

## Diel horizontal migration and swarm formation in *Daphnia* in response to *Chaoborus*

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### Abstract

Dense swarms of *Daphnia longispina* (up to 4000 animals  $l^{-1}$ ) were recorded along the littoral zone in a lake where *Chaoborus flavicans* is considered the main predator. *D. longispina* coexisted with *D. pulex*, but there were no *D. pulex* in the littoral swarms. Swarms were less dense at night (about 1/10 the density), and *D. longispina* exhibited diel horizontal migrations by aggregating in the littoral during the day and spreading out at night. Laboratory experiments showed that *Chaoborus* capture efficiency on juvenile daphnids was higher in the light compared to darkness, and that *Daphnia* exhibited a behavioural response to water that had previously contained *Chaoborus*. We conclude that predation from *Chaoborus* can be an important factor affecting the distribution patterns of *Daphnia* observed in this lake. The behavioural experiments indicated that this influence might be partly mediated by chemical agents.

### Introduction

Flocking is a well-known behaviour to avoid predation in terrestrial vertebrates (Humpries & Driver, 1967; Hamilton, 1971; Pulliam, 1973; Treisman, 1975a, b; and Caraco *et al.*, 1980). The same mechanisms operate among fish and in fish-zooplankton interactions (Neill & Cullen, 1974; Milinski & Heller, 1978; Heller & Milinski, 1979; Milinski, 1979; Jakobsen & Johnsen, 1987, 1988a). The efficiency of swarming to avoid predation is due to two effects: a) confusion, used against visual predators, and b) dilution, i.e. reduction in the chances of a given individual being eaten. The main cost of swarming is considered to be a rapid reduction in the concentration of food within the swarm, and it has been shown that zooplankton conspecifics tend to be more aggregated under excess food compared to low food conditions (Jakobsen & Johnsen, 1988b).

The impetus of our work was based on observations of dense swarms of the waterflea *Daphnia longispina* O.F. Müller (Crustacea: Cladocera) within and along the littoral zone of a mesotrophic lake: Myra-

vatn, Bergen, Norway (Giske, 1986). In Myravatn, *D. longispina* coexists with *Daphnia pulex* Leydig, and it is assumed that there is habitat segregation between the two species. There are no planktivorous fish in the lake. According to Giske (1986), the phantom midge *Chaoborus flavicans* Meigen is the main factor regulating the *Daphnia* population in Myravatn. Because it is subjected to low predation pressure in Myravatn, *Chaoborus* is able to go through two generations per year, in contrast to one generation in lakes in the same area inhibited by planktivorous fish. The *Chaoborus* in Myravatn perform diel vertical migration, but to a lesser extent than would be expected in a lake with planktivorous fish (pers. comm., Jan Wivegh, University of Bergen). *Chaoborus* feed on juvenile daphnids, but since *Daphnia* release offspring approximately every third day (at 20 °C), one might assume that also adult egg-bearing females distribute themselves in response to the predator, in order to protect their offspring just after birth.

Davies (1985) reported diel horizontal migration in *Daphnia hyalina lacustris* with aggregations of > 1000 animals  $l^{-1}$  in the littoral. He considered these

dense concentrations of daphnids in the littoral to be an escape response from fish predators in the pelagial. De Meester *et al.* (1993) also described a similar type of diel horizontal migration in *Scapholebris mucronata*, with  $> 1000$  individuals  $l^{-1}$  swarming in the littoral zone during day and dispersing at night. These distribution patterns are also considered to be the result of active avoidance from fish predators. Whether *D. longispina* exhibit similar mechanisms to avoid predation from *Chaoborus*, depends partly on the question of modes of predation. *Chaoborus* is generally considered to be a tactile sit-and-wait predator (Pastorok, 1978), and Swift and Forward (1981) did not find that *Chaoborus* had a higher strike efficiency in the light compared to dark conditions when feeding on copepods. It should be noted however, that the 'confusion effect' of swarming can apply even if olfactory or tactile senses are used to detect the prey item, and that the dilution effect will be the same.

During the last decade, several authors have demonstrated that chemical signals can be important factors regulating aquatic environments, especially concerning predator-prey interactions. A review of chemical communication in planktonic animals is given by Larsson and Dodson (1993). Dodson (1988) demonstrated experimentally that some *Daphnia* species responded to chemical signals from *Chaoborus americanus* by changing their vertical migration pattern. We therefore assumed that the *Daphnia* distributions in Myravatn could be affected by the detection of chemical substances released by *Chaoborus*.

