

Inducible defenses: The relevance of chemical alarm cues in *Daphnia*

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Abstract

Many aquatic organisms use chemical cues to recognize predators and to activate inducible defenses. In zooplankton, most of these cues are thought to be kairomones released by specific predators. However, in several other organisms, e.g., fish and amphibians, alarm signals from conspecifics also provide reliable cues. We tested whether alarm substances also act as chemical cues in *Daphnia* and assessed their relevance compared with predator kairomones. Water-borne cues from macerated conspecifics altered morphology in all *Daphnia* species tested (*Daphnia cucullata*, *Daphnia longicephala*, and *Daphnia lumholtzi*). However, kairomones released by feeding predators had distinctly stronger effects. In *D. cucullata*, we tested for the relevance of predator diet and found that the increase in relative helmet length was most pronounced when the predator was fed with conspecifics instead of heterospecifics. Cross-species experiments with alarm substances also revealed that *D. cucullata* is more sensitive to info-chemicals from macerated conspecifics than to chemical cues from *D. magna*. Unspecific alarm cues together with cues released by predators consuming prey may form a blend of chemicals that indicates predation risk and activates inducible defenses in nature.

Chemical information transfer is widespread in inter- and intraspecific communication, especially in aquatic ecosystems in which turbidity can make visual cues less detectable. Sources of biotic information include factors such as food quality, mating-partner density, or predation impact (Atema et al. 1988), and chemical cues are often used to trigger inducible defenses. Antipredator responses frequently incur costs, and a driving factor behind many inducible defense systems is that these costs can be saved when a particular defense is not needed (Tollrian and Harvell 1999). Thus, the specificity and reliability of the information about the predation risk is crucial to maximize the benefit of a defense (Gabriel et al. 2005).

Both info-chemicals, predator-released kairomones and prey-released alarm cues, can provide important information about the current risk of predation for prey. Chemical substances released by predators, defined as kairomones (Brown et al. 1970), lead to phenotypic changes in some of their prey. These chemically mediated antipredator responses have been shown in a variety of aquatic organisms (for review, see Tollrian and Harvell 1999; Lass and Spaak 2003). Also prey-released alarm cues are important factors for the activation of antipredator responses in terrestrial and aquatic habitats. Although numerous examples are reported to evoke behavioral changes (e.g., Brown and Godin 1997; Wisenden et al. 2001), sparse work on the

relevance of alarm substances for the induction of morphological defenses has been done so far (reviewed in Chivers and Smith 1998).

Many cladoceran species form predator-induced defenses (reviewed in Tollrian and Dodson 1999). Because no detectable effect of chemical stimuli of crushed conspecifics could be demonstrated in *Daphnia pulex* (Walls and Ketola 1989; Parejko and Dodson 1990) and *Daphnia galeata mendotae* (Stirling 1995), most researchers working on inducible defenses in daphnids focused on predator kairomones as cues (e.g., Krueger and Dodson 1981; Hanazato 1991; Laforsch and Tollrian 2004). However, plankton organisms are often lethally or nonlethally injured during attack because larval and juvenile fish still have to learn how to feed on their prey (Ibrahim and Huntingford 1992) and several invertebrate predators crush their prey items during feeding (Branstrator 1998). Therefore, alarm cues could also provide relevant information in *Daphnia*. Two studies on *Daphnia magna* indicated that the behavior and life history was affected by cues from homogenized conspecifics (Pijanowska 1997; Pijanowska and Kowalczewski 1997). Furthermore, Stabell et al. (2003) suggested in *Daphnia galeata* that latent alarm signals, activated by intestinal enzymes of planktivorous fish, could cause morphological changes in nature.

The aim of our study was to test the importance of broadly defined alarm info-chemicals in *Daphnia* (in daphnids, potential alarm cues are most likely not released by specific glands, but rather consist of cellular fluids). We used three *Daphnia* species for our induction experiments, all well known for inducible morphological defenses. *Daphnia cucullata* develop high helmets (Agrawal et al. 1999, Laforsch and Tollrian 2004), *Daphnia longicephala*

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produces huge crests (Grant and Bayly 1981), and *Daphnia lumholtzi* show a remarkable elongation of their head and tail spines (Tollrian 1994). Using *D. cucullata* as a model system, we also investigated whether the cues are concentration dependent and whether they are species specific. Furthermore, we tested in all experimental species the difference between kairomones released by feeding predators and cues from homogenized daphnids to estimate the potential ecological relevance.

