Hide, rest or die: a light-mediated diapause response in *Daphnia magna* to the threat of fish predation

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**SUMMARY**

1. In a laboratory batch culture experiment, a diapause response of *Daphnia magna* to a simulated threat of fish predation was tested at various light intensities, which under natural conditions determine potential vulnerability of *Daphnia* to visual planktivorous fish.

2. Under moderate light intensity (1.4 μmol m\(^{-2}\) s\(^{-1}\)) that allows effective predation by fish, the proportion of females producing dormant eggs was significantly higher than under dim light conditions (0.001 μmol m\(^{-2}\) s\(^{-1}\)) that are not favourable for visual detection of prey. Production of dormant eggs was not observed in complete darkness or in treatments missing fish kairomones, irrespective of tested light conditions.

3. The observed phenomenon is interpreted as a flexible response of prey to the conditional risk of predation assessed by *Daphnia* according to the presence of fish-derived cues on the one hand and the presence of dark refugia on the other. Irrespective of the presence of fish kairomones, *Daphnia* may not produce resting eggs as long as a safe, dark, bottom zone is accessible.

**Keywords:** chemical communication, ephippia, kairomone, predator avoidance, refuge

**Introduction**

The relatively homogenous environment of open water does not offer pelagic organisms many places to hide from visually orienting predators. As light attenuates with depth in the water column, affecting the visual detection abilities of aquatic animals, many pelagic organisms diminish predator encounter rates by descending to the relatively safe, dark, bottom zone before sunrise and only ascending to subsurface water to feed after sunset (for a review see De Meester *et al.*, 1999). However, the effectiveness of this diurnal mechanism may be affected by various environmental factors. The presence of a safe aphotic zone during the daylight hours depends on water depth and on the rate of light attenuation in the water column, which is affected by the quantity of suspended matter and watercolour. Depending on water transparency, the depth at which the safe, dark, refugium zone may be reached during daylight hours may range from tens of centimetres in turbid waters to tens or hundreds of meters in clear lakes and marine environments (Dodson, 1990; Ringelberg, 1995). Moreover, the daylight conditions may change substantially during the course of the season because of temporal changes in water transparency and seasonal or weather-dependent changes in light intensity impinging the water surface. In early spring, because of low algae production, or in June because of high grazing pressure on algae by abundant large-sized herbivorous zooplankton, light can penetrate deep into the water column. In late spring, summer, and autumn, water transparency is typically strongly reduced by abundant phytoplankton in eutrophic lakes (Sommer *et al.*, 1986). In shallow lakes, water transparency may be additionally affected by resuspension of sediments on windy days. The presence of a dark refuge may also be determined by water level, which may change substantially during the course of the season in shallow
water basins or man-made lakes. Even when a dark refuge is present, access to this zone may be permanently or temporarily obstructed by hostile conditions, such as the oxygen depletion which regularly occurs in the profundal zone of lakes of higher trophic status in winter or summer stratification periods (Sommer et al., 1986).

When the effectiveness of diel vertical migration is reduced, to survive periods of predation in shallow or clear waterbodies, planktonic organisms should apply alternative defence mechanisms such as production of dormant forms (Hairston, 1987). The production of dormant eggs to avoid fish predation has been shown in a single species of calanoid copepod (Hairston, 1987) and in the cladoceran Daphnia (Słusarczyk, 1995). Dormant forms of planktonic species may be relatively safe if they are resistant to ingestion or digestion or when deposited in places which are not accessible to predators (e.g. in the periodically deoxygenated hypolimnion). Dormant (’ephippial’) eggs of Daphnia are highly resistant to digestion (Mellors, 1975) and can be found in large quantities in bottom sediments where they can stay viable for years or decades (Caceres, 1998). Although diapause may allow persistence in periods of low survival probability, it may reduce fitness of individuals because of extension of generation time and temporal retardation of development and reproduction (Cohen, 1966). Therefore, diapause seems to be an ultimate defence utilised only in extreme cases when active defence mechanisms are ineffective or cannot be used.

According to recent findings, poor food conditions (Słusarczyk, 2001) as well as low yet tolerable temperature (Słusarczyk, unpublished results) may determine the decision of D. magna to produce dormant eggs under a risk of fish predation. This reaction was claimed to be adaptive, as both lower food levels and low temperature may diminish the relative benefit of production of active offspring compared with the formation of dormant eggs when at risk of fish predation. Light conditions may influence this balance in another way, by determining the level of predation risk at particular densities of visual predators.

