Swimming behaviour of *Daphnia* clones: differentiation through predator infochemicals

**ANKE WEBER**1,3,* AND **ARIE VAN NOORDWIJK**2

1NETHERLANDS INSTITUTE FOR ECOLOGY, CENTRE FOR LIMNOLOGY (NIOO-CL), PO BOX 1299, 3600 BG MAARSSEN, THE NETHERLANDS
2NETHERLANDS INSTITUTE FOR ECOLOGY, CENTRE FOR TERRESTRIAL ECOLOGY (NIOO-CTO), PO BOX 40, 6866 ZG HETEREN, THE NETHERLANDS
3PRESENT ADDRESS: TNO ENVIRONMENT, ENERGY AND PROCESS INNOVATION, PO BOX 57, 1780-AR DEN HELDER, THE NETHERLANDS

*CORRESPONDING AUTHOR: a.weber@mep.tno.nl

We studied variation in small-scale swimming behavior (SSB) in four clones of *Daphnia galeata* (water flea) in response to predator infochemicals. The aim of this study was 3-fold. First, we tested for differences in SSB in *Daphnia*; second, we examined the potential of differences in SSB to explain survival probability under predation; and third, we tested the effect of differences in SSB on survival under predation. Four treatments were applied: one kairomone-free control, one Chaoborus- (phantom midge) and one *Perca* (perch)-conditioned treatment, and a mixed treatment containing both infochemicals. All of the three tested behavioral parameters (swimming speed, trajectory length and vertical distribution) were affected by the presence of the Chaoborus infochemical, and swimming speed and vertical distribution were also affected by the presence of *Perca* infochemical. The effect of the treatment was interfered with by a clone effect: genetic differences were pronounced in all traits. These results illustrate that clones can be responsive in only a subset of traits. The general theory that clones are either responsive or non-responsive is not valid for SSB. The outcome of the predation trial confirms that a decrease in activity is a main factor in lowering *Daphnia* vulnerability to Chaoborus predation.

**INTRODUCTION**

Various characteristics of population and community dynamics of freshwater zooplankton have been studied in the past, in attempts to increase our knowledge of how freshwater ecosystems function. A major leap forward in disentangling ecosystem processes was the discovery of communication based on chemical cues (infochemicals) in the early 1980s. Infochemicals were found to affect many aspects of zooplankton ecology, such as life history, morphology, physiology and behavior [see reviews by (Harvell, 1990; Larsson and Dodson, 1993; Tollrian and Dodson, 1998; Boersma et al., 1999)].

Swimming behavior is an important factor in the predator–prey relationship (Gerritsen, 1980; Pastorok, 1980, 1981; Kinsen et al., 1984) because it affects the probability and the frequency with which prey and potential predators encounter each other. Encounter is the central process in governing direct and indirect interactions between prey and predators. The mechanism behind behavioral strategies of both prey and predator becomes clear with the help of theoretical models. According to the empirical model of Gerritsen, the encounter rate of a predator with its prey is a function of prey density and the mean speed of both predator and prey (Gerritsen, 1980). The encounter rate depends on the encounter radius, density of prey, and swimming speeds of both predators and prey. The model predicts two optimal tactics for efficient predators: the ambush tactic (often found in invertebrates) and cruising (found in planktivorous fish). For both tactics, swimming speed is a key trait in determining vulnerability of prey to predators. Either an increase in predator speed or an increase in prey speed, or both, can increase encounter rate. The greater of the two speeds has the strongest effect. This is important for slow animals, because a further decrease in speed of a slow-moving prey would result in a negligible decrease of its encounter probability with a fast-moving predator. The same holds for the reversed situation with a slow-moving predator and fast-moving prey.
Depending on the nature of predation (visual or tactile, size preferences), different behavioral strategies have evolved in prey organisms to minimize encounter rates. For example, the ambush predator Chaoborus (phantom midge) assumes the ‘sit-and-wait-strategy’ and detects disturbances in the water caused by prey with its mechanoreceptors. Attacks are restricted to the ‘strike volume’, the area in which prey can be attacked. The strike volume differs between prey species (Riessen et al., 1984; Mort, 1986). The strike volume of Chaoborus is a cylinder of ~6–8 mm in diameter along its longitudinal axes (Riessen et al., 1984). In the case of Chaoborus, the encounter rate is simply a linear function of prey velocity (Dodson, 1996). Chaoborus can exhibit nocturnal diel vertical migration (DVM), remaining in or near the bottom during the day when co-existing with fish (Berdonoky and O’Brien, 1993), but in the absence of fish, Chaoborus is usually randomly distributed. The best behavioral strategy for prey to avoid this tactile predator is therefore (i) to minimize swimming speed in order to minimize encounter probability (Dodson, 1996) or (ii) to minimize the time of being in the strike volume of the predator. The latter can be achieved by increasing the trajectory length traveled in one direction (Riessen et al., 1984).

