
SHORT COMMUNICATION

How do migrating daphnids cope with fish predation risk in the epilimnion under anoxic conditions in the hypolimnion?

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Abstract. The vertical distribution of *Daphnia* in experimental tubes is influenced by fish kairomone concentration in the 'epilimnion' and oxygen conditions in the 'hypolimnion'. Daphnids trade off reduced predation mortality in a refuge against disadvantages due to unfavourable oxygen conditions.

Diel vertical migration (DVM) is a common behaviour in zooplankton and occurs in all kinds of water bodies ranging from small ponds to oceans (Forward, 1988; Haney, 1988). The proximate factor inducing this behaviour is light, or, more specifically, the relative changes in light intensity induce vertical migration (Ringelberg, 1987). Many studies have also concentrated on the ultimate causes of DVM, and it has become evident that DVM is mostly an adaptation to visual predation by planktivorous fish (Zaret and Suffern, 1976; Stich and Lampert, 1981; Gliwicz, 1986; Bollens and Frost, 1989). However, DVM is a complex behavioural trait and cannot be explained by any single factor (Salonen and Lehtovaara, 1992). The vertical migration pattern of the cladoceran *Daphnia*, for example, is modified by several other environmental parameters, such as food availability (Gasol *et al.*, 1995; Van Gool and Ringelberg, 1995), temperature (Haney, 1993) and dissolved oxygen (Gasol *et al.*, 1995).

Wright and Shapiro stated that the concentration of oxygen plays a paramount role in the determination of the vertical distribution of daphnids (Wright and Shapiro, 1990). In contrast to other aquatic invertebrates, e.g. the phantom midge *Chaoborus*, cladocerans are unable to withstand anoxic conditions for long. Filtering and respiration rates of *D.pulex*, for example, decrease dramatically below an oxygen threshold concentration of around 3.5 mg O₂ l⁻¹ (Weider and Lampert, 1985). However, several *Daphnia* species can counteract low oxygen conditions to a certain degree by producing haemoglobin (Fox *et al.*, 1951; Landon and Stasiak, 1983; Engle, 1985; Sell, 1996). When *D.pulex* has a high enough haemoglobin content it is able to survive oxygen concentrations as low as 0.5 mg O₂ l⁻¹ (Weider and Lampert, 1985; Sell, 1998).

Oxygen depletion is a common phenomenon in the hypolimnion of many eutrophic lakes, especially during the summer months. This poses a problem for zooplankters performing vertical migration as the lack of oxygen experienced by the animals during the daytime results in a reduced growth rate (Hanazato and Dodson, 1995) and a lower reproductive output (Hanazato *et al.*, 1989). However, zooplankters are still found in low-oxygen layers (Hanazato *et al.*, 1989;

Hanazato, 1992; Duncan *et al.*, 1993). A possible explanation for this phenomenon might be that these oxygen-depleted hypolimnetic layers provide not only a light refuge, because the most important planktivorous fish are visual predators, but also an oxygen refuge, as most fish are obligate aerobes and therefore unable to penetrate low-oxygen layers for longer periods (Hanazato *et al.*, 1989; Wright and Shapiro, 1990).

In this study, we set out to investigate the trade-off between low oxygen in the hypolimnion and high fish predation pressure in the epilimnion. Migrating down results in a reduced mortality risk due to fish predators but unfavourable oxygen conditions, whereas non-migrators trade off favourable oxygen conditions against increased predation mortality. Therefore, in an experiment with different oxygen concentrations and different fish predation pressures (expressed as different concentrations of fish kairomones), we would expect to find an interaction between these two factors on the vertical distribution of the daphnids. Under high fish predation pressure, the animals are expected to be deeper, even if the oxygen concentrations are bad, whereas they should not enter the anoxic layers if the predation pressure is low. In this way, they would be able to balance benefits against disadvantages to optimise their fitness. Therefore, in this study, we determined the behaviour of *Daphnia galeata* × *hyalina* when experimentally exposed to different concentrations of fish kairomones and oxygen concentrations. Different migration strategies result in differences in environmental conditions experienced by the animals. Hence, one would expect life-history characteristics to reflect different behaviours. We chose to assess these life-history consequences in a system with conditions as close to the ambient environmental conditions in the field as possible. As a consequence, the hypolimnion was not only partly oxygen depleted, but was also colder and contained less food. Furthermore, predator kairomones were present only in the epilimnion. This means that we were not aiming to quantify the direct effects of differences in oxygen and kairomone concentrations, as these effects have been well studied [e.g. (Weider and Lampert, 1985; Sell, 1998; De Meester and Weider, 1999)], but rather, the more ecologically relevant changes caused by the whole suite of environmental variables changing with depth.

