Experimental evidence of a low-oxygen refuge for large zooplankton

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Abstract

We tested the hypothesis that hypoxic zones in the metalimnion and hypolimnion of lakes can provide a refuge against fish predation for large zooplankton. Experiments were run in a large indoor mesocosm system (Plön Plankton Towers). We compared mortality rates of Daphnia pulicaria due to free-ranging fish in mesocosms with either oxic or hypoxic hypolimnion. In the presence of fish Daphnia moved down below the thermocline. Under hypoxic conditions their distribution peaked in the upper hypolimnion at a concentration of approximately 1 mg O₂ L⁻¹. The mortality rate of Daphnia in the hypoxic treatment was only one third of that in the oxic treatment. The hypoxic habitat provided a refuge, as Daphnia tolerated lower oxygen concentrations than did fish. However, there may be demographic costs associated with living in low-oxygen conditions. Hence, the importance of a hypoxic refuge under natural conditions will most likely depend on the trade-off between predation risk and cost of living in hypoxic waters.

Deep-water refuges are important for large zooplankton in the presence of visually hunting planktivorous fish, as they offer an opportunity for hiding in the dark during daytime hours (Lampert 1993). It has frequently been shown (Leibold and Tessier 1991) that habitat partitioning and diel vertical migration of differently sized Daphnia species depend on the availability of a refuge from fish predation. Deep lakes usually provide a good refuge, in particular if their vertical light profile is steep as a result of mesotrophic or eutrophic conditions. However, such lakes often develop poor oxygen conditions in the hypolimnion during summer stratification. Consequently, the hypolimnetic refuge zone shrinks from the bottom upward. The loss of a refuge renders large zooplankton in particular more vulnerable, and this may cause changes in zooplankton community composition, competitive relationships, and community grazing (Wright and Shapiro 1990; Tessier and Welser 1991; Hu and Tessier 1995). These studies found a strong correlation between hypolimnetic oxygen decline and a shift from large cladocerans (Daphnia pulicaria) to intermediate-sized (Daphnia galeata mendotae) and small species. Wright and Shapiro (1990) presented some experimental evidence for the mechanism causing the shift. They added bluegill sunfish to 6.5-m-long enclosures with a mesh bottom in a lake that was anoxic below 2.5 m. In two of the four enclosures, they oxygenated the lower part (i.e., they expanded the refuge zone). All three Daphnia species in the enclosures survived better in the presence of a refuge, and the effect was most pronounced for the largest species, D. pulicaria. Field and Prepas (1997) aerated the hypolimnion in one basin (34-m depth) of a Canadian lake while a second basin was not aerated. After aeration, Daphnia longiremis started with diel vertical migration, while they did not migrate in the non-treated basin (i.e., the Daphnia used the refuge).

However, an alternative hypothesis was suggested at the same time, proposing that oxygen depletion may even provide a refuge for zooplankton (Hanazato et al. 1989; Horppila 1997). This hypothesis was based on the observation that several Daphnia species had been found to be physiological regulators with respect to respiration (Heisey and Porter 1977; Kobayashi 1982) and could tolerate ambient oxygen concentrations below 1 mg L⁻¹ (Weider and Lampert 1985). On the contrary, most pelagic fish species have been found to avoid oxygen concentrations < 2–4 mg L⁻¹ (Eby and Crowder 2002; Klumb et al. 2004). Consequently, to avoid fish predation zooplankton may migrate into hypoxic waters where they can still survive, although at a cost. Support for this hypothesis comes from various recent studies (Hembre and Megard 2003; Vanderploeg et al. 2009a,b).

