

# The effect of ambient oxygen concentration on filtering and respiration rates of *Daphnia galeata mendotae* and *Daphnia magna*<sup>1</sup>

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## Abstract

The filtering and respiration rates of *Daphnia magna* and *Daphnia galeata mendotae* were measured in oxygen concentrations ranging from air saturation to near zero, using animals that had lived in air-saturated conditions so that facultative hemoglobin synthesis was not initiated. *Daphnia magna*'s filtering and respiration rates are independent of oxygen concentrations above 3.0 mg liter<sup>-3</sup>; below this, rates decline rapidly. *Daphnia galeata mendotae*'s filtering and respiration rates exhibit a linear dependence on oxygen concentrations. It is suggested that *D. magna*'s relatively greater tolerance of low oxygen concentrations is due largely to its higher basal levels of hemoglobin. *Daphnia* may often be oxygen limited in nature.

The importance of *Daphnia* in aquatic food webs (Edmondson 1974) has motivated studies of environmental factors influencing its feeding and respiration rates. Physical factors affecting these include temperature, light, and pH (Kibby 1971; McMahon 1965; Buikema 1972, 1973; Ivanova and Klekowski 1972; Kring and O'Brien 1976a). The effect of ambient oxygen concentration, which is often quite variable in nature, is less well understood.

Hoshi (1957) has shown that low oxygen concentrations depress the respiration rates of *Simocephalus vetulus*; apparently no similar data exist for *Daphnia*. Fox et al. (1951) demonstrated that the rate at which *Daphnia obtusa* cleared graphite suspensions was oxygen dependent, and more recently Kring and O'Brien (1976b) have demonstrated that filtering rates of *Daphnia pulex* are oxygen dependent at levels below 3.0 mg liter<sup>-1</sup>. Animals that have had prolonged exposure to low oxygen concentrations respond by facultatively increasing their hemoglobin levels (Fox et al. 1951; Kring and O'Brien 1976b). This enhances such functions as filtering rate, egg production, general activity, and survivor-

ship of *Daphnia* at low oxygen concentrations. We have investigated the effect of ambient oxygen concentrations on the filtering and respiration rates of a pond zooplankter, *Daphnia magna*, and a lake zooplankter, *Daphnia galeata mendotae*, before facultative hemoglobin synthesis is initiated.

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## Materials and methods

*Daphnia magna* was grown at 24°C in aquaria with aged, aerated tapwater under 12:12 LD fluorescent lighting. *Ankistrodesmus falcatus* and *Chlamydomonas reinhardtii* were supplied as food. *Daphnia galeata mendotae* was collected in early summer from an epilimnetic population in Baseline Lake, Michigan, and acclimated for 48 h under the same laboratory conditions as *D. magna*. The epilimnetic waters are air saturated and usually between 20°–25°C at this time of year (Hall 1964; pers. obs.). Determinations of respiration and filtering rate were made on *D. galeata mendotae* from single collections.

Animals were sieved and visually sorted into three size classes before use. Sizes were determined at 60× magnification as

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the carapace length from the anteriormost portion of the head to the base of the tail spine in subsamples of 20 animals from each size class. Size classes were large *D. magna* ( $\langle x \rangle = 2.34$  mm, SE = 0.06 mm), small *D. magna* ( $\langle x \rangle = 1.54$  mm, SE = 0.02 mm), and *D. galeata mendotae* ( $\langle x \rangle = 1.57$  mm, SE = 0.03 mm). About 50% of the large *D. magna* and *D. galeata mendotae* were ovigerous, while few of the small *D. magna* were.

*Ankistrodesmus falcatus* used in the filtering rate determinations was grown axenically in a modified Woods Hole MBL pH 7.2 medium (Stein 1973) with glycylglycine buffer and without silicate. Algae were labeled by adding 20  $\mu\text{Ci}$  [ $^{14}\text{C}$ ]NaHCO<sub>3</sub> per 100 ml of algal culture at least 24 h before use. The radioactive algae were then centrifuged, washed, and counted by Klett colorimetry (Hall 1964; Arnold 1971).

