTER 4

Survivorship In Alternate Environments: Transplant Experiments

Introduction

Long Lake provided a unique opportunity to experimentally assess physiological effects with clones of *D. pulicaria* that commonly occurred in two day time layers. The shallow layer always had concentrations of dissolved oxygen well above 3 mg/liter; the deep layer was located in the hypolimnion where dissolved oxygen concentrations often dropped below 3 mg/liter. The 3 mg/liter concentration of dissolved oxygen was significant because it has been reported to be a threshold for nutritional and physiological changes in other large and closely related species of *Daphnia.* Kring and O'Brien (1976) found that the filtering (feeding) rate in *D. pulex* declined sharply at dissolved oxygen concentrations below 3 mg/liter. Heisey and Porter (1977) reported similar results for *D. magna* but, they found that *D. galeata*, a smaller species, was more tolerant of low levels of dissolved oxygen perhaps do to higher concentrations of hemoglobin in the latter species. Engle (1985) showed experimentally that hemoglobin formation was induced in *D. pulex* by reducing oxygen concentrations to 3 mg/liter.

I hypothesized that *D. pulicaria* from the deep layer, acclimated to lower levels of food and dissolved oxygen (Chapter 1, Fig. 2), might show increased survival when moved to the upper layer, if protected from predation. Conversely, I anticipated that *D. pulicaria* acclimated to higher concentrations of food and dissolved oxygen might exhibit a decreased survival when moved to the deep layer. I was particularly interested in clone specific differences in survival in the transplant experiments as a potential explanation for the genetic structuring observed in middle to late summer in chapter 1. Weider and Lampert (1985) had summarized that, "Dissolved oxygen may be an important selective force influencing the clonal composition of natural populations".

Methods

The general experimental design was to confine *D. pulicaria* in plastic bags, permeable to dissolved gasses, within each layer. These were termed controls. Second, *D. pulicaria* confined in bags were transplanted from one layer to the other. The transplants were intended to assess physiological stresses. These stresses were imposed by an environment that was conceivably available to, but not used (during daylight hours) by *D. pulicaria* from the respective layers.

Zooplankton tows were made through discrete layers of zooplankton detected with sonar at the southeast sampling location. In 1992 the upper layer extended from approximately 9 m to 14 m and the lower layer was located from 16 m to 19 m. In 1993 the upper layer extended from approximately 10 m to 14 m and the lower layer was located from 16.5 m to 19.5 m. *D. pulicaria* collected with vertical tows of a closing plankton net, were temporarily placed in 100 ml sample vials filled with water from the depths where the collections were made. Samples from each layer were randomized mechanically by shaking the vials. Individual *D. pulicaria* were drawn from the vial with a pipette and alternately assigned to control or transplant groups. *D. pulicaria* and 3.8 L of water from the destination depth were placed in a 7.6 L 'Ziploc' brand recloseable plastic bag; the excess air was squeezed out and the bag sealed. The top lip of each bag was reinforced with water-proof construction tape, a hole was punched in the reinforcement and the bag was attached to a snap clip. This clip was then attached to a line stretched between an anchor and a marker buoy. Double bags were used the second year. Transplants and controls were incubated at 11 m (shallow) and 17 m (deep) both years. Transplants and controls were incubated for 66 hours between 3-6 August, 1991 and between 2-5 August, 1992.

Sample sizes varied between the years. In 1992, 100 *D. pulicaria* were enclosed in each bag (26 individuals/liter). In 1993, when population densities were lower only 50 *D. pulicaria* were added to each treatment and each control bag resulting in a density of 13 individuals/liter. Sample densities were somewhat arbitrary. They were 10-20 times larger than the densities of the natural population. These concentrations compared to peak concentrations of

20-100 animals/liter reported for the genus *Daphnia* (Hebert, 1978) and to peak concentrations of 30-50 *D. pulex*/liter in Minnesota (Helgen, 1982).

Results

Long Lake exhibited typical summer thermal stratification in early August 1992 and 1993 (Fig. 1) when the transplant experiments were conducted. Oxygen profiles were positive heterograde both years, but more pronounced in 1993. Two zooplankton layers of were present both years at approximately the same depths (Fig 1). Dissolved oxygen concentrations were generally higher in 1993, especially at the lower zooplankton layer. In 1992 dissolved oxygen ranged from 0.5-2.5 mg/liter in the depths occupied by the hypolimnetic layer of zooplankton, in 1993 the values ranged from 2.5-5.5 mg/liter. Quantitative data on densities were not collected, however, the densities of *D. pulicaria* in both layers were noticeably lower in 1993 when many tows through each layer were necessary to provide a sample size of 50 individuals per treatment per layer.

Deep Transplants

The SS clones survived transplanting from the deep layer to the shallow layer significantly better than the more abundant FS clone (Table 1). In 1992, 6 of 7 (86%) SS individuals survived when transplanted from the deep to the shallow layer; in 1993 2 of 3 (66%) SS individuals survived. This compared to a survivorship of 14 out of 29 (48%) FS individuals in 1992 and 2 out of 13 (15%) in 1993 when transferred to the shallower environment. SS to FS survivorship ratios were 1.8 : 1 in 1992 and 1.4 : 1 in 1993. Indeed, 94% (15 of 16) of all the mortality in the 1992 transplant group and 85% (11 of 13) of the mortality in the 1993 transplant group was attributed to the FS clone. This clone made up 58% of the deep population in 1992 and 52% of the deep population in 1993.

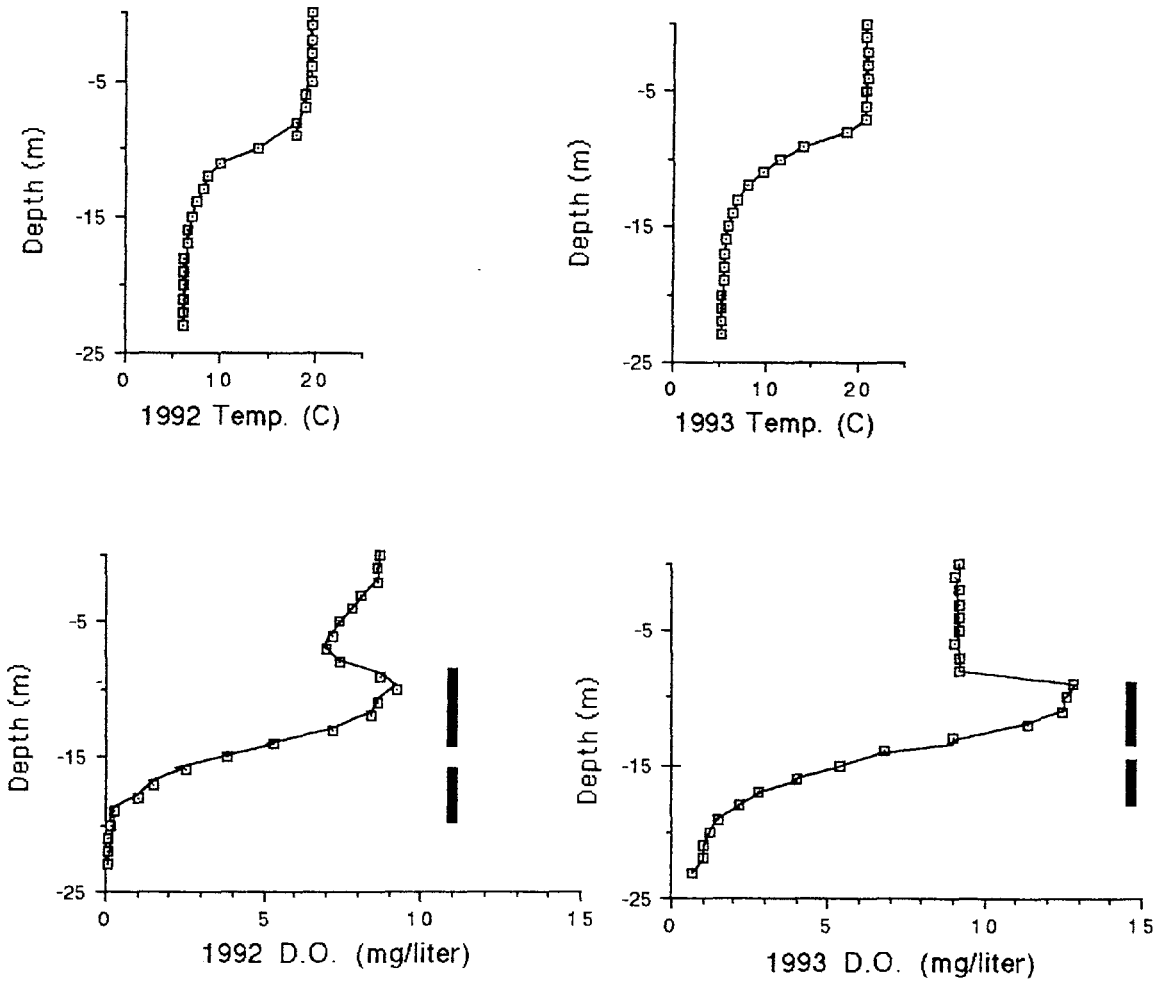


Figure 1. Temperature and dissolved oxygen profiles from Long Lake during transplant experiments in early August 1992 and 1993. The dark vertical bars indicate the position of the shallow and deep layers of zooplankton.

Table 1. Categorization of D. pulicaria at the termination of the deep transplant experiments.

CONDITION	YEAR	TREATMENT	*PGI* CLONE FF	FS	SS	MFS	Total
Alive	1992	Deep Control	0	11	13	5	29
		Deep -> Shallow	4	14	6	0	24
	1993	Deep Control	3	7	3	3	16
		Deep -> Shallow	1	2	2	5	10
Dead	1992	Deep Control	0	0	0	0	0
		Deep -> Shallow	0	15	1	0	16
	1993	Deep Control	1	3	1	0	5
		Deep -> Shallow	0	11	1	1	13

Log-linear modeling of the deep transplant data (Table 2) indicated that eleven models fit the data (Chi-square $P \geq 0.05$). Model selection was more ambiguous compared to the analysis of the shallow data (below). The model selection procedure of information criteria pointed to the first model in table 4: [123] [134] [24], however, the principal of parsimony pointed to the second model: [123] [14] [24] [34]. Plotting information criteria against the number of model parameters was not definitive in deciding between these two models. Both of these models included clone type as a significant component. However, the first model contrasted nicely with the model selected to explain the shallow transplants (below) differing only by one term, the inclusion of clone type. Specifically the [123] [134] [24] model states that the response variable: [4] (survival) was related to the explanatory variable cluster [123] (treatment, clone and year) by treatment, year and the treatment-year interaction [134]; and independently by clone type [24]. The final term of the model accepted as the best explanation of the data in the deep transplant experiments, the clone effect [24], indicated that the SS homozygotes had a significantly higher survival rate compared to the FS heterozygotes.

