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Vertical migration of *Chaoborus* larvae is induced by the presence of fish

Abstract—Diel changes in the depth distribution of *Chaoborus flavicans* larvae in thermally stratified aquaria with a distinct light gradient revealed that the larvae responded behaviorally to the presence of fish. Both the midday and midnight mean depths of the population were greater in the presence of fish. Most fish-treated larvae found a daily refuge in bottom sediments. It appears that the stimulus for vertical migration is chemical, not visual or mechanical. The fish effect persisted for more than 15 d but was reversible. Those individuals that previously had been exposed to fish factor were considerably more sensitive to light than untreated larvae and showed a panic response when suddenly illuminated.

There is increasing evidence that vertical migration in planktonic animals is an adaptive response to avoid predation by visual predators (Zaret and Suffern 1976; Stich and Lampert 1981; Gliwicz 1986). It appears that at least in some species the range of diel vertical migration is correlated with predation intensity (Gliwicz 1986). Luecke (1986) reported that the appearance of migratory behavior in previously nonmigrating larvae of *Chaoborus flavicans* coincided with cutthroat trout introduction to Lake Lenore, Washington. Two mechanisms could be involved in this behavioral shift. First, in a behaviorally polymorphic population, selective predation may account for mortality of nonmigratory individuals. Second, the presence of a predator may induce behavioral change through visual or chemical cues (Dodson 1988) that are likely similar to those inducing morphological changes (*see* Havel 1987).

The aim of our study was to find out which of these two mechanisms could be responsible for migratory or nonmigratory behavior of *Chaoborus* larvae. We tested in the laboratory whether the animals could respond behaviorally to the presence of fish by changing their distribution pattern and sensitivity to light.

In our experiments we used 4th instar larvae of *C. flavicans*, which are migratory in most lakes of the temperate zone. The larvae were collected from mesoeutrophic Lake Roś (northeastern Poland), where they were exposed to fish. Before we started the experiments, larvae were kept for 2 weeks in lake water at 6°C, in complete darkness. We used three-spined stickleback (*Gasterosteus aculeatus* L.) from a small stream as our predatory fish. The experiment was performed in thermally stratified aquaria with light gradients to simulate the conditions experienced by migratory animals in a stratified lake.

Before fish were introduced the initial vertical distribution of *Chaoborus* was observed for 24 h. The fish were then introduced and subsequently removed after 4 d. The distribution of larvae was followed for 12 d more to determine whether the fish effect was reversible and how long it lasted. To determine whether the stimulus for migration was chemical, we monitored the distribution and sensitivity to light of larvae exposed to fish-treated water after fish removal.

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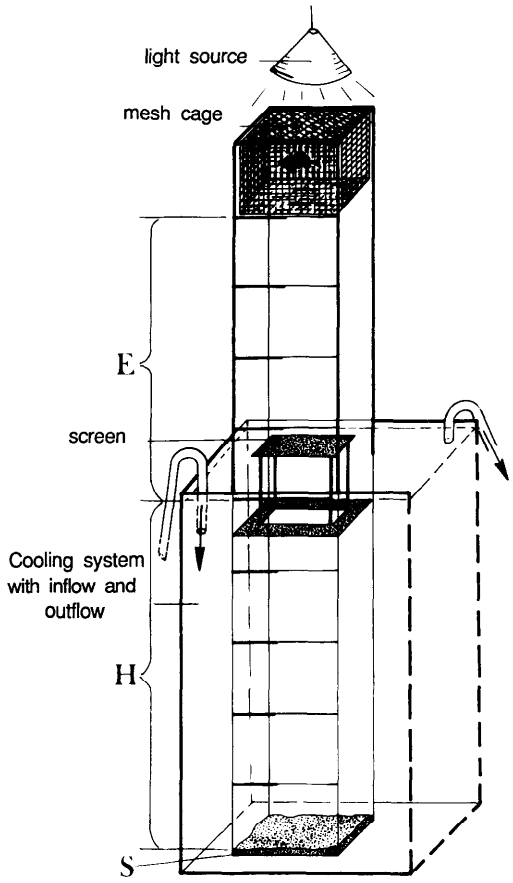

Fig. 1. The experiment setup. Six aquaria were placed in the large one, which served as a cooling system. Single fish were introduced to three aquaria, and the other three remained fishless. Fish and no-fish aquaria alternated in the large aquarium.