Filtering rates were determined in a feeding suspension of 0.45- $\mu$  HA Millipore-filtered, aged tapwater to which enough unlabeled *A. falcatus* had been added to obtain a cell concentration of  $5 \times 10^3$  cells cc<sup>-1</sup>. Oxygen in this feeding suspension was adjusted to the levels desired by bubbling nitrogen gas through it. This had a negligible effect on pH. The medium was then siphoned into 300-ml BOD bottles and the oxygen concentration determined with a calibrated oxygen electrode with BOD stirrer (Yellow Springs).

Thirty to 50 *Daphnia* were then pipetted into the BOD bottles, which were stoppered and placed on a plankton wheel (1 rpm) for a 15-min acclimation period. One milliliter of concentrated  $^{14}\text{C}$ -labeled *Ankistrodesmus* was then quickly injected into the bottles. This resulted in a final cell concentration of  $5.6 \times 10^3$  cells cc<sup>-1</sup>, a food level at which filtering rates are independent of food concentration (Rigler 1961). After 15 min of feeding on the labeled suspension, the animals were collected on a small mesh screen, dipped in boiling water, rinsed with 5% HCl and distilled water, and placed in liquid scintillation vials. Aliquots (10 ml) of the radioactive feeding

suspension were deposited on Reeve Angel 984H glass fiber filters and placed in scintillation vials, in triplicate for each BOD bottle. All samples were digested with 1 ml of NCS (Amersham/Searle) at 50°C overnight, and 10 ml of scintillation fluid (6.6 g PPO:82.5 mg POPOP:1 liter toluene) was added to the cooled samples. Samples dark-adapted for 24 h were counted with a Beckman LS-230 liquid scintillation counter and the efficiencies were estimated by the external standards method.

Filtering rates (ml animal<sup>-1</sup> h<sup>-1</sup>) were calculated as

$$f = \frac{\text{dpm}(\text{individual } Daphnia)^{-1}}{\text{dpm}(1 \text{ ml of medium})^{-1}} \times \frac{1}{15 \text{ min}} \times \frac{60 \text{ min}}{1 \text{ h}}.$$

Respiration rates at different oxygen concentrations were measured at 24°C in 300-ml BOD bottles filled with filtered, autoclaved, aged tapwater in which the oxygen concentration was again adjusted with nitrogen and measured. Twenty-two to 40 *Daphnia* were added and left to incubate for 4 h at 24°C, after which the final oxygen concentrations were determined. We used *D. magna* between 1.5 and 2.7 mm long and *D. galeata mendotae* of the same size as we used in the filtering rate determinations. Again, about 50% of the animals were ovigerous. The bottles were not rotated, as Schindler (1968) noted that this had no detectable effect on respiration. Several bottles without animals served as controls. Dry weights were determined by collecting the animals from each BOD bottle on previously tared glass fiber filters which were then dried at 55°C for 48 h and weighed on a Cahn electrobalance.

The ambient oxygen concentration in the respiration bottles was considered to be the mean of the initial and final oxygen concentrations. Animals gave erratic responses at concentrations below 0.5 mg liter<sup>-1</sup> O<sub>2</sub>, presumably because of high mortality: these points were not included in the statistical analysis of the results.

Table 1. Regression equations and correlation coefficients ( $r$ ) expressing various relationships between filtering ( $f$ ) and respiration ( $R$ ) rates and ambient oxygen concentration for two species of *Daphnia*.