The experiment was carried out in a multiple cylinder set-up, which was similar to that described by Dawidowicz (Dawidowicz, 1993), and included Perspex tubes (1 m in length, 1.5 cm in diameter, closed at the bottom) placed inside three transparent water baths with half of each cylinder submerged in the water. A thermal stratification was established in the cylinders with 10°C in the lower, and 20°C in the upper part. The set-up was illuminated from above with two rows of halogen lamps. A long-day photoperiod (16L:8D) was established during the experiment, with both sunrise and sunset simulated by increasing or decreasing the light intensity stepwise over one hour. The experimental tubes were subjected to the following treatments in a full factorial design: three concentrations of 'fish water' in the upper part of the cylinders ('epilimnion') and three different oxygen concentrations in the lower part of the tubes ('hypolimnion'). This set-up resulted in a total of 3 (kairomone concentrations) × 3 (oxygen concentrations) × 3 (replicates) = 27 experimental tubes.

Chemostat-grown *Scenedesmus obliquus* (1.5 mg C l⁻¹) was provided as food in the upper part of the tubes only. This was done for several reasons. Under normal circumstances in lowland lakes, food conditions in the epilimnion are considerably better than in the hypolimnion [e.g. (Guisande *et al.*, 1991)], and we wanted to mimic natural conditions. Moreover, algae added to the hypolimnion would have increased the oxygen concentration in the lower part of the tubes. The kairomone water was obtained from a 25 l aquarium containing three *Carassius carassius* which were fed *D. magna* daily. Water was collected from this aquarium every day, filtered (0.45 µm), aerated, and mixed with aged filtered (0.45 µm) water from the nearby mesotrophic Lake Schöhsee to obtain three different kairomone concentrations (10, 50 and 100% 'fish water'). The aquarium was refilled with unfiltered Schöhsee water. Only the 'epilimnion' water in our tubes contained the fish kairomone, again to mimic natural conditions as closely as possible. For the 'hypolimnion', water with three different oxygen concentrations was prepared (10, 2 and 0.5 mg O₂ l⁻¹). To remove the oxygen, filtered (0.45 µm) and cooled (10°C) Schöhsee water was bubbled with nitrogen. The experimental tubes were half filled with this 'hypolimnion' water and then filled up carefully with the prepared 'epilimnion' water (20°C). Measurements of the oxygen concentration in three parallel cylinders without any daphnids showed that the oxygen conditions remained almost constant over 24 h. The water in the tubes was changed daily and the tubes were rearranged randomly in the water baths every day.

The experiment was done with a *D. galeata* × *hyalina* clone, caught in Lake Plußsee, a eutrophic lake in Northern Germany with regular summer anoxia. Individual mothers were kept in 200 ml vessels at 21°C, a 16L:8D rhythm, and a food concentration of 1.5 mg C l⁻¹. From these cultures, five newborns (fourth, fifth or sixth brood) were put into each experimental tube to start the experiment. During the experiment, we measured the daytime depth daily (11:00 h) to assess the differences in behaviour. Moreover, in order to establish the effect of these differences in behaviour on other important traits, we measured body length and number of eggs. Repeated measurements ANOVA was used to test for treatment effects on the daytime depth, body size of adult instars 1–5, and number of eggs in these instars. Two *post hoc* tests (Duncan's multiple range test) were carried out to compare means of daytime depth and body length.

The mean daytime depth of the daphnids increased significantly with increasing fish kairomone concentration in the upper part of the cylinder (Figure 1A, Table I). Higher oxygen concentration in the lower part of the cylinder also led to a significantly deeper daytime depth of the daphnids (Figure 1A). The mean daytime depth increased with increasing age of the animals and so instar had a significant effect on the depth distribution of the daphnids (Table I, Figure 2). The large error bars in Figure 1A result from the fact that the means and standard errors were calculated using all observed depths. The interaction of kairomone and oxygen concentration on the daytime depth was only marginally significant (Table I). However, the animals in the low oxygen treatments were always found in the upper part of the cylinders (Figure 1A). The *post hoc* test on the daytime depth showed that there was no significant difference between the

