Predators can induce swarming behaviour and locomotory responses in *Daphnia*

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SUMMARY

1. Exposure to chemical cues released by fish induced a tendency to form and maintain aggregations in two *Daphnia* clones originating from habitats inhabited by fish (and invertebrate predators), while *Daphnia* from a clone originating from a fishless habitat did not aggregate in response to fish cues.

2. *Daphnia* from one of the two clones responsive to fish cues, also aggregated in response to chemical cues released by invertebrate predators or to a substance released from homogenized *Daphnia*.

3. Chemical cues released by predators and the substance released from homogenized conspecifics induced a significant decrease in *Daphnia*'s swimming speed and an increased readiness to perform a somersaulting escape behaviour, especially in response to light cues.

Introduction

Aggregating animals are protected against predation in different ways (Hamilton, 1971; Milinski, 1977a, 1979; Pulliam & Caraco, 1984). First, there is confusion of the predator whose attention, when approaching a group of prey, is distracted by the great number of moving targets, visible simultaneously, and whose hesitation period is thus longer, giving a lower capture rate and a greater probability of escape for a given prey item. The other is the dilution effect; that is, the lower probability of a particular prey item being captured by a predator when hidden among a large number of similar-looking conspecifics. High swarm density and uniformity of swarm members enhance both effects (Milinski, 1977b).

The effectiveness of swarming as an antipredator behaviour has been documented for planktonic crustaceans (Heller & Milinski, 1979; Jakobsen & Johnsen, 1988a). In organisms that partially reproduce via parthenogenesis, such as *Daphnia*, swarm formation among clone-mates may be favoured over solitary behaviour once both individual- and group-related benefits are involved (Young *et al.*, 1994). However, association of kin-preferential selection within a

swarm of *Daphnia* does not seem to be a general phenomenon (Mitchell *et al.*, 1995).

There is considerable evidence, well supported within the last decade, that antipredator morphological and behavioural responses, as well as life-history modifications, can be directly induced in planktonic animals by substances released to the environment by either fish or invertebrate predators (for a recent review see Larsson & Dodson, 1993). Preliminary evidence of swarm induction through the chemical cues released by fish is given by Pijanowska (1994). In this paper, the approach is developed further, with comparisons made of the responsiveness of *Daphnia magna* originating from three clones from habitats representing different predation regimes and different sensitivity to fish kairomones. Also tested is the readiness of *Daphnia* to aggregate in the presence of invertebrate cues and a chemical substance released from homogenized *Daphnia*, applied to mimic a cue released by injured conspecifics. A similar mechanism is well described in fish (Von Frisch, 1941; Lima & Dill, 1990; Smith, 1992), but has not yet been recognized in planktonic crustaceans. We also quantify the differ-

ences in swimming patterns between animals exposed and not exposed to predator cues and, thus, showing different tendencies to aggregate.

Methods

Use was made of *D. magna* Straus clones originating from three habitats: a fish pond in Żabieniec near Warsaw (clone *Z*), an experimental pond in Mikołajki where fish are kept occasionally (clone *M*), and a fishless pond near Warsaw from which one clone has been kept in our laboratory cultures for the past 15 years (clone *W*).

Experimental design

Effects of chemicals released by predators on the spatial distribution of *Daphnia* were tested in flat-bottomed, round bowls ($r = 20$ cm, 25 l capacity). Each experiment was run in six vessels, three filled with medium conditioned by predators and three filled with control medium. Sixty *Daphnia* of different sizes were introduced to each of six bowls, filled with 6 l of medium, so the water level did not exceed 5 cm. Flat-bottomed, cylindrical bowls were used to eliminate the tendency of *Daphnia* to aggregate in the corners of rectangular aquaria. Each bowl was illuminated from above through opaque paper to diffuse the light by a 40-W bulb. Light conditions were constant, and mean water temperature was between 16 and 19 °C.