Our aim in this study was to: a) describe the distribution patterns and the swarming phenomenon in Myravatn; b) conduct experiments to test whether *Chaoborus* had higher predation success on daphnids in the light rather than in darkness; and c) conduct experiments focusing on the role of chemical substances from *Chaoborus*, which affect the horizontal migration of *Daphnia*.

## Materials and methods

### Field

The lake studied, Myravatn, is located 8 km south of Bergen, Norway. It is a small ( $0.55 \text{ km}^2$ ) temperate mesotrophic lake without planktivorous fish. The littoral vegetation is well developed, and consists of a continuous vegetation belt around most of the lake,

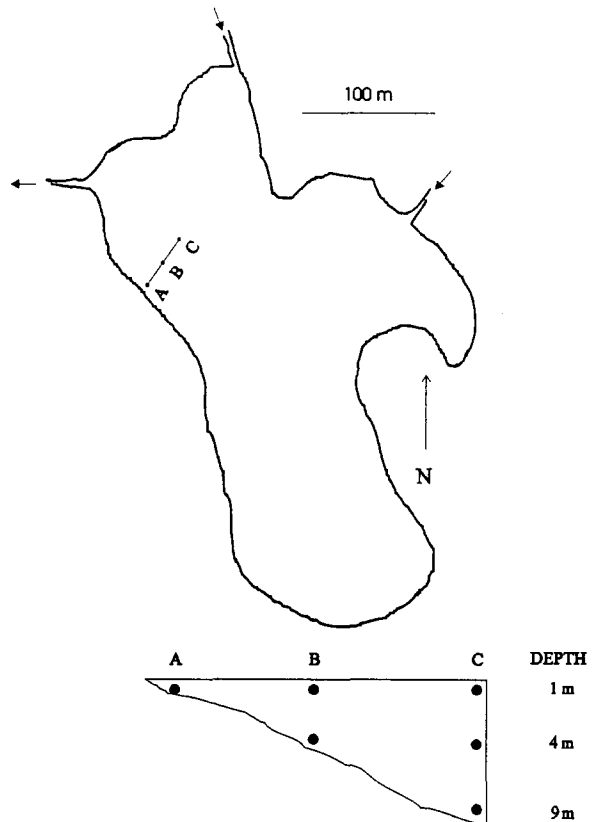


Fig. 1. Map of Myravatn with the sampling stations A, B and C, and a cross-section showing the six locations where the day and night samples were collected.

composed mainly of *Equisetum fluviatile*, *Nymphaea alba*, *Nuphar lutuem*, and *Potamogeton natans*.

The horizontal and vertical distribution of daphnids in Myravatn was analysed by two kinds of samples. Firstly, day and night series of plankton samples were taken at the end of every month from May to September. We collected at three stations at the northern end of the lake (Fig. 1): station A was at the edge of the vegetation zone; station B was 15 m offshore; and station C was 30 m offshore (at the deepest point of the basin). At each station, samples were taken at each of the following depths: 1 m at station A, 1 and 4 m at station B, and 1, 4 and 9 m at station C. Twenty samples were taken at each location. Secondly, cross-sectional samples from station A to C were taken in the middle of the summer to ensure that there was no area where aggregations or swarms were not accounted for in the monthly samples. In the cross-sections, a total of 121 samples were collected in a transect from the shore to the deepest point of the basin; samples were taken