The aim of the present research was to test whether the diapause response of large-bodied, planktonic Daphnia magna to fish predation risk may be altered by light conditions, mimicking the presence or absence of a safe dark refugium in aquatic environments. Several other environmental factors have already been recognised as triggers of Daphnia diapause, namely: a short photoperiod (Stross, 1987), low temperature (Mortimer, 1935), and low food concentration when associated with other signals of environmental hostility such as crowding (Banta, 1939; Kleiven, Larsson & Hobaek, 1992; Alekseev & Lampert, 2001).

**Methods**

A clone of D. magna from Binnensee (Schleswig Holstein, N. Germany), a shallow, eutrophic lake in which D. magna coexists with fish (Lampert, 1991), was utilised in the experiment. Daphnia magna does not coexist with fish in many lakes as its large size makes it extremely vulnerable to visually feeding predators. The clone used in the experiment has been shown to respond to chemical signals associated with fish predation by changing its behaviour (Loose, von Ellert & Dawidowicz, 1993; Pijanowska & Kowalczewski, 1997), life-history (Weider & Pijanowska, 1993) and the induction of dormant egg formation (Słusarczyk, 1995; Pijanowska & Stolpe, 1996). Daphnia magna from Binnensee is the only species known so far to produce dormant propagules in response to the presence of predatory kairomones.

Two experiments were performed. The first experiment tested an effect of light conditions and presence/absence of fish-derived chemical cues on a decision of Daphnia to produce dormant eggs. Experimental females were exposed to fish kairomones or water without addition of fish cues under three different light conditions: permanent darkness, permanent light, and the ‘summer’ daylight cycle (16 L : 8 D). The summer daylight treatment was intended to simulate light conditions of environments inhabited by Daphnia and lacking a dark refuge, while the dark treatment was intended to simulate light conditions faced by vertically migrating Daphnia in deep or turbid lakes. When hiding from visually hunting fish, Daphnia may face dark conditions permanently. It commonly spends nights in the subsurface zone and daytime hours in a dark, deep refuge. The permanent light treatment was used in the experiment to control for potential consequences of a change of photoperiod experienced by animals in the dark treatment that were moved from the summer daylight cycle (faced as embryos during pre-experimental period) to permanent dark conditions.
permanent light conditions may be experienced by Daphnia in summer at higher latitudes. Light intensity was set at a moderate level (1.4 μmol m⁻² s⁻¹) in the experimental boxes during daylight hours. Such a light intensity should allow visually hunting planktivorous to detect prey in natural conditions (Blaxter, 1970). The experiment was run two times and employed equal number of fish and fish-free jars. Each treatment had six replicates. Data from the two runs were pooled and analysed together.

The second experiment tested more specifically the effect of different light intensities on dormant egg production by Daphnia when exposed to fish kairomones at a summer photoperiod of 16 L : 8 D. Three light intensities were tested: 1.4 μmol m⁻² s⁻¹ (called moderate light), 0.001 μmol m⁻² s⁻¹ (called dim light) and 0.000 μmol m⁻² s⁻¹ (called dark). The first treatment mimicked light intensities that should allow effective predation by visually hunting planktivorous fish (Blaxter, 1970). The second treatment imitated the light intensity close to the lower threshold (0.02–0.0002 μmol m⁻² s⁻¹) indicated by Blaxter (1970) for visual predation by most tested planktivorous fish. Such light conditions may be found at a few meters depth in murky water of eutrophic lakes or at tens of meters in more transparent oligotrophic ones during daylight hours (personal observation). In the last treatment, experimental animals stayed in complete darkness over the study period. During the experiment, Daphnia were exposed to fish-derived chemical cues. Five replicates of each treatment were used. Moreover single batch cultures with no addition of fish cues were utilised for reference at each of the light conditions but were not considered as treatments in the statistical analysis because of lack of replication.

Experimental animals were enclosed in 0.9 L glass jars placed in cardboard boxes of predetermined light transparency at 22 ± 1 °C. Each cardboard box enclosed six glass jars. A single light source, a 120 W incandescent bulb, was placed 1.5 m over the experimental boxes. The cardboard boxes were opaque on the sides but were covered by lids with windows, the transparency of which was modulated by leaving a given number of sheets of white photocopy paper over it. This resulted in different light intensity in the different boxes.