Media

In the control, water taken from eutrophic Lake Roś, and aged for at least 7 days, was aerated for 24 h prior to use and filtered through netting of 30 μm mesh size. The fish medium was prepared by incubating two roach (*Rutilus rutilus* L.), of total length not exceeding 12 cm, in 20 l of lake water for 24 h and then filtering the medium through Whatman GF/C glass filters. The invertebrate media were prepared by incubating one adult *Dytiscus marginalis* L. or one adult *Notonecta glauca* L. in 3 l of water, and ten fourth instar *Chaoborus flavicans* (Meigen) or *Chaobous crystallinus* (de Geer) larvae in 10 l for 24 h. While conditioning the water, neither fish nor invertebrates were fed.

The *Daphnia* medium was prepared by homogenizing 200 *Daphnia* (1.8–2.2 mm) in 250 ml of lake water,

filtering the suspension through Millipore membrane filters (0.2 μm), and diffusing the filtrate within 18 l of water (three bowls × 6 l), filtered through Whatman GF/C. The concentration of *Daphnia* substance thus mimicked the effect of releasing the whole tissue content of about ten individuals into 1 l of water. Although adult fish usually ingest *Daphnia* whole, newborn fish and invertebrate predators are 'sloppy' feeders, which release a portion of *Daphnia* tissue into the water. Under natural conditions, an unknown amount of *Daphnia* tissue content is excreted by a predator after passing through the gut, but the quality of this cue may differ from that directly released from killed specimens. We intended to mimic the situation where predators (invertebrate or young fish) prey on *Daphnia*, and thus release a certain amount of tissue to the environment.

In each experiment, the food level for *Daphnia* was initially set high, above 2 mg C l^{-1}, by adding a suspension of lake algae smaller than 30 μm to the water.

Standard procedure

As part of a standard procedure, the first observation was made 1 h after *Daphnia* had been introduced to the freshly made media. At each observation, all animals were counted from above, in a grid of sixteen fields (of equal surface area), painted on the bottom of each vessel and readily visible through the water.

To reduce error related to the movement of individuals across boundary lines, animals were usually counted three to five times (only occasionally once), with counts following one another in quick succession.

We first checked the stability of distribution of animals during a period of 5 h, by counting animals from a fish pond (clone *Z*) every 15–20 min in the fields of the grid. In the longest, 50-h-long experiment with the same clone, animals from both fish and control treatments were transferred to freshly prepared control media after 24 h, kept there for 15 h and then re-exposed to freshly prepared versions of the previous treatments. Animals were counted every 1 or 2 h, with a total of twelve counts within 10 h in the invertebrate media. Other experiments routinely lasted 24 h, with the distribution of animals being examined every 1–2 h.

During experiments, *Daphnia* were not provided with additional food, but the water was mixed after

every other observation to redistribute the remaining algae in each bowl.

The degree of aggregation was estimated by calculating the density independent patchiness index Pi (Lloyd, 1967):

$$Pi = \frac{\sigma}{x^2} - \frac{1}{x} + 1,$$

where x is the number of individuals in a square of the grid and σ is the sample variance. $Pi < 1$ describes an even distribution, $Pi = 1$ random one, and $Pi > 1$ a patchy distribution (the higher the value, the greater the degree of patchiness).