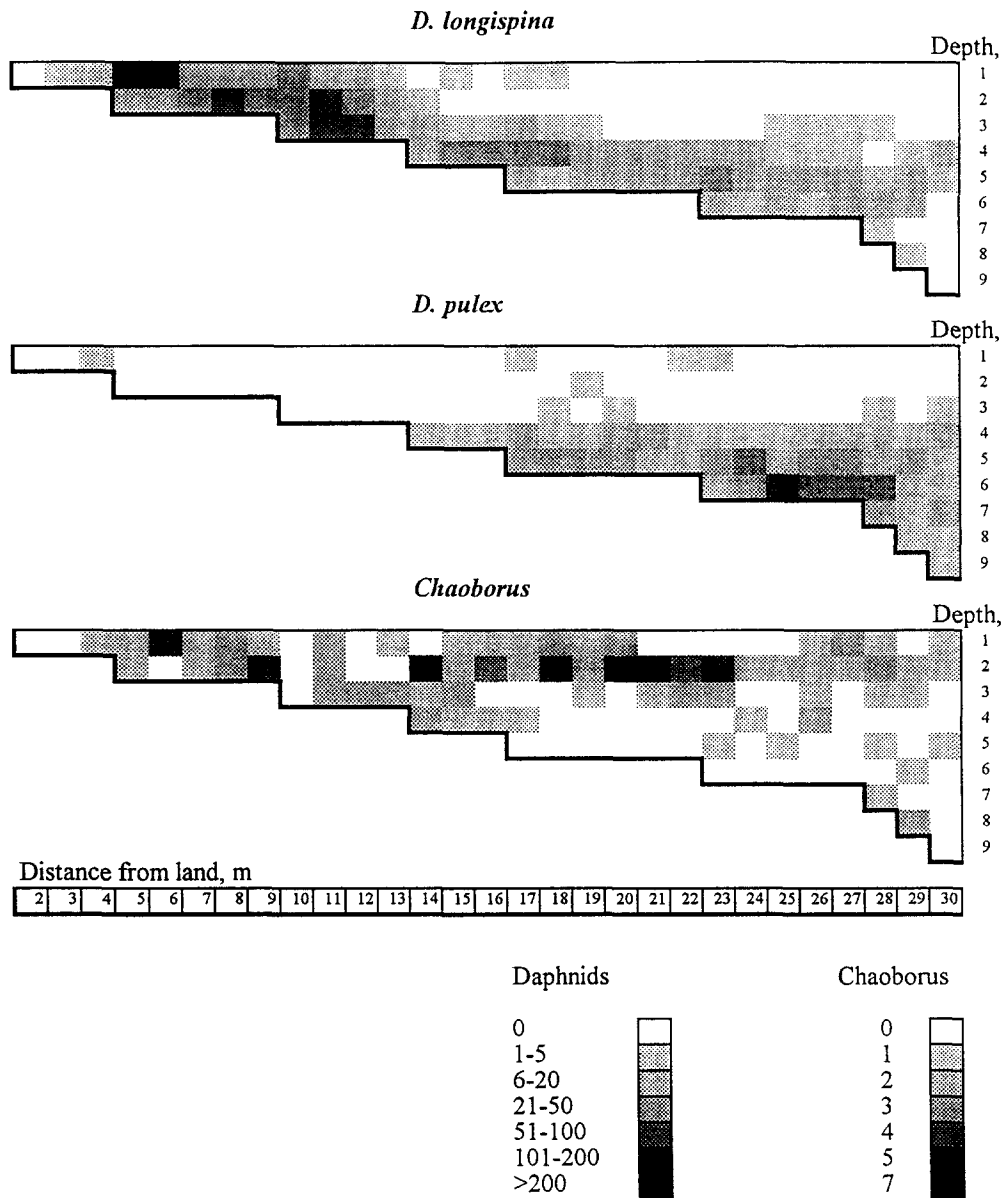


Fig. 2. Depiction of the distribution patterns of *D. longispina*, *D. pulex* and *Chaoborus* in Myravatn on 28 July 1990. The level of shading indicates the number of animals  $l^{-1}$ .

at 1 m intervals, both horizontally and vertically (Fig. 2).

All samples were collected with a 1-liter Ruttner water sampler, filtered through a plankton net, and preserved in 96% ethanol. Because of very clear distribution patterns, the daphnids were only counted in five randomly chosen samples from the total of 20 parallels for each depth/station in the day and night samples, whereas all samples were screened for *Chaoborus*. The Ruttner sampler is not a very good device for quantify-

ing *Chaoborus*, because it will underestimate the total density. Still we decided to include the results, because they indicated a trend in the proportions between the different locations. Because only the large larvae are a threat to the daphnids, only third and fourth instars of *Chaoborus* were counted. The cross-sectional sampling in the middle of the summer was tested by a Kolmogorov-Smirnov two-sample test to determine if the populations of *D. pulex*, *D. longispina*, and *Chaoborus* had divergent distributions. The numbers