The experiment was performed in six aquaria (100 cm high × 15 cm × 10 cm), each containing ~12 liters (Fig. 1). Forty *C. flavicans* larvae were introduced to each of the six aquaria filled with tapwater. Soft-bottom sediment (2 cm thick) from a fish-free habitat was added to provide a refuge for the larvae. The lower parts of the aquaria were cooled (Fig. 1) to produce a gradient in temperature between surface and bottom layers. The "epilimnetic" temperature was maintained at 22°C as compared to 14°C in the "hypolimnion." At the end of the experiment the dissolved oxygen concentration was 9.8 mg O_2 liter^{-1} at the surface and 4.2 in the near-bottom layer.

The experiment was performed in a dark room, but each aquarium was illuminated from the top. A microscope lamp was installed 10 cm above the water surface of each aquarium. In the middle of each aquarium a segmented screen was installed (Fig. 1) to create a light gradient between the surface and deeper waters, but allowing larvae to pass. The light intensity at the surface was 4.4 × 10^{-2} μEinst cm^2 s^{-1} as compared to 4.3 × 10^{-4} close to the bottom. The light cycle was 9 : 15 L/D. The light was switched on and off gradually, via four intermediate intensities, to prevent the animals from being startled. The change from complete darkness to full intensity and the reverse took 30 min. The larvae were counted twice a day: at 0900 hours after 15 h of darkness, and at 1800 hours after 9 h of light. On days 2 and 11 larval distribution was monitored every 4–5 h for 24 h. A repeated-measures ANOVA (Sokal and Rohlf 1969) was used to test the significance of differences in vertical distributions between treatments.

The larvae were counted from the surface to the bottom in each 10-cm interval. As reported by Swift and Forward (1980), *Chaoborus* larvae have relatively low responsiveness to red light; therefore we used a dim red flashlight for night counting and for counting in the darker deep layers. The number of individuals in bottom sediments was calculated as the difference between the number of individuals introduced to the aquaria and the individuals occupying the water column. When the experiment was over, all larvae were counted again; no mortality was detected. The larvae were fed cyclopoid copepods, three prey per predator daily, which were introduced through the plastic tube to the surface water layer of each of the aquaria just before the light was switched off.

In each aquarium a net cage of 100-μm mesh was installed at the surface, and in three of the six cages individual three-spined sticklebacks were introduced. They were fed twice a day with ~200 cyclopoid copepods. We removed the fish in the evening of day 4 of the experiment, when the effect of fish presence on *Chaoborus* distribution was clearly evident. Routine counting of the larvae was continued for 12 d until the first larvae started to pupate.