Swimming activity

To compare the swimming activities of the control and experimental animals (in fish and *Daphnia* treatments) measurements were made of the distance covered by a single animal (from clone *Z* which proved to be responsive to predator and *Daphnia* cues) in 1 min, in glass bowls ($r = 7.5$ cm, 0.5 l capacity, water level 3.5 cm), with a bottom surface divided into sixteen fields, of about 11 cm^2 each. Fish, *Daphnia* and control media were prepared in the manner described above. Thirty animals of different sizes were introduced to each of the nine bowls (three replicates in each treatment). A single animal out of thirty was selected randomly after 1 h of exposure and its route followed for 1 min as it swam between the fields of the grid. One of us tracked the swimming animal and announced its position by giving the symbol of the field, while the other registered the data. The scorer was unaware of the treatment being tested. Each change of *Daphnia* position was registered; a displacement between neighbouring fields was assumed to be equal to the distance between their centres. Altogether forty-eight randomly selected animals were examined one by one in each treatment. The observed animal, after completing its task, was replaced by another, treated up to that moment in exactly the same way. While watching large numbers of *Daphnia* exposed to the predator kairomones, we noticed a clear tendency for them to somersault. In an effort to quantify this behaviour, we also counted in the same vessels the number of somersaulting (looping) individuals, under constant light, and after a sudden change in light conditions (a stress to stimulate the animals to perform
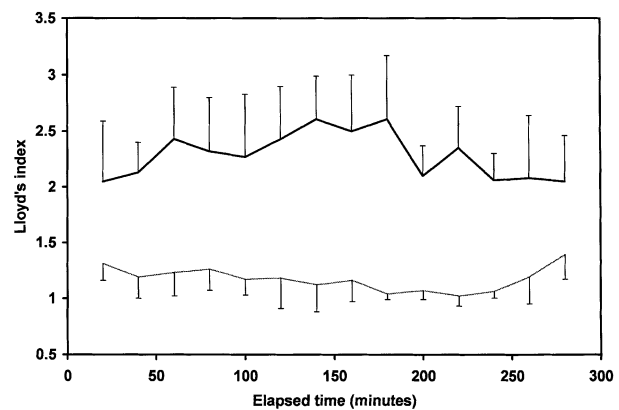
Fig. 1 Short-term survey of aggregation patterns (Lloyd's index, means and standard deviations) of *Daphnia* from clone Z originating from a fish pond. Animals were observed for 5 h by recording their distribution every 15–20 min. Thick line = fish treatment, thin line = control treatment.

unusual movements), which was produced by covering the vessels with a black lid for 3 min, and then removing it suddenly. Four times within 12 h (in three successive counts at each observation), we counted how many of the thirty animals somersaulted.

Results

Aggregation patterns in the presence of a cue released by fish

Daphnia from a fish pond (clone Z) when exposed to a substance released by a fish predator exhibited an increase in aggregation (Fig. 1; $P = 0.00016$, $F_{1,83} = 187.44$); the distribution of animals was stable over a 5-h period (there was no significant effect of time, $P = 0.9587$, $F_{13, 83} = 0.40$; repeated measurements of ANOVA). The difference was already established by the beginning of data collection, within the first hour of pre-exposure of animals to their treatments.

When the same clone was tested in another experiment for 50 h, the effect persisted for 24 h. After the animals from both fish and control treatments were transferred for the next 15 h to the control media, the aggregation tendency clearly decreased in the formerly fish-treated animals, and then increased again after another exposure to fresh fish media. In the control treatment, aggregation patterns remained stable (Fig. 2).