Field studies on low-oxygen refuges for zooplankton are usually qualitative. They report that large zooplankton species are present when a hypoxic zone develops and are absent when the hypoxic zone disappears, or they describe diurnal population maxima within the hypoxic zone. A quantitative assessment of the net effect of a low-oxygen refuge is probably impossible under the uncontrolled, variable conditions in the field. Even the results of field enclosure experiments (Wright and Shapiro 1990) were partly unexplained. Hence, we set up experiments in large indoor mesocosms with a controlled vertical temperature, food, and oxygen environment to gain quantitative data on the effect of a low-oxygen refuge. The advantage of this system was the full control over abiotic factors and phytoplankton distribution that it afforded (i.e., these factors remained constant regardless of the presence or absence of fish). Hence, fish effects could be separated from environmental effects. Our hypothesis was that the presence of a hypoxic (but not anoxic) hypolimnion will protect a large Daphnia species from fish predation, reduce zooplankton mortality, and result in improved net population growth.

Methods

The experiments were carried out with a clone of D. pulicaria in the Plön Plankton Towers (Lampert and Loose...
1992) in two 11.5-m-high indoor cylinders with an inner diameter of 0.86 m made of stainless steel. A temperature control system allowed for the establishment of a stable temperature profile with 50-cm vertical resolution. Once the temperature profile was stable, it was also possible to control the vertical distribution of food algae and oxygen. The towers were filled with filtered (5 μm) water from a nearby mesotrophic lake. A thermocline was established at 2.5 m in depth, separating the warm (20°C) epilimnion from the upper hypolimnion (10°C). To stabilize the upper hypolimnion we added a second, weaker thermocline at 5.5 m in depth, which produced a lower hypolimnion of approximately 8°C. The temperature profile remained identical in all treatments.

Artificial light was provided by lamps situated above the columns for 12 h per day. As a result of the restricted angular distribution caused by the walls the vertical light gradient was compressed. The 1% light level was at about 5 m in depth (Lampert and Loose 1992). Incident light at the surface measured ~ 350 μmol quanta m⁻² s⁻². Light conditions were identical for both columns and for the three experiments.

The hypolimnion was made hypoxic by bubbling with pure nitrogen gas before the lower thermocline had been established. We used an airlift system constructed from two long polyvinyl chloride tubes hung into the towers. A thin tube (2-cm diameter) was placed inside a larger (5-cm) outer tube. The outer tube reached 30 cm above the water surface and ended about 2 m above the bottom. The inner tube reached only to the water surface but ended about 1 m deeper than the outer tube. Nitrogen from a tank was finely distributed through a bubble stone fixed inside the inner tube about 50 cm above the lower end. The nitrogen bubbles then rushed toward the water surface, transporting a continuous stream of water upward and extracting oxygen from the uprising water. When the water flow reached the surface, the gas escaped to the atmosphere, while the oxygen-depleted water circulated back to the bottom through the outer tube. Hence, the deep water was circulated and bubbled without disturbing the epilimnetic water. The hypolimnion reached oxygen concentrations below 1 mg L⁻¹ within 24 h. Only then was the lower thermocline established. Food algae (Scenedesmus obliquus) were cultured in 10-liter batch cultures in diluted (1:4) Z4 medium (Zehnder and Gorham 1960). The density of the stock algal suspension was determined by measuring the light extinction (800 nm) in a filter photometer and reading the equivalent concentration of particulate carbon from a pre-established calibration curve (extinction vs. carbon concentration). The appropriate amount of algae calculated to reach the aimed-at final algal concentration was then introduced through a tube into each vertical section.

Vertical profiles of temperature and oxygen were checked daily with probes lowered from the water surface. Algae and zooplankton were sampled through specific ports in the cylinder wall (Lampert and Loose 1992) with a vertical distance of approximately 50 cm. Algae were sampled daily through syringe needles reaching 10 cm into the water column. Approximately 50 mL of water was taken from each of 12 ports. The algal density was determined with a particle counter (CASY, Schärfe) and converted to carbon; algae removed by grazing and sinking were replaced every day. Daphnia were sampled at 10–11 depths by pumping 48 liters of water through a glass trap equipped with a 250-μm mesh (described in Lampert and Loose [1992]). All Daphnia traps at each of the two towers were operated simultaneously. The location of the respective sampling ports for algae and zooplankton are described in Fig. 1. Daphnia were preserved in sucrose formaldehyde (Haney and Hall 1973). They were later counted and sized by the bench-top version of the Optical Plankton Counter (Focal Technologies) and divided into four body size classes (0.6–1.0 mm, 1.01–1.5 mm, 1.51–2.0 mm, and 2.0–3.5 mm). The suitability of this method for counting individuals of a monospecific Daphnia population has been tested by Kessler and Lampert (2003).