Visual predators, such as fish, select for large prey individuals. At least four behavioral strategies have been identified in planktonic prey to avoid visual predation: alertness, swarming, reduced conspicuousness and DVM (Kerfoot, 1977, 1978). DVM reduces prey vulnerability because small fish depend on light to locate their prey. A natural refuge is, therefore, provided for by darker parts of the habitat where contrasts are reduced (Van Goor and Ringelberg, 1995; De Meester and Weider, 1999). An increased alertness triggered by the presence of fish infochemicals (De Meester and Pijanowska, 1996; Van Goor and Ringelberg, 1997) has been shown to decrease predation success on Daphnia. By forming swarms, the distraction of the predator by conspecifics increases the survival chance of the individual within the group (‘dilution effect’; Milinski, 1977). A reduction of conspicuousness can be achieved by prey behavior that does not conform to the selection pattern (Pastorok, 1980, 1981) that governs predation behavior in fish (Brewer and Coughlin, 1996). For example, horizontal movements are better perceived than vertical ones (Ingle, 1968). Animals moving in a vertical direction will, therefore, bear a lower predation risk, and slower movements decrease conspicuousness since fish are better able to locate fast-moving items than slow-moving ones (Brewer and Coughlin, 1996).

The encounter rate depends mainly on prey velocity, since the predator is generally the faster moving party. As a consequence, if both predator and prey are cruising, low prey velocities will result in a constant encounter rate. If prey velocities are relatively high, the encounter rate will depend mainly on, and linearly increase with, prey velocity (Dodson, 1996). Consequently, the best strategy adopted by prey to minimize the encounter rate and, thus, avoid fish predation, is expected to be a decrease in swimming speed and a modification of the trajectory lengths moved in the same direction in order to deviate from the prey behavior selection pattern of the fish (Macchiusi and Baker, 1993).

In the past, most attention in the study of zooplankton behavior has been paid to migration [e.g. (De Meester, 1989, 1993; Van Goor and Ringelberg, 1995)]. Migration behavior is highly variable and is strongly dependent on species, sex and season [e.g. (Cushing, 1951)]. DVM can be triggered by the presence of fish (Van Goor and Ringelberg, 1995) or Chaoborus kairomone (Leibold, 1990), which alter behavioral responses to light, thereby triggering migration (Van Goor and Ringelberg, 1995). DVM is generally studied on a scale of meters to tens of meters. However, the understanding of small-scale swimming behavior (SSB) has received relatively little attention (Jacobs, 1961; Kerfoot, 1978; Dodson et al., 1995; O’Keefe et al., 1998). Movements on a scale of millimeters to centimeters can, however, affect visibility (Buskey, 1992) and the energy budget of an individual (Stich and Lampert, 2000). Therefore, SSB is essential in determining predator–prey relationships and for our complete comprehension of ecosystem functioning.

The lack of research into the role of SSB in predator–prey interactions stems from an inability to monitor prey swimming behavior in predation trials prior to consumption by the predator. However, this problem can presently be overcome by the indirect simulation of predator presence via exposure of prey to predator infochemicals. Moreover, recent advances in the technology of video imaging make it possible to collect digitized video records of free-swimming zooplankton. Recent advances in the study of SSB have identified a typical swimming pattern of zooplankton that consists of a series of small-scale behaviors (Dodson et al., 1995). Additionally, sex-specific mating behavior was reported [e.g. Cyclops (Gerritsen, 1980) and Daphnia (Brewer, 1998)]. The above illustrates that phenotypic plasticity in SSB in response to infochemicals might offer great advantages to an organism that experiences fluctuating predation regimes, e.g. during its life cycle or during the seasons. In spite of the increasing number of studies addressing questions on SSB in various planktonic animals (Brewer, 1998; O’Keefe et al., 1998), our knowledge is still not sufficient to understand the significance of modifications in prey swimming behavior in response to predators.
were fed
and occurring infochemicals. The predators (Perca fluviatilis equal in size and with a total length of 7–8 cm) were kept in two separate aquaria, each of them containing 10 l of water. In one of the aquaria, two perch equal in size and with a total length of 7–8 cm were fed fresh oligochaete worms (Tubifex) daily. Two hundred fourth instar Chaoborus larvae were fed Daphnia (~5 individuals (ind.) 1 l–1) daily in the other aquarium. From each predator aquarium, 7 l were taken each day and replaced by sand-filtered water. Four treatments were prepared: (i) a control, solely consisting of sand-filtered water, without any predator infochemical; (ii) a Chaoborus treatment, a 1:1 mixture of control water and water inhabited by Chaoborus (0.2 ind. l–1) prior to the trial; (iii) a mixed treatment, a 1:1 mixture of water inhabited by Chaoborus (0.5 ind. l–1) and water inhabited by Perca (0.2 ind. l–1) prior to the trial; (iv) a Perca treatment, a 1:1 mixture of control water with water inhabited by Perca prior to the trial. Water for each treatment was filtered through 0.45 µm membrane filters prior to use, to eliminate bacteria and feces from the predators. The green alga Scenedesmus acutus was added as food source in a concentration of 1 mg C l–1, which is well above incipient limiting level (Lampert, 1977). The algae were cultured at high nutrient levels in a continuous culture with a growth rate of 1.3 day–1. The algal concentration was established photometrically. The treatments were refreshed daily to keep food and infochemical concentrations sufficiently high (Weber, 2001a, 2001b). The photoperiod consisted of a 16 h light:8 h dark cycle. Ambient temperatures were kept constant at 17.5°C. For further details, see Weber and Declerck (Weber and Declerck, 1997).