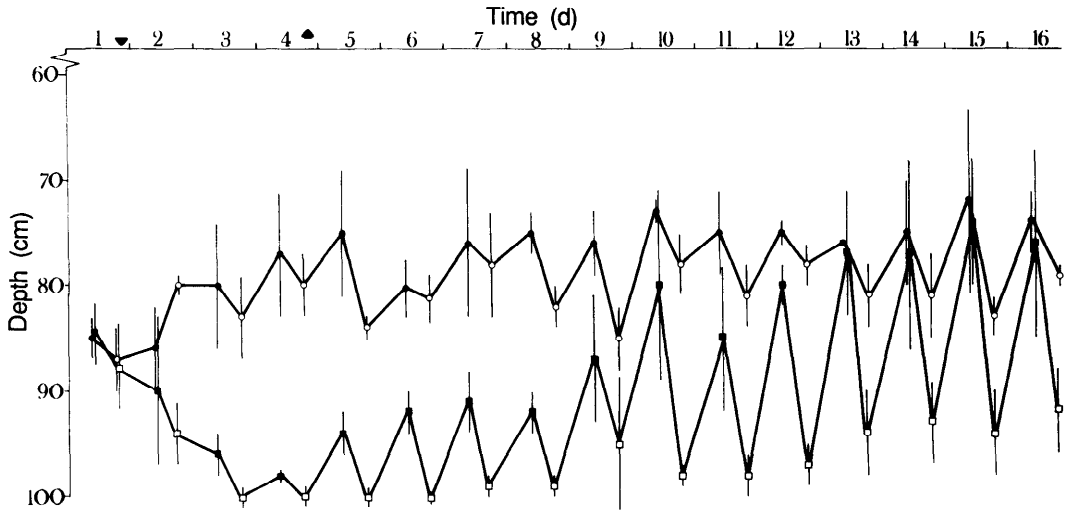

Fig. 2. *Chaoborus* distribution (mean depth ±1 SD) in fish-free (○, ●) and fish-stocked aquaria (□, ■) during the day (○, □) and at night (●, ■) over 15 d. The effect of treatment on population depth is significant at $P < 0.0001$ (df = 1, $F = 419.51$, ANOVA), and the time-by-treatment interaction is significant at $P = 0.0001$ (df = 14, $F = 149.78$, ANOVA). Arrowheads indicate times fish were introduced and removed.

The same experimental design was used to determine whether the effect of fish on *Chaoborus* behavior was produced by visual, mechanical, or chemical stimuli. In three of the six aquaria we held fish for 12 h, which was enough to produce a clear effect on *Chaoborus* distribution (Fig. 2). The larvae were introduced to all six aquaria. The vertical distribution of the larvae was followed until the first effect of fish factor was demonstrated.

During our routine counting we observed differences in *Chaoborus* sensitivity to light between individuals that were or were not exposed to fish. We therefore compared the responses of untreated vs. fish-treated larvae to sudden illumination. Larvae were placed individually, 160 per treatment, into small glass vessels (50-ml capacity), half of them filled with water from untreated and half from fish-treated aquaria. Half of the individuals in each treatment came from the fish-exposed tanks. Low-intensity light was used to illuminate the vessels, each of them individually. Observations were made in a dark room. The number of individuals that showed a panic response (abrupt, randomly directed movements) when illuminated was recorded.

Before fish were introduced, the vertical distribution of *Chaoborus* larvae was similar in all six aquaria. The larvae remained at ~85-cm depth at night and moved deeper during the day (Fig. 2). Less than 50% of each population remained in the sediments and <10% in the epilimnion during both the day and night (Fig. 3).

Twenty hours after fish were introduced, a clear difference between fish-free and fish-treated populations appeared. The fish-treated populations moved about 15 cm deeper, and this difference later increased to ~20 cm d^{-1} (Fig. 2). At the same time the mean depth of the control population decreased ~10 cm, probably resulting from acclimation to experimental conditions. The deeper location of fish-treated larvae persisted after the fish were removed. The difference between the mean depth of fish-free and fish-treated populations during the day remained >10 cm at the end of the experiment. The initial difference of ~15–20 cm between the night position of fish-free and fish-treated populations gradually decreased soon after the fish were removed and reached 2 cm at the end of the experiment.

Fish-free and fish-treated larvae migrated vertically; they descended during the day toward the bottom and ascended during the night toward the surface (Fig. 2). In the fish-free populations the amplitude of migration