A similar response to a fish kairomone was observed in another clone from a pond inhabited by fish— clone *M* (Fig. 3). A significant effect of treatment was
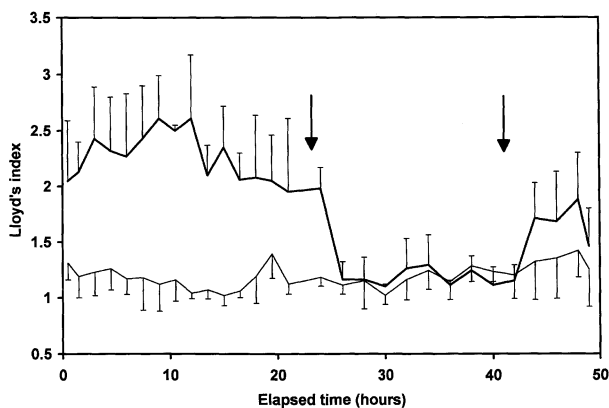
Fig. 2 Long-term aggregation patterns (Lloyd's index, means and standard deviations) of *Daphnia* from clone *Z* originating from a fish pond. Arrows indicate when animals were transferred to fresh medium: first, to medium free of any predator signal; second, back to original medium, freshly prepared. Thick line = fish treatment, thin line = control.
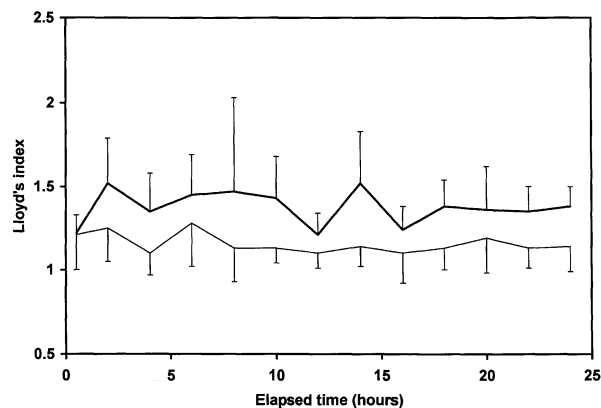

Fig. 3 Aggregation patterns (Lloyd's index, means and standard deviations) of *Daphnia* from clone *M* originating from an experimental pond, occasionally inhabited by fish. Thick line = fish treatment, thin line = control treatment.

Fig. 4 Aggregation patterns (Lloyd's index, means and standard deviations) of *Daphnia* from clone *W* originating from a fishless pond. Thick line = fish treatment, thin line = control treatment.

Fig. 5 Aggregation patterns (Lloyd's index, means and standard deviations) of *Daphnia* from clone *Z* originating from a fish pond in the presence of invertebrate predators.
1, *Dytiscus marginalis*; 2, *Notonecta glauca*; 3, *Chaoborus flavicans*; 4, *Chaoborus cristallinus*; 5, control.

observed ($P = 0.0285$, $F_{1,233} = 4.89$, repeated measurements of ANOVA), with no effect of time ($P = 0.3797$, $F_{12,233} = 1.09$).

In contrast, clone *W* animals, which originated from a fishless environment, did not show any significant response to fish kairomone (Fig. 4). Neither the effect of treatment ($P = 0.6382$, $F_{1,269} = 0.22$), nor the effect of time ($P = 0.4958$, $F_{14,269} = 0.96$, repeated measurements of ANOVA) were significant.