Experimental design

The four selected D. galeata clones were cultured either in infochemical-free water (control); or in water conditioned with Chaoborus infochemical, Perca infochemical or a mixture of both. In total, the experimental set-up consisted of 160 individual cultures for four clones under four treatments, with 10 replicates per clone and per treatment. In order to minimize interference from maternal effects (Lampert, 1993), the mothers of the experimental animals were raised under experimental conditions. Newborns (neonates) originating from the third brood of the mother were raised as experimental animals in 100 ml tubes. When the experimental daphnids had reached their second adult instar, they were used for the swimming analysis. For additional details, see Weber and Declerck (Weber and Declerck, 1997).

Swimming experiment

Video recording

A glass beaker (diameter 12 cm) containing 500 ml of water from one of the four treatments was surrounded with black cardboard from two adjacent sides and from below. On the remaining two sides, two video cameras (handycam) were placed orthogonally at a distance of 30 cm from the beaker. This set-up allowed the three-dimensional recording of an individual swimming in the beaker at all times. A light source was installed directly above the beaker (fluorescent bulb, light intensity: 10.32 µmol m–2 s–1 ± 1.38 SE). Daphnia individuals were allowed to acclimatize to the new environment for 10

Brewer, 1998; O’Keefe et al., 1998). There are in fact several studies on Daphnia SSB in which the behavior was unexpected, given what is known to date about Daphnia ecology. Based on standard encounter rate models, Dods et al. predicted that Daphnia swimming velocity would decrease in the presence of Chaoborus, since slower swimming velocity increases survival probability (Dods et al., 1995). However, this theory was not confirmed, as the velocity of one Daphnia pulex clone remained unchanged in the presence of Chaoborus (Dods et al., 1995). This lack of response is unexpected, but might point to a benefit of swimming that balances increased predation risk (Dodson and O’Keefe, 1998). There are in fact unexpected, given what is known to date about D. pulex swimming velocity increases survival probability (Dods et al., 1995). However, this theory was not confirmed, as the velocity of one Daphnia pulex clone remained unchanged in the presence of Chaoborus (Dods et al., 1995). This lack of response is unexpected, but might point to a benefit of swimming that balances increased predation risk (Dodson and O’Keefe, 1998).
min before the recordings were started. From each experimental *Daphnia*, recordings of 4 min were made. The sequence of clones and treatments was fully randomized. The experimental beaker was thoroughly rinsed and filled with fresh treatment water before each session. The recordings were made between 09:00 and 17:00 h to exclude diurnal effects. In total, 4 days were needed to tape the 10 replicates for each of the four clones in each of the four treatments. Each *Daphnia* was only used once.

**Data analysis**

The video recordings were randomly analyzed with a video system [Mitsubishi HS-E82 (X)] connected to a computer as described in Van Goor and Ringelberg (Van Goor and Ringelberg, 1997). The swimming *Daphnia* individuals were traced on a video screen, and coordinates on an X-Y scale were recorded every 2 s and at every change in movement direction. To check for the error between different measurement runs, the same videotape section was analyzed twice. No significant differences between the different runs were found (correlation coefficient 0.98). We defined trajectory length as the distance covered in one direction without a change of bearing. Based on the X-Y coordinates, two-dimensional trajectory length was calculated per individual *Daphnia* swimming track. For each pair of two-dimensional trajectory lengths, one real trajectory length (three-dimensional trajectory length was converted into a trajectory length index [modified after De Meester and Gool and Ringelberg (Van Goor and Ringelberg, 1997)] for the vertical distribution: \( \frac{T - B}{T + H + B} \), where \( T \) is the total sum of recordings in the top 3 cm, \( B \) is the total sum of recordings in the bottom 3 cm and \( H \) is the total sum of recordings in the middle 6 cm of the experimental vessel. A positive value of the index indicated that the *Daphnia* individual was more frequently found in the top 3 cm than in the bottom layer of the experimental vessel.

Prior to statistical analysis, all data were tested for normality with the Wík–Shapiro procedure after checking for equality of variance with Levene’s test. The effects of the independent fixed variables *Chaoborus*, *Perca* and ‘clone’ were tested by a three-way analysis of variance (ANOVA). Significant clone effects or interaction effects between treatments were further explored with the Tukey HSD for unequal sample size (Sokal and Rohlf, 1981). When significant clone \( \times \) treatment interactions were found, two-way ANOVAs were performed in order to determine which clones differed in their response to the treatments.