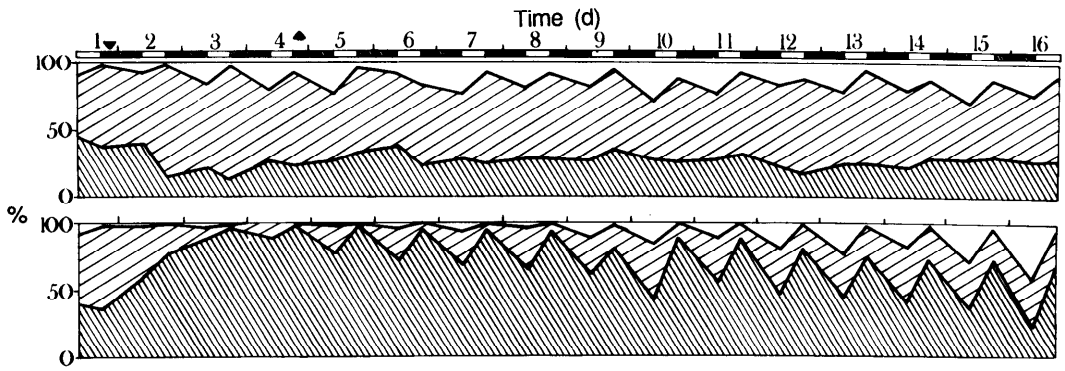

Fig. 3. Percent distribution of *Chaoborus* larvae in the epilimnion (clear), hypolimnion (wide hatching), and bottom sediments (narrow hatching) in fish-free (above) and fish-treated (below) aquaria over 15 d. Arrowheads as in Fig. 2.

remained similar over the 2-week period; the average day depth of the larvae was 81 ± 2 (SD) cm as compared to 77 ± 4 cm at night. The mean day depth of fish-treated larvae increased to 100 cm soon after fish were introduced. More than 90% of the population remained in the bottom sediments during the day. The bottom certainly acted as a barrier to downward migration in this case. Whereas the mean day depth of the fish-treated population decreased very slowly after fish were removed, the night depth decreased steeply to reach the night position of untreated larvae at the end of the experiment.

In the fish-free treatments a nearly constant fraction of the population remained in the bottom sediments during the day and at night, rarely >30% of the total number (Fig. 3). It is not clear, however, whether the same individuals always occupied the bottom strata. The relative abundance of the epi- and the hypolimnetic fractions changed diurnally; during the day more larvae remained in deeper waters than at night. In fish-treated aquaria an increase in the fraction of larvae at the bottom was observed after fish were introduced (Fig. 3). The epilimnetic fraction was never abundant. No larvae were found in the upper 40-cm layer, and 100% of the larvae took daily refuge in the sediments. After fish were removed, more and more individuals appeared in the epilimnion at night, but no more than 4% of the population stayed there during the day. After fish removal, regular diel displacements were observed among all three layers, with a repeated pattern of leaving the sediments and ascending toward the surface at night, then descending toward the bottom and entering the sediments during the day.

A clear tendency of the epilimnetic fraction to increase and the bottom fraction to decrease was observed after fish were removed, but the initial day state was not reached until the end of the experiment. The night distribution after 1 week was the same as it was at the beginning of the experiment.

When *Chaoborus* larvae were introduced to aquaria in which fish had been kept for 12 h and then removed, the effect was the same (Table 1). Twenty-four hours after the larvae were introduced, the mean day depth of the population exposed to the fish factor was 15 cm lower than that of untreated larvae (the difference being significant at $P = 0.001$, Mann-Whitney U-test). Almost 60% of the population was in the bottom sediments during the day compared to 10% for the untreated larvae. These results clearly show that the stimulus from fish is chemical.

The number of panic responses to sudden illumination was higher in fish-treated than in untreated larvae (Table 2). Only the difference between the frequency of responses of fish-treated larvae in fish-treated water and untreated larvae in untreated water (65.0% as opposed to 37.5%) was statistically significant ($P = 0.001$, χ^2-test, 1 df), however.

Table 1. The mean daytime depth and the percent distribution of *Chaoborus* larvae after 24 h of exposure in untreated and fish-treated water (E—epilimnion; H—hypolimnion; S—bottom sediments).

	Untreated water*			Fish-treated water†		
	E	H	S	E	H	S
Larvae (%)	5	85	10	0	41	59

* Mean depth (cm ±1 SD), 81±13.
† Mean depth (cm ±1 SD), 90±5.

The pattern of timing of diel migration was similar in fish-free and fish-treated larvae during both 24-h cycles analyzed (Fig. 4). The fish-treated population remained deeper during the whole 24-h period, at least before fish removal. This migration pattern occurred deeper in these aquaria than with control larvae. Ten days after fish removal the timing of migration remained similar, but the night position of both populations was already almost identical and the day position was closer to the surface than during the first diel cycle.

Upward migration tended to occur after the light was already switched off, and the larvae began to migrate downward long before it was switched on (Fig. 4). It appears that downward movements are governed by an internal clock, whereas upward displacements are adjusted to the present light regime.