Material and methods

Laboratory-cultured clones of daphnids were used for the experiments. We chose a clonal line of *D. cucullata* collected from Lake Thalersee (Germany). *D. magna* was isolated from Ismaninger Speichersee (Germany). We used a clone of *D. longicephala* originating from Lara pond (Australia) and a clone of *D. lumholtzi* originating from Fairfield Reservoir (Texas). Additional experimental animals, *Chaoborus flavicans* and *Bosmina longirostris*, were taken from Lake Klostersee (Germany). We collected *Notonecta glauca* from a pond in the garden of the Department of Biology II, Ludwig-Maximilians University, Munich. All tests were conducted in the laboratory under constant conditions at 20°C in 1.5-liter glass beakers, filled with 1 liter of synthetic medium based on ultrapure water, trace elements, and phosphate buffer (Jeschke and Tollrian 2000). The medium was changed weekly. Fluorescent light was used to simulate a constant day–night rhythm (16 h day : 8 h night). The daphnids were fed daily with *Scenedesmus obliquus* at a concentration of 1.5 mg C L⁻¹ to avoid food limitation. Experiments conducted with *D. cucullata* were started with 25 randomly chosen ovigerous daphnids originating from the third clutch of age-synchronized daphnids. Experiments with *D. lumholtzi* were started with 10, experiments on *D. longicephala* with 18 neonates of age-synchronized mothers per beaker. All beakers contained a cylindrical cage made of an acryl frame (10-cm diameter and 12-cm length) covered by nylon mesh (100- μ m mesh size). The cages prevented direct contact between crushed conspecifics or predators and daphnids, but still enabled the exchange of chemical cues. To produce alarm cues, the daphnids were macerated, resuspended, and diluted to the appropriate concentration. Subsequently, the homogenate was poured into the net cages. Alarm cues were added daily into the beakers. All experiments were replicated three times.

Induction with alarm cues released by D. cucullata and concentration dependence—In the first experiment, we tested whether *D. cucullata* shows a helmet induction with chemical cues released by macerated conspecifics. A suspension of 100 crushed *D. cucullata* was added daily to each beaker. A similar volume of synthetic medium was added to the control beakers.

The second experiment was conducted to show whether the inductive effect of alarm cues is concentration dependent. Solutions of 0, 20, 40, 60, and 80 macerated conspecifics of *D. cucullata* L⁻¹ were added.

Species specificity of the cues—To test whether *D. cucullata* reacts only to alarm substances released by conspecifics, we investigated the induction effect of alarm cues released by *D. magna* on *D. cucullata*. In the first treatment, 80 crushed *D. cucullata* per liter were used. This amount was based on our results from the concentration-dependent induction experiment. In the second treatment, we added the suspension of 40 macerated adult *D. magna* L⁻¹. The third treatment, without any addition, served as control. We added 100 μ L L⁻¹ ampicillin daily to each beaker to reduce putrescence in the beaker. Preliminary experiments had shown that the antibiotic ampicillin has no effect on helmet growth per se. The dry-weight of *D. magna* (160 μ g) is, in our experiment, approximately 27-fold higher compared with *D. cucullata* (5.86 μ g). Based on our prior experiments, we chose a distinctly higher biomass of *D. magna* to ensure that a possibly stronger response to conspecific cues could not be caused by a higher *D. cucullata* biomass.

Kairomones released by predators consuming prey versus alarm cues—The last experiment was designed to show whether kairomones released by actively feeding predators and alarm cues from macerated conspecifics have similar effects on the magnitude of plastic morphology of different *Daphnia* species.

D. cucullata: In the first treatment, a solution of 40 crushed *D. cucullata* was poured daily into each beaker (40c). In the second treatment, 10 *Chaoborus* larvae, which were starved and stored in a climate chamber for at least 4 weeks before the experiments, were fed daily with 40 individuals of *D. cucullata* (ch_cuc). Ten *Chaoborus* larvae were fed every day with 300 individuals of *Bosmina longirostris* in the third treatment (ch_bos), because the dry weight of one adult *D. cucullata* (5.86 μ g) is, in our experiment, equivalent to the dry weight of approximately eight *B. longirostris* (8 \times 0.70 μ g = 5.6 μ g). A fourth treatment, without supplement, served as control. One hundred μ L L⁻¹ ampicillin were added to each beaker daily.