Aggregation patterns in the presence of a cue released by invertebrates

A significant difference was observed between the four treatments involving invertebrate predators
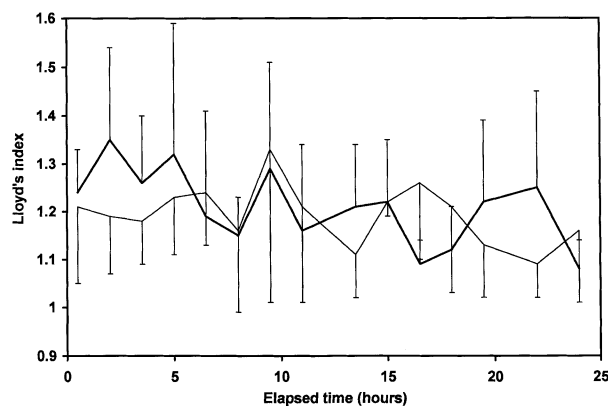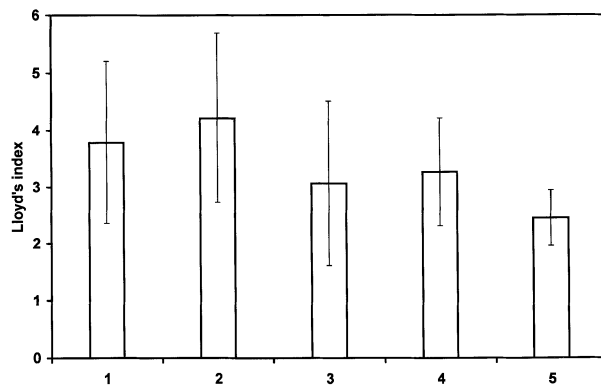
(1. *Dytiscus marginalis*, 2. *Notonecta glauca*, 3. *Chaoborus flavicans*, 4. *Chaoborus crystallinus*) and 5. the control ($P = 0.0001$, $F_{4,59} = 4.82$), with the within-treatments variance being generally very high (Fig. 5). Tukey (HSD) pairwise comparisons revealed significant differences between three groups (1, 2, 3 vs. 2, 3, 4 and vs. 3, 4, 5, at $P = 0.05$), as well as between the control and either predator treatment ($P = 0.05$). High variances within treatments together with the significant time-replication effect ($P = 0.0001$, $F_{11,59} = 5.92$), reflected a decreasing tendency to aggregate with time. The differences between invertebrate treatments may be related to differences in the kairomone concentration, which is more or less blindly adjusted as long as its chemical nature remains unknown. After 9 h, about
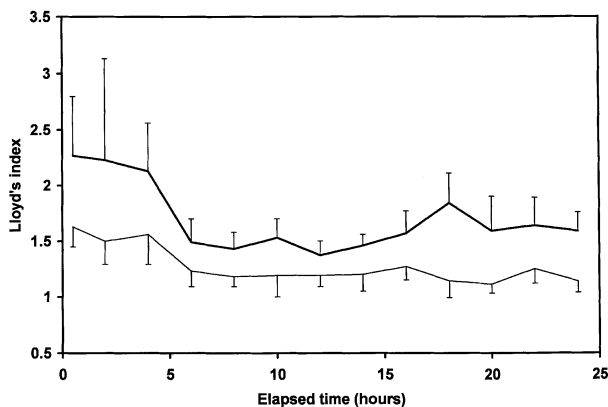
Fig. 6 Aggregation patterns (Lloyd's index, means and standard deviations) of *Daphnia* from clone Z originating from a fish pond in the presence of a signal from crushed conspecifics. Thick line = *Daphnia* treatment, thin line = control treatment.

Table 1 Average distance in cm (mean ± 1 SD), travelled by *Daphnia* during 1 min, before and during their exposure to the chemical substances released by fish and from crushed conspecifics

	Before	After 0.5 h	After 1 h	After 6 h
Fish	20.6 ± 11.5	14.1 ± 10.6	10.6 ± 11.0	11.1 ± 12.1
Daphnia	18.9 ± 17.7	10.2 ± 7.0	8.9 ± 8.0	10.5 ± 8.6
Control	19.8 ± 19.5	18.6 ± 9.6	25.9 ± 15.1	20.2 ± 17.9

15% of the experimental animals had died and the experiment was terminated.

Aggregation patterns in the presence of a substance released from crushed Daphnia

The exposure of *Daphnia* from a fish pond (clone Z) to a cue originating from crushed conspecifics also caused an increased tendency to aggregate relative to the control treatment (Fig. 6; $P = 0.00001$, $F_{1,233} = 132.02$, repeated measurements of ANOVA).

Induced locomotory response

The distances covered by animals from a fish pond (clone Z) differed significantly between treatments ($P = 0.0003$, $F_{2,143} = 9.06$, ANOVA; Table 1). Observations made after 6 h of exposure to the experimental media indicated that control animals covered distances on average 1.6 times greater than those exposed to the cue released by fish, and 1.9 times greater than those exposed to the *Daphnia* factor (the differences between the control and the two other treatments were significant at $P = 0.01$; Tukey (HSD) pairwise comparisons of means). Within each treatment, standard deviations from the mean distances covered by *Daphnia* were high, most probably as a result of apparent differences in swimming speeds between animals of different sizes which were observed but not quantified.