Before fish removal, a small and almost constant fraction of the control population remained in the bottom sediments during the whole 24-h period, and the migration occurred between the epi- and the hypolimnion (Fig. 5). On the contrary, the fish-treated population remained almost entirely in the bottom sediments during the day and appeared in the epilimnion in very low numbers only under complete darkness. After fish removal, however, the buried fraction decreased and the typical migration occurred among all three layers. Untreated larvae showed a similar pattern of migration.

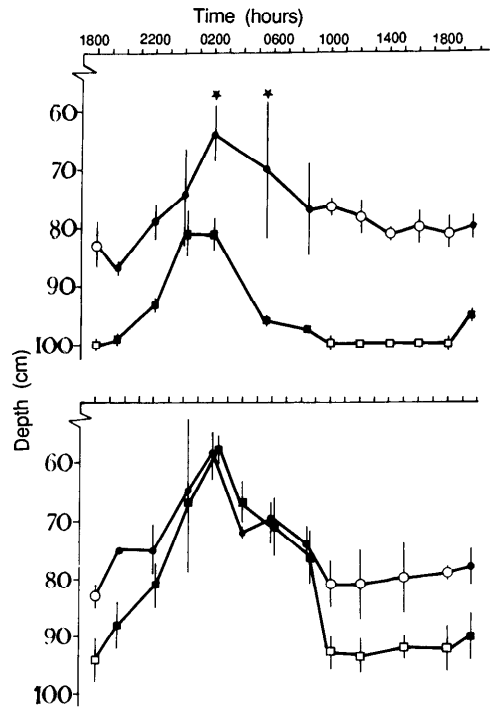

Fig. 4. Diel changes in *Chaoborus* distribution (mean depth ±1 SD) in fish-free (○, ●) and fish-treated (□, ■) aquaria, during the day (○, □) and at night (●, ■) 2 d after fish were introduced (above) and 11 d after they were removed (below). In both cases the effect of treatment ($P > 0.0001$, df = 1, $F = 93.10$, ANOVA) and the time of day ($P < 0.0001$, df = 11, $F = 6.50$, ANOVA) on the mean depth of populations is highly significant.

Table 2. Light sensitivity of untreated and fish-treated larvae, measured as the number of panic responses per 80 individuals, after sudden illumination.

	Untreated larvae	Fish-treated larvae
Untreated water	30	36
Fish-treated water	33	52

Chaoborus flavicans larvae showed two clear behavioral responses to the presence of fish. The first was avoidance of visual predation by descending toward the bottom for the day before laboratory "sunrise." The second was increased sensitivity to light, manifested by a panic response to sudden illumination.

It appears that the stimulus released by fish is a water-soluble compound. Although reversible, its effect lasted for at least 15 d. Assuming a linear decline in the fish-factor effect, we would expect its persistence for ~2 weeks longer. Thus, the predator effect was much more durable than that recently described by Dodson (1988). Two possibilities might account for this persistence. The