1. Relationship between filtering rate, $f$ (ml/animal/h), and ambient dissolved oxygen concentration, $O_2$ (mg/liter), for small (1.54 mm) <i>D. magna</i> (Fig. 1).					
$f = -0.229(\pm 0.091) + 0.554(\pm 0.069)O_2^*$	for $O_2 < 3.0$ mg/liter	$r = 0.96$	$df = 7$	$P = 0.0002^\dagger$	
$f = 1.015(\pm 0.122) + 0.032(\pm 0.022)O_2$	for $O_2 > 3.0$ mg/liter	$r = 0.63$	$df = 4$	$P = 0.2531$	
2. Relationship between filtering rate, $f$ (ml/animal/h), and ambient dissolved oxygen concentration, $O_2$ (mg/liter), for large (2.34 mm) <i>D. magna</i> (Fig. 1).					
$f = -1.036(\pm 0.386) + 2.259(\pm 0.303)O_2$	for $O_2 < 3.0$ mg/liter	$r = 0.93$	$df = 9$	$P = 0.0001$	
$f = 3.482(\pm 0.772) + 0.101(\pm 0.136)O_2$	for $O_2 > 3.0$ mg/liter	$r = 0.39$	$df = 4$	$P = 0.5127$	
3. Relationship between filtering rate, $f$ (ml/animal/h), and ambient dissolved oxygen concentration, $O_2$ (mg/liter), for <i>D. galeata mendotae</i> (Fig. 3).					
$f = -0.019(\pm 0.077) + 0.169(\pm 0.016)O_2$		$r = 0.98$	$df = 7$	$P < 0.0001$	
4. Relationship between respiration rate, $R$ ( $\mu$ l $O_2$ /mg dry wt <i>Daphnia</i> /h), and ambient dissolved oxygen concentration, $O_2$ (mg/liter), for <i>D. magna</i> (Fig. 2).					
$R = 2.743(\pm 0.263) + 1.177(\pm 0.239)\ln O_2$		$r = 0.83$	$df = 12$	$P = 0.0004$	
5. Relationship between respiration rate, $R$ ( $\mu$ l $O_2$ /mg dry wt <i>Daphnia</i> /h), and ambient dissolved oxygen concentration, $O_2$ (mg/liter), for <i>D. galeata mendotae</i> (Fig. 4).					
$R = 3.998(\pm 1.207) + 1.411(\pm 0.261)O_2$		$r = 0.83$	$df = 14$	$P = 0.0001$	

\*Numbers in parentheses are the standard errors of the regression coefficients.

$^\dagger P$  is the probability level obtained for the regressions (F-test).

## Results

Descriptive functions were selected and fitted to the data by statistical routines available in the University of Michigan-Michigan Terminal System (MTS:MIDAS). All equations were fitted by least-squares linear regression. Except when a rectilinear function appeared to be the most appropriate, the descriptive function was chosen from several reasonable alternative functions by a forward stepwise selection process (Draper and Smith 1966). Equations selected, correlation coefficients ( $r$ ), and probability levels attained ( $P$ ) are given in Table 1.

Figure 1 illustrates the filtering rate responses observed for two size classes of *D. magna* over a range of oxygen concentrations. The initial sharp rise and the rela-

tively abrupt plateau appeared to be best fitted with a rectilinear model, which suggests that there is an incipient limiting oxygen concentration below which filtering rate is oxygen dependent. The slopes of the lines regressed to the points above 3.0 mg liter<sup>-1</sup>  $O_2$  are not significant, so although there appears to be some dependency between oxygen concentration and filtering rate at the higher oxygen concentrations, we do not have statistical confidence that this is so.

The similarity of the plots for the two size classes of *D. magna* indicate that the general trends are not size specific; both have their incipient limiting concentration between 2.5 and 3.0 mg liter<sup>-1</sup>  $O_2$ . The maximum filtering rates are somewhat higher than the values of Burns (1969) for these sizes of *D. magna*.

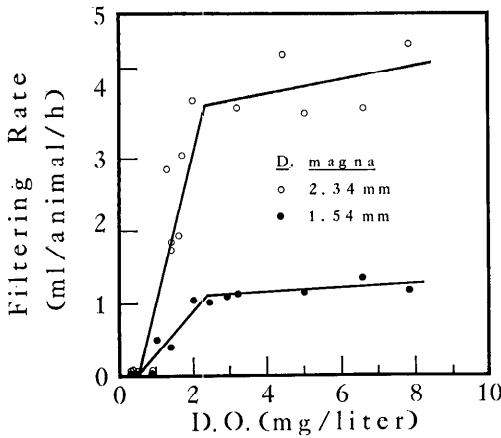


Fig. 1. Relationship between filtering rate and ambient dissolved oxygen concentration (D.O.) for two size classes of *Daphnia magna*.