The predators, fourth-instar larvae of *Chaoborus flavicans*, were placed into the cages described above. Pupate *Chaoborus* larvae and dead predators were replaced, and the cages were cleaned daily. Beakers without predators served as control. In this experiment, we measured daphnids of the second and third body-length size class for analysis because *D. cucullata* shows the longest absolute helmet length in these size classes.

All induction experiments with *D. cucullata* lasted 3–5 weeks to take possible transgenerational effects into account (Agrawal et al. 1999).

D. longicephala: We applied two concentrations of homogenized conspecifics in the *D. longicephala* experiment: Ten daphnids (10c) were crushed and added daily to the first treatment. In the second treatment, a concentration of 50 homogenized *D. longicephala* L⁻¹ was used (50c). One *Notonecta*, maintained with *D. magna* as food source prior to experiments, was placed into a net cage and fed daily with 10 *D. magna* in the third treatment (noto). We used *D.*

magna as food source to find the reaction to cues released by predators independent of *D. longicephala* alarm cues. The cages were covered by a net to prevent backswimmers from escaping. The cages were treated as described in the *D. cucullata* experiment. Beakers without any addition served as control. The daphnids were measured after having reached maturity, which is defined as the first appearance of eggs in the brood pouch.

D. lumholtzi: Treatments were similar to those of the *D. longicephala* experiment (10c; 50c; control). We used deep-frozen fish kairomones from *Phoxinus phoxinus* for the predator treatment in a concentration of 1 fish 3 L⁻¹ d⁻¹. We produced the fish kairomone by keeping 10 fish for 24 h in 10 liters of medium. Thereafter, the fish were removed and the medium was filtered (Schleicher and Schuell: Excelon PES 20/3HC; 0.2 μm) and frozen (-60°C). This medium was diluted for the experiments to the appropriate concentration. Prior to this procedure, the fish were fed exclusively with chironomid larvae, which were tested in preliminary experiments to be ineffective in inducing morphological changes in cladocerans. The daphnids were measured after having reached maturity.

Daphnids from each beaker were measured under a dissecting microscope with a digital image-analysis system (Soft Imaging System, Analysis Pro). The morphological parameters, recorded from *D. cucullata*, *D. lumholtzi*, and *D. longicephala*, were the helmet length (defined as the length between the tip of the helmet to the upper edge of the compound eye) and the body length (defined as the length between the upper edge of the compound eye to the base of the tail spine). For *D. longicephala*, we also recorded the crest height (defined as the distance between the outer edge of the compound eye to the most dorsal point of the crest). Prior to analysis, we divided *D. cucullata* into four body-length size classes (sc1: <600 μm; sc2: 600–799 μm; sc3: 800–1,000 μm; sc4: >1,000 μm). Body-length size classes were analyzed separately because helmet growth is negatively allometric after the third size class and alarm cues may have different effects in different ontogenetic stages, as has been described for predator kairomones in *D. cucullata* (Laforsch and Tollrian 2004). We calculated the relative helmet length and relative crest height (helmet length or crest height/body length × 100), to compensate for size-dependent changes in helmet length and crest height within the classes. The relative values were arcsin-square-root transformed prior to analysis (Sokal and Rohlf 1995). Data were tested for normal distribution and homogeneity of variances. A nested analysis of variance (ANOVA), with three replicates per treatment as random factor, was performed for the experiments to analyze for treatment effects between animals faced with alarm cues and control daphnids among size classes. A Tamhane post hoc test, which is insensitive to unequal variances, was used for pairwise comparisons.