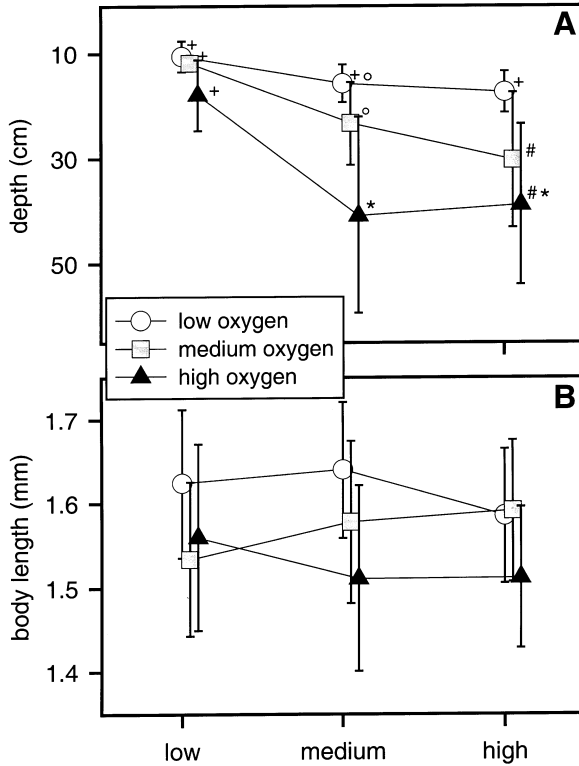


Fig. 1. (A) Mean daytime depth of adult instars 1–5 of *Daphnia galeata × hyalina* in response to different combinations of fish kairomone concentrations in the upper part and oxygen concentrations in the lower part of the experimental cylinders. A *post hoc* test (Duncan's multiple range test) showed that there are no significant differences between means with the same symbol. (B) Mean body length of all five instars. Error bars indicate standard errors.

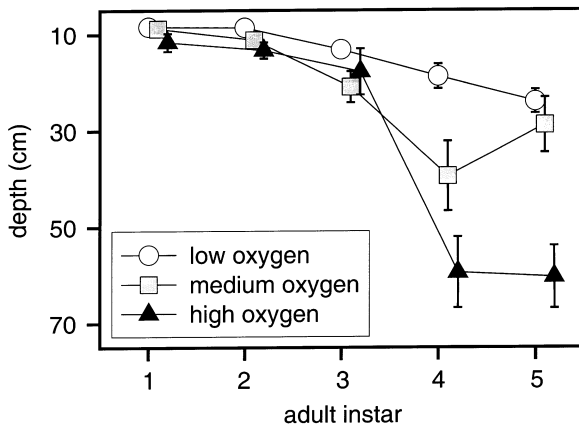


Fig. 2. Mean daytime depth at different oxygen concentrations during the first five adult instars in *Daphnia galeata × hyalina*. Error bars indicate standard errors.

Table I. Results of the repeated measurements analysis of variance of the mean daytime depth of *Daphnia galeata* × *hyalina* in response to different kairomone concentrations in the upper part and different oxygen concentrations in the lower part of the experimental tubes

Effect	d.f.	MS	F	P-level
O ₂	2	2898	22.10	<0.001
Kairomone	2	2666	20.33	<0.001
Instar	4	4824	24.78	<0.001
O ₂ × kairomone	4	322	2.45	0.091
O ₂ × instar	8	748	3.84	0.001
Kairomone × instar	8	570	2.93	0.008
O ₂ × kairomone × instar	16	238	1.22	0.280

different oxygen treatments at low kairomone concentrations, whereas at medium kairomone concentrations, the daphnids in the high oxygen treatment were found significantly deeper than the animals in the low and medium oxygen treatments (Figure 1A). Under high kairomone conditions, the animals in the medium and high oxygen treatments exhibited a deeper daytime depth than the daphnids in the low oxygen treatment. Hence, we show that a trade-off exists in the migration reaction of *Daphnia* under different fish kairomone concentrations in the 'epilimnion' and different oxygen concentrations in the 'hypolimnion'. The animals traded reduced predation mortality for a fitness loss due to unfavourable environmental conditions such as an anoxic hypolimnion. Therefore, the animals were not found in deeper depths when the oxygen conditions were too unfavourable, even if the kairomone concentration was high at the surface. This has also been found in field studies where the downward distribution of *Daphnia* populations is limited by oxygen depletion in the hypolimnion (Wright and Shapiro, 1990; Hanazato, 1992). Thus, the progressive depletion of hypolimnetic oxygen through the spring and summer first creates an oxygen refuge for zooplankters, excluding fish predators (Hanazato, 1992), but this refuge becomes unavailable for cladocerans when the oxygen concentration declines to very low levels. Due to the anoxia, the daphnids are finally restricted to a small portion of the lake and forced into a zone with increasing fish predation pressure (Wright *et al.*, 1980).