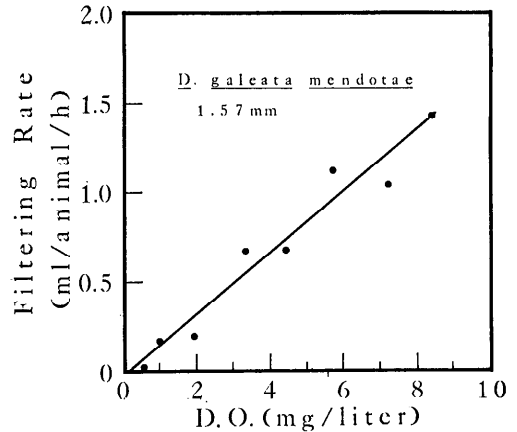


Fig. 3. Relationship between filtering rate and ambient dissolved oxygen concentration (D.O.) for *Daphnia galeata mendotae*.

Figure 2 suggests that a trend similar to the filtering rate responses also exists in *D. magna* for respiration rates over a range of oxygen concentrations. A sharp initial rise and later leveling off is seen. However, a less distinct breaking point in the data resulted in selection of a log function, although many other curvilinear models would probably have fit equally well. A hyperbolic-type response such as this, indicating considerable regulatory ability, is

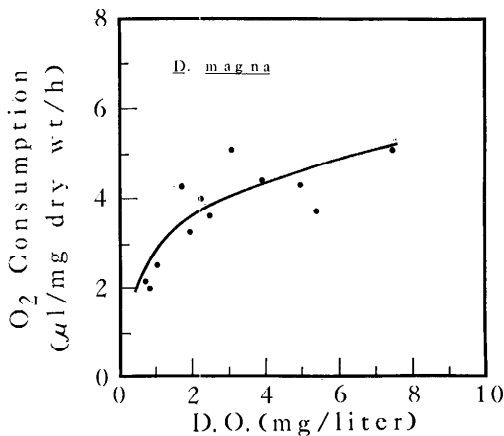


Fig. 2. Relationship between respiration rate and ambient dissolved oxygen concentration (D.O.) for *Daphnia magna*.

typical of organisms inhabiting low oxygen environments (Callow 1975) and is similar to the type-2 curve reported by Berg et al. (1962) for certain profundal animals. The maximum respiration rates appear to be slightly higher than those reported by Schindler (1968) for this species.

Figure 3 illustrates the influence of ambient oxygen concentration on filtering rates of *D. galeata mendotae*. This species exhibits a linear dependency of filtering rate on oxygen concentration at all oxygen levels tested. Maximum filtering rates appear to be similar for *D. magna* and *D. galeata mendotae* of equivalent sizes (the sizes of the small *D. magna* and *D. galeata mendotae* were not significantly different when tested using the Mann-Whitney U test).

As with *D. magna*, the respiration response of *D. galeata mendotae* (Fig. 4) shows a trend similar to that of its filtering rates, being best fitted with a linear function. A linear respiration response with a constant dependence on ambient oxygen concentration shows that the animal has little ability to regulate its oxygen consumption.

The maximum respiration rates for *D. galeata mendotae* are about three times higher than those for *D. magna* and are

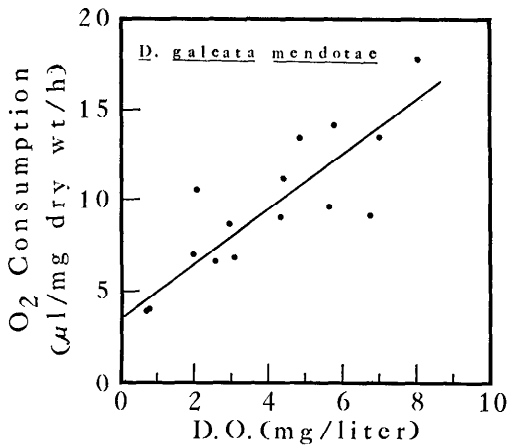


Fig. 4. Relationship between respiration rate and ambient dissolved oxygen concentration (D.O.) for *Daphnia galeata mendotae*.

comparable to the value reported by LaRow et al. (1975). This higher rate is in part due to the fact the *D. galeata mendotae* used for the respiration determinations were smaller than the *D. magna* (see Zeuthen 1953); it may also reflect differences in other factors such as general activity or maintenance energy expenditures.

#### Discussion

The classical explanation for the different responses shown for the two species is that the greater regulatory ability of *D. magna* is an adaptive response to an environment in which the oxygen concentration may typically be lower or more variable than that of *D. galeata mendotae*. The oxygen regimes of the animals' habitats are largely consistent with this explanation. The eutrophic temporary pond habitats of *D. magna* often have large fluctuations in oxygen concentrations, while the epilimnetic habitat of *D. galeata mendotae* is often near saturation or above. However, *D. galeata mendotae* is also sometimes found in oxygen-poor zones near the thermocline (Brooks 1962; Suffern 1973; pers. obs.). These differences in regulatory ability may be due largely to differences in the basal levels of hemoglobin in these animals. A

similar mechanism for differences in oxygen tolerance in various branchiopods was proposed by Moore and Burn (1968).

Kring and O'Brien (1976b) found that *D. pulex* did not initiate hemoglobin synthesis until 12 to 14 h after exposure to low oxygen and suggested that this prevents an animal that diurnally migrates into low oxygen zones from becoming pigmented and vulnerable to fish predation. If this is generally true for *Daphnia* that undergo diurnal migrations in oxygen-stratified lakes, oxygen stress before hemoglobin synthesis may occur quite commonly in nature and could be significant in limiting the growth of *Daphnia* populations.

Zooplankters that are susceptible to visual predation may rely on deep water as a refuge from predation (Zaret and Suffern 1976). Light attenuation at the greater depths apparently decreases the effectiveness of visual predators. If these zones become deoxygenated, *Daphnia* populations may be excluded from them and exposed to intense fish predation. The larger, more visible species such as *D. pulex*, which must migrate deeper to avoid fish predation, may generally have a greater tolerance to low oxygen than epilimnetic species, but cannot tolerate anoxia or the toxic compounds that may accumulate in an anoxic hypolimnion. These animals are the first to lose their deep water refuge when the anoxic zone shifts toward the surface of a lake and subsequently may be decimated by fish. This interaction of oxygen stratification and predation could result in the species more tolerant of low oxygen disappearing as a lake develops an even greater oxygen deficiency.

Brynildson (1958) suggested that such an interaction played a major role in determining seasonal abundances of *Daphnia* species in the lakes he studied. Fast (1971) observed that when the anoxic hypolimnion of a meromictic lake was artificially aerated, the *D. pulex* population present increased 88-fold. Fast felt that this was the result of making available a refuge from trout predation. Kerfoot (1972) noted

that the disappearance of large *Daphnia* remains in Frains Lake sediments corresponded with the time at which the surrounding land was cleared and agricultural runoff greatly increased, resulting in hypolimnetic deoxygenation. Although an increase in planktivore density may have occurred, the hypolimnetic oxygen deficiency probably also resulted in deterioration of the quality of the deep water refuge for the large *Daphnia*. Unfortunately, there appear to be few studies that provide data adequate to allow a detailed examination of the role of the interaction of oxygen stratification and fish predation in controlling *Daphnia* population dynamics. However, in many situations it may not be adequate to view size-selective predation pressure as merely a function of planktivore density; it may also depend on such other factors as the depth of a lake, its oxygen stratification, and its light transmitting qualities.

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