Results

D. cucullata exposed to a high concentration (100 daphnids L⁻¹) of crushed conspecifics showed a significant

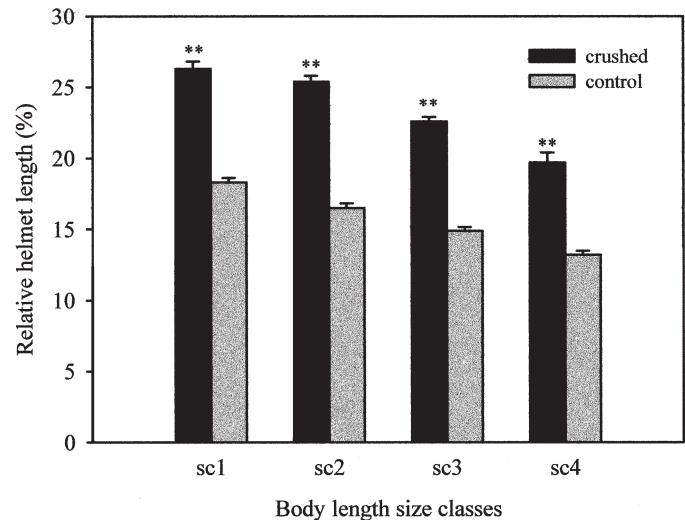


Fig. 1. Mean relative helmet length (%) and standard error (SE) of *D. cucullata* raised with (crushed: 100 daphnids L⁻¹) and without (control) chemical cues released from crushed conspecifics. The daphnids were divided into four body-length size classes (sc1: <600 μm; sc2: 600–800 μm; sc3: 800–1,000 μm; sc4: >1,000 μm) for analysis. A nested ANOVA with three replicates per treatment as random factor was performed to compare size classes for treatment effects. Asterisks indicate significant differences between the two treatments (**, $p < 0.01$).

helmet elongation in each body-length size class (nested ANOVA: sc1: $F_{1,4} = 39.24$, $p = 0.003$; sc2: $F_{1,4} = 30.55$, $p = 0.005$, sc3: $F_{1,4} = 36.92$, $p = 0.004$, sc4: $F_{1,5} = 40.59$, $p = 0.001$; Fig. 1).

A test of the concentration dependence revealed significant inductions already at 20 crushed *Daphnia* L⁻¹. Helmet length of *D. cucullata* differed significantly between concentration treatments in body-length size classes 2–4 in this experiment (nested ANOVA: sc1: $F_{4,10} = 2.57$, not significant [ns]; sc2: $F_{4,10} = 6.69$, $p = 0.007$; sc3: $F_{4,10} = 7.61$, $p = 0.004$; sc4: $F_{4,10} = 8.81$, $p = 0.002$). Tamhane multiple-comparison tests show that helmet length of *D. cucullata* was, in each induction treatment and in each body-length size class, significantly longer than that of the control, with the only exception being the first size class in the treatment with the lowest concentration of 20 crushed daphnids (Tamhane post hoc tests: control vs. 20c; sc1: $p = 0.130$; sc2: $p = 0.005$; sc3: $p = 0.010$; sc4: $p < 0.001$; control vs. 40c, 60c, 80c; sc1–4: all $p < 0.001$). The dose–response curve revealed a maximum at approximately 60 crushed *Daphnia* L⁻¹, followed by a plateau (Tamhane post hoc tests: 60c vs. 80c; sc1–4: all ns; Fig. 2).

Cross-species experiments showed that *D. cucullata* also respond to alarm cues from other *Daphnia* species, but the inductive effect is stronger when animals are exposed to chemical cues from macerated conspecifics. Helmet induction differed significantly between treatments in each body-length size class (nested ANOVA: sc1: $F_{2,6} = 20.48$, $p = 0.002$; sc2: $F_{2,6} = 6.47$, $p = 0.031$; sc3: $F_{2,6} = 10.76$, $p = 0.010$; sc4: $F_{2,6} = 53.96$, $p < 0.001$). Helmet length of both induction treatments with *D. cucullata* and *D. magna* were in each body-length size class significantly longer than that

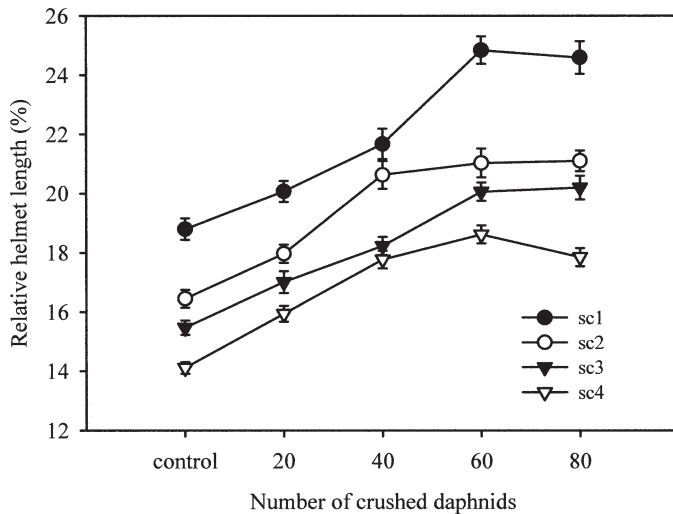


Fig. 2. Concentration-dependent helmet induction in *D. cucullata* with chemical cues released by crushed conspecifics (20 crushed daphnids L^{-1} ; 40 crushed daphnids L^{-1} ; 60 crushed daphnids L^{-1} ; 80 crushed daphnids L^{-1}). Symbols with standard error bars indicate the mean relative helmet length (%). The daphnids were divided into four body-length size classes (sc1: $< 600 \mu m$; sc2: 600–800 μm ; sc3: 800–1,000 μm ; sc4: $> 1,000 \mu m$) for analysis.

of the control (Tamhane post hoc tests: all $p < 0.001$). *D. cucullata* showed in size classes 2–4 significantly longer helmets in response to conspecific cues compared with alarm cues from *D. magna* (Tamhane post hoc tests: sc1 = ns; sc2–3: $p < 0.001$; sc4: $p = 0.004$; Fig. 3).

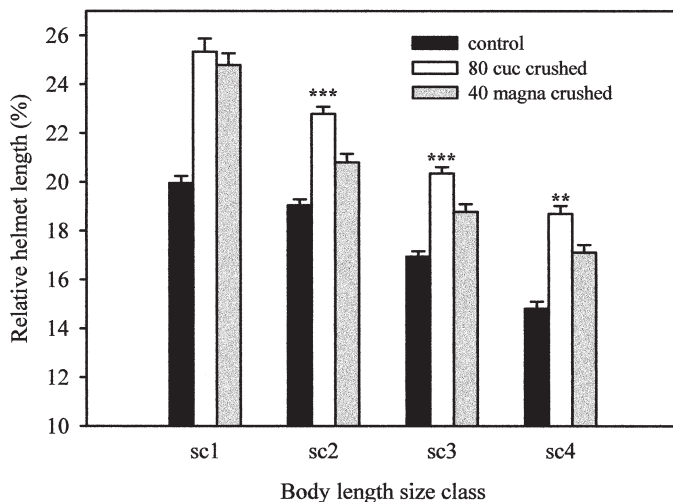


Fig. 3. Helmet induction in *D. cucullata* with chemical cues released by crushed daphnids (80 cuc crushed = 80 crushed *D. cucullata* L^{-1} ; 40 magna crushed = 40 crushed *D. magna* L^{-1}) from different species. Standard error bars indicate the mean relative helmet length (%). The daphnids were divided into four body-length size classes (sc1: $< 600 \mu m$; sc2: 600–800 μm ; sc3: 800–1,000 μm ; sc4: $> 1,000 \mu m$) for analysis. Asterisks indicate significant differences between the two treatments: 80 crushed *D. cucullata* and 40 crushed *D. magna* (***, $p < 0.001$; **, $p < 0.01$). The differences from the control were significant ($p < 0.001$) in all cases.

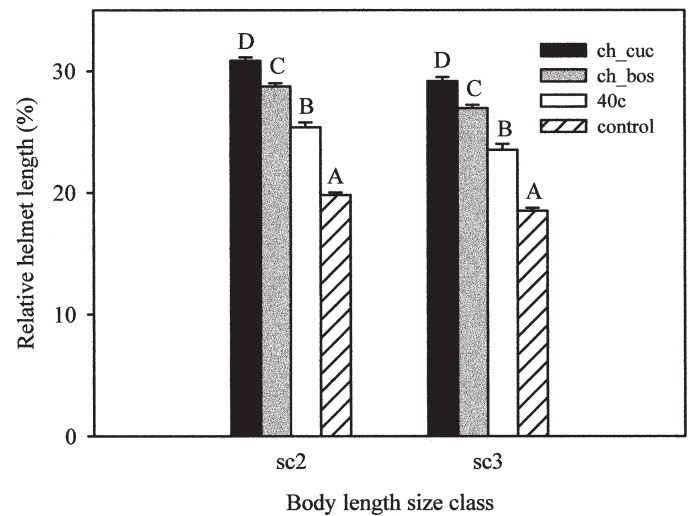


Fig. 4. Mean relative helmet length (%) of *D. cucullata* induced with chemical cues released by predators compared with alarm cues released by crushed conspecifics (ch_cuc: *Chaoborus* larvae fed daily with 40 *D. cucullata*; ch_bos: *Chaoborus* larvae fed daily with 300 *Bosmina longirostris*; 40c: 40 crushed *D. cucullata* L^{-1} ; control without any addition). Two body-length size classes were used for analysis (sc2: 600–800 μm ; sc3: 800–1,000 μm). Arcsin-square-root-transformed data were tested using a nested ANOVA. Homogeneous groups (A–D; all $p < 0.001$) are based on Tamhane post hoc tests.

Experiments with predator kairomones revealed a distinctly stronger induction of the plastic traits compared with alarm cue induction in all tested *Daphnia* species.

Helmet length of *D. cucullata* differed significantly between treatments in both body-length size classes in the predator kairomone experiment (nested ANOVA: sc2: $F_{3,8} = 30.05$, $p < 0.001$; sc3: $F_{3,8} = 23.46$, $p < 0.001$; Fig. 4). *D. cucullata* showed the strongest helmet elongation in both body-length size classes in the treatment with phantom midge larvae fed with conspecifics (Tamhane post hoc test: all $p < 0.001$). Also, helmets of *D. cucullata* induced with kairomones released by *Chaoborus* larvae fed with *Bosmina* were significantly longer than helmets induced with alarm substances from *D. cucullata* in each size class (Tamhane post hoc test: all $p < 0.001$). Additionally, helmets of *D. cucullata* induced with alarm substances were significantly longer than that of the control (Tamhane post hoc test: all $p < 0.001$).

Similarly, *D. longicephala* showed in the predator and in the alarm-cue treatment significantly longer helmets and crests than the control animals (nested ANOVA: relative helmet length: $F_{3,4} = 443.14$, $p < 0.001$; relative crest height: $F_{3,4} = 936.35$, $p = 0.001$; Tamhane post hoc test: all $p < 0.001$; Fig. 5). Both the relative helmet length and the relative crest height of *D. longicephala* were distinctly larger in the predator treatment as compared with the alarm-cue and the control treatments (Tamhane post hoc test: all $p < 0.001$). We observed no difference in any of the morphological traits between both alarm cue concentrations (Tamhane post hoc test: ns).

Also, in the experiment with *D. lumholtzi*, the daphnids induced by predator kairomones showed significantly

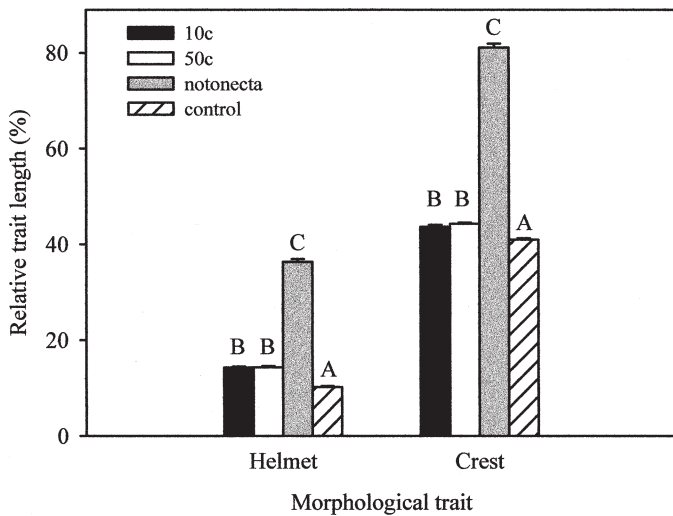


Fig. 5. Mean relative helmet length (%) and relative crest height of *D. longicephala* induced with chemical cues released by the invertebrate predator *Notonecta* compared with different concentrations of alarm cues released by crushed conspecifics (10c: 10 crushed *D. longicephala* L⁻¹; 50c: 50 crushed *D. longicephala* L⁻¹; notonecta: one *Notonecta* fed daily with 10 *D. magna*; control without any addition). Morphological parameters measured were the relative helmet length (Helmet) and the relative crest height (Crest). Arcsin-square-root-transformed data were tested using a nested ANOVA. Homogeneous groups (A–B; all $p < 0.001$) are based on Tamhane post hoc tests.

longer helmets than both control animals and the animals exposed to different concentrations of alarm cues (nested ANOVA: $F_{3,4} = 34.78$, $p = 0.002$; Tamhane post hoc test: all $p < 0.001$). Only the treatment with 10 macerated daphnids showed a significant difference from the control (Tamhane post hoc test: $p = 0.006$). The treatments with 50 crushed animals showed no significant difference in the relative helmet length in this experiment.

Discussion

Our experiments revealed that the morphologically plastic traits of different *Daphnia* species are indeed affected by chemical cues from macerated conspecifics. All three *Daphnia* species responded with the induction of their defenses. Induction of the plastic traits resembled a saturation-dose-response curve (Fig. 2) as it is known from responses to predator cues (Tollrian 1993). However, all tested daphnids showed a distinctly stronger induction effect when exposed to chemical cues released by their predators. A particular *Daphnia* species exposed to alarm stimuli may be able to sense a current predation risk, but the chemical information gathered is not specific to any predator. Therefore, a predator-specific response cannot be activated. In this context, alarm cues are unspecific, which is important if predators with contrasting selectivity are present, e.g., invertebrates and fish. However, even if alarm cues provide no particular information about the predator, they could be relevant in situations where the predators supply insufficient information or where many predator species with similar selectivity co-occur in low densities.

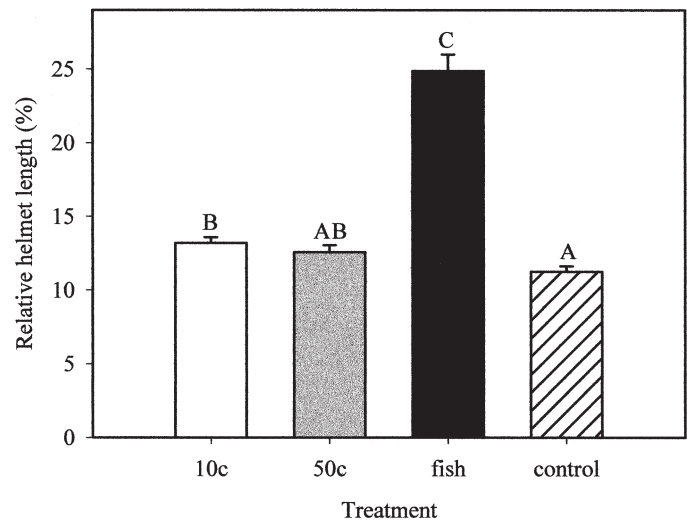


Fig. 6. Mean relative helmet length (relhel %) of *D. lumholtzi* induced with chemical cues released by fish compared with different concentrations of alarm cues released by crushed conspecifics (10c: 10 crushed *D. lumholtzi* L⁻¹; 50c: 50 crushed *D. lumholtzi* L⁻¹; fish: 1 fish 3 L⁻¹; control without any addition). Arcsin-square-root-transformed data were tested using a nested ANOVA. Homogeneous groups (A–C, all between $p < 0.01$ and $p < 0.001$) are based on Tamhane post hoc tests.

Potentially, their specific cues would not reach a sufficient concentration, but the combined alarm cues could indicate a significant predation risk. Another possible adaptive value of these damage-released cues could be that alarm cues lead to a general alertness state. Induced alertness has been indicated by the results of Pijanowska (1997), who showed that *D. magna* induced with crushed conspecifics avoided attacks by fish significantly more often than naïve daphnids. Similarly, Hews (1988) considered that prey alertness induced by alarm signals may be responsible for different capture efficiencies by a predatory dragonfly on tadpoles and low concentrations of chemical alarm cues in fish were shown to increase vigilance toward optical secondary cues (Brown et al. 2004). In this alertness state, daphnids can decide how to respond when the most likely source of danger is identified.