We observed a significant effect of the oxygen concentration in the hypolimnion on the size of the animals (Table II). The animals were largest in the tubes with the lowest oxygen concentrations and were, on average, smallest in the treatment with oxygen saturation in the hypolimnion (Figure 1B). Again, the large error bars in Figure 1B result from the fact that body size increased significantly with instar (Figure 2) and means and standard errors were calculated from all measurements. The *post hoc* test showed that the animals were significantly larger under low oxygen conditions in the low and medium kairomone concentrations than under high oxygen conditions in the medium and high oxygen concentrations. However, neither a significant kairomone effect nor an interaction of oxygen and kairomone concentration on body size was observed (Table II). The number of eggs in the brood pouch of the daphnids was not significantly influenced by either of the two parameters (Table II).

Table II. Results of repeated ANOVA measurements of the body size and the number of eggs in the first five adult instars of *Daphnia galeata* × *hyalina* in response to different kairomone concentrations in the upper part, and different oxygen concentrations in the lower part of the experimental tubes

Effect	d.f.	Body length		No. of eggs	
		MS	F	MS	F
O ₂	2	0.073	5.71*	6.009	0.42
Kairomone	2	0.002	0.14	1.679	0.12
Instar	4	0.609	137.25**	139.431	23.92**
O ₂ × kairomone	4	0.014	1.07	10.145	0.72
O ₂ × instar	8	0.003	0.77	9.339	1.60
Kairomone × instar	8	0.006	1.25	5.855	1.00
O ₂ × kairomone × instar	16	0.003	0.77	3.993	0.68

*p < 0.05

**p < 0.001

It is difficult to pinpoint exactly why the animals were significantly larger in the low oxygen treatment. We wanted to simulate the natural differences between epilimnion and hypolimnion as much as possible and offered food only in the upper part of the cylinder combined with a higher temperature in the 'epilimnion'. Therefore, the larger body size in animals that stayed closer to the surface could result from either better food conditions or higher temperatures. Laboratory experiments show that growth rate is reduced under low oxygen conditions and thus, predict a smaller size with decreasing oxygen conditions (Hanazato and Dodson, 1995). This is in contrast to our results with relatively larger animals in the low oxygen treatments. However, the daphnids in our low oxygen treatments stayed higher up in the tubes and therefore did not experience the low oxygen concentrations in the 'hypolimnion'. It is more likely that the reduced growth rate in the high oxygen treatments is not a direct oxygen effect but results from the lack of food in the 'hypolimnion'. The alternative explanation, that the animals experienced lower temperature in the 'hypolimnion', is unlikely as lower temperatures normally lead to larger animals (Atkinson, 1994). Most likely, the reduced growth rate is a constraint resulting from the migratory behaviour to unfavourable food conditions and is not a direct result of the oxygen concentration.

In this study, we set out to investigate the effects of differences in oxygen level in the hypolimnion for migrating *Daphnia*. The behavioural reactions are straightforward, with higher levels of fish kairomones being needed to induce vertical migration behaviour when oxygen levels in the hypolimnion are lower. The resulting effects on the life-history traits, however, are much less easy to predict. Animals residing in the epilimnion all day, as was the case with the lowest oxygen levels, are exposed to the fish kairomones for the whole 24 h period. This could mean that the effect of the fish kairomones on the life-history traits of these animals is greatest. In general, daphnids react to the presence of fish kairomones by decreasing their size (Stibor, 1992). We observed the opposite, i.e., animals that stayed in the epilimnion all day had the largest body size. Other studies have

shown that larger animals migrate deeper relative to smaller ones of the same species [e.g. (De Meester *et al.*, 1995)]. In this study, we have demonstrated that the absence of oxygen in the hypolimnion could result in exactly the opposite. Hence, our results imply that *Daphnia* in the typical summer situation of many eutrophic lakes, with higher temperature, oxygen and food supply in the epilimnion (Hanazato and Yasuno, 1987; Guisande *et al.*, 1991), experience a higher fish predation pressure not only because they do not migrate into the low oxygen levels but also due to their larger body size.

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