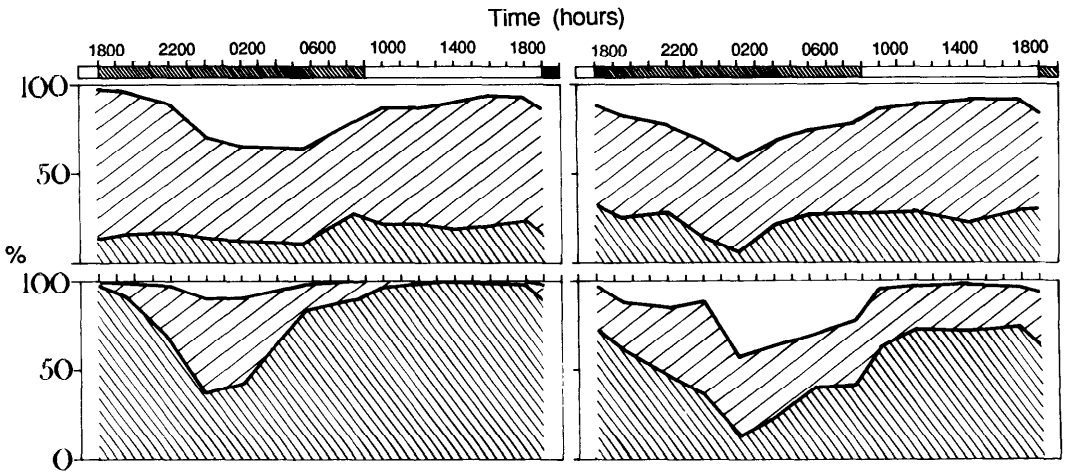

Fig. 5. Diel changes in percent distribution of *Chaoborus* larvae in the epilimnion (clear), hypolimnion (wide hatching), and bottom sediments (narrow hatching) in fish-free (above) and fish-stocked (below) aquaria 2 d after fish were introduced (left) and 11 d after they were removed (right).

fish-factor concentration in our experiment was probably unusually high. The fish density was 1 individual per 12 liters, which is ~100 times more than in Lake Roś (Jachner 1988), the lake from which the experimental larvae were collected. Another reason for the persistence of the fish effect is also possible: a *Chaoborus* memory effect could underlie the persistence of the behavioral response after fish removal.

Our results suggest that the behavior of vertical migration may appear as an immediate response to fish. Luecke (1986) attributed the shift from nonmigratory to migratory behavior in *Chaoborus* to a genetic change in the population, evoked by fish predation; an isolated fraction of this population continued to migrate for 2 months after contact with fish. Although dismissed by Luecke, it might have represented a long-lasting effect caused by a chemical factor released by the fish. Our results indicated that *Chaoborus* larvae exhibited continuous propensity to enter the sediments during the day 2 weeks after the fish were removed.

The mediation by predation of a plastic behavioral reaction to light intensity seems to be advantageous under unpredictable predation regimes. *Chaoborus* larvae originate from eggs deposited randomly in lakes of varying predation pressure by parents coming from habitats with severe or weak predation pressure. Behavioral plasticity may also be advantageous under seasonally changing predation pressure (Dodson 1989). Both fish population densities and fish feeding activity are seasonally variable in the temperate zone (*see* Gliwicz and Pijanowska 1989). Under severe predation pressure, the risk of being eaten is reduced in migratory individuals, possibly counterbalancing the fitness disadvantages of leaving warm, food-rich, surface waters (Stich and Lampert 1984). Under weak predation pressure it is better not to pay the costs of vertical displacement and to stay at those depths that offer better food conditions (Gabriel and Thomas 1988; Lampert 1989).

Our data support the hypothesis that the amplitude of diel vertical migration is related to the intensity of fish predation or to the concentration of fish factor. The larvae we used originated from a lake containing dense fish populations. The larvae showed a readiness to migrate vertically, which continued for 2 weeks in fish-free habitats but was amplified when the larvae were exposed to fish factor. The amplitudes of diel vertical migrations of *Cyclops abyssorum tatricus* in Tatra lakes were attributed by Gliwicz (1986) to the history of artificially introduced predation, i.e. to the duration of selective pressure against the nonmigratory genotypes. An alternative explanation is that *Cyclops* migration is related behaviorally to fish population densities, i.e. to the concen-

tration of fish factor. Our results indicate that the presence of predators, often considered an ultimate cause of vertical migration, may simultaneously serve as a proximal cue for *Chaoborus* larvae, or at least that it may increase sensitivity to light cues.

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Microdistribution and diel vertical migration of flagellated vs. gas-vacuolate purple sulfur bacteria in a stratified water body

Abstract—The diel vertical distribution of purple sulfur bacteria was followed in Lake Cisó. A syringe system provided simultaneous samples every 3 cm. One of the bacteria, *Chromatium minus*, was flagellated and showed vertical migration of ~35 cm. This daily excursion was enough to maintain itself in the uppermost anaerobic part of the metalimnion. The swimming speeds necessary to explain such migration were within the range of values previously recorded for these bacteria in the field. The other bacterium, *Amoebobacter* M3, had gas vesicles but did not seem to move vertically. Thus, vertical migration by a planktonic bacterium was demonstrated in nature.

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