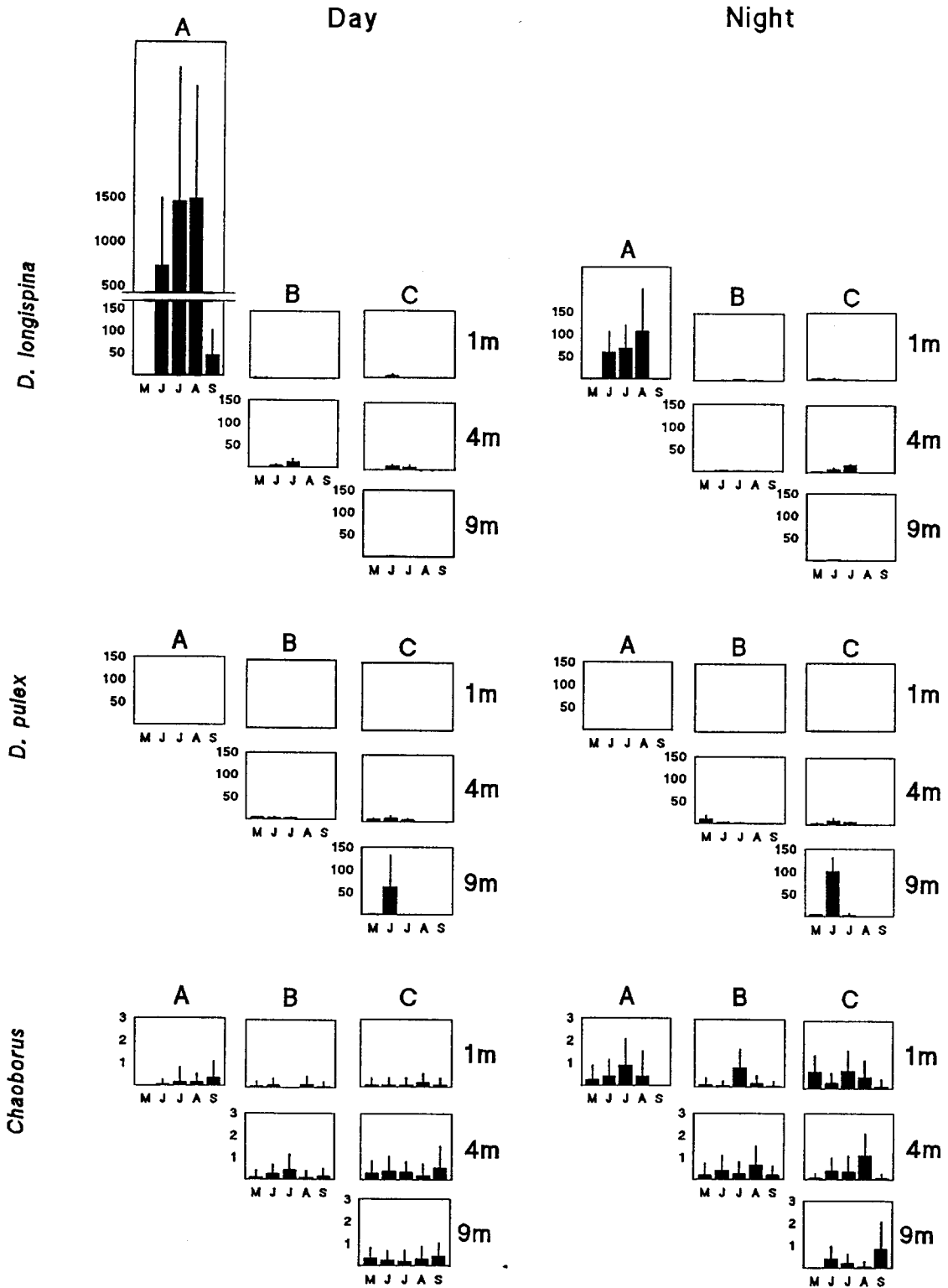


Fig. 3. The distributions of *D. longispina*, *D. pulex* and *Chaoborus* in day and night routine samples at stations A, B and C, at depths 1, 4 and 9 m. Each histogram shows the number of animals  $l^{-1}$  in samples taken at the end of May, June, July, August and September.

of the different species in each sample were tested in pairwise comparisons. Because of clear results (Fig. 3), tests for swarming and segregation between the two *Daphnia* species were not conducted for the monthly samples.