**Predation trial**

The trial always consisted of four second instar neonates (two from clone 26 and two from clone 27) originating from mothers who were adapted to different infochemical treatments (control, *Chaoborus*, *Perca*). By using two clones that could be distinguished by means of allozyme electrophoresis (Hoback *et al.*, 1998; Giesler, 1997), the survivors of each trial were identified a posteriori. This set-up, therefore, allows effectiveness to be distinguished among the clone-specific phenotypes. Neonates from the third broods of the clones were used for the predation trials. They were transferred into fresh water until they had reached their second juvenile instar. Subsequently, body length (from the top of the eye to the base of the tail spine), helmet length (top of the eye to tip of the head), spine length (base to tip of the tail spine) and carapace width (perpendicular to body axis at the widest distance) were measured before daphnids were grouped according to similar sizes. This resulted in six combinations of clone-specific phenotypes. Trials were started by introducing four individuals (two per clone) into a Petri dish (10 cm in diameter, filled with 1 cm of treatment water). They were allowed to adapt to the conditions for 10 min before one pre-starved, fourth instar *C. americanus* larva was introduced. The phantom midge larvae had been starved for 1 day prior to the experiment. This ensured that they were hungry enough to actively prey, but not too starved to become relatively unselective. The trials were carried out at ambient light conditions (light intensity 10.32 µmol m\(^{-2}\) s\(^{-1}\) ± 1.38 SD) at 17.3°C and ended when two of the four daphnids had been eaten by...
the predator. In five out of all trials (namely, 26P: 27P (1×), 26P: 27Ch (1×), 26c: 27Ch (1×)), only one Daphnia could be recovered. In three out of all trials (26c: 27c (2×), 26P: 27c (1×)), the experiment was stopped after 5 h with three survivors. In seven trials (26c: 27c (7×)), only one of the survivors could be reliably identified as one of the two clones by electrophoresis. These trials, leaving one or three survivors or one survivor unidentified, were excluded from the analysis, which resulted in the exclusion of the combination clone 26 control adapted–clone 27 control adapted daphnids. A total of 20 predation trials spread over five clone-specific phenotype combinations, replicating each combination four times, resulted in five trials with one survivor unidentifiable, thus reducing the number of survivors. Based on the above predation trials, an index representing the vulnerability to Chaoborus predation (vulnerability index) was calculated. The index was derived from the average number of survivors found for each clone-specific phenotype in the different combinations. The two clone-specific phenotypes that showed the same survival (clone-specific phenotypes 26F and 27c) were characterized by the vulnerability index of 1 indicating their equalness. Based on these values, the vulnerability indices for all other clone-specific phenotypes could be calculated. For example, in the trial 26F–27F, on average two individuals of 26F and three individuals of 27F survived. This means that the vulnerability of 26F is higher (fewer survivors) as compared with 27F. If the vulnerability for 26F is 1, then that for 27F is smaller, namely 2/3 of 26F, which results in a vulnerability index of 0.66 for this clone-specific phenotype.

**RESULTS**

**Trajectory length**

Clones responded differently to the presence of *Chaoborus* infochemical (clone × *Chaoborus* interaction; Table 1a, Figure 1a). In contrast to the other clones, clone 25 responded to the cue by decreasing the proportion of long trajectories (<4 mm) (Figure 1b). Moreover, genotype-dependent reaction to the simultaneous exposure to both infochemicals was observed. This interaction was not additive (three-way interaction; Table 1a and b): trajectories of clone 27 were shorter in the *Chaoborus* and mixed treatments than in the control (*P* < 0.05), but were longer in the *Perca* treatment. Long distances (>17 mm) were observed significantly more in the *Perca* and mix treatments than in the control (*P* < 0.05; clones 17 and 26) or in the *Chaoborus* and mix treatments (*P* < 0.05; clone 27). The frequency of short trajectories (<3 mm) generally differed little between the control and the infochemical-containing treatments (Figure 1b).

**Swimming speed**

The swimming speed varied significantly between clones (Table 1; Figure 2). A post hoc test (Tukey HSD) indicates that clone 17 generally swam faster than clone 25 (*P* < 0.05). Moreover, a significant clone-specific response to the presence of *Chaoborus* infochemical was found (*Chaoborus* × clone interaction; Table 1a and b). The swimming speed of clone 25 was reduced in the presence of *Perca* infochemical.