The experimental media were prepared from aged lake water aerated for at least 48 h to remove potential predatory cues originating from the lake. This method was found effective in fish kairomone elimination (Loose et al., 1993). The aerated water of the fish treatments was exposed to a small cyprinid fish, sunbleak (Leucaspius delineatus), at a density of two fish per 20 L of water, for 24 h prior to use. The two fish were fed with 20 chironomid larvae in total after the experimental media were prepared and water in a fish aquarium refilled. As at least two chemical cues, fish kairomones and alarm substance originated from injured Daphnia, were found necessary to induce diapause in D. magna (Ślusarczyk, 1999), the fish water was ‘enriched’ with prefiltred (GFC Whatman filter) homogenate of 10 D. magna per 1 L of experimental medium. Prior to use, experimental media were supplied with algae (Scenedesmus obliquus) at a concentration of 0.7 mg C L⁻¹. According to a previous study (Ślusarczyk, 2001), these food conditions should favour ephippia production in Daphnia exposed to fish kairomones when reared in batch cultures. Fo the first 9 days of the experiment, the volume of water was increased gradually over time as experimental animals grew (from 11 mL per individual at the first day of the experiment to 30 mL per specimen on the ninth day of the experiment and afterwards) and their filtration rates increased. Experimental media were changed daily.

All experimental females were likely to be genetically identical as they came from a line of females reproducing parthenogenetically. Experimental females, randomly chosen from a group of synchronously hatched offspring derived from a single female, were placed in experimental beakers within 20 h of birth. Twenty-eight neonate females were used initially in each experimental beaker. The mother females of experimental animals were kept at favourable food conditions (>2 mg C L⁻¹), summer photoperiod (16 L : 8 D) and a light intensity higher than 1.4 μmol m⁻² s⁻¹ before the experiments were started.

The number of females producing ephippia (the protective structures enclosing dormant eggs) and the number of newborn offspring were recorded daily. Females having ephippia were removed without replacement from cultures daily when the media were changed. This procedure allowed for reliable calculations of the frequency of ephippial females in each experimental jar and prevented miscalculation because of the possible multiple production of dormant eggs by a single female. Animal densities were maintained constant throughout the reproductive
period by adjusting the water volume (30 mL per female) when individuals were removed. Daily manipulations of experimental animals from dark and dim light treatments were performed under red light illumination which, according to Young (1974), is hardly visible to Daphnia. The first experiment was continued until all females released their fourth-brood offspring, while the second one was finished when every animal had released its fifth brood.

Nonparametric Kruskal–Wallis ANOVA for ranks with sequential Bonferroni correction (Rice, 1989) was used for pairwise comparison of the proportion of ephippia-producing daphnids in experimental treatments.

**Results**

The results of both experiments indicate that light conditions may modulate a decision of D. magna to produce resting eggs when exposed to chemical cues of fish predation.

The first experiment indicated that the tested light conditions were alone an insufficient cue to stimulate production of dormant eggs in experimental clone of D. magna in the fish-free environment. Ephippia were not observed in any treatment lacking fish kairomones irrespective of light conditions (Fig. 1). However, when combined with fish-derived cues, the applied light conditions modulated diapause response in experimental Daphnia. In the two treatments where experimental females faced fish kairomones and moderate light intensity, production of dormant eggs was recorded. Under summer photoperiod, ephippial production was observed in all experimental jars in 26 ± 12% (mean ± 1 SE) of experimental females, whereas under permanent light conditions dormant eggs were recorded in four of six experimental jars in 24 ± 15% (mean ± 1 SE) of experimental females (Fig. 1). This recorded difference in diapause response between the two treatments was statistically insignificant. Concurrently, no ephippia production was observed in any fish jars kept in permanent darkness (Fig. 1).

A higher proportion of ephippial females was recorded in the second experiment, which tested diapause response of Daphnia to different light intensities at summer photoperiod and presence of fish kairomones. Ephippial production was observed in all experimental cultures where Daphnia was exposed to fish-derived cues in the moderate light treatment, with 62.0 ± 7.8% (mean ± 1 SE) of females forming ephippia, on average (Fig. 2). Under dim light conditions in the presence of fish kairomones the average proportion of females producing ephippia (8.7 ± 1.6%, mean ± 1 SE) was significantly smaller (Kruskal–Wallis ANOVA for ranks $H = 6.9, P = 0.009$). Ephippial females still occurred in all replicates. The proportion of ephippial females in the dim light treatment differed significantly (Kruskal–Wallis ANOVA for ranks $H = 7.8, P = 0.005$) from that in the dark treatment, in which diapause was not observed during the study period. Similarly, dormant eggs were not observed in the jars without predatory cues in any of the tested light conditions.