### Experiment 1

To test whether *Chaoborus* had a higher predation success on daphnids in the light compared to darkness, a series of simple laboratory experiments was set up. One fourth instar *Chaoborus* larva was added to 1 litre jars containing 10 new-born *D. longispina* and kept there for four hours. The daphnids were given surplus food (1 mg dry weight of *Scenedesmus cf. acutus*  $l^{-1}$ ), while the chaoborid was starved for 24–48 hours prior to each experiment. Each experiment consisted of two jars, one of the glasses was placed in the light, whereas the other was kept in total darkness. After four hours, the remaining daphnids in each jar were counted. A total of 13 replicates was run. To control for mortality, which was not related to predation, and to ensure that none of the small neonates were able to avoid our detection, jars without *Chaoborus* were also set up.

The basic medium for all the laboratory experiments was 'Standard water', deionized water to which irradiated and filtered seasalt and  $CaCO_3$  were added to a conductivity of about 400 mS  $cm^{-1}$  (Hobæk & Larsson, 1990). All experiments were conducted with *Daphnia*-clones from Myravatn that had been cultured in the laboratory for more than two years. The *Chaoborus* were collected directly from the lake and conditioned for some days prior to the experiments.

### Experiment 2

To test possible behavioural reactions of *D. longispina* and *D. pulex* to chemicals released from *Chaoborus*, experiments were conducted in an experimental set-up described by Kleiven *et al.* (1994). Five flow-through chambers (plastic cell culture flasks 135 × 11 × 4.5 cm) were glued together and interconnected with an opening (1.5 × 3 cm) large enough for adult daphnids to traverse. Ten adult daphnids with eggs were placed in each chamber, and water that had previously contained *Chaoborus* was added in chamber #1. The other chambers received an input of Standard water. In the controls, Standard water was added to all chambers. The flow-through system created a gradient of experimental water from chamber #1 to chamber #5, which was tested by adding coloured water to chamber #1.

The experiments lasted for four hours, and during this time, experimental water from chamber #1 was able to mix gradually with the water in chambers #2 and #3. Small amounts of experimental water were detected in chamber #4, but none of this water was detected in chamber #5.

To avoid microbial degradation of the predator substance (Loose *et al.*, 1993), we used fresh medium. *Chaoborus* were allowed to feed on *D. longispina* and *D. pulex* juveniles. After four hours of feeding, *Chaoborus* were pipetted into a tank with Standard water at a density of 10 *Chaoborus*  $l^{-1}$ . The 'Chaoborus water' was pumped from the tank directly into the chamber. To prevent *Chaoborus* from getting into the chambers, a plankton net was used at the inlet of the peristaltic pump. All experiments were conducted with excess food (1 mg dry weight of *Scenedesmus cf. acutus*  $l^{-1}$ ) and a flow-through rate of 4 ml  $min^{-1}$ . The experiments were conducted both under light and dark conditions. In each replicate, one row of chambers containing *D. longispina* and one row containing *D. pulex* were run simultaneously.

To test whether there was any escape response from the experimental water, a regression analysis between the numbers of daphnids in the five chambers was calculated for each replicate. The mean regression line of all replicates was then tested against a deviation from a mean value of zero (no horizontal displacement) using a t-test.

## Results

### Field

The day and night routine samples revealed that the swarms of *D. longispina* were about ten times denser during the day than during night (Fig. 3). *Chaoborus* performed a weak diel vertical migration, while *D. pulex* was found mainly in the deeper water strata. The dense aggregations of *D. longispina* along the littoral persisted from June until the end of August, and visual observations made it clear that the aggregations were formed as a 1–2 m broad belt of swarms all the way around the lake. The animals were not evenly distributed within this belt, but occurred in local swarms. The maximum number of adult *D. longispina* found in a 1-l sample was 4038 animals, while the maximum number of juveniles was 4392 animals. The juvenile *Daphnia* distribution was similar to the adult *D. longispina* distribution, but was not included in the figures because

Table 1. *Chaoborus* predation efficiency on juvenile *D. longispina*

Exp. #	Light	Dark
	Proportion captured	Proportion captured
1	0.7	0.2
2	0.6	0.2
3	0.6	0.2
4	0.6	0.3
5	0.4	0.4
6	0.7	0.2
8	0.4	0.3
9	0.2	0.2
10	0.4	0.1
11	0.3	0.5
12	0.4	0
13	0.4	0.2

littoral, while *D. pulex* are found at greater depths. *Chaoborus* are more evenly distributed in the water column than would be expected in a lake with planktivorous fish. The Kolmogorov-Smirnov two-sample test revealed that the three populations had divergent distributions ( $p < 0.05$ ). From the cross-sectional samples, it seems that the most important aspects of the distribution patterns were detected in the day and night routine samples.