Table 2. Summary of log-linear models applied to the data in table 1.

Model	Pearson Chi-sq.	*df*	G sq.	Change in G sq. from base	Change in *df* from base
[123] [134] [24]	4.47	7	4.51	45.66	6
[123] [14] [24] [34]	7.27	8	7.34	42.83	5
[123] [124] [134]	0.09	4	0.08	50.09	9
[123] [124] [34]	1.69	5	2.44	47.73	8
[123] [134] [234]	2.83	4	3.23	46.94	9
[123] [234] [14]	5.2	5	6.11	44.06	8
[123] [124] [134] [234]	0	1	0	50.17	12
[123] [134]	15.23	10	18.2	31.97	3
[123] [124] [234]	1.69	2	2.44	47.73	11
[123] [14] [34]	19.5	11	22.5	27.67	2
[123] [124]	11.9	6	13.05	37.12	7
[123] [4]	41.31	13	50.17	Base Model	

[1] -> Treatment = Explanatory Variable
[2] -> Clone = Explanatory Variable
[3] -> Year = Explanatory Variable
[4] -> Condition = Response Variable

Model Selected: [123] [134] [24]

Shallow Transplants

Results of the shallow transplant experiments (Table 3) indicated that the FS heterozygote dominated the shallow layer both years. Combining data from both years indicated that survival was greater in the control group (63%) than the group that was transplanted to the deep layer (55%), but, the trends were not consistent from year to year. In 1992 transplant survival (47%) was less than that of the control group (82%), in 1993 transplant survival (60%) was greater than control survival (38%). In contrast to the deep transplant experiments (above) there was no evidence of a clone specific pattern of survivorship.

Log-linear modeling of the shallow transplant data (Table 4) indicated that five models fit the data (Chi-square $P \geq 0.05$). Model selection procedures of information criteria alone, information criteria plotted against the number of model parameters and parsimony all converged on the [123] [134] model. This model indicated that the response variable: [4] (condition) was related to the explanatory variable cluster: [123] (treatment, clone and year), by the main effects of treatment [1] and year [3], as well as the treatment-year interaction. Significantly, this model suggested that the variable, clone type, was not required to explain the outcome of the shallow transplant experiments.

While, the combined 1992-1993 deep transplant mortality rate (46%) was almost identical to that of the combined shallow transplants (45%), mortality in the combined deep controls (10%) was much lower than the combined shallow controls (37%). In 1993 the mortality rates in both the deep control (24%) group and the deep transplant (57%) group increased compared to 1992 (0% control, 40% transplant). This contrasted to the inconsistent results between years in the shallow experiment.

Table 3. Categorization of D. pulicaria at the termination of the shallow transplant experiments.

CONDITION	YEAR	TREATMENT	*PGI* CLONE FF	FS	SS	MFS	Total
Alive	1992	Shallow Control	2	40	3	2	47
		Shallow -> Deep	0	17	2	0	19
	1993	Shallow Control	0	15	1	1	17
		Shallow -> Deep	1	21	2	5	29
Dead	1992	Shallow Control	0	8	1	1	10
		Shallow -> Deep	0	19	1	1	21
	1993	Shallow Control	1	22	1	4	28
		Shallow -> Deep	0	12	7	0	19

Table 4. Summary of log-linear models applied to the data in table 3.

Model	Pearson Chi-sq.	*df*	G sq.	Change in G sq. from base	Change in *df* from base
[123] [134]	13.56	11	16.68	24.88	3
[123] [134] [24]	11.48	8	14.11	27.45	6
[123] [134 [234]	8.48	5	9.71	31.85	9
[123] [134] [124]	9.08	5	9.75	31.81	9
[123] [134] [124] [234]	5.04	2	5.62	35.94	12
[123] [4]	36.55	14	41.56	Base Model	

[1] -> Treatment = Explanatory Variable
[2] -> Clone = Explanatory Variable
[3] -> Year = Explanatory Variable
[4] -> Condition = Response Variable

Model Selected: [123] [134]

Discussion

It is particularly interesting that the SS clone preferentially tolerated transplanting from the deep layer to the shallow layer because it was also the clone that was more likely to migrate upward after sunset (Chapter 5). These results suggest that the FS clone was particularly vulnerable when it was transplanted from the deep layer to the shallow layer. This result is puzzling because the FS clone has consistently dominated the shallow layer. However, this clone specific response to changing environmental conditions

may account for the differences observed in diel vertical migration (DVM) among *Daphnia* clones (Weider, 1984; Muller and Seitz, 1993).

A combination of factors likely contributed to a lack of sensitivity in this experimental design. These included the uneven distribution of clones and smaller than anticipated sample size. With this design it was not possible to know the distribution of clones in any sample prior to the initiation of the experiments. *Daphnia* are small enough that the entire animal must be homogenized for the electrophoresis techniques used in this study. Conceivably, a pre-experimental set of observations could have estimated the distribution of clones, however, time constraints precluded this approach. The relatively unbalanced design, in which the FS clone made up a much larger proportion in the samples than any of the other clones, could have failed to detect interclonal differences.

Only slightly more than one third (303 of 800) of the planned data points were actually realized. Low densities of *D. pulicaria* in 1993 resulted in 50 individuals per sample compared to the 100 individuals per sample in 1992. Additionally because these experiments were carried out in conjunction with the ecological genetics class each year, much of the electrophoresis work was conducted by personnel with very limited experience, therefore, some gels were uninterpretable. While this resulted in a lower than anticipated sample size, it did not bias the results because error in gel technique was independent of other experimental variables.

All of the factors cited above could have contributed to inability of these experiments to detect additional interclonal physiological responses in the transplant groups. I detected neither the clone specific response in to low dissolved oxygen concentrations reported by Weider and Lampert (1985) for *D. pulex*, nor, the differential temperature tolerance reported by Laberge and Hann (1990) for clones of the same species. The shallow transplants were going from an warm, oxygen rich environment to one much lower in oxygen and temperature. This was particularly true in 1992 when oxygen levels were lower in the hypolimnion than in 1993. The mortality rate among the shallow transplants was greater in 1992 but this difference could not be ascribed to a particular clone.

References

ENGLE. D. L. 1985. The production of haemoglobin by small pond *Daphnia pulex* : intraspecific variation and its relation to habitat. *Freshwater Biology.* 15, 631-638.

HEBERT, P. D. N. 1978. The population biology of *Daphnia* (Crustacae, Daphnidae). *Biol. Rev.* 53, 387-426.

HEISEY, D. AND PORTER, K. G. 1977. The effect of ambient oxygen concentration on filtering and respiration rates of *Daphnia galeata mendotae* and *Daphnia magna. Limnol. Oceanogr.* 22, 839-845.

HELGEN, J. C, 1982. *Effects of Crowding on Reproduction and Feeding Rates in the Cladoceran Daphnia pulex Leydig.* Ph. D. Thesis, University of Minnesota.

KRING, R. L. AND O'BRIEN, W. J. 1976. Effect of varying oxygen concentrations on the filtering rate of *Daphnia pulex. Ecology* 57, 808-814.

LABERGE, S. AND HANN, B. J. 1990. Acute temperature and oxygen stress among genotypes of *Daphnia pulex* and *Simocephalus vetulus* (Cladocera, Daphiniidae) in relation to environmental conditions. *Can. J. Zool.*, 68, 2257-2263.

MULLER, J. AND SEITZ, A. 1993. Habitat partitioning and differential vertical migration of some *Daphnia* genotypes in a lake. *Arch. Hydrobiol. Beith.,* 39, 167-174.

WEIDER, L. J. 1984. Spatial heterogeneity of *Daphnia* genotypes: Vertical migration and habitat partitioning. *Limnol. Oceanogr.* 29, 225-235.

WEIDER, L. J. AND LAMPERT, W. 1985. Differential response of *Daphnia* genotypes to oxygen stress: respiration rates, hemoglobin content and low-oxygen tolerance. *Oecologia* 65, 487-491.

CHAPTER 5

Vertical Migration

Introduction

No other topic has captured the attention of biological limnologists more than diel vertical migration (DVM) in zooplankton. Hardy (1956, cited in Dini and Carpenter, 1992) described DVM as "the planktonic problem No. 1". In his pivotal contribution McLaren (1963) stated, "The literature on the vertical migration of zooplankton is enormous, complex and full of conflicting observations and generalizations". Beginning with Cuvier's 1817 description of *Daphnia* vertical migration (Bayly's 1986 translation in Dodson and Frey, 1991) and the work of pioneering north American limnologists such as Birge (1895) and continuing to the present, the phenomena of DVM has fascinated limnologists. Even the number of review papers is becoming large (Ringelberg, 1993a). Comprehensive citations of review papers, hypotheses and models can be found in McLaren (1963) and Gabriel (1993).

DVM refers to the daily vertical movement of zooplankton, especially the larger cladocerans, toward surface waters at night and returning to deeper waters during daylight. This behavior usually takes grazing zooplankton from a warmer, relatively well illuminated and food rich environment, where they are more vulnerable to visual predators, to an environment that is colder, darker and less productive but where the risk of predation is reduced. These migrations are typically on the order of 2 m to 20 m (Dodson and Frey, 1991; Pennak, 1989). A brief synopsis of factors contributing to zooplankton DVM was provided by Guisande et. al. (1991). They pointed out the following three generalizations: 1. In lakes without significant predation, diurnal vertical migrations do not take place; 2. Where food is uniformly available at all depths and the risk of predation is high, again migration does not take place; 3. In a lake with some risk of predation and differences in food quality with depth, zooplankton have two options. If food is of sufficient quantity to prevent starvation, animals may adopt the strategy of 'better hungry than dead' (Lampert, 1989) and migrate to deeper waters by day. Conversely, with lower food levels large vulnerable animals may adopt the strategy of 'better dead than unfed' (Johnsen

and Jakobsen, 1987) and remain in more productive but riskier shallow waters during daylight hours. However, to claim that there has been a lack of consensus in the literature on these generalizations would be an understatement indeed.

Early hypotheses proposed to explain the phenomena of DVM included optimal light levels, the notion that DVM acted to mix populations thereby preventing over specialization within a species (McLaren 1963) and Wynne-Edward's (1962) social control ideas. Although I will not pursue these hypotheses in any further detail, it is worth noting that the ideas of David (1961) for sexually-reproducing populations are exactly the converse of the viewpoint of this thesis for asexually-reproducing populations. He suggested that genetic diversity could be preserved via recombination in a population of zooplankton reproducing sexually if there was a continual vertical mixing of the population. I suggested in chapter 1 that in the absence of recombination, spatial and temporal isolation along with fluctuating selection can preserve genetic diversity.

As McLaren (1963) noted explicitly (quoted above) and implicitly, by the tone of frustration throughout his introduction, there was a definite lack of consensus among early investigations regarding the cause of DVM. Pennak (1944) noted, "the problem is exceedingly complex and difficult of solution". This lack of consensus can be ascribed to several interesting causes. Pennak (1989) cautioned against transposing results between species and habitats. However, generalizations have been attempted with phylogenetically disparate organisms often occurring in widely varying habitats from both marine and freshwater environments. While the role of predation has long been recognized, historically zooplankton DVM was associated with aspects of the physical and chemical environments such as light, phosphorus and oxygen. Chief among these was the idea that zooplankton migrated to maintain an optimum light environment as a end in itself (McLaren, 1963).

In the early 1960s limnologists were starting to focus on biological explanations for phenomena observed in fresh water habitats (Chapter 2). Similarly, recent hypotheses that have been proposed to explain the phenomena of DVM have focused on biological explanations (Gabriel, 1993). Most of the points of view converge on two competing but not necessarily mutually exclusive lines of

thought that have been termed 'metabolic hypotheses' and 'predation hypotheses'. The metabolic model first postulated by McLaren (1963) essentially states that there is a metabolic advantage for migrating zooplankton because they can ingest food in warm productive surface waters at night while digesting food in relatively cool deeper water during the day. Because zooplankton are poikilotherms or thermal conformers, metabolic rates drop in cooler waters, therefore, more intake energy can be partitioned to growth and reproduction and less to maintenance metabolism. The predation model articulated by Zaret and Suffern (1976) claims that vertical migrations are an adaptation to selective pressures from visually dependent predators. Zooplankton retreat to less illuminated waters during daylight hours to lower the probability of detection. Wright and Shapiro (1990) concluded that refuges from predation were a significant factor alleviating zooplanktivore-induced mortality.

The topic of DVM has been plagued with its share of cloudy thinking and obscure logic. In retrospect, it is not surprising that early investigations of DVM focusing almost exclusively on abiotic factors were frustrated by inconsistent results. The situation is a clear example of the need to be aware of important hidden variables (in this case the biotic factors of predation and metabolism energetics) when attempting to assign cause and effect based upon correlative research. Additionally, Ringelberg (1980) pointed out that causal (proximate cues) and teleological (ultimate adaptations) aspects of DVM have often been confused in the literature. Gabriel (1993) reiterated the plea for clear and logical thinking with respect to competing DVM hypotheses and cautioned that one should never misuse the consequences of DVM as an explanation for DVM without *proving the selection element.* This last phrase may be over stating the case somewhat, possibly do to a differences arising in translation to English; *proving selection* can be a formidable task indeed. I believe that what was intended was something closer to *demonstrating adaptation.* Gabriel (1993) also attempted to dispose of DVM hypotheses that relied on group selection. He pointed out in general terms, that due to the different time scales involved, any effects of group selection would be so small (orders of magnitude less than effects of individual selection) that if group selection acted in concert with individual selection it could not be detected, and, if group selection acted counter to individual selection it would very quickly be overwhelmed.

Perhaps the only generally agreed upon aspect of DVM is that light, more specifically the rate of change in light intensity, constitutes the proximate cue for zooplankton DVM. *Daphnia* are maximally sensitive to light at wavelengths from 530 nm to 540 nm (Waterman, 1961). Ringelberg (1993b) recently reconfirmed observations made by Kikuchi (1937), McNaught and Hasler (1964) and others that the rate of change of light was integral to phototaxis in *Daphnia*. Stearns (1975) observed that *D. pulex* activity responses to light peaked in the morning and declined through the day, he further reported that the responses were primarily in the vertical dimension at wavelengths between 480 nm and 760 nm. Smith and Baylor (1953) reported that *D. magna* responded to bright blue light by swimming down and responded to dim blue and red light by swimming up. They further observed that a shift in spectral composition to blue light caused downward swimming while a shift to red light resulted in upward swimming. Haney and Hall (1975) reported that both DVM and feeding rates were stimulated in *D. pulex* by the rate of change in light intensity. All of these observations are consistent with the concept that light is the basic proximate cue for DVM.

Secondary factors can augment the light stimulus. McNaught and Hasler (1964) reported that *Daphnia's* response to light was increased at higher temperatures. Gerritsen (1982) reported that three species of *Daphnia* responded to increases in temperature by changing the duration and frequency of upward swimming in proportion to the change in temperature and tended to sink when exposed to falling temperatures. Thus, once started, upward and downward migrations could be self-reinforcing behaviors in lakes that are thermally stratified. Haney (1993) reiterated that zooplankton responded to the rate of change of light and responded to increases in temperature by swimming upward. He further reported that chemical cues released by fish and invertebrate predators could cause an enhanced DVM by increasing light sensitivity and inducing negative phototaxis in *Daphnia*.

Investigations dealing with the two persistent biotic hypotheses for DVM, metabolic advantage and predator avoidance, reveal that they may be viewed as a hierarchy of adaptations (Dini and Carpenter, 1992). The predation hypothesis is currently favored

as the dominant force driving zooplankton DVM. Energetic advantages proposed by the metabolic hypotheses (McLaren, 1963; Enright, 1977) have either not been found (Swift, 1976; Orcutt and Porter, 1983 Lampert, 1987; and Guisande et. al., 1991) or been found to be equivocal and dependent on developmental stage (Enright and Honegger, 1977). Indeed Swift (1976), Orcutt and Porter (1983), Lampert (1987) and Guisande et al. (1991) all documented metabolic advantages associated with continuous residence in conditions usually found in shallow, warmer more productive surface waters. It was thought that perhaps the energy demands of swimming involved with DVM, might more than offset the advantages confered by residing in cooler waters for part of the day. But Dawidowicz and Loose (1992) failed to find metabolic costs in DVM simulations in the laboratory. Johnsen and Jakobsen (1987) reported that DVM was a function of food availability and represented a trade-off between predation risk and energy demands. Although some questions remain, the lack of support for the metabolic hypothesis as the major force behind DVM is evident.

Concurrent with the lack of support for the metabolic hypothesis, a growing body of research clearly supports the predation hypothesis as the primary force driving zooplankton DVM especially when predator avoidance is combined with recent contributions detailing genetic effects associated with zooplankton distribution and behavior. Lampert' s (1987) results not only failed to support the metabolic hypothesis but also suggested that DVM was a strategy to reduce mortality caused by fish predation. Recent research with water columns under controlled laboratory conditions (Haney, 1993; Loose, 1993) and field studies (Duncan et. al., 1993) have clearly shown that *Daphnia* DVM is a response to predators and predator-mediated chemical cues. The argument for the predation hypothesis was further corroborated by the seasonal and annual DVM patterns observed in nature. Stirling et. al. (1990) reported that the amplitude of DVM in *D. galeata* doubled in years when zooplanktivorous fish were more abundant. Stich (1989) reported that the onset of zooplankton DVM coincided seasonally with the hatching of fish larvae. Ringelberg et. al. (1991) reported that the appearance of large shoals of juvenile perch *(Perca fluviatilis)* initiated DVM behavior in *Daphnia.* But Flik and Ringelberg (1993) found that the seasonal onset of DVM in *Daphnia,* in response to the

appearance of large shoals of juvenile perch, could be delayed by a month when chlorophyll concentrations (*Daphnia* food) were low.

In an elegant series of laboratory experiments with *D. magma* De Meester and Dumont reconfirmed the effect of fish mediated chemicals on DVM behavior and demonstrated that phototactic behavior:

1. Was genetically determined (De Mester and Dumont. 1988).
2. Was an inherited character in asexually as well as sexually reproducing individuals (De Meester, 1989a, 1991).
3. Varied with both hunger and genotype but that there was no hunger-genotype interaction (De Meester, 1989b).
4. Varied with both fish-mediated chemicals and genotype and that there was a significant interaction between the effects of genotype and fish mediated chemicals (De Meester, 1993a).

Many of these observations were confirmed under more natural conditions with outdoor tanks (De Meester, 1993b). Watt and Young (1992) observed that adult *D. magna* exhibited clone-specific depth responses and that clones remaining near the surface suffered the greatest predation. When results from the aforementioned studies, conducted under controlled conditions, are combined with field studies on the clone-specific DVM differences in *D. pulex* (Weider, 1984), *D. galeata* and *D. galeata x cuculatta* (Muller and Seitz, 1993) and *D. longispina* (King and Miracle, 1995); a powerful, well-developed and internally consistent hypothesis emerges that explains DVM in terms of predator avoidance.

Johnsen and Jakobsen (1987) claimed that genetic differences were not needed to account for variation in DVM behavior exhibited by *Daphnia.* They concluded that DVM represented a trade-off between predation and starvation in *D. longispina* and argued that phenotypic plasticity in behavior by itself, as a response to hunger, could alter DVM. This is a puzzling claim because apparently the *Daphnia* were introduced to experimental columns from a natural population that was not genetically screened and was almost certainly genetically diverse. Therefore, it would seem to be difficult to eliminate variation due to genetic factors from their 'phenotypic' plasticity. Loose (1993) reported that individuals from a single *Daphnia* clone could exhibit a phenotypically plastic response in depth selection in response to predators in an experimental plankton tower. However, he acknowledged that his finding did not

refute the possibility of a genetic component to DVM. Ringelberg et. al. (1991) concluded that the rapid seasonal onset of DVM behavior in *Daphnia* indicated a phenotypic response as opposed to a genetic change in the population. Even if phenotypic plasticity does play a role in the variation among individuals in DVM behavior, these characters and the control of their expression are ultimately genetically determined and simply expressed differently with differing environmental stimuli.

In a recent paper entitled: 'Ultimate causes of diel vertical migration of zooplankton: New evidence for the predator-avoidance hypothesis'; Lampert (1993) States:

> "The strongest support comes from the recent discovery that DVM is an inducible response triggered by a cue (mostly chemical) from the predator. DVM is modified by environmental factors such as food and oxygen and also the strength of the trade-off between maximum protection and energetic input. However, avoidance of light dependent mortality (presumably visual predation) seems to provide a unifying concept to explain the evolution of this widespread behavior".

An even stronger and more unified case can be made for the predation hypothesis by incorporating the genetic basis of DVM behavior. The insights by De Meester (1993) on clone-specific differences in phototactic behavior provided a basis for unifying the proximate cue and the ultimate selection for DVM. He reported that not only do *Daphnia* clones respond differently to light and a fish-mediated chemical cue, but also that there was a significant interaction between these two stimuli.

This indicated that the commonly agreed upon primary cue (light induced negative phototaxis) could be significantly and differentially (by clone) enhanced by the presence of predators as a secondary cue. Thus, those individuals that responded more negatively to light would seem to have a selective advantage in avoiding light-dependent visual predators. While the metabolic hypothesis has not been eliminated, the preponderance of recent research has certainly relegated it to a secondary position in a hierarchy that needs to include predation, genotype and the interaction of predation with genotype as primary factors in the explanation of DVM.

While DVM was not a major focus of my research and my observations on this fascinating topic were limited, my intention in

this chapter was to use DVM observations as a basis to begin to tie together the results from chapters 1 through 4. Data analysis was limited to the observations made on the deep layer of *D. pulicaria* before and after the upward migration that followed sunset on a single night. I did not know the densities in any layer before or after sunset and, therefore, could not estimate the changes in clone frequencies. However, because we did not observe any downward migration into the deep layer, a simple comparison of pre and post sunset clone frequencies provided an estimate of clone specific tendencies to migrate upward from this layer following sunset. Additionally it was the DVM behavior of clones in the deep layer that I was most interested in as a potential part of the explanation for the increased frequency of *PGI*$_{SS}$ individuals in the hypolimnion (Chapter 1).

My objective was to measure the proportions of clones and color morphs in the deep layer before and after the post-sunset upward migration to test the hypotheses of differential migration in these groups.

Methods

Observations and collections were conducted between 1830 on 10 August and 0600 on 11 August, 1992 at the northwest sampling location (Chapter 1). Pre migration samples were collected at 1900 from a depth of 15-17 m. Post migration samples were collected from the same depth interval at 0035. Sunset occurred at 1941. All times are central standard (CST).

Zooplankton were monitored continuously throughout the period of observation with the acoustic equipment described in Chapter 1. The major difference in technique was that the observations were recorded while anchored at a fixed position. The result was a record of the change of zooplankton vertical distribution with time compared to the spatial records described in Chapter 1.

Limnological observations, *Daphnia* collection, electrophoresis techniques and observations on pigmentation remained as described in the preceding chapters.

Results

Long Lake was thermally stratified. The epilimnion (21.0 C) extended from the surface to 7 m. Water temperature declined rapidly in the metalimnion from 18.0 C at 8 m to 8.0 C at 13 m. Temperatures declined slightly in the hypolimnion from 7.5 C at 14 m to 6.0 C at a depth of 20 m. Dissolved oxygen exhibited a positive heterograde profile. Concentrations varied from 8.4 mg/L to 8.8 mg/l in the epilimnion, increased to a maximum of 10.8 mg/L at 10 m in the metalimnion and declined to a minimum of 0.3 mg/L at 20 m.

Water transparency (Fig. 1) indicated that phytoplankton densities were greatest in the metalimnion where light transmission values dropped to 45%. A secondary minimum was observed near the bottom at 20 m perhaps due to a layer of bacteria in these relatively anoxic waters. Comparing a pre-sunset profile (1900) and a night profile (0035), indicated that a maximum light transmission increase of 8% occurred a depth of 3 m after sunset. This was probably due to the patchy nature of phytoplankton rather than a systematic temporal change.

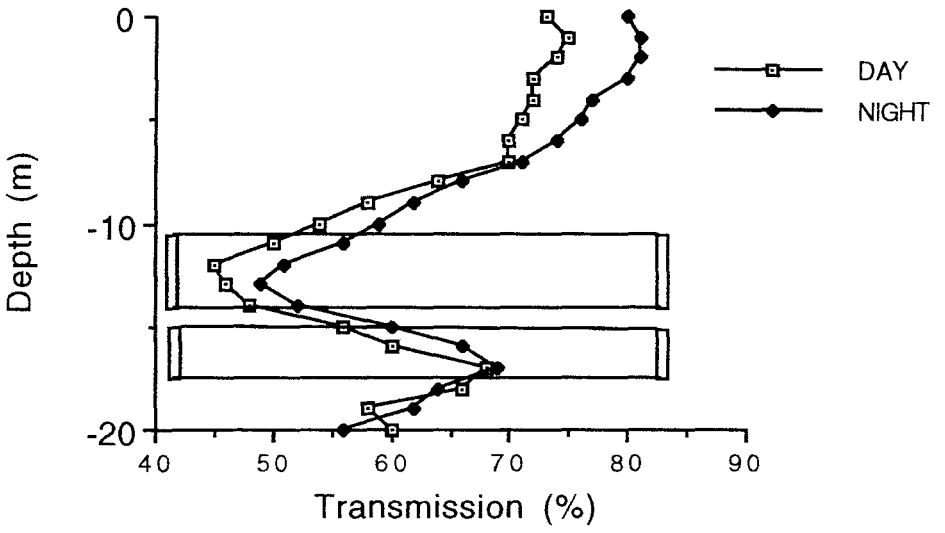


Figure 1. Water transparency at 1900 and 0035. Horizontal rectangles indicate layers of zooplankton.

Sonar echograms indicated that zooplankton were most abundant in two layers prior to sunset (Fig. 1). An upper layer occurred in the metalimnion between 11 m and 14 m, a deeper layer occurred in the hypolimnion from 15 m to 17 m. Upward movement of the upper layer began at approximately 2015, one-half hour after sunset. The layer became diffuse and separated into migrants and non-migrants. Most of the migrants moved into depths between 5 m to 8 m, although there was an indication of a very diffuse shallower layer from 1 m to 4 m that developed by 2100. The pre-sunset lower layer (15-17 m) appeared to ascend slightly, perhaps 1/2 m, at 1955.

Between 2100 and 2400 the vertical distribution consolidated into 4 layers and stabilized for the rest of the night. The resulting nighttime distribution consisted of a diffuse very shallow layer (1 to 4 m) and the above-mentioned 5-8 m layer that expanded and descended slightly to form a stable layer from 6 to 10 m. A third layer comparable to the pre-sunset shallow layer, (11 to 14 m) remained at the same depth, although it was lower in density. The deepest layer (15-17 m, nominal) remained intact, but lower in density, and oscillated vertically 1/2 - 1 m. By midnight very little if any temporal change in vertical distribution was observed.

A dramatic descent of the upper nighttime layers began at 0415 with the first diffuse daylight almost an hour prior to sunrise at 0513. By 0600 the vertical distribution of zooplankton had returned to the two-layer configuration that was observed prior to sunset the previous day.

Zooplankton layers were not made up entirely of *D. pulicaria,* the densities in any given layer were unknown, and I could not track individuals from their original daytime layers to nighttime layers. Therefore, I only analyzed genetic composition and hemoglobin pigmentation in pre- and post-migration samples from the deepest (15-17 m) layer. After sunset part of this layer migrated upward but there was no downward migration from the shallower (11-14 m) daytime layer Additionally the deep daytime layer had become the focus of interest because most the variation in the proportions of *D. pulicaria* clones occurred in this layer.

D. pulicaria pigmented with hemoglobin were clearly more abundant in the daytime sample, which indicated that these individuals were much more likely to migrate upward at night than transparent morphs. Pigmented morphs outnumbered transparent morphs approximately 2:1 during the day in the deep layer while the converse was evident at night (Fig. 2). PGI_{SS} individuals made up a significantly larger proportion of the daytime population compared to the nighttime proportion indicating that individuals of this clone were more likely to migrate upward after sunset.

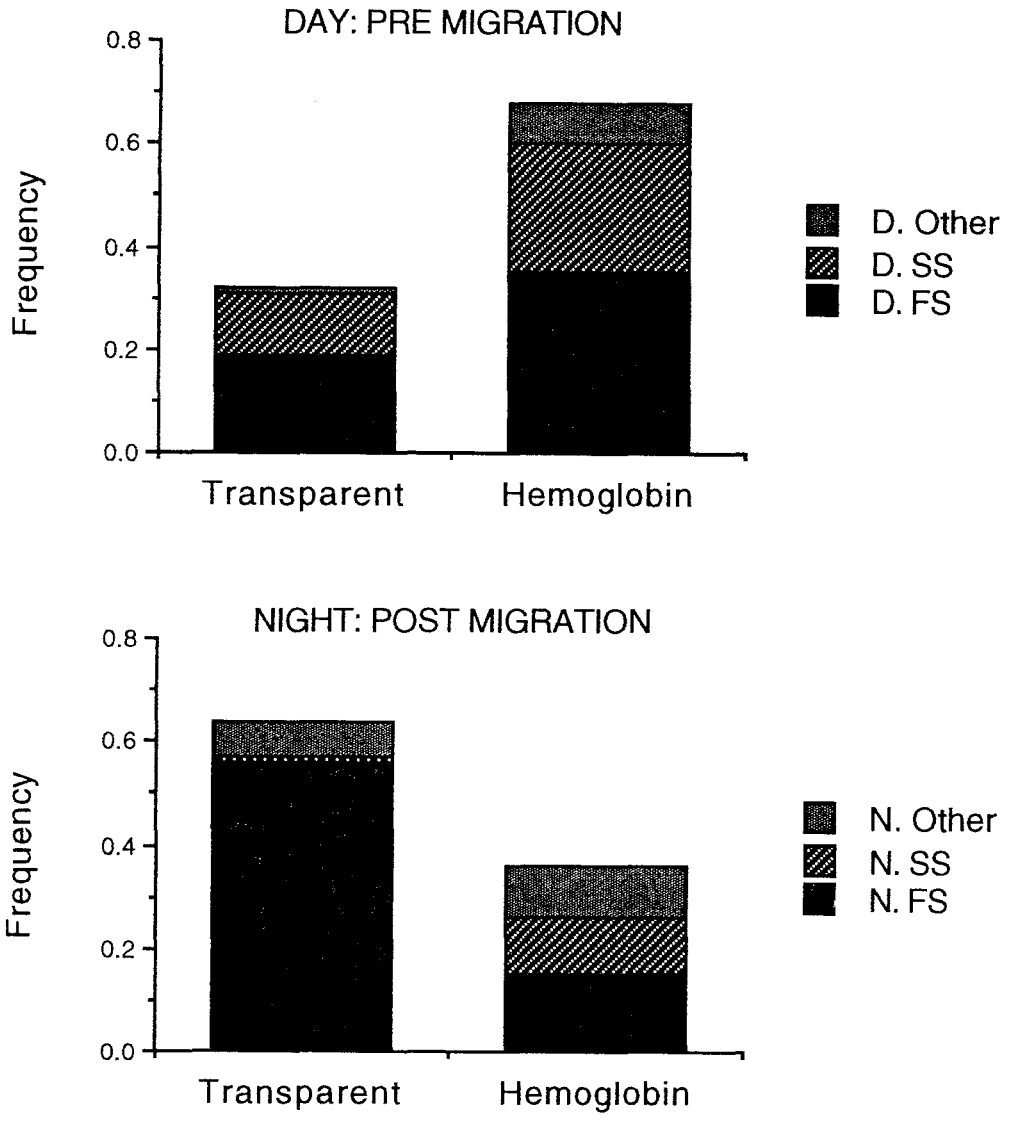


Figure 2. Relative frequencies of *PGI* clones from the deep zooplankton layer.

However, there were more complex factors. A three dimensional log-linear analysis of the data in table one indicated that these complex relationships were significant. All main effects, and the second order effect needed to be included in the log-linear model (the saturated model) to account for the observed temporal proportions of clones and color morphs. For example the proportion of SS clones was lower in the transparent category of the post migrants (night) indicating that this clone migrated with a greater probability than would have been predicted based upon the association of color and genotype alone.

Table 1. Numbers of *D. pulicaria* categorized by clone type and color morph in the deep layer of zooplankton before (day) and after (night) the post-sunset vertical migration. Proportions are in parentheses.

		PGI CLONES			
TIME	COLOR	FS	SS	OTHER	TOTALS
Day	Transparent	18 (0.19)	11 (0.12)	1 (0.01)	30 (0.32)
					93 (1.00)
	Hemoglobin	33 (0.35)	23 (0.25)	7 (0.08)	63 (0.68)
Night	Transparent	40 (0.56)	1 (0.01)	5 (0.07)	46 (0.64)
					72 (1.00)
	Hemoglobin	11 (0.15)	8 (0.11)	7 (0.10)	26 (0.36)

The differences between the daytime and nighttime proportions of the clones and color morphs illustrated these complex relationships (Fig. 3). In this construct positive values indicated a tendency to migrate upward after sunset, negative values indicated a tendency to remain in the deep layer overnight. Clearly the transparent morphs of the FS clone were not as likely to migrate compared to any other group. Individuals containing hemoglobin were more likely to migrate than transparent individuals, but SS individuals tended to migrate regardless of color morph. Because densities were unknown it was not possible to construct a 'migrating population' by simply subtracting nighttime frequencies from the daytime frequencies.

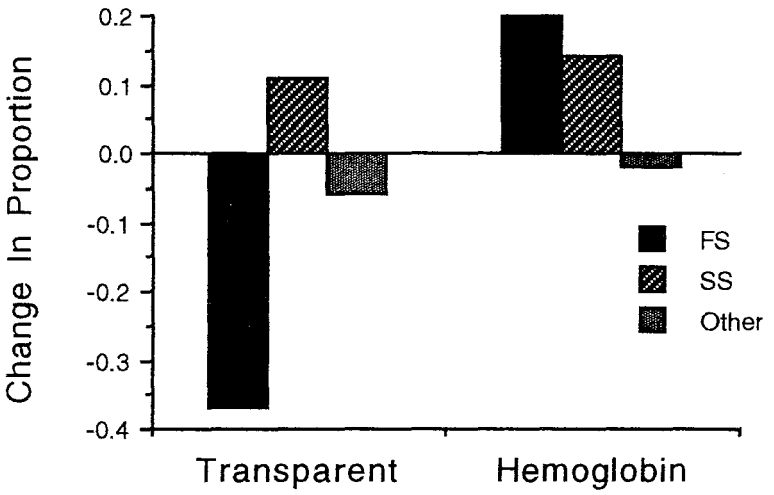


Figure 3. Daytime proportions minus nighttime proportions of *D. pulicaria* in the deep layer.

Discussion

Integration of the results obtained at Long Lake with the vast DVM literature was made difficult by the limited scope of my observations, by the existence of multiple daytime and nighttime zooplankton layers, and by the complex interactions among color morphs and genotypes with migrators and non-migrators.

Neither of the daytime zooplankton layers nor the densest food concentrations occurred in the warm waters of the epilimnion where the metabolic advantages reported in the literature would be greater (Swift, 1976; Orcutt and Porter, 1983; Lampert, 1987; Guisande et al., 1991). The upper daytime layer coincided with a the maximum phytoplankton density as indicated by the low transparency between 11 m and 14 m. While neither the species composition nor the nutritional value of the algae were known, this relatively dense layer of phytoplankton resulted in a typical mid-summer surplus of dissolved oxygen in the metalimnion. The deep zooplankton layer (15-17 m) occurred immediately above a distinct deep phytoplankton minimum at 17 m. The slight differences (< 8%) between day and night turbidity observations suggested that major diel shifts in food resources did not occur.

The upward migration after sunset brought deep migrants up to, and perhaps through, more abundant food resources. Incident light at the lake surface was not measured so I could not rigorously compare my results relative to the exact timing cues reported by McNaught and Hasler (1964) and others. Significantly, however, the migration did not begin until well after sunset. Enright and Honegger (1977) noted that metabolic advantages could be maximized if upward migration began before sunset.

My results indicating that the evening migration began after sunset and that the morning migration began before sunrise were in good agreement with Ringelberg et. al. (1991). They found that *Daphnia* migration was associated with the relative change in light intensity rather than the absolute change in light intensity. Relative changes in light intensity (the change in intensity divided by the intensity) were greatest after sunset and before sunrise (Ringelberg et. al., 1991).

The downward migration before sunrise was much faster than the upward post sunset migration. This may have been a stronger response to visual predation that was about to be substantially increased with increasing daylight. But other factors need to be considered. Ringelberg et. al. (1991) suggested that differences in upward and downward migration might be do to differences in the sensitivity of light adapted *Daphnia* to declining light at sunset compared to dark adapted *Daphnia* responding to increasing light at sunrise. *Daphnia* are slightly negatively buoyant. Perhaps the downward movement was simply reinforced by gravity. The abrupt downward migration was not likely caused by predators consuming slower *Daphnia* because fish did not appear on the echolocator.

The disproportionate decrease of pigmented and PGI_{SS} *D. pulicaria* in the deep layer after sunset indicated that this morph and this genotype were more likely to migrate upward at night. These findings were consistent with observations reported on clone-specific vertical migration (Weider, 1984; Muller and Seitz, 1993; King and Miracle, 1995). My observations were also consistent with Lampert's (1993) conclusion that the most conspicuous individuals were the most pronounced migrators because they could gain greatest benefit by avoiding detection from visual predators. Hembre (Pers. Com.) noticed that hemoglobin pigmentation was accelerated in Long Lake *D. pulicaria,* routinely held at cool temperatures in the laboratory, when they were inadvertently allowed to warm up to room temperature overnight. This overnight warm up likely simulated the temperatures encountered on upward vertical migration. Perhaps the diel increase in metabolic rates experienced by migrating individuals enhanced the synthesis of hemoglobin.

The deep daytime layer of zooplankton was clearly made up migrating and non-migrating individuals. Guisande et. al. (1991) reported, in a slightly different context, that these two behaviors could coexist simultaneously and further that the coexistence was an example of an evolutionary stable strategy. They reported that a low proportion of a *Daphnia* population, mostly small individuals with low risk of predation, remained in food-rich surface waters continuously, while the majority of the population exhibited typical DVM behavior. Lampert (1993) reported that 8-9% of *Daphnia* adopted a non-migratory strategy and remained in the hypolimnion. He

further noted the need for physiological adaptation for long-term residence in low oxygen conditions. The situation with the non-migrating *D. pulicaria* in the deep layer in Long Lake is puzzling because this group is clearly skewed away from hemoglobin formation, an important physiological adaptation to low oxygen conditions. The disproportionately high number of transparent morphs remaining in the deep layer cannot be explained at this time. However, it should be noted that the PGI_{FS} genotype, over-represented in the transparent morph, was also much more likely to succumb when transplanted to the shallow layer (Chapter 4). Perhaps this numerically abundant genotype was less thermally tolerant, thus, 'trapped' in hypolimnetic conditions once acclimated to cooler temperatures.

While this admittedly brief glimpse into the fascinating topic of diel vertical migration left many questions unanswered, it did provide evidence for another adaptation within the PGI_{SS} clone(s) of *D. pulicaria* in Long Lake. This clone which has been shown to be both more vulnerable to visual predation (Chapter 2) and more likely to form hemoglobin (Chapter 3), was more likely to adopt a behavior of DVM. PGI_{SS} clones occurring in the hypolimnion were able to exploit a physiological refuge (Chapter 3) during daylight hours while moving into food- and oxygen-rich waters at night, thus minimizing exposure to light-dependent visual predators. Although specific explanations were lacking, apparently the numerically dominant PGI_{FS} clone was not able to adopt these strategies as effectively as the PGI_{SS} clone. Clearly additional work is needed on the topic of DVM in order to more adequately understand the complex relationships among genotypes, physiological adaptations and behavior.

References

BAYLY I. A. E. 1986. Aspects of diel vertical migration in zooplankton, and its enigma variations. Pages 349-368 In. Deckker, P. and Williams, W. D. (eds) *Limnology In Australia.* CSIRO, Melbourne. Junk, Dordrecht, Netherlands.

BIRGE, E. A. 1895. Plankton studies on Lake Mendota. I. The vertical distribution of the pelagic crustacae during July, 1894. *Trans. Wisc. Acad. Sci. Arts Lett.,* 10, 421-484.

DAVID, P. M. 1961 The influence of vertical migration on speciation in the oceanic plankton. *Systematic Zoology,* 10, 10-16.

DAWIDOWICZ, P. AND LOOSE, C. J. 1992. Cost of swimming by *Daphnia* during diel vertical migration. *Limnol. Oceanogr.* 37, 665-669.

DE MEESTER, L. AND H. J. DUMONT. 1988. The genetics of phototaxis in *Daphnia magna:* Existence of three phenotypes for vertical migration among parthenogenetic females. *Hydrobiologia* 162, 47-55.

DE MEESTER, L. 1989a. An estimation of the heritability of phototaxis in *Daphnia magna* Straus. *Oecologia* 78, 142-144.

DE MEESTER, L. 1989b. Phototaxis in *Daphnia:* Interaction of hunger and genotype. *Limnol. Oceanogr.* 34, 1322-1325.

DE MEESTER, L. 1991. An analysis of the phototactic behavior of *Daphnia magna* clones and there sexual descendants. *Hydrobiologia* 225, 217-227.

DE MEESTER, L. 1993a. Genotype, fish mediated chemicals, and phototactic behavior in *Daphnia magna. Ecology* 74, 1467-1474.

DE MEESTER, L. 1993b. The vertical distribution of *Daphnia magna* genotypes selected for different phototactic behavior: Outdoor experiments. In: Ringelberg (ed) *Diel vertical migration of zooplankton. Arch. Hydrobiol. Beih.: Advances in Limnology.* 39, 137-155.

DINI, M. L. AND CARPENTER, S. R. 1992. Fish predators, food availability and diel vertical migration in *Daphnia. J. Plankton Res.,* 14, 359-377.

DODSON, S. I. AND FREY, D. G. 1991. Cladocera and other Branchiopoda. In: Thorp, J. H. and Covich, A. P. (eds) *Ecology and Classification of North American Freshwater Invertebrates.* Chapter 20, pp 723-786. Academic Press, San Diego.

DUNCAN, A., GUISANDE, C. AND LAMPERT, W. 1993. Further trade-offs in *Daphnia* vertical migration strategies. In: Ringelberg (ed) *Diel vertical migration of zooplankton. Arch. Hydrobiol. Beih.: Advances in Limnology.* 39, 99-108.

ENRIGHT, J. T. 1977. Diurnal vertical migration adaptive significance and timing. Part I. Selective advantage: A metabolic model. *Limnol Oceanogr.* 22, 856-872.

ENRIGHT, J. T. AND HONEGGER, H. W. 1977. Diurnal vertical migration adaptive significance and timing. Part II. A test of the model: Details of timing. *Limnol Oceanogr.* 22, 873-886.

FLIK B. J. G. AND RINGELBERG, J. 1993. Influence of food availability on the initiation of diel vertical migration (DVM) in Lake Marrsseveen. In: Ringelberg (ed) *Diel vertical migration of zooplankton. Arch. Hydrobiol. Beih.: Advances in Limnology.* 39, 57-65.

GABRIEL, W. 1993. Models on diel vertical migration. In: Ringelberg (ed) *Diel vertical migration of zooplankton. Arch. Hydrobiol. Beih.: Advances in Limnology.* 39, 123-136.

GERRITSEN, J. 1982. Behavioral response of *Daphnia* to rate of temperature change: possible enhancement of vertical migration. *Limnol. Oceanogr.* 27, 254-261.

GUISANDE, C., DUNCAN, A. AND LAMPERT, W. 1991. Trade-offs in vertical migration strategies. *Oecologia* 87, 357-359.

HANEY, J. F. 1993. Environmental control of diel vertical migration behaviour. In: Ringelberg (ed) *Diel vertical migration of zooplankton. Arch. Hydrobiol. Beih.: Advances in Limnology.* 39, 1-17.

HANEY, J. F. AND HALL, D. J. 1975. Diel vertical migration and filter-feeding activities of *Daphnia. Archiv f. Hydrobiologie, Bd.,* 75, 413-441.

HARDY, A. C. 1956. *The Open Sea-Its natural History: The World Of Plankton.* Houghton Mifflin, Boston.

JOHNSEN, G. H. AND JAKOBSEN, P. J. 1987. The effect of food limitation on vertical migration in *Daphnia longispina. Limnol. Oceanogr.* 32, 873-880.

KIKUCHI, K. 1937. Studies on the vertical distribution of the plankton Crustacae, II: The reversal of phototropic and geotropic signs of the plankton Crustacae with reference to vertical movement. *Rec. Oceanogr. Works Jap.,* 17-41.

KING, C. E. AND MIRACLE, M. R. 1995. Diel vertical migration by *Daphnia longispina* in a Spanish lake: Genetic sources of distributional variation. *Limnol. Oceanogr.* 40, 226-231.

LAMPERT, W. 1987. Vertical migration of freshwater zooplankton: Indirect effects of vertebrate predators on algal communities. In: Kerfoot, W. C. and Sih, A. (eds) *PREDATION: Direct and Indirect Impacts on Aquatic Communities.* pp. 291-299. University Press of New England, Hanover and London.

LAMPERT, W. 1989. The adaptive significance of diel vertical migration. Funct. Ecol. 3, 21-27.

LAMPERT, W. 1993. Ultimate causes of diel vertical migration of zooplankton: New evidence for the predator-avoidance hypothesis. In: Ringelberg (ed) *Diel vertical migration of zooplankton. Arch. Hydrobiol. Beih.: Advances in Limnology.* 39, 79-78.

LOOSE, C. J. 1993. *Daphnia* diel vertical migration behavior: Response to vertebrate predator abundance. In: Ringelberg (ed) *Diel vertical migration of zooplankton. Arch. Hydrobiol. Beih.: Advances in Limnology.* 39, 29-36.

MCLAREN, I. A. 1963. Effects of temperature on growth of zooplankton, and the adaptive value of vertical migration. *J. Fish. Res. Bd. Canada,* 20, 685-727.

MCNAUGHT, D. C. AND HASLER, A. D. 1964. Rate of movement of populations of *Daphnia* in relation to changes in light intensity. *J. Fish. Res. Bd. Canada.* 21, 291-318.

MULLER, J. AND SEITZ, A. 1993. Habitat partitioning and differantial vertical migration of some *Daphnia* genotypes in a lake. In: Ringelberg (ed) *Diel vertical migration of zooplankton. Arch. Hydrobiol. Beih.: Advances in Limnology.* 39,167-174.

ORCUTT, J. D. AND PORTER, K. G. 1983. Diel vertical migration by zooplankton: Constant and fluctuating temperature effects on life history parameters of *Daphnia. Limnol. Oceanogr.* 28, 720-730.

PENNAK, R. W. 1944. Diurnal movements of zooplankton organisms in some Colorado mountain lakes. *Ecology,* 25, 387- 403.

PENNAK, R. W. 1989. *Fresh-Water Invertebrates Of The United States.* John Wiley and Sons, New York.

RINGELBERG, J. 1980. Introductory remarks: Causal and teleological aspects of diurnal vertical migration. In: Kerfoot, C. W. (ed) *Evolution and Ecology of Zooplankton Communities.* pp. 91-97. University Press of New England, Hanover and London.

RINGELBERG, J. 1993a. (ed). *Advances in Limnology: Diel vertical migration of zooplankton.* Proc. Intl. Symposium, Lelystad, The Netherlands. *Arch. Hydrobiol. Beih.*

RINGELBERG, J. 1993b. Phototaxis as a behavioral component of diel vertical migration in a pelagic *Daphnia.* In: Ringelberg (ed) *Diel vertical migration of zooplankton. Arch. Hydrobiol. Beih.: Advances in Limnology.* 39, 45-55.

RINGELBERG, J., FLIK, B. J. G., LINDENAAR, D. AND ROYACKERS, K. 1991. Diel vertical migration of *Daphnia hyalina* (sensu latiori) in Lake Maarsseveen: Part 1. Aspects of seasonal and daily timing. *Arch. Hydrobiol.,* 121, 129-145.

SMITH, F. E. AND BAYLOR, E. R. 1953. Color responses in the Cladocera and their ecological significance. *Am. Nat.,* 87, 49-55.

STEARNS, S. C. 1975. Light responses of *Daphnia pulex. Limnol. Oceanogr.,* 20, 564-570.

STICH, H. B. 1989. Seasonal changes of diel vertical migrations of crustacean plankton in Lake Constance. *Arch. Hydrobiol. Suppl.* 83, 355-405.

STIRLING, D. G., MCQUEEN, D, J. AND M. R. S. JOHANNES. 1990. Vertical migration in *Daphnia galeata mendotae* (Brooks): Demographic responses to changes in planktivore abundance. *Can. J. Fish. Aquat. Sci.* 47, 395-400.

SWIFT, M. C. 1976. Energetics of vertical migration in *Chaoborus trivittatus* larvae. *Ecology.* 57, 900-914.

WATERMAN, T. H. 1961. Light sensitivity and vision. In: Waterman, T. H. (ed) *The Physiology of Crustacae, Vol II: Sense Organs, Integration and Behavior.* pp. 1-53. Academic Press, New York.

WATT, P. J. AND YOUNG S. 1992. Genetic control of predator avoidance behavior in *Daphnia. Freshwater Biology* 28, 363-367.

WEIDER, L. J. 1984. Spatial heterogeneity of *Daphnia* genotypes: Vertical migration and habitat partitioning. *Limnol. Oceanogr.* 29, 225-235.

WRIGHT, D. AND SHAPIRO, J. 1990. Refuge availability: a key to understanding the summer disappearance of *Daphnia. Freshwater Biol.,* 24, 43-62.

WYNNE-EDWARDS, V. C. 1962. *Animal Dispersal In Relation To Social Behavior.* Oliver and Boyd, Edinburgh.

ZARET, T. M. 1980. *Predation and Freshwater Communities.* Yale University Press, New Haven and London.

ZARET, T. M. AND SUFFERN, J. S. 1976. Vertical migration in zooplankton as a predator avoidance mechanism. *Limnol. Oceanog.* 21, 804-813.

CHAPTER 6

Effects Of Predation And Oxygen Concentration: Contrasting 1990-1992 With 1993-1994

Introduction

Adaptation to predation and the ability to exploit a low oxygen environment have been postulated as mechanisms responsible for the annual development of genetic structure in the *D. pulicaria* population in Long Lake (Chapter 1). The ideas concerning these mechanisms (Chapters 2-5) were developed during the first three years of this study, from 1990 through 1992. Beginning with an increase in rainbow trout stocking in the fall of 1992, and continuing for the next two summers when oxygen depletion in the hypolimnion was less, the two principle parameters hypothesized to be responsible for genetic structuring changed significantly. In this chapter I will contrast distribution, hemoglobin pigmentation, clone dynamics and behavior of *D. pulicaria* related to the changes in limnological conditions and predator abundance.

Before 1993 the oxycline was steep; dissolved oxygen averaged less than 2 mg/liter in the hypolimnion during mid-summer (Table 1). Oxygen concentrations approached 3 mg/liter in 1993 and exceeded 3 mg/liter in 1994. The concentration of 3 mg/liter dissolved oxygen is significant because it is threshold below which hemoglobin is produced in *D. pulex,* a closely related species (Engle, 1985).

Table 1. Average dissolved oxygen concentrations (mg/liter) in the hypolimnion (15-20 m) at Long Lake during July and August.

	1991	1992	1993	1994
$\bar{x}$	1.5	1.7	2.6	3.9
n	6	42	54	17

Rainbow trout were first planted in Long Lake in 1961 (Moyle, 1969). The lake was stocked with trout in October each year during this study. The stocking rate was at stable 7500-8000 fingerlings per year since 1985 (Table 2). The rate increased to 14,500 fingerlings in October 1992 and to 22,550 fingerlings in 1993. Population densities are dependent on the number stocked, angling pressure and natural mortality because rainbow trout do not reproduce naturally in the Lake.

Table 2. Rainbow trout stocked in Long Lake.

YEAR	NUMBER	YEAR	NUMBER
1981	7500	1988	7500
1982	7805	1989	8000
1983	7644	1990	7500
1984	882	1991	7500
1985	7505	1992	14500
1986	7500	1993	22500
1987	7500	1994	14500

Methods

Field methods including limnological and zooplankton collecting techniques; laboratory procedures involved with recognition of hemoglobin pigmentation and electrophoresis; and data analysis techniques have been described in previous chapters. In 1993 most of the density data were qualitative, inferred from field notes and casual observations made during the predation and transplant experiments. In 1994 *D. pulicaria* density estimates were made by counting all of the individuals in each sample without replication. Data on rainbow trout stocking rates were provided by the Minnesota Department of Natural Resources, Section of Fisheries. Vertical migration observations were conducted at the mid-lake sampling station with acoustic techniques described in Chapter 5.

Results

The distribution of *D. pulicaria* changed significantly in 1993 compared to the three previous years. The daytime shallow (metalimnetic) layer did not develop until late in June; it never reached the densities observed in 1990-1992, and it disappeared in early August. The deep layer developed and persisted as usual, but the densities were lower than in previous years.

D. pulicaria virtually disappeared from Long Lake between March 1, 1994 and June 21,1994. The shallow layer of *D. pulicaria* never developed. In late June populations temporarily increased in the deep layer at both sampling locations, but again, densities were lower than 1990-1992 levels. Initially densities were 0.10 per liter at the mid-lake station and 0.004 per liter at the southeast station (Fig. 1, Chapter 1). By mid July densities increased to 0.23 and 0.48 per liter at the mid-lake and southeast stations respectively, before declining to 0.070 and 0.016 per liter by August 1. They disappeared from these two stations by August 16, 1994. On this date a layer of zooplankton between 14-17 m at the northwest end of Long Lake contained 2.3 *D. pulicaria* per liter. *D. pulicaria* were normally not found in the northwest end of Long Lake except in late August 1993 and 1994. *Daphnia galeata* replaced *D. pulicaria* as the dominant cladoceran in Long Lake for most of the summer in 1994.

As in previous summers, the distribution of *D. pulicaria* clones was complex during the summer in 1993 (Table 3). However, in contrast to 1990-1992, the proportions of SS clones in the hypolimnion did not increase in 1993. Log-linear modeling revealed that on the three mid-summer dates when two layers existed (Table 3) significant ($P \geq 0.05$) differences in clone proportions were associated with date, location, a date-location interaction, and independently, clone proportions were associated with depth. By the summer of 1993 the previously rare Medium Fast-Slow (MFS) clone became a significant part of the *D. pulicaria* population especially in the daytime hypolimnetic layer.

Table 3. *PGI* genotypes (= clones) of *D. pulicaria* collected at two depths at each of two locations in Long Lake during 1993.

DEPTH	LOCATION	CLONE	DATE 20 May	28 June	21 July	1 August	18 August	31 August
Metalimnion	South East	FF		2	5	3		
		FS		69	61	55		
		SS		5	8	5		
		MFS		13	8	7		
		Other		3	2	0		
	Mid-Lake	FF		22	10	0		
		FS		45	52	63		
		SS		5	12	3		
		MFS		19	7	28		
		Other		5	0	2		
Hypolimnion	South East	FF	8	2	1	1	0	0
		FS	71	52	45	64	67	64
		SS	3	12	2	19	10	6
		MFS	9	21	16	8	15	25
		Other	0	0	0	1	1	1
	Mid-Lake	FF	8	12	8	0	3	1
		FS	77	58	56	62	55	56
		SS	8	5	18	11	8	5
		MFS	2	15	12	19	21	29
		Other	0	4	1	0	0	0

For several reasons the remaining comparisons were limited to the deep layer at the mid-lake station in August. Earlier analyses (above and Chapter 1) indicated that the complex interactions associating clone distribution with time, depth and location could confound comparisons made between years unless the comparisons were from similar times, depths and locations. I had the most complete set of comparative data from the mid-lake location. I was particularly interested in adaptations within the SS clone to low oxygen conditions and reduced predation in the deep layer. By late 1993 the shallow layer had disappeared. Analysis of the genotypes from the August 1993 samples in the hypolimnion at the mid-lake station (Table 3) revealed that clone proportions were independent of date (Chi-square $P = 0.20$) therefore, these samples were pooled for the multi-year analysis in table 4.

PGI clone proportions and hemoglobin pigmentation varied significantly and systematically from 1991-1994 (Table 4). Log-linear modeling of the data in table 4 indicated that all possible pairwise associations (year-pigmentation, year-clonal composition, and pigmentation-clonal composition) were significant and that the data could be best explained without higher order interactions among these variables. The pairwise associations (Fig. 1) indicated a decreasing trend in the proportion of *D. pulicaria* with hemoglobin, ending in 1994, when there were no pigmented individuals in the deep layer in August. Although MFS individuals were unusually abundant in 1993, the major trend was toward increased proportions of heterozygous (FS) individuals and decreased proportions of homozygous slow (SS) individuals from 1991 to 1994. By 1994 the SS clone, which previously was most likely to develop pigmentation, did not produce hemoglobin, even by late summer in the deep layer.

Table 4. Numbers of *D. pulicaria* clones that were collected from the deep layer of zooplankton at the mid-lake sampling location in August 1991-1994, categorized by hemoglobin pigmentation.

DATE	PIGMENTATION	*PGI* CLONES FF	FS	SS	MFS	OTHER	TOTAL
10 Aug. 1991	Transparent	0	13	3	0	0	16
	Hemoglobin	3	37	37	0	0	77
2 Aug. 1992	Transparent	0	18	11	1	0	30
	Hemoglobin	7	33	23	0	0	63
Aug. 1993 Totals	Transparent	1	136	14	50	0	201
	Hemoglobin	3	37	10	19	0	69
1 Aug. 1994	Transparent	0	67	8	2	6	83
	Hemoglobin	0	0	0	0	0	0

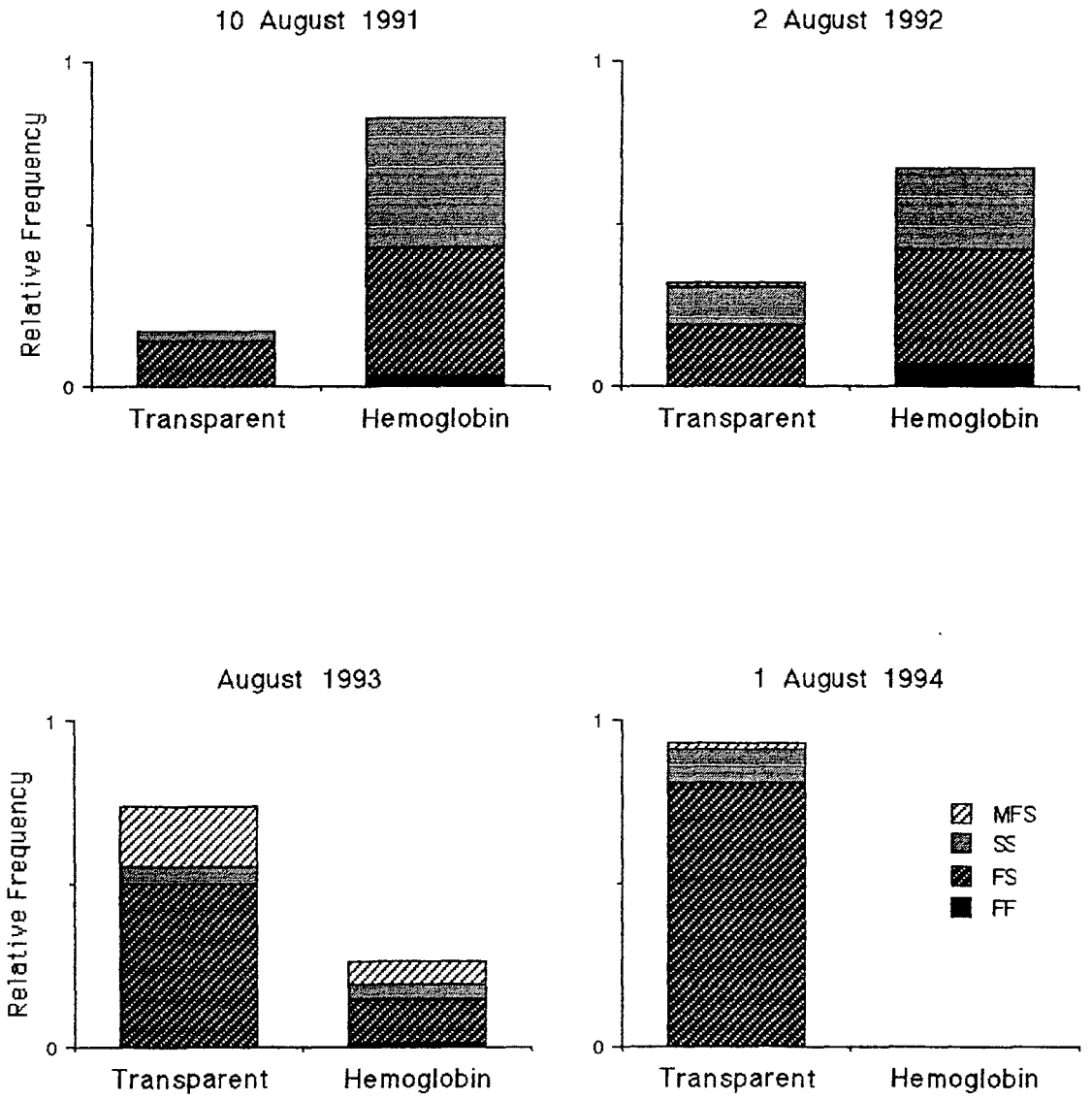


Figure 1. Proportions of *D. pulicaria* clones collected from the deep zooplankton layer at the mid-lake sampling location in August 1991-1994, categorized by hemoglobin pigmentation.