**Discussion**

While summer diapause of aquatic invertebrates inhabiting temporary waterbodies is commonly regarded as the mechanism of survival of periods of

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Fig. 1 The overall frequency (mean ± 1 SE) of ephippial females exposed to different light conditions (different photoperiod at moderate light intensity) in the presence or absence of fish kairomones.

Fig. 2 The overall frequency of ephippial females (mean ± 1 SE) at the three different light intensities and summer photoperiod in environments with or without fish kairomones.

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deterioration of abiotic conditions (Williams, 1998), it has less straightforward explanations in permanent lakes well buffered against abiotic changes. Summer diapause of planktonic crustaceans in permanent environments has been commonly attributed to two major biotic factors: deterioration of food resources because of intense intra- or interspecific competition (Banta, 1939; Santer & Lampert, 1995) or predation by fish or invertebrate predators (Threlkeld, 1979; Hairston, 1987). This seasonal phenomenon may be triggered by various environmental cues, which are directly (low food concentration, fish kairomones or metabolites of overcrowded population) or indirectly (specific photoperiod or water temperature) related to the ultimate forces. All these possible triggers may coincide during periods of summer diapause of the freshwater cladoceran *Daphnia*. This makes it difficult to judge the ultimate reason of this seasonal event under natural conditions (Threlkeld, 1979). Our experimental study extends the list of environmental factors that may affect the decision of *D. magna* to produce ephippial (dormant) eggs in summer.

The overall results of the two experiments indicate that light intensity may play an important role in the process of induction of dormant egg formation in *Daphnia* while under a threat of fish predation. As visually predating fish hunt more effectively in illuminated than in dim environments (Blaxter, 1970) the predation risk, and hence the need for prey defences, must be stronger under better light conditions. Chemical cues associated with fish predation may inform zooplankton on a potential danger of fish predation, which becomes regarded as a real threat by *Daphnia* when forced to stay in a bright environment. *Daphnia* may have a high likelihood of surviving in an active stage as long as a dark refuge is available. When the dark refuge is not present, or access to this zone obstructed by hostile conditions, the production of dormant eggs may become the ultimate defence assuring persistence of *Daphnia*. This modulation of the predator-induced responses by light intensity may be a more general phenomenon and could apply to other defence mechanisms too.

The presented results may explain the occurrence of multiple periods of dormant egg formation within a single summer, as observed by Lampert (1991) in the Binnensee, the lake from which the experimental clone was isolated. According to Lampert (1991) periods of dormant egg formation by *D. magna* coincided with a marked increase in water transparency and low food concentrations, likely caused by intense grazing of a dense *Daphnia* population. An increase in water transparency could have eliminated dark refugium zones in this shallow lake and hence could have exposed *Daphnia* to a higher risk of predation by fish. This agrees with the increased mortality at these times (Lampert, 1991). More intense predation by fish together with low food concentration could have diminished chances for *Daphnia* to survive and reproduce to below a threshold value where the production of dormant eggs becomes more beneficial than formation of active offspring (Ślusarczyk, 2001).

Although the experimental set-up did not allow us to test explicitly the occurrence of a trade-off between diel vertical migration and diapause in *Daphnia*, the presented results suggest this. The two mechanisms are likely triggered by the same ultimate force (danger of fish predation) and similar proximate factors (light intensity and presence of fish-derived chemical cues), which allow announcement of the danger in space and time. In the presence of visually hunting predators, we may expect prey to migrate downwards to a dark refugium. If for some reason (shallowness, high water transparency or oxygen depletion) a dark refuge is not accessible, the ultimate defence – production of dormant eggs – may be employed instead.

According to the results of our study, we may expect summer diapause of planktonic invertebrates to be favoured in shallow or transparent permanent lakes inhabited by fish, where the effectiveness of vertical migration is low. Factors like fish density, water transparency and temperature, as well as food conditions may fluctuate unpredictably in temperate lakes. This may explain why the mechanism of induction of summer diapause in *D. magna* may be modulated by so many environmental factors (Ślusarczyk, 2004).

**Acknowledgments**

We thank R. Jones, H. van Leeuwen and two anonymous reviewers for comments on the manuscript and for linguistic help and B. Niedomagala for help in running the experiments. The research was supported by grant KBN-6 PO4C03718 from Polish State Committee for Scientific Research.
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(Manuscript accepted 11 October 2004)