#### Experiment 1

*Chaoborus* capture efficiency on juvenile *D. longispina* under light and dark conditions are given (Table 1). After four hours, *Chaoborus* demonstrated a significantly higher success rate in detecting and eating juvenile daphnids in the light compared to dark conditions (t-test,  $p < 0.005$ ). In the control jars, all the animals were found, and no mortality was detected.

#### Experiment 2

The experiments, which tested the response of *D. longispina* and *D. pulex* to water that had previously contained *Chaoborus*, revealed that the daphnids were able to distribute themselves horizontally according to chemical cues from *Chaoborus* (Fig. 4). The experiments also indicated a difference in behavioural response between light and dark conditions. In darkness, both *D. pulex* and *D. longispina* demonstrated a significant escape response ( $p < 0.05$ ) from the chamber which received water from *Chaoborus*, i.e. they increased their distance from the input of 'Chaoborus water'. In the light, both *D. longispina* and *D. pulex* responded by aggregating in the middle chambers, but showed no escape response (Fig. 4). The same tendency towards aggregating in the middle was also found in the control experiments.

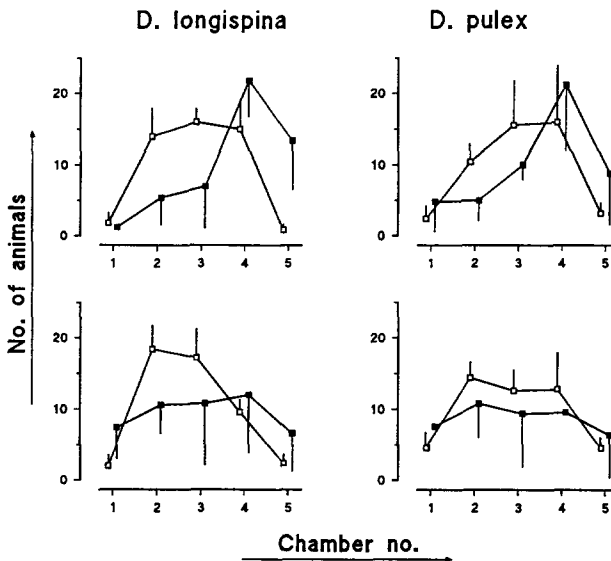


Fig. 4. Horizontal distributions of *D. longispina* and *D. pulex* in experiments conducted under light (open squares) and dark conditions (filled squares). The squares represent the average  $\pm$  (S.D.) number of animals in each chamber. Upper figures are experiments with input of 'Chaoborus-water' into chamber #1, lower figures are control experiments with Standard water input into all chambers.

of difficulties in determining with certainty the species identity for juveniles.

The cross-sectional sampling series from the littoral to the deepest point in the lake (Fig. 2) gives the trends of the distribution patterns in Myravatn. *D. longispina* exhibits dense swarms within and along the

#### Discussion

Swarming along the littoral vegetation in Myravatn is contrary to the horizontal distribution patterns observed in many other lakes. There are several examples in the literature where *Daphnia* avoid the littoral zone. This is commonly referred to as shore-avoidance. Siebeck (1980) suggested that optical orientation towards the land shadow was a mechanism that could lead to different distributions among different species. Pennak (1973) discussed the effects of

repellents from macrophytes on *Daphnia*, and Gliwicz and Rykowska (1992) considered predation from littoral fish, as the ultimate factor responsible for shore-avoidance in *Daphnia*.

The fact that the swarms in Myrvatn are less dense at night (about 1/10 of the density during daytime), indicates that *D. longispina* exhibits a diel horizontal migration similar to the migration observed by Davies (1985) and De Meester *et al.* (1993). Our sampling does not show where the animals go when the swarms disintegrate, but it seems likely that the daphnids spread out into the pelagial, as described in the studies mentioned above. If the dense swarms spread into the much larger volume of water in the pelagial during the night, then one would not be able to detect an increase in numbers anywhere in the pelagial, which would correspond to the reduction in density in the littoral.