Although all examined *Daphnia* species showed a significant response to damage-released alarm cues, this effect was definitely moderate in comparison with the induction by water-borne cues from predators (Figs. 4–6). Our data revealed that *D. cucullata* shows significantly longer helmets in the induction treatment with *Chaoborus* larvae fed with conspecifics as compared with the treatment of *Chaoborus* larvae fed with *Bosmina*. However, *D. cucullata* responded to *Chaoborus* larvae feeding on *Bosmina* much stronger than to conspecifics alarm cues. Taking into account that *D. cucullata* and *Bosmina* share the same habitat and are threatened by similar predators, it is tempting to speculate that it would be adaptive to respond even to cues from a prey species of a slightly lower size range, because *Chaoborus* larvae switch quickly to a bigger prey-size range while growing, and even in their last instar feed on both types of prey. The stronger effect of

Chaoborus fed with *D. cucullata* should not have been a food concentration effect because we had provided equal prey biomass and all prey were consumed. Thus, alarm cues should have been nearly identical in both experiments. Hence, our data suggest that *D. cucullata* is able to discriminate between cues released from predators consuming conspecifics or heterospecifics. Similarly, our cross-species experiments suggest that daphnids can partly distinguish between alarm cues from conspecifics and from related species. However, we cannot rule out the possibility that the amount of latent cues in the tissues differs between species.

Similar cross-species responses on injury-released alarm cues have been reported in a variety of aquatic animals (e.g., tadpoles, Pfeiffer 1966; fish, Smith 1982; crayfish, Hazlett 1994). Thus, our results suggest that daphnids can differentiate between predators foraging on any kind of prey and a predator feeding on conspecifics. Predators feeding on a relatively large zooplankton species, such as *D. magna*, usually show a different size selectivity as compared with predators foraging on *D. cucullata*. In this context, alarm cues from *D. magna* should not be as relevant as alarm cues from conspecifics for *D. cucullata* in assessing the predation risk. Although *D. cucullata* and *D. magna* are not sharing the same environment, the morphological changes in *D. cucullata* as a response to body fluids from *D. magna* may be based on their phylogenetic relatedness, as has been reported for tadpoles (Schoeppner and Relyea 2005).

There is no evidence that *Daphnia* produce a specific chemical substance that has evolved as an alarm signal for daphnids, as reported for fish tissue (e.g., Smith 2000; Bryer et al. 2001). It seems more likely that daphnids evolved sensitivity to detect general components of crushed conspecifics. So far, still little is known about the molecular structure of the chemical compounds responsible for activating morphological plasticity in cladocerans in nature, although some progress has been made to identify the chemical characteristics of kairomones for *Chaoborus* (Tollrian and von Elert 1994) and fish (von Elert and Loose 1996). Therefore, two possible explanations for the enhanced effect on morphological defenses in *Daphnia* of damage-released alarm cues and predator kairomones can be hypothesized. Either there is a synergistic effect when both cues are given simultaneously, or predators produce a different quality of kairomone when feeding on conspecific prey. Hereby, the alarm cues may be modified and activated by enzymatic processes in the gut of a predator. That kind of predator labeling, where alarm substances accumulate inside the predator and are released subsequently in relevant concentrations, is reported for many predator-prey interactions (e.g., cnidaria, Howe and Harris 1978; insects, Chivers et al. 1996; fish, Stabell and Lwin 1997; echinoderms, Hagen et al. 2002). Similarly, even alien fish species were shown to be labeled by *Daphnia*-specific cues, indicating that cellular fluids of prey were altered by intestine enzymes, resulting in the actually chemically active compound (Stabell et al. 2003). Nevertheless, what is true for a fish predator-prey system is not necessarily transferable to an invertebrate system. Jacobsen and Stabell (2004)

demonstrated in a marine gastropod that both cues, tissue fluids and latent conspecifics chemicals modified by a predatory crab, are responsible for antipredator behavior. Therefore, also in *Daphnia*, a mixture of several components may constitute the active substance, including injury-released alarm cues. Similarly, Slusarczyk (1999) concluded in his experiments that kairomones and chemical alarm cues act synergistically to induce resting-egg production in *D. magna*. Likewise, Appleton and Palmer (1988) revealed for snails threatened by a predatory crab that damage-released alarm cues combined with predator-released cues are more effective in inducing morphological defenses. These combined chemical cues certainly increase the information quality and the reliability of the predation risk estimation. Taking into account that alarm cues from crushed conspecifics and kairomones released by predators, which might be labeled by a specific prey, are responsible for the activation of inducible defenses, might lead to a new impulse to investigate the chemical compounds involved in this process in nature and will have an influence on our knowledge of regulating mechanisms in population dynamics, especially on the role of information-transfer networks that overlay the classical food webs.

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