The number of somersaulting animals in constant light conditions differed between the treatments (Fig. 7a; $P < 0.00001$, $F_{2,62} = 20.86$, ANOVA). Disturbance involving the lifting of black lids from bowls suddenly after 3 min resulted in a significant increase in the number of somersaulting individuals (Fig. 7b; the effect of sudden illumination was significant at $P < 0.00001$, $F_{1,62} = 74.05$). Because the fraction of somersaulting animals also increased significantly within the group of control animals, no significant interaction was found between treatment and the effect of a sudden change in light conditions ($P = 0.094$, $F_{2,62} = 2.48$).

Discussion

Exposure to the chemical cues released by fish and by invertebrate predators induced a tendency to form aggregations in two *Daphnia* clones (Z and M) originating from habitats where both fish and invertebrate predators are present (Figs 1–3). It is not clear whether such aggregations form because individuals aggregate, each of them autonomously performing a similar reaction to the presence of a cue, or whether individuals are responding behaviourally to one another in a gregarious manner once danger is recognized. In contrast, individuals from clone W, which originated from a fishless habitat, did not aggregate in response to the cue released by fish (Fig. 4), making another addition to the list of examples of interclonal behavioural variability in response to the presence of a predator (Pijanowska *et al.*, 1993; DeMeester, Weider & Tollrian, 1995; DeMeester & Pijanowska, 1996).

Preliminary data indicate that *Daphnia* from clone W respond to a cue from invertebrates and from injured conspecifics, but these data are too ambiguous to be presented at this stage. In another study it was shown that individuals from clone W are not induced by the presence of fish kairomone to perform either vertical migration (Pijanowska *et al.*, 1993) or escape
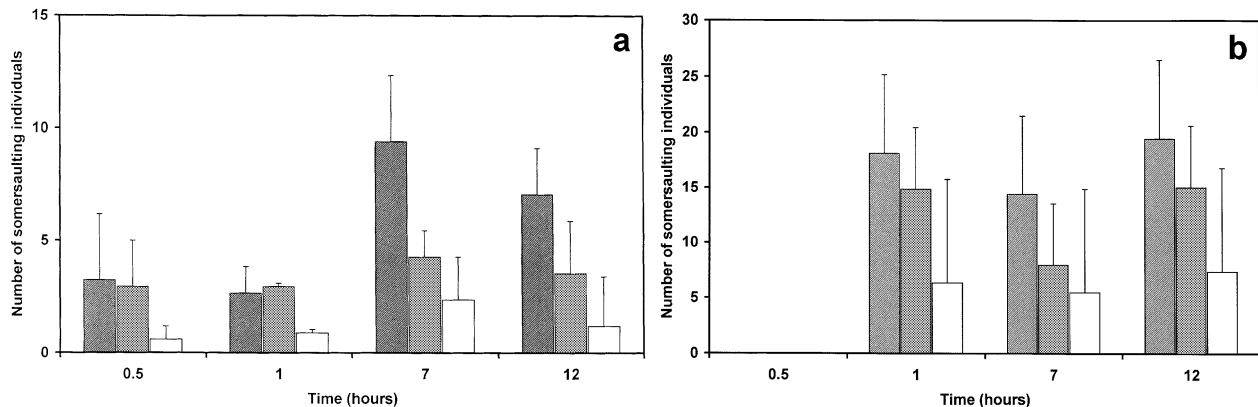
Fig. 7 Number of somersaulting *Daphnia* from clone Z originating from a fish pond (means and standard deviations): (a) in constant light conditions; (b) after light disturbance. Black bars, fish; grey bars, crushed *Daphnia*; white bars, control treatment.

responses (DeMeester & Pijanowska, 1997); both considered behavioural adaptations to avoid predation. In this way they differ from individuals originating from clones that experienced fish predation in their native habitat. On the other hand, life histories within clone W (e.g. size and age at first reproduction, offspring number and size) can, to a certain extent, be modified by a cue from a fish predator (Weider & Pijanowska, 1993). This suggests that *Daphnia*'s life history modifications in response to potential danger are more conservative features than the behavioural ones.