The dispersion of swarms at night indicates an avoidance behaviour in response to a visual or day-active predator. The feeding-experiments show that *Chaoborus* eats significantly more daphnids in the light, and this illustrates the importance of visual prey detection. This is in contrast to the results of Swift & Forward (1981), who did not find differences in *Chaoborus* capture efficiency on copepods under light and dark conditions. This contradiction might be attributed to the differences in criteria for measuring *Chaoborus* predation and/or the different modes of swimming in copepods compared to daphnids. If *Chaoborus* is a more efficient predator in the light, it will induce a higher degree of swarming during the day, when compared to the night. *Chaoborus* in Myrvatn exhibit some diel vertical migration (Fig. 3), but to a lesser extent than in lakes with planktivorous fish (Jan Wivegh, pers. comm.). Hence, a larger number of *Chaoborus* is present in the upper layers of the lake during the day than in lakes with planktivorous fish, and the potential swarm-inducing effect from *Chaoborus* on daphnids during the day will be more pronounced than in other lakes.

Gradually, a reduction in food concentration within a swarm will force the animals to disperse. The aggregations of *D. longispina* in Myrvatn are very dense (up to 4000 animals  $l^{-1}$ ), and it is not likely that phytoplankton production is great enough to support continuous swarming. Therefore, the animals have to disperse at certain time intervals. Since phytoplankton production is reduced at night, food is also a factor that will enhance swarming during the day and dispersing during the night.

Both the cross-sectional samples and the day and night routine samples showed that *D. longispina* and *D. pulex* had divergent distributions, and that *D. longispina* exhibited denser swarms than *D. pulex*. These differences in distribution patterns between the two species of *Daphnia* might be caused by different modes of predation defense. Morphological defenses like neckteeth formation are found in both *D. pulex* and *D. longispina* in Myrvatn (Giske, 1986), but they are commonly more pronounced in *D. pulex*. We can not say unequivocally that *D. pulex* use more morphological defenses, while *D. longispina* rely more on behavioural predation avoidance. Still, if the smaller size of juvenile *D. longispina* makes them vulnerable to *Chaoborus* predation for a longer time period than the larger *D. pulex*, and if the larger *D. pulex* has higher food requirements, there would be a difference in the cost/benefit ratio of swarming between the two species. Therefore, swarming behaviour might be favoured in *D. longispina*, while morphological defenses might be favoured in *D. pulex*.

The distribution of daphnids in Myrvatn might also be influenced by other predators, but we did not conduct experiments with predators other than *Chaoborus*. In addition to *Chaoborus*, there are also pike (*Esox lucius*), water mites, Notonectidae and Corixidae in the lake. Pike are not considered planktivores, and zooplankton have not been found in the stomach contents of pike from this lake (Nilsen, 1980). Water mites can be a dominant predator in lakes (Butler & Burns, 1993), and Notonectidae can exhibit high predation rates as well (Murdoch *et al.*, 1984). However, since *Chaoborus* is by far the most abundant invertebrate predator in Myrvatn, we still believe that the distribution patterns of daphnids are influenced primarily by this predator.

Our behavioural experiments revealed differences in escape response between light and dark conditions. In darkness, both species responded with a significant escape response away from the '*Chaoborus* water'. This indicates that the daphnids distribute themselves horizontally according to chemical signals from *Chaoborus*. In the light, however, the daphnids responded by aggregating in the middle chamber even when there were chemical substances that indicated the presence of a predator (Fig. 4). Aggregations in the light and in the presence of surplus food are in accordance with the findings of Jakobsen and Johnsen (1988b). A stronger tendency to swarm in the light compared to darkness might also explain the aggre-

gation in the middle chamber, instead of an escape response when chemical substances are present.

The results of both the field work and the experiments indicate that *Chaoborus* is an important factor in explaining the atypical *Daphnia* distribution patterns found in Myrvatn. The behavioural experiments showed that daphnids are able to respond adequately to chemical substances emitted from their main predator in Myrvatn. Therefore, we suggest strongly that the *Daphnia* distributions observed in Myrvatn are partly mediated by chemical substances from *Chaoborus*.

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