

Extreme helmet formation in *Daphnia cucullata* induced by small-scale turbulence

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*Although *Daphnia cucullata* is used as a textbook example for cyclomorphosis, distinct helmet development, as shown in the field, has not been demonstrated in the laboratory until now. We show for the first time that small-scale turbulence is able to induce the maximum response of morphological plasticity in *D. cucullata*. Helmet elongation reached magnitudes as extreme as observed in the field. Different modes of generating small-scale turbulence caused different levels of helmet formation. Small-scale turbulence alone may not be responsible for cyclomorphosis in nature because field data show that two nearby lakes with similar morphