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Differential response of *Daphnia* genotypes to oxygen stress: respiration rates, hemoglobin content and low-oxygen tolerance

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Summary. Laboratory respiration rate experiments using three electrophoretically identified clones of the fresh water, planktonic cladoceran, *Daphnia pulex*, from an eutrophic farm pond, indicated that clones acclimated to both low and high oxygen levels, regulated oxygen consumption across a wide range of oxygen concentrations (1.0–9.0 mg·liter^{-1}). A "threshold" oxygen level of 0.5–1.0 mg·liter^{-1} was reached, where animals succumbed to oxygen stress, regardless of hemoglobin content. No significant clonal differences in respiration rates were found. These data suggest that members of this *Daphnia* population are able to regulate oxygen metabolism across a wide range of ambient oxygen concentrations, and indicate a well-adapted respiratory system.

Low-oxygen tolerance experiments and hemoglobin measurements indicated further that physiological differences indeed exist between clones; one clone produced the lowest amount of hemoglobin and was least tolerant of low oxygen levels. These data imply that spatial and temporal changes in dissolved oxygen concentration may be an important selective force influencing the clonal (genotypic) composition of natural cladoceran populations.

In aquatic habitats, dissolved oxygen is known to be a critical factor influencing the composition of communities and distribution of species (Hutchinson 1967). Differences in low oxygen tolerance (Davis 1975) and hemoglobin synthesis (Terwilliger 1980) have been examined for a variety of aquatic organisms, primarily aquatic invertebrates (Fox 1945, 1954, Weber 1980). One group in particular, the fresh water Cladocera, has been studied extensively with the majority of studies concentrating on the genus, *Daphnia* (Fox 1948, Green 1956, Kobayashi 1982a, b). Interspecific and intraspecific differences in low oxygen tolerance (Herbert 1954), hemoglobin synthesis (Chandler 1954, Kobayashi 1981, Carvalho 1984), and respiration rates (Heisey and Porter 1977) have been observed in a number of *Daphnia* species. Very few studies have examined clonal (genotypic) differences in respiration rates, low-oxygen tolerance, and hemoglobin synthesis within a given population (Obreshkove and Banta 1930).

In a study of the temporal and spatial distribution of electrophoretically distinct clones of *Daphnia pulex* in an eutrophic farm pond, Weider (1984a) observed considerable variation in the vertical distribution and vertical migration behavior of different clones, as well as seasonal displacement of clones. Low dissolved oxygen was proposed as being a potentially important ecological factor influencing the spatial distributions, temporal composition, and overall abundances of clones in this population. For example, clones may respond differentially to low-oxygen levels, which could explain the observed differences in vertical distribution and migration through the oxycline, or explain partially the temporal (seasonal) heterogeneity of clones (Weider 1984b).

Our purpose is to compare and contrast respiration rates, low-oxygen tolerance (survivorship), and hemoglobin content between three electrophoretically and ecologically distinct clones of *Daphnia pulex* from Smith Big Pond (Weider 1984a, b), in an attempt to elucidate the effect(s) that low oxygen levels have on clonal composition of planktonic cladoceran populations.

Materials and methods

The three clones (here after referred to as clones 1, 3, and 5, as described in Weider 1984a), were isolated from samples collected in early spring 1982 from Smith Big Pond (Vermillion County, Illinois, Weider 1984a) and maintained in the laboratory in mass cultures (1.5 and 10-liter glass flasks) during the course of the experiments. Artificial pond water (see Lynch et al. 1985) served as medium; *Scenedesmus* sp. was added to obtain a concentration of 1.0 mg C·l^{-1}, twice weekly. About one-third volume of the flasks was replaced with fresh medium, biweekly. Two experimental groups of animals (of each clone) were maintained at 2.7–3.3 mg O_2·l^{-1} (low-oxygen group) and 8.5–9.0 mg O_2·l^{-1} (high-oxygen group). Oxygen levels were monitored daily using a digital oxygen meter, and adjusted by bubbling either nitrogen gas or compressed air into the medium. This technique provided relatively stable oxygen readings ($\pm$0.5 mg O_2·l^{-1}·day^{-1}), and also served to resuspend settled food (algae).

Respiration rate experiments

A series of respiration rate experiments were conducted over a broad range of ambient dissolved oxygen concentrations (0.5–9.0 mg·l^{-1}) using animals from both low and high-oxygen groups to determine if quantitative and/or

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qualitative differences in respiration rates exist between clones.

All experiments were conducted at 20° C in a walk-in environmental chamber set on a 12:12 h light-dark cycle (light intensity of 5.6 $\mu E \cdot m^{-2} \cdot s^{-1}$). Animals were exposed to medium of a given test oxygen concentration for one h, prior to beginning experiments. Medium of any desired oxygen concentration could be produced by bubbling with a preselected mixture of compressed air and nitrogen (provided in the appropriate proportions) by an automatic, volumetric gas mixer (Dräger Labomix). Seven to ten animals/clone (depending on size) were transferred to 25-ml ground-glass stoppered bottles containing membrane-filtered (0.2 µm) artificial pond medium of the desired initial oxygen concentration (lacking *Scenedesmus*). Before the bottles were stoppered, a 2 ml subsample was withdrawn from each bottle using a glass syringe, and injected into a water-jacketed, temperature-controlled measuring chamber of a Radiometer PHM72 oxygen analyzer to obtain initial oxygen concentrations (The use of a plastic syringe, as suggested by the manufacturer, produced considerable errors at low oxygen partial pressures). Two ml of medium of equivalent oxygen content were added to replenish each bottle; care was taken to prevent the introduction of air bubbles. The oxygen electrode was calibrated with oxygen-equilibrated distilled water. Control bottles (containing only filtered medium) were run simultaneously to measure "background" respiration, i.e. bacteria. Since a few drops of medium from the vessel in which animals had been "preacclimated" (for one h) were introduced into the experimental bottles together with the animals, an equal amount of this medium (undoubtedly containing some bacteria) was added to control bottles. Bottles were placed in a water bath (20 °C), and checked frequently for dead animals. After four h, bottles were opened carefully and water samples from the center of each bottle were taken to obtain final oxygen concentrations. The precision of oxygen measurements was better than 0.01 mg $O_2 \cdot l^{-1}$. Oxygen concentrations in the experimental bottles decreased during the experiments by 0.5 to 1.0 mg $O_2 \cdot l^{-1}$, varying according to the weight and activity of the animals. Controls showed a decrease in oxygen of 0.01 to 0.1 mg $O_2 \cdot l^{-1}$. The average oxygen concentration during an experiment was calculated as the mean of initial and final concentrations.

Animals were collected, dried in an oven at 102 °C for 3–4 h, placed in a desicator for 12-h, and weighed to the nearest 0.001 mg on a Cahn electrobalance. Respiration rates were calculated on a per dry weight basis as described in Lampert (1984). Since the individual weight of animals varied between 40 and 120 µg, the weight specific respiration rates were recalculated for animals of 60 µg individual weight using an exponent of −0.25.

Low oxygen tolerance

Two low-oxygen tolerance experiments were conducted at 20° C to compare clonal survivorships, and simultaneously measure the decrease in oxygen concentration (due to respiration) during the course of the experiments. Animals from the low and high-oxygen acclimated groups were examined in separate experiments. Fifteen adult animals/clone (1.80–2.00 mm size class) were placed in 100-ml stoppered bottles containing filtered medium, with initial oxygen concentrations of 0.8–1.0 mg $\cdot l^{-1}$ (low-oxygen group), and 1.8–2.0 mg $\cdot l^{-1}$ (high-oxygen group). Bottles were run in triplicate, along with controls (3.0 mg $O_2 \cdot l^{-1}$ low-oxygen control; 8.5–9.0 mg $O_2 \cdot l^{-1}$ high-oxygen control). Survivorships were monitored at half-h to one-h intervals, and 2.0 ml subsamples were withdrawn from each bottle for oxygen analysis as described above, to monitor changes in dissolved oxygen levels throughout the experiments. The experiments were terminated when survivorships fell below 20%. Total time needed to reach this level varied between 9 and 15 h, depending on clone. Animals from clone 1 (high-oxygen group) were considerably smaller than animals from clones 3 and 5. Subsequently, they did not respire at the same rate and thus, did not deplete the oxygen concentrations in the bottles to the same extent. Therefore, we excluded clone 1 from the high-oxygen analysis.

Hemoglobin analyses

Hemoglobin measurements were determined for individuals from the low-oxygen acclimated group. (Production of hemoglobin in the high-oxygen group was below detectable levels). Adult females of each clone were pipetted on a 150 µm mesh screen, measured from the top of the head to the base of the tail spine, brood size counted, and groups of 25–35 animals were placed in 1.0 ml plastic Eppendorf test tubes containing 0.1 ml of distilled water. Animals were macerated with a teflon grinding rod, diluted with an additional 0.4 ml of distilled water, and centrifuged for five min at 12,000 rpm in a refrigerated centrifuge (4° C). One-half ml of supernatant was removed and placed in a cuvette containing 2.0 ml distilled water and 0.075 ml of 0.1% KCN (modification of the methods of Landon and Stasiak 1983). Hemoglobin absorption was read after five min at 415 nm (wavelength of peak hemoglobin absorption) on a Shimadzu double-beam digital spectrophotometer. Absorption values were standardized against crystallized rabbit hemoglobin (Sigma # H7255) over a range of 0.01–10.0 mg $\cdot l^{-1}$. A length-dry weight (log-log) regression was determined for five groups of *Daphnia* (size classes of 1.60–1.79, 1.80–1.99, 2.00–2.19, 2.20–2.39, and 2.40–2.59 mm; $N=100$). Each daphnid was measured to the nearest 0.04 mm using an ocular micrometer on a Wild dissecting microscope, grouped in the proper size class, and then dry weights were determined as described above. Dry weight (mg) of the *Daphnia* as a function of length (mm) was:

$$\text{dry weight (mg)} = 2.048\ (\text{length})^{2.997}$$

($r=0.988$). Hemoglobin values were divided by the dry weights of the *Daphnia* and converted to mg hemoglobin $\cdot$ g dry weight^{-1}.

Results

Respiration rates

Results from respiration rate experiments (Fig. 1) indicated that all three clones were able to regulate oxygen consumption across a wide range of oxygen concentrations. There was no reduction of the respiratory rate at low oxygen concentrations until animals started to succumb. However, mortality during the five h period (one h "preacclimation", four h experiment) occurred at considerably lower oxygen levels for the low-oxygen acclimated daphnids. It usually

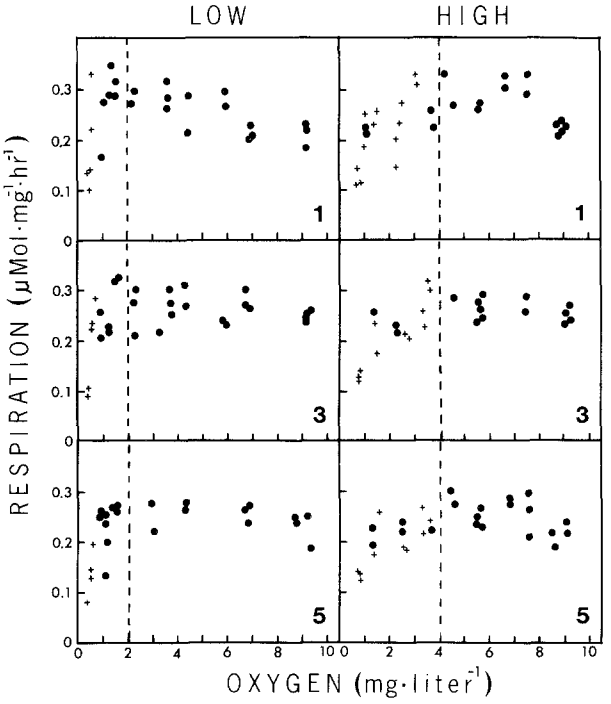

Fig. 1. Weight specific respiration rates of three clones of *Daphnia pulex* at different dissolved oxygen concentrations. Animals were acclimated to either low oxygen (2.7–3.3 mg l^{-1}, left panel), or high oxygen (8.5–9.0 mg l^{-1}, right panel). Numbers 1, 3, and 5 denote clones. Crosses (+) indicate that mortality occurred during the four h of incubation. No mortality was observed in the range of oxygen concentrations to the right of the broken line; i.e. "safe" range

started around 0.5 mg $O_2 \cdot l^{-1}$. When animals were acclimated to high oxygen, dead animals were observed initially at about 3.5 mg $O_2 \cdot l^{-1}$. Clone 3 suffered the highest mortality, often reaching 80% or greater, especially for the high-oxygen acclimated animals.

In the lowest range of oxygen concentrations, respiratory rates were occasionally reduced, even though no animals died during a given experiment. This may indicate that some animals were already seriously weakened, and probably would have died if the experiments had been extended. For comparison of respiratory rates, we therefore used only data from the "safe" range; i.e. oxygen concentrations greater than 2 mg $\cdot l^{-1}$ for the low-oxygen group, and greater than 4 mg $\cdot l^{-1}$ for high-oxygen acclimated animals.

If respiratory rates in these ranges are pooled, the results are remarkably similar (Table 1). There are no significant clonal differences in respiration rates within or between oxygen treatments (two-way analysis of variance, Statistical Analysis System, General Linear Model, Ray 1982; $F_{5,81} = 0.62$, n.s.). Respiration rates increased slightly towards the lower limit of the "safe" range. All linear regressions of respiration rate vs. oxygen concentration had negative slopes (b); however, the only significant regression was for clone 1 acclimated to low oxygen ($P < 0.05$, $b = -0.416$, $r = 0.723$, $N = 15$).

Low oxygen tolerance

The protocol for one survivorship experiment (clone 1, low-oxygen group) is presented as an example (Fig. 2). During

Table 1. Mean (±1 S.D.) respiratory rates of *Daphnia pulex* (individual dry weight 60 μg) clones acclimated to low and high oxygen levels. Respiration rates were taken from the "safe" range, where no mortality occurred (see Fig. 1). (N = sample size)

Treatment	Clone	Range	N	Respiratory rate (μmol $O_2 \cdot mg^{-1} \cdot h^{-1}$)
Low O_2	1	>2	15	0.252±0.043
	3	>2	18	0.263±0.028
	5	>2	11	0.248±0.027
High O_2	1	>4	16	0.265±0.042
	3	>4	12	0.262±0.020
	5	>4	15	0.251±0.034

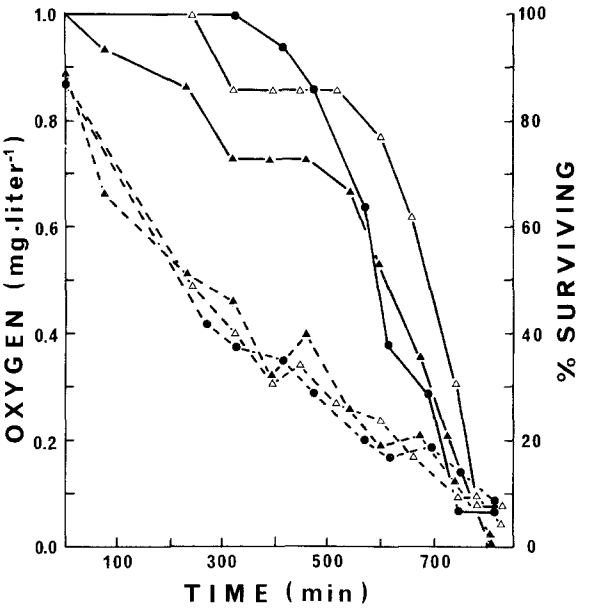

Fig. 2. Protocol of one survivorship experiment (clone 1, low-oxygen acclimated group). Symbols represent three replicate bottles. Broken line: oxygen concentration; solid line: percentage of surviving animals

the first 300 min, the rate of oxygen consumption in the three bottles is nearly constant, since mortality levels are low. As oxygen levels decrease below the 5% saturation level (0.45 mg $O_2 \cdot l^{-1}$), mortality increases. The rate of oxygen depletion eventually decreases as fewer animals are left alive in the bottles.

If one compares the results from the low-oxygen acclimated experiment, it is clear that clone 3 has a lower survivorship than clones 1 and 5 at <1.0 mg $O_2 l^{-1}$ (Fig. 3). Clone 3 reached the 50% mortality level approximately 4 h before either clone 1 or 5 (6.1±0.5 h for clone 3, 10.5±0.8 h for clone 1, and 9.6±0.8 h for clone 5). The "lethal concentration" of oxygen for clone 3 at the 50% mortality point (LC_{50}) was 0.34±0.01 mg l^{-1}, while the values for clones 1 and 5 were 0.19±0.04 and 0.17±0.01 mg l^{-1}, respectively. An one-way analysis of variance confirmed that the 50% mortality points for the clones were significantly different from each other ($F_{2,6} = 30.19$, $P < 0.001$), as where the critical low oxygen concentrations ($F_{2,6} = 42.12$, $P < 0.005$).

For the high-oxygen acclimated experiment (Fig. 4), there were no significant differences in survivorship between

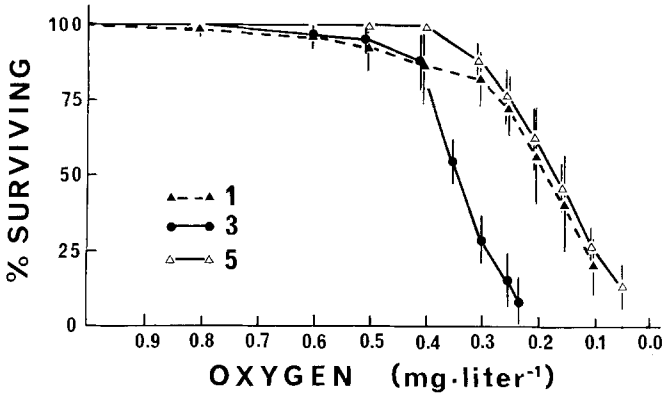

Fig. 3. Survivorship curves (mean ± 1 S.E.) of clones 1, 3, and 5 when acclimated to low oxygen levels

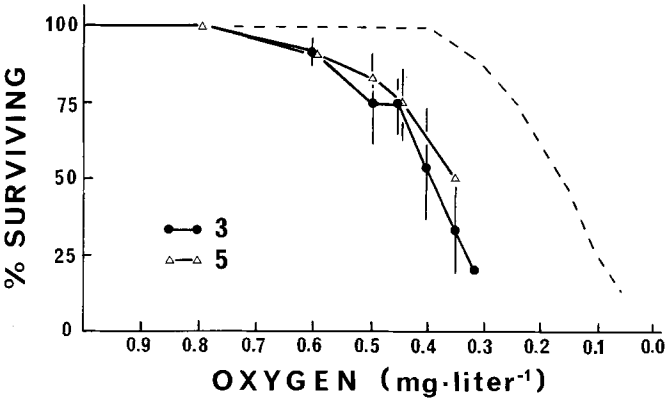

Fig. 4. Survivorship curves (mean ± 1 S.E.) for clones 3 and 5 when acclimated to high oxygen levels. The dotted line indicates the respective curve for clone 5 when acclimated to low-oxygen (same as in Fig. 3)

clones 3 and 5, based on either the 50% mortality points ($F_{1,4}=2.086$, n.s.), or the critical low oxygen level ($F_{1,4}=0.415$, n.s.). The survivorship curve for clone 3 (Fig. 4) was similar (in shape) to its survivorship curve for low-oxygen acclimated group (Fig. 3), while clone 5 had a greatly depressed survivorship curve (high-oxygen group), when compared with its low-oxygen acclimated curve (Fig. 4).

Hemoglobin analyses

Hemoglobin measurements showed significant clonal differences in hemoglobin content after exposure to low-oxygen levels (clone 5 28.00 ± 3.29 mg·g dry wt^{-1}; clone 1 20.95 ± 3.56, clone 3 17.04 ± 1.78; $F_{2,12}=17.392$, $P<0.005$). Clone 5 produced more hemoglobin (on average) than either clones 1 or 3 (Scheffe's multiple comparison test, $P<0.05$, Sokal and Rohlf 1981); there was no significant difference in hemoglobin content between clones 1 and 3.

Discussion

Results from this study indicate that *Daphnia pulex* clones from Smith Big Pond are able to regulate oxygen consumption across a wide range of ambient dissolved oxygen concentrations, indicating a well-adapted respiratory system that is relatively "insensitive" to changing oxygen levels, at least above 1.0 mg $O_2 \cdot l^{-1}$. It is not surprising to find that many aquatic organisms can regulate their metabolic rate quite precisely, as dissolved oxygen levels change (Childress 1968, 1971, 1975, 1976, Belman and Childress 1976, Vos et al. 1979). Vos et al. (1979) observed that *Artemia salina* was a respiratory regulator across a wide range of dissolved oxygen concentrations (1.0–5.0 mg·l^{-1}). A critical oxygen tension existed (0.5–1.0 mg·l^{-1}), and decreased with acclimation to lower oxygen concentrations. These findings are similar to our results (Fig. 1). Further, Heisey and Porter (1977) reported that pond-dwelling *Daphnia magna's* respiration rate was independent of oxygen concentrations above 3.0 mg·l^{-1}, but declined rapidly below this level. In contrast, they found that lake-dwelling *Daphnia galeata mendotae's* respiration rate exhibited a linear dependence on oxygen concentration. They attributed the interspecific difference to higher basal levels of hemoglobin for *D. magna*, and suggested that it is an adaptive response to living in eutrophic, temporary ponds that experience large fluctuations in oxygen concentrations.