---

**Table 1a: Results of three-way ANOVAs for the SSB response of *D. galeata* to predator infochemicals are shown**

<table>
<thead>
<tr>
<th>(1) Trajectory length</th>
<th>(2) Swimming speed</th>
<th>(3) Vertical distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d.f.</td>
<td>MS</td>
</tr>
<tr>
<td></td>
<td>error</td>
<td></td>
</tr>
<tr>
<td>cl</td>
<td>3</td>
<td>134</td>
</tr>
<tr>
<td>Ch</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>P</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>cl × Ch</td>
<td>3</td>
<td>134</td>
</tr>
<tr>
<td>cl × P</td>
<td>3</td>
<td>134</td>
</tr>
<tr>
<td>Ch × P</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>cl × Ch × P</td>
<td>3</td>
<td>134</td>
</tr>
</tbody>
</table>

Measured traits were (1) trajectory length, (2) swimming speed and (3) vertical distribution. Independent factors were ‘clone’ (cl), presence of *Chaoborus* (Ch) and *Perca* (P) infochemical.
of Chaoborus, in contrast to any other clone in any treatment (Tukey HSD: \( P < 0.05 \); Table Ia and b). ANOVAs performed on data of the separate clones show that clone 25 swam slower in the presence of Chaoborus (Tukey HSD: \( P < 0.01 \)) and faster in the presence of Perca (Table Ib). Also, clone 26 swam faster in the presence of the Perca cue (Tukey HSD: \( P < 0.01 \)). Clone 27, however, swam faster in the presence of Chaoborus infochemical (Tukey HSD: \( P < 0.01 \)). The swimming speed of clone 26 is lowest in the Chaoborus treatment compared with the other treatments (Chaoborus × Perca interaction, Tukey HSD: \( P < 0.05 \)). Clone 17 does not respond to the presence of either infochemical (Tukey HSD: not significant).

### Vertical distribution

Results for vertical distribution were the same for both cameras (\( P = 0.720 \)). Therefore, the results of only one camera (right) will be discussed.

The vertical distribution in the experimental vessel was strongly clone dependent (Table Ia; Figure 3). Clone 26 remained nearer the bottom than clones 25 and 27 (Tukey HSD: \( P < 0.01 \)). Moreover, a significant clone × Chaoborus effect (Table Ia) identifies clone-specific responses to Chaoborus infochemical. ANOVAs performed on the data of each separate clone revealed that clone 27 responded with a significant preference for the upper water layer to the presence of Chaoborus (Tukey HSD: \( P < 0.05 \)), while the opposite was found for clone 25 (Table Ib).

---

**Table Ib:** Overview of significant differences per trait as identified by a post hoc test (Tukey HSD for unequal sample sizes)

<table>
<thead>
<tr>
<th>Trait</th>
<th>ANOVAs per trait</th>
<th>Treatment differences</th>
<th>ANOVAs (Ch/H11003 P) per clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Trajectory length</td>
<td>Ch &gt; clone</td>
<td>Ch × P &lt; clone</td>
<td>Ch × P &lt; 0.0314* Chaoborus, mix more short trajectories than Perca, control</td>
</tr>
<tr>
<td>(2) Swimming speed</td>
<td>clone 17&gt;26*, 27*</td>
<td>Ch × P &lt; 0.0196* Chaoborus, mix &lt; control, Perca</td>
<td></td>
</tr>
<tr>
<td>(3) Vertical distribution</td>
<td>clone 26 more frequently at the bottom than 25**, 27***</td>
<td>Ch × P &lt; 0.0004*** Chaoborus, mix deeper than control, Perca</td>
<td></td>
</tr>
</tbody>
</table>

**Table Ib:** Overview of significant differences per trait as identified by a post hoc test (Tukey HSD for unequal sample sizes)

---

Daphnia individual small-scale swimming behaviour was studied in four infochemical treatments: Chaoborus (Ch), Perca (P), mixture of Chaoborus and Perca (m), and an infochemical-free control (c). Numbers in the shaded fields represent clones that differ significantly in their response to treatment. In the column of ANOVAs, the main factors inducing the change in trait value are given.
Predation trials

Significant differences in the number of survivors were found in two of the four statistically tested combinations of clone-specific phenotypes (Figure 4). Clone 26 control treatment daphnids survived predation significantly more often than daphnids from clone 27 raised in the Chaoborus treatment (Tukey HSD: \( P < 0.05 \)). Furthermore, Perca-conditioned clone 26 individuals survived predation more frequently than Chaoborus-conditioned clone 27 individuals (Tukey HSD: \( P < 0.05 \)). No difference was found in the survival frequency of clone 26 Perca-conditioned daphnids and clone 27 control-conditioned daphnids (Figure 4). The difference in vulnerability to Chaoborus predation was therefore pronounced (Table II). The two clones showed a different pattern. Clone 26 was most vulnerable to predation after the adaptation to Perca (vulnerability index = 1) and least after the adaptation to Chaoborus (vulnerability index = 0.15), with the control taking on an intermediate value (vulnerability index = 0.77). Clone 27, on the contrary, was most vulnerable after adaptation to Chaoborus infochemical (vulnerability index = 2.34) and least vulnerable after adaptation to Perca infochemical (vulnerability index = 0.66), with the control treatment daphnids taking on an intermediate...
vulnerability (vulnerability index = 1). Consequently, the two Chaoborus-conditioned treatments led to the most (clone 27) and least (clone 26) vulnerable daphnids of all combinations tested.