A cue from crushed tissue of *Daphnia* induced a similar reaction among conspecifics to one released by predators, in terms of both swarming (Fig. 6) and locomotory behaviour (Table 1, Fig. 7). This cue alone (even with no additional cues from either predator) possibly gives more realistic information on the extent of a danger than those released by predators. Our experiments do not indicate whether this cue is interpreted as danger from fish or invertebrates. Both young fish and invertebrates can damage their prey while handling an item, thus releasing tissues into the water. In our experiment, we mimicked the release of the whole tissue content of about ten specimens into 1 l of water. Under natural conditions, a substantial amount of prey tissue is most probably assimilated by a predator. Thus, the concentration of *Daphnia* signal was probably quite high in our experiment. However, if the animals respond to high concentrations of a signal from crushed conspecifics in an adaptive manner, they can probably respond to lower concentrations as well. Also, the specificity of the response to homogenized *Daphnia* is as yet unclear.

As was recently described by Stirling (1995), the most reliable information for a potential prey item is, most probably, a combination of two kinds of information: a signal advertising the death or injury of conspecifics and a cue from a predator. Responses to signals from conspecifics are not well documented in cladocerans (Dodson *et al.*, 1994), but both behavioural and life-history responses to the cues released by injured conspecifics have been described for fish (Von Frisch, 1941; Smith, 1992).

The aggregation response was fast, taking place soon after animals were exposed to a predator. However, those from clones Z and M could possibly have experienced predator cues in their recent 'biographies' before the experiments. In another study on *Daphnia* aggregations, Pijanowska (1994) preconditioned animals to the presence of a cue for 24 h prior to an experiment. Aggregations persisted for 24 h at least, most probably for as long as the cue is active or animals do not habituate to its presence, a phenomenon recently documented for isopods in the presence of fish cues (Holomozuki & Hatchett, 1994).

Another question, not considered here, concerns the costs related to aggregating. Enhanced protection from predators gained by individual swarm members can bring costs related to the increased competition resulting from joining such a group (Alexander, 1974; Pulliam & Caraco, 1984). Under our experimental conditions, food was present until the end of the period of exposure. High densities of cladocerans can, however, rapidly deplete local resources (Jakobsen & Johnsen, 1988b) and even at high food levels, the permanent maintenance of a microscale patch could affect the growth rate and clutch size of aggregating

individuals (Burns, 1995), most probably due to a reduction in the feeding rate (Hayward & Gallup, 1976; Helgen, 1987; Matveev, 1993). Our experiments, however, were not designed to evaluate the long-term metabolic costs of living in a group.

A readiness to stay within aggregations was associated with reduced mobility (for the relationship between aggregation patterns and motion in cladocerans see also Young & Taylor, 1990). Although animals exposed to the presence of predator stimuli can potentially escape faster than those not exposed, they manifest this escape response only while endangered (e.g. disturbed by a sudden change in light conditions). With no additional cues they reduce their general mobility. This may somehow be related to their readiness to stay within an aggregation once it is formed. On the other hand, animals alerted by predator chemicals somersault more readily than unexposed animals, especially if disturbed either by light (this paper) or mechanical stimuli (J. Pijanowska & L. W. Weider, unpublished data), which may be associated with approaching danger. The efficiency of predator attacks on *Daphnia* exposed to chemicals from predators is lower than on those never exposed (DeMeester & Pijanowska, 1996) and a part of this reduced predator attack efficiency is related to somersaulting behaviour, as noted by J. Pijanowska & L.W. Weider (unpublished data), and supported by our own observations.

Acknowledgments

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