Vertical migration was measured only in 1992 and 1993. Inter-annual comparisons of clone specific migration behavior were made difficult not only by changes in clone composition between 1992 and 1993, but also, by significant differences between the 7 July and 10 August in 1993. In 1992 the SS clone migrated upward after sunset with a greater frequency than the other clones (Fig. 2). This resulted in a lower proportion of the SS clone in the night category compared to the day category of the 10 August 1992 sample (Fig. 2). A consistent day-night difference of comparable magnitude was not observed in 1993. Log-linear modeling of the data in table 5 indicated that the saturated model provided the only acceptable ($P \geq 0.05$) fit. The interpretation of this model was that the clonal composition of the deep layer differed not only by time of day, but also, among the three days on which observations were made.

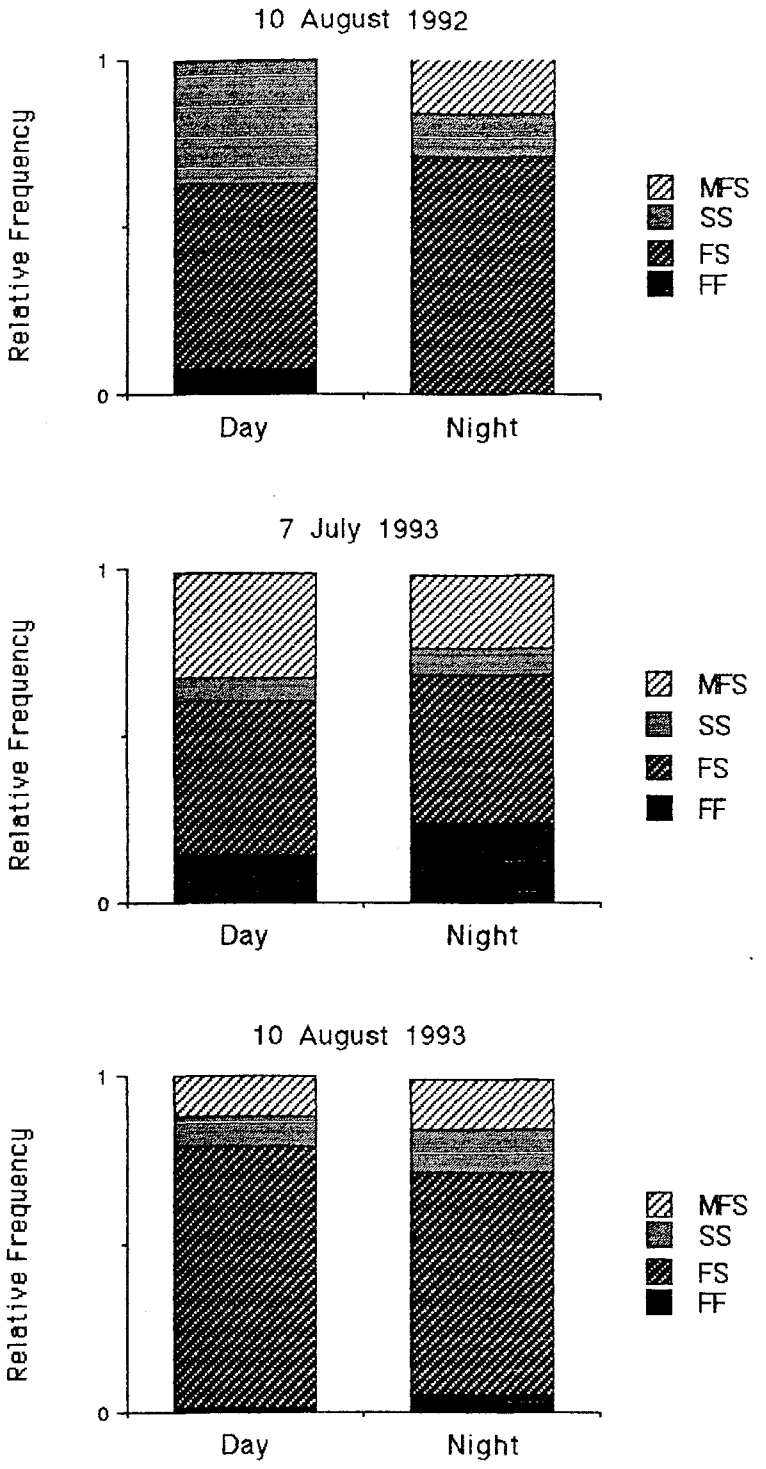


Figure 2. Clonal composition of the deep layer of *D. pulicaria* before (day), and after (night), the post sunset vertical migration on three dates.

Table 5. Numbers of *D. pulicaria* clones that were collected from the deep layer of zooplankton before (day), and after, (night), the post sunset vertical migration on three dates.

DATE	TIME	*PGI* CLONES FF	FS	SS	MFS	OTHER	TOTAL
10 Aug. 1992	Day	7	51	34	1	0	93
	Night	0	51	9	12	0	72
7 July 1993	Day	13	44	7	30	1	95
	Night	23	42	8	21	2	96
10 Aug. 1993	Day	1	71	8	11	0	91
	Night	5	61	12	14	1	93

Discussion

Significant changes in predation and dissolved oxygen during the last two summers of this study allowed me to compare the effects of these parameters with the previous three years. Predation and dissolved oxygen had been postulated as the primary factors responsible for the genetic structure of the population during the first three years of my research.

The lower frequency of *D. pulicaria*, pigmented with hemoglobin in 1993, and the complete absence of the colored morph from the population in 1994 can be attributed to increased oxygen levels in the hypolimnion. Hemoglobin has been shown to be an inducible trait in *Daphnia*. Concentrations of dissolved oxygen must fall to 3 mg/liter (Engle, 1985) or less (Fox and Phear, 1953) to stimulate hemoglobin production.

The increased level of dissolved oxygen in the hypolimnion could have been due to several factors. A protracted, cool and windy spring might have resulted in a more complete mixing of the relatively steep and protected basin. Cooler than normal summer temperatures could have resulted in thermal stratification that was less intense or shorter in duration. The amount of dissolved oxygen was also due to the dynamic equilibrium that existed between community photosynthesis and community respiration in the hypolimnion. But, because I did not have data on many of these parameters, I could not discern the relative contributions to the above normal oxygen concentrations in the hypolimnion in 1993 and 1994.

Work associated with the development of the trophic cascade paradigm has thoroughly documented the ubiquitous effects of predation in aquatic systems (Zaret, 1980; Kerfoot and Sih, 1987). My observation that increased predation caused both distribution and population changes of *D. pulicaria* in Long Lake very closely parallels the observations on *D. pulicaria* in Wisconsin lakes. Leibold (1990) reported that the vertical distribution of *D. pulicaria* was shifted significantly deeper in experimental enclosures containing fish predators compared to fishless enclosures. In a survey of seven lakes, Leibold and Tessier (1991) found that *D. pulicaria* were restricted to the hypolimnion under high fish predation but used

shallower waters in the lakes with reduced predation. The shift from *D. pulicaria* to the smaller *D. galeata*, especially in the shallow layer, was a typical response to increased predation that has been reported repeatedly in the trophic cascade literature (Hrbacek et. al., 1961; Brooks and Dodson, 1965; Carpenter et. al., 1985).

Reduction in pigmentation has also been shown to be an adaptation to light dependent visual predators (Zaret, 1980). But because both the increase in dissolved oxygen and the increase in predation occurred simultaneously and acted in the same direction I could not determine the relative magnitude of the effects of these two stimuli.

D. pulicaria continued to migrate vertically in 1993. However, clone specific comparisons in migration behavior were obscured by shifts in the clonal composition of the population between 1992 and 1993. But, the disproportionately large change in day versus night distribution of the SS clone, so apparent in 1992, had disappeared by 1993.

The failure of the SS clone to increase in the hypolimnetic population in 1993 and 1994 compared to previous years likely resulted from the absence of the conditions to which this clone was particularly well-adapted. Because this clone was more likely to form hemoglobin and more likely to migrate, it was particularly well-suited to daytime refuge from predation in deeper, darker and oxygen-depleted waters. Due to increased levels of hemoglobin, SS individuals could extract oxygen from hypolimnetic areas more efficiently than the FS clone which was much less likely to have elevated hemoglobin levels. But this clone-specific advantage largely disappeared with the increased oxygen levels in the hypolimnion in 1993 and 1994. Furthermore, given the clarity of Long Lake, hemoglobin pigmentation very likely became an greater liability even in deep water after the introduction of more visual predators in 1993 and 1994. Thus, the SS clone lost a physiological advantage that made it uniquely suited to the seasonal anoxic habitat deep in the lake prior to 1993. And simultaneously in the presence of more predators, decreased visual detection due to less pigmentation became more important.

References

BROOKS, J. L. AND DODSON, S. I. 1965. Predation, body size and composition of plankton. *Science,* 150, 28-35.

CARPENTER, S. R., KITCHELL, J. F. AND J. R. HODGSON. 1985. Cascading trophic interactions and lake productivity. *BioScience,* 35, 634-639.

ENGLE. D. L. 1985. The production of haemoglobin by small pond *Daphnia pulex* : intraspecific variation and its relation to habitat. *Freshwater Biology.* 15, 631-638.

FOX, H. M. AND PHEAR, E. A. 1953. Factors influencing haemoglobin synthesis by *Daphnia. Proc. Roy. Soc. Lond.* B. 141, 179-189.

HRBACEK, J., DVORAKOVA, M., KORINEK, V. AND PROCHAZKOVA. L. 1961. Demonstration of the effect of the fish stock on the species composition of zooplankton and the intensity of metabolism of the whole plankton assemblage. *Verh. Int. Ver. Theoret. Angew. Limnol.,* 14, 192-195.

KERFOOT, W. C. AND SIH. A. 1987. *PREDATION: Direct and Indirect Impacts on Aquatic Communities.* University Press of New England, Hanover and London.

LEIBOLD, M. 1990. Resources and predators can affect the vertical distributions of zooplankton. *Limnol. Oceanogr.,* 35, 938-944.

LEIBOLD, M. AND TESSIER, A. J. 1991. Contrasting patterns of body size for *Daphnia* species that segregate by habitat. *Oecologia,* 86, 342-348.

MOYLE, P. B. 1969. *Ecology of the Fishes of a Minnesota Lake with Special Reference to the Cyprinidae.* Ph. D. Thesis, Uninersity of Minnesota.

ZARET, T. M. 1980. *Predation and Freshwater Communities.* Yale University Press, New Haven and London.