In comparison, our *Daphnia pulex* clones, acclimated to low oxygen levels, exhibited a wider capacity for respiratory regulation than has been previously reported in the Cladocera (Lampert 1984). Only recently Kobayashi and Hoshi (1984) reported curves demonstrating the effect of ambient oxygen concentration on the respiratory rate of *D. magna* which are very similar to our results. Our experiments suggest that respiratory rate and regulatory capacity are in a wide range independent of hemoglobin content. It is difficult to compare our results with those of Heisey and Porter (1977) because of considerable differences in experimental design, which may influence the response of the daphnids. We used *D. pulex* clones, the oxygen concentrations in our bottles changed only slightly, and the experimental time was constant, so that an equivalent amount of excretory products should have been produced in all bottles. In contrast, Heisey and Porter (1977) used *D. magna* and *D. galeata mendotae* that had been acclimated to air-saturated water. They used variable incubation times (from 4 to 24 h), and did not report the magnitude of the oxygen decrease in the bottles during the incubation period.

Although there were no clonal differences in respiration rates in our experiments, clones did show significant differences in low-oxygen tolerance. Clone 3 produced the least amount of hemoglobin, and subsequently was least tolerant of low oxygen, when compared with clones 1 and 5. Our survivorship experiments did not allow us to determine whether hemoglobin-rich clones are able to persist at very low O_2 levels for extended periods of time, i.e. weeks or months, or whether they are able to survive longer than hemoglobin-poor clones because of greater oxygen stores in the hemoglobin. Interestingly, clone 1 exhibited a similar survivorship curve (Fig. 3) to clone 5, when acclimated to low oxygen, despite producing less hemoglobin than clone 5. Apparently, other important physiological and/or behavioral differences exist between clones (e.g. kinetic differences in oxygen affinities of hemoglobins, Lehninger 1975). The data, however, suggest strongly that hemoglobin production in this population is an important adaptation to low oxygen, as demonstrated by differences in clonal survivorships (Fig. 3). Further, the ability of certain clones (genotypes) in a population to respond to low-oxygen conditions by producing more hemoglobin, may provide a competitive advantage over other clones that are unable to pro-

duce the same quantity or quality of hemoglobin. Results from a previous study (Weider in prep.) indicated that competitive interactions between clones can be influenced by oxygen concentration. Clone 3 displaced clone 5 when raised together at 5.0 mg $O_2 \cdot l^{-1}$, but the reverse was true at 1.0 mg $O_2 \cdot l^{-1}$.

The Smith Big Pond *Daphnia* population is exposed to very large changes in dissolved oxygen concentration, both spatially and temporally (Weider 1984b, in prep.). For example, during a two and one-half week period in April–May 1982, mean dissolved oxygen concentration in the pond decreased from 10.4 to 2.1 mg $O_2 \cdot l^{-1}$ (Weider 1984b). During July and August 1982, dissolved oxygen levels decreased further (1.0–1.5 mg $O_2 \cdot l^{-1}$ at 1 m depth; 0.1 mg $O_2 \cdot l^{-1}$ at 6 m depth), coinciding with a drastic population decrease, with the majority of animals confined to the top 2 m of the water column. Changes in the clonal composition of the population also occurred; clone 3 decreased to below detectable levels, while clones 1 and 5 were able to persist at low densities. These findings imply that spatial and temporal shifts in the clonal (genotypic) composition of the Smith Big Pond population may be influenced greatly by changes in dissolved oxygen levels. It seems plausible that clone 3 declined during this period because of poor survivorship and decreased competitive ability at low oxygen levels, as supported by the aforementioned laboratory studies. Clones that are less tolerant of low oxygen may be restricted temporally to better-oxygenated water strata, and may be unable to colonize low-oxygenated regions (hypolimnion) during certain portions of the year, i.e. summer.

These results suggest strongly that spatial and temporal fluctuations in dissolved oxygen may be an important selective force influencing the clonal (genotypic) composition of natural planktonic cladoceran populations (Hebert and Crease 1980, Lynch 1983, Weider 1984b, in prep.). Future field and laboratory studies need to elucidate the exact physiological/ecological mechanisms of low oxygen tolerance among genotypes in a given population.

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