**DISCUSSION**

A significant, clone-specific effect of predator infochemicals on SSB is noticed in all of the tested parameters.
Genetic variability

Clonal differences are enhanced both by the presence of Chaoborus infochemical (all three traits) and Perca infochemical (trajectory length, vertical distribution). For two traits (trajectory length, swimming speed), the clonal responses to a mixture of infochemicals are not additive (Figure 5). The most plastic clone (clone 27) adapts every parameter to the treatments, whereas another clone (clone 17) does not respond in any of the traits. Some clones respond in a particular trait only to the presence of Chaoborus infochemical, while others react only to Perca infochemical (e.g. swimming speed, clones 27 and 26; Table Ib). This is not in agreement with Dodson et al., who found no effect of Chaoborus infochemical on behavior of D. pulex ('null behavior') (Dodson et al., 1995). However, their study did not use daphnids from pre-adapted mothers, which is known to affect sensitivity to the infochemical. Moreover, the vessels used by Dodson et al. were very small (183 ml) compared with the 500 ml used in this study, which might account for at least part of the differences in responsiveness (Dodson et al., 1995). Another potential explanation is a species-specific difference: the bigger species D. pulex is less prone to predation by this invertebrate predator, because Chaoborus selects for smaller prey items. Our results illustrate that 'genotype' is an important parameter in determining relative fitness in environments with fluctuating predation pressures.

Perca treatment

A significant response of the D. galeata clones to Perca infochemical is observed in swimming speed and trajectory length. However, vertical distribution remains unaffected by the treatments. This is not in accordance with the literature, where it is shown that the presence of fish infochemicals alters vertical preference in Daphnia (Ringelberg, 1991; De Meester, 1993; Loose et al., 1993; Loose and Dawidowicz, 1994). The lack of response may simply be a consequence of experimental scale. Changes in vertical distribution are usually detected in studies of (diel) vertical migration, which generally takes place on a scale of meters to tens of meters. In the present experiment, however, a distance of only 12 cm was studied, so potential modifications of the mean average depth may have been undetectable.

The infochemical induces a genotype-dependent shift towards either longer or shorter trajectories relative to the control (Figure 1b). This points to a clone-characteristic swimming path (O'Keefe et al., 1998). Apparently, various clone-specific swimming patterns can exist in a single population. This can blur the pre-cognitive prey selection pattern (Pastorok, 1980, 1981; Prete et al., 1993) of the fish predator. Having more than one SSB pattern within one species and one population might be a way to decrease predation by fish, which has not been described previously.
In two of the four tested *D. galeata* clones, swimming speed is significantly increased in the *Perca* treatment (Table Ib). This is a rather unexpected result since fish are known to detect fast-moving objects better than slow-moving ones (Brewer and Coughlin, 1996; O'Keefe et al., 1998). An acceleration of swimming in the presence of fish infochemical has been described previously [e.g. (Macchiusi and Baker, 1991; Dodson et al., 1997; O'Keefe et al., 1998)]. A potential explanation for this accelerated swimming is that faster (vertical) movement is less prone to detection by fish and that it consequently makes individuals less liable to be predated by fish than either sinking or moderate vertical swimming (Dodson et al., 1997).

**Mixed treatment**

In the present study, *Perca* and *Chaoborus* infochemicals are found to interact for two traits: trajectory length and swimming speed (three-way interaction; Table Ia and b). In some cases, the effect of the mixed infochemicals resembles the response found in the *Perca* treatment, but occasionally the presence of the *Chaoborus* infochemical dominates the response in this treatment (Table Ib). For trajectory length (Table Ib, clone 27), the interplay of the infochemicals in the mixed treatment leads to a trait value significantly different from that of either *Chaoborus* or *Perca* treatments. This non-additive effect might be an indicator of stress when presented with both types of predators simultaneously. A similar effect is found for life history traits (Weber and Declerck, 1997), which confirms the stress hypothesis. The effect of the simultaneous exposure of two infochemicals on the SSB of *Daphnia* has not been analyzed previously. The only studies investigating this combination have concentrated on life history traits (Lüning, 1992; Black, 1993; Weber and Declerck, 1997). The study by Weber and Declerck (Weber and Declerck, 1997) was carried out with the same clones and predators as in the present study. They report that effects on life history induced by the *Chaoborus* cue are neutralized by the fish infochemical in the mixed treatment. We cannot confirm this finding for SSB. Life history and SSB responses to predator infochemicals are not affected in the same way. The uncoupling of these two traits suggests that a wide array of strategies can evolve in response to fluctuating predation pressure.

**Chaoborus treatment**

The response of *D. galeata* to *Chaoborus* infochemical is in accordance with the literature, in that no clear-cut pattern

Fig. 5. Graph showing changes in swimming speed and trajectory length of *D. galeata* in response to predator kairomones. The X-axis shows the swimming speed (mm s⁻¹); the Y-axis shows the trajectory length as calculated by an index. A more positive index indicates that relatively more short trajectories were traveled as compared with long trajectories.
could be established. The shift towards shorter trajectories is found in the same clones that significantly altered their vertical position in the presence of the invertebrate infochemical. The results agree with previous findings (Riessen et al., 1984), where Daphnia, in contrast to Diaphanosoma, are found to be unable to escape the strike volume of Chaoborus due to its slow and ineffective evasion reaction. The observed changes in trajectory length are difficult to interpret with respect to escape from the predator's strike volume. To minimize time spent in the strike volume, traveling longer trajectories appears to be more rewarding. However, the number of observations of long trajectories may have been too small to reveal statistically significant changes in the frequency of long trajectories per treatment. This does not hold for short distances, since they were observed much more frequently (n = 20 ± 3.3 SD) than long distances (n = 6 ± 1.9 SD) during the experimental period of 4 min.

The mean swimming speed calculated for the control and Chaoborus treatment (4.3 mm s⁻¹ ± 0.4 SD) is in accordance with average swimming speeds reported in the literature [3.5 mm s⁻¹ (Porter et al., 1982); 5.4 mm s⁻¹ (Dodson et al., 1997)]. The observed decrease in swimming speed (Table Ib, clones 25 and 26) in response to the Chaoborus treatment has been reported previously (Gerritsen, 1980; Dodson, 1996). Surprisingly, Chaoborus infochemical was responsible for an increase in swimming speed (clone 27, Table Ib). Although Daphnia has been reported to show an accelerated evasion reaction after Chaoborus attack (Kerfoot, 1977, 1978), an increase in swimming speed triggered by this infochemical has not been reported before and seems maladaptive. This unexpected result might point toward a benefit of swimming that balances the increased predation risk (Dodson, 1996). The general strategy to minimize encounter probability with a Chaoborus predator appears to be to decrease travel distance rather than travel speed (but see the section ‘Vulnerability to Chaoborus predation’). This strategy may be useful in distracting the tactile predator by stimulating multiple mechanoreceptors at the same time, which might affect the precision of the attack. The vertical position remains unaffected by Chaoborus infochemical in two clones (clones 17 and 26), which is in accordance with a study by Leibold (Leibold, 1990). In contrast to this lack of response, Chaoborus infochemical is known to induce aggregation in the top layer (Leibold, 1990; Ramcharan et al., 1992) and a clear swim-away reaction (Vetti Kvam and Kleiven, 1995). This response is observed in one of the clones (clone 27). Chaoborus infochemical is also responsible for a decrease in vertical position relative to the Perca and control treatments (clone 25), which has not been previously reported in the literature.

**Correlation among traits**

The correlation between swimming speed and trajectory length is negative, irrespective of clone and treatment (Figure 5; Table II). Thus, long distances correlate with high swimming speeds. This points towards a general pattern, where the number of movements per unit time is relatively constant but the trajectory length is flexible. A similar finding leads to the hypothesis that faster movements make prey less liable to fish predation (Dodson et al., 1997).

**Vulnerability to Chaoborus predation**

Vulnerability to Chaoborus predation is determined by the size of the prey individual and its behavior (e.g. Vetti Kvam and Kleiven, 1995; Nesbitt et al., 1996; Wissel and Benndorf, 1996). High swimming speed increases the encounter probability and, therefore, predation risk. In the predation trial presented here, prey size was standardized and differences in SSB determined vulnerability to predation. The most important trait in determining vulnerability to Chaoborus predation is swimming speed, and trajectory length is of least importance. The fastest swimmer is also the phenotype with the highest vulnerability (clone 27—Chaoborus pre-adaptation; Figures 3 and 5). Conversely, the phenotype with the lowest vulnerability to predation (clone 26—control pre-adaptation) displays the lowest swimming speed (Figures 3 and 5). An overview of the relationship between vulnerability, swimming speed and trajectory length is given in Table II (see also Figure 5).

For clone 26, a 100% positive correlation between vulnerability and swimming speed illustrates the relevance of this parameter in affecting vulnerability. A positive relationship between vulnerability and swimming speed is also found in two out of three phenotype combinations (Table II, clone 27). The strong positive correlations between vulnerability and swimming speed found for both clones (Table II) indicate that swimming speed plays an important role in decreasing or increasing the vulnerability to Chaoborus predation. Trajectory length appears to be of less significance in lowering predation risk. In some cases, vulnerability of daphnids is even negatively correlated to trajectory length, i.e. swimming longer trajectories makes the prey animals more vulnerable to Chaoborus predation and shorter trajectories decrease vulnerability (clone 27, Table II). Although trajectory lengths are similar for the clones across the treatments (Table II, column ‘Trajectory length’), the vulnerability between clones differs strikingly (Table II, column ‘Vulnerability’). Consequently, there appears to be no clear advantage in swimming either long or short trajectories, but rather a clone-intrinsic trajectory length, which does not necessarily lead to a decrease in vulnerability to
Chaoborus predation. This is confirmed by the correlation pattern between swimming speed and trajectory length (Table II; Figure 5). Both positive and negative correlations exist between swimming speed and trajectory length (positive: one phenotype has a high swimming speed and moves long trajectories relative to the other, Table II; negative: one phenotype has a slow swimming speed and swims long trajectories or vice versa, Table II). In some cases, only one of the two traits is modified by the treatment, while the other remains unchanged (e.g. clone 27, c relative to P, Table II), indicating no direct relationship between the traits. The above findings illustrate that the clone- and treatment-dependent differences in behavior are not random, but can indeed account for differences in vulnerability to Chaoborus predation, which are not foreseeable by simply looking at the results of swimming speed and trajectory length changes alone.

Our results show that a wide range of genotype-dependent phenotypic plasticity in SSB is present within a population of a single Daphnia species, which illustrates the effect of plasticity on competition and co-existence. Individual behavior differs within a species and cannot be adequately described by looking at one clone. Therefore, current models of prey selection can be improved by the incorporation of both clone-specific differences (e.g. life history) and SSB, because they are important factors determining prey vulnerability. We also show that infochemical-induced changes in SSB play an important role in determining vulnerability to Chaoborus predation. Given a choice between two clones of equal size, Chaoborus preys more successfully on individuals from the faster swimming clone. These findings show that predator-induced alterations of clone-specific swimming behavior indeed significantly influence vulnerability to predation.

ACKNOWLEDGEMENTS

We wish to thank Steven Declerck and Ralph Lehnert for valuable comments on earlier versions of the manuscript. We are grateful to Peter McGillivary, Klaas Swiersen and Guus Postema for practical assistance. Erik Van Gool kindly provided the video system for the analysis.

REFERENCES


Table II: Correlation between relative vulnerability to Chaoborus predation and changes in swimming speed and trajectory length

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vulnerability</th>
<th>Swimming speed</th>
<th>Trajectory length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clone 26</td>
<td>c relative to P</td>
<td>↑↑ ↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>Clone 26</td>
<td>c relative to Ch</td>
<td>↓↓ ↓↓</td>
<td>↓↓</td>
</tr>
<tr>
<td>Clone 27</td>
<td>c relative to P</td>
<td>↓↓ ↓↓</td>
<td>↓↓</td>
</tr>
<tr>
<td>Clone 27</td>
<td>c relative to Ch</td>
<td>↑↑ ↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>Clone 27</td>
<td>P relative to Ch</td>
<td>↑↑ ↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>Combined 26c–27c</td>
<td>(↑) (↑)</td>
<td>(↑)</td>
<td>(↑)</td>
</tr>
<tr>
<td>Combined 26c–27P</td>
<td>(↓) (↓)</td>
<td>(↓)</td>
<td>(↓)</td>
</tr>
<tr>
<td>Combined 26c–27Ch</td>
<td>(↓)</td>
<td>(↓)</td>
<td>(↓)</td>
</tr>
<tr>
<td>Combined 26c–27P</td>
<td>(↓) (↓)</td>
<td>(↓)</td>
<td>(↓)</td>
</tr>
<tr>
<td>Combined 26c–27Ch</td>
<td>(↓)</td>
<td>(↓)</td>
<td>(↓)</td>
</tr>
<tr>
<td>26c–27c</td>
<td>(↑) (↑)</td>
<td>(↑)</td>
<td>(↑)</td>
</tr>
<tr>
<td>26c–27P</td>
<td>(↓) (↓)</td>
<td>(↓)</td>
<td>(↓)</td>
</tr>
<tr>
<td>26c–27Ch</td>
<td>(↑)</td>
<td>(↑)</td>
<td>(↑)</td>
</tr>
<tr>
<td>26c–27P</td>
<td>(↓)</td>
<td>(↓)</td>
<td>(↓)</td>
</tr>
</tbody>
</table>

The treatments to which the Daphnia were pre-adapted are abbreviated as follows: c, infochemical-free control; Ch, conditioned with Chaoborus infochemical; P, conditioned with Perca infochemical. In the column ‘treatment’, the characteristics (vulnerability, swimming speed, trajectory length) of two individuals of the same clone pre-adapted to different treatments are compared, for example: ‘c relative to P’ means that daphnids pre-adapted to the control treatment are compared with daphnids pre-adapted to the Perca treatment. Arrows indicate the direction of change in a parameter from the first relative to the second ‘treatment’. Parentheses indicate small trends and double arrows strong relative changes.


Received on June 28, 2002; accepted on September 2, 2002