We carried out three experiments that differed in terms of environmental conditions (Fig. 1). In all three experiments we established a deep-water algal maximum in the upper hypolimnion. This prevented confounding estimates of fish predation by starvation of zooplankton that escaped the epilimnion in response to fish. In experiment (Expt) 1 the hypolimnion was hypoxic, and the algal concentration at the maximum was 0.36 mg carbon (C) L⁻¹, which is slightly below the incipient limiting concentration (ILC), while the algal concentration in the epilimnion (0.07 mg C L⁻¹) was strongly limiting for Daphnia (Lampert 1987). Expt 2 also involved a hypoxic hypolimnion, but different food conditions. The algal concentration was approximately 0.5 mg C L⁻¹, which is near the ILC, both in the epilimnion and the upper hypolimnion. In Expt 3, the hypolimnion had normal oxygen concentrations, and the food concentration in the hypolimnion (1 mg C L⁻¹) was above the ICL, while it was limiting (0.13 mg C L⁻¹) in the epilimnion. Consequently, the feeding rates of Daphnia in the upper hypolimnion should have been identical in Expts 2 and 3 and only slightly lower in Expt 1. Hence, Expts 1 and 2 had identical oxygen but differing food conditions, while Expts 1 and 3 had food concentrations at or above the ILC but different oxygen conditions. The experiments were run under identical conditions in the two towers simultaneously.

The three experiments followed the same pattern. After the temperature, oxygen, and food conditions had been established, both towers were inoculated with D. pulicaria. They were maintained for 2–3 weeks to build up a sufficient Daphnia population. Then each tower received five small cyprinid fish (Leuciscus idus, red ide; 2–6 g in wet weight, 46–76 mm in length). These fish have the advantage of displaying a yellowish color, which makes them visible from the water surface so that missing fish could be replaced during the experiment and all fish could be recovered from the towers after the experiments. They are omnivores feeding on Daphnia if available.

The total population size of D. pulicaria was monitored on the last pre-fish day (day 0) and then following the fish introduction. The vertical profiles of Daphnia were integrated to calculate the number per tower in each of the four size classes. As the starting densities differed between towers and experiments, the data were normalized.
by setting the population size at the day before the fish introduction (day 0) to 1. Only one of the two towers could be considered on day 6 of Expt 2, as the total number of *Daphnia* caught was too low to calculate a reasonable vertical profile. The net population growth rate for each experiment was determined from logarithmic regressions of the normalized data over time, combining results of both towers. The slope of these regressions gives the net growth

Fig. 1. Vertical distributions of *Daphnia pulicaria* (% of total population), food algae, and oxygen concentrations in the plankton towers after introduction of fish in the three experiments (numbers in left panels). Means of three to four measurements from each of the two towers (± SE). Invisible error bars are smaller than symbol diameter. Temperature conditions were identical in all experiments. The broken horizontal lines indicate thermoclines separating three layers: the epilimnion (20 °C), the upper hypolimnion (10 °C), and the lower hypolimnion (8 °C).
rate (d\(^{-1}\)) under different conditions. Differences between the slopes of the regressions were tested using the Statistix 7.0 package (Analytical Software), after Bartlett’s test for equal variances.

**Results**

The environmental conditions during the experiments are depicted in Fig. 1. Oxygen profiles were very stable over time. The epilimnion was always oxygen saturated. In Expts 1 and 2, the oxygen concentration dropped to approximately 1 mg L\(^{-1}\) in the upper hypolimnion, and it decreased further to 0.3–0.5 mg L\(^{-1}\) with depth. No oxygen depletion occurred in Expt 3; the oxygen concentration even increased with depth as a result of the lower temperatures. Although the food concentrations varied a little more as a result of depletion and replenishment of algae during a diel cycle, the vertical profiles conformed very well with the expectations, showing deep-water maxima in Expts 1 and 3 and similar concentrations in the two upper layers in Expt 2.

The vertical distribution of *Daphnia* differed between experiments. In both experiments with oxygen depletion, the *Daphnia* maximum was found just below the thermocline at an oxygen concentration of approximately 1 mg L\(^{-1}\). The larger error bars in Expt 2 indicate a random movement between 2.5 m (thermocline) and 3 m (optimum) in depth. The lowest oxygen concentrations at which *Daphnia* were found corresponded to 0.65–0.75 mg L\(^{-1}\). On the contrary, *Daphnia* distributed evenly in the upper hypolimnion when oxygen was not limiting (Expt 3).

Figure 2 presents the distributions of *Daphnia* on day 0, before the introduction of fish. *Daphnia* were distributed as predicted by the ‘ideal free distribution with costs’ (Lampert et al. 2003; Lampert 2005). Data from the two towers were very consistent, except at 2.5 m and 3 m in depth in Expt 1. This difference between the towers is caused by small-scale changes in the depth of the maximum between the two sampling depths.

The starting populations of *Daphnia* before the introduction of fish varied between 30,000 and 70,000 per tower in Expts 1 and 2, but reached 98,000 in Expt 3 for both towers. As soon as the fish had been added, *Daphnia* populations declined (Fig. 3). The decline was particularly rapid in Expt 3. Regression statistics are given in Table 1. Overall, the slopes of the three regressions are significantly different (\(F_{2,19} = 37.94, p < 0.0001\)). However, pair-wise comparisons show that the slope for Expt 3 differs significantly from the slopes in both Expt 1 (\(F_{1,14} = 94.90, p < 0.0001\)) and Expt 2 (\(F_{1,11} = 36.85, p < 0.001\)), while the difference between the slopes for Expts 1 and 2 is marginally non-significant (\(F_{1,13} = 4.51, p = 0.053\)).

The slope of the regressions reflects the net population growth rate comprising the reproductive rate and the mortality rate. While the mortality rate is influenced by fish predation, the reproductive rate depends on the rate of egg production, which is affected by the rate of food uptake and conversion, and on the egg development rate, which is mainly controlled by the temperature. Consequently, the net population growth rate may have been influenced by the different environmental conditions in the three experiments independent of fish predation. Unfortunately, we were not able to estimate the reproductive rate of *Daphnia* directly to calculate the true mortality rate.

**Discussion**

This experiment provides clear support for the hypothesis that low-oxygen zones can function as a refuge against fish predation for relatively large zooplankton such as *D. pulicaria*. Under otherwise-identical conditions, the rate of extinction of *Daphnia* by fish was much lower if the
hypothesis of an ‘ideal free distribution with costs.’ Many distributions have been published describing *Daphnia* behavior in the absence of fish in response to food, temperature, size, day–night cycle, and population density (Lampert et al. 2003; Kessler and Lampert 2004; Lampert 2005). The distributions on day 0 (Fig. 2) could have been predicted perfectly by the earlier work. In Expt 1, *Daphnia* traded high temperature in the epilimnion for food in the hypolimnion. However, the distribution was truncated in the deeper hypolimnion because of the additional costs of hypoxia. As epilimnetic food was available in Expt 2, the greatest fitness gain was in the epilimnion. Consequently, *Daphnia* did not penetrate the hypolimnion. In Expt 3 there was again a trade-off between high temperature in the epilimnion and high food in the hypolimnion. As predicted, the majority of *Daphnia* dwelled in the hypolimnion, exploiting deeper layers in the presence of oxygen. A comparison of the hypolimnetic distributions in Expts 1 and 3 nicely demonstrates the effect of hypoxia.

Reproductive activities inferred from day 0 distributions do not indicate a strong effect on the loss rates after fish introduction (Fig. 3). Any explanation of the large difference between slopes in Expts 1 and 3 based on natural mortality and reproductive rates would require compensation for the losses through very high reproductive rates in Expt 1. On the contrary, the day 0 distributions indicate the highest population growth rates for Expt 2 (high temperature and food) and the lowest for Expt 1. Greater food availability and the lack of direct hypoxia stress must have resulted in higher reproductive rates in Expt 3 compared to Expt 1. In addition to restricting food availability, low oxygen concentrations in Expt 1 likely had a direct negative effect on the reproductive rate. Earlier studies reported that oxygen depletion reduced the feeding rate of *Daphnia* (Kring and O’Brien 1976; Heisey and Porter 1977), as well as its growth and reproduction (Homer and Waller 1983). All of these experimental studies reported critical lower oxygen levels at ~ 3 mg L$^{-1}$. There are controversies about the improvement of fecundity due to hemoglobin production at very low oxygen concentrations (discussion in Sell [1998]), although there is no doubt that living at those oxygen levels normally avoided by fish bears costs, but still provides effective protection (Lass et al. 2000). The clone used in our experiments did not produce visible amounts of hemoglobin. Consequently, there is even more reason to assume that the reproductive rates were higher in Expt 3 than in Expt 1, which means that our estimate of high mortality in Expt 3 may even be conservative.

The vertical distribution of *Daphnia* after fish introduction, as depicted in Fig. 1, is a compromise between food availability and temperature (Lampert et al. 2003), oxygen

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**Table 1.** Regression statistics for the log-transformed, normalized population size of *Daphnia pulicaria* after fish introduction in the three experiments (cf. Fig. 3). Data are from both towers combined. MS, mean square.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>$n$</th>
<th>Intercept</th>
<th>Slope</th>
<th>MS</th>
<th>$R^2$</th>
<th>$p$</th>
</tr>
</thead>
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<td>-0.0583</td>
<td>0.0018</td>
<td>0.916</td>
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<td>0.0046</td>
<td>0.899</td>
<td>0.001</td>
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<tr>
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<td>-0.2236</td>
<td>0.0031</td>
<td>0.964</td>
<td>&lt;0.001</td>
</tr>
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concentration, and predation threat. While the effect of predation threat is clearly documented in Figs. 1 and 2, the contribution of oxygen can best be seen by comparing vertical profiles in Experiments 1 and 3. If oxygen is available, *Daphnia* distribute evenly in the upper hypolimnion. They only trade fish predation against low temperature. In the oxygen gradient they form a pronounced maximum just below the thermocline, as they must trade low temperature and low oxygen against fish predation. Such strongly compressed distributions of zooplankton have been detected by hydroacoustic surveys under similar conditions in situ (Ludsin et al. 2009). It is important to note that the *Daphnia* distributions shown in Figs. 1 and 2 are not static but rather dynamic. Individual *Daphnia* pictured at a certain depth do not dwell there permanently. Rather, they migrate up and down randomly, and the distribution reflects the average time an individual stays at a particular point (Lampert and Grey 2003). Consequently, individuals caught at the lowest oxygen concentrations (approximately 0.5 mg L$^{-1}$) will, on average, experience slightly higher oxygen levels. Nevertheless, individuals in both experiments spent most of their time at a concentration of approximately 1.0 mg L$^{-1}$, which is consistent with the tolerance level reported by Weider and Lampert (1985).

It is not surprising that hypolimnetic oxygen depletion has been reported to be a disadvantage (Wright and Shapiro 1990; Tessier and Welcer 1991; Field and Prepas 1997) or a refuge (Hanazato et al. 1989; Hembre and Megard 2003; Vanderpluog et al. 2009) for large cladocerans. The importance of an oxygen refuge must be context dependent. For example, an oxygen refuge can be an alternative to a light refuge. In a deep lake with oxygenated hypolimnion, zooplankton can migrate deep enough to exist in a light refuge, while this may be impossible in a shallow lake, where an oxygen refuge may be more important. In addition, the function of an oxygen refuge can depend on the fish species living in a particular lake. Salmonid fishes are known to be dependent on high oxygen concentrations. Consequently, Hembre and Megard (2003) found *D. pulicaria* to dwell in a refuge zone between 3 and 5 mg O$_2$ L$^{-1}$, too low for the rainbow trout populating this Minnesota lake. The clupeid *Alosa pseudoharengus* in Lake Ontario avoided oxygen concentrations $< 4$ mg L$^{-1}$. A limit of 3 mg O$_2$ L$^{-1}$ has been reported for fish in Lake Erie (Vanderpluog et al. 2009). In the presence of more low-oxygen–tolerant fishes (e.g., cyprinids), the refuge will be much more compressed. For the Neuse River estuary, Eby and Crowder (2002) reported that the minimum oxygen concentration for 10 of 11 fish species was approximately 2 mg O$_2$ L$^{-1}$. Under such conditions, the extension of the refuge (1–2 mg O$_2$ L$^{-1}$) depends on the steepness of the oxygen gradient.

Lower oxygen limits for zooplankton found in the field (Hanazato et al. 1989; Vanderpluog et al. 2009) are usually consistent with our observations with *Daphnia* in the plankton towers. Although they spent part of their time at 0.5 mg O$_2$ L$^{-1}$, the majority were found at approximately 1 mg L$^{-1}$. *Daphnia* seems to be a good model for many other zooplankton (Vanderpluog et al. 2009), except for *Chaoborus* larvae, which can penetrate completely anoxic waters for many hours. *Chaoborus* has also been reported to use a metalimnetic oxygen minimum as refuge against predation by smell (*Osmerus eperlanus*). The protection is particularly effective if combined with moderate levels of clay, which increase turbidity (Liljendahl-Nurminen et al. 2008).

An oxygen refuge does not provide perfect protection, as some fish are able to make short excursions to feed in hypoxic waters. Mudminnows (*Umbrina limi*) seem to be effective foragers on *Chaoborus* larvae in anoxic waters, although they cannot stay there for extended time periods (Rahel and Naiman 1994). Yellow perch (*Perca flavescens*) in Lake Erie avoid water with oxygen concentrations $< 2$ mg L$^{-1}$, but occasionally they ‘dive’ into hypoxic waters to feed on bottom fauna (Roberts et al. 2009). Using hydroacoustic equipment that discriminated between fish and *Chaoborus* larvae, Knudsen and Larsson (2009) observed individual fish penetrating the hypolimnion to feed. However, for crustacean zooplankton, the risk of being eaten is much smaller in hypoxic than in oxic waters if fish spend only a fraction of their time foraging in the refuge. It may be more profitable for the fish to ‘hold their breath’ if they find dense populations of large *Chaoborus* prey.

In the presence of fish the population development of *D. pulicaria* in our experiments was negative, even when an oxygen refuge was available. This cannot be explained by natural mortality, as the populations were growing before the fish were added. However, not all *Daphnia* specimens stayed in the refuge; there was always a small part of the population in the epilimnion taking the risk. As the vertical distribution reflects the fitness in the different habitats (Kessler and Lampert 2004), the individuals trade the predation risk against the developmental costs of low temperature and reduced oxygen in the hypolimnion. This implies a certain degree of predation loss in the epilimnion. *Leuciscus idus*, used in our experiment, is a cyprinid, and although we do not have measurements, we assume that it is relatively tolerant of hypoxia. Hence, it is likely that the fish foraged occasionally in deeper layers, in particular as the oxygen gradient in our experiments was rather steep. Fish species more sensitive to oxygen depletion would not be able to forage that deep (i.e., the oxygen refuge would even be more effective).

Nevertheless, the mortality rate is strikingly different in the treatments with and without an oxygen refuge. The availability of an oxygen refuge reduces the mortality to one third its normal level. We believe that our study provides strong experimental support for the existence of oxygen refuges for zooplankton depending on lake properties such as the fish community and the vertical gradients of temperature, chemistry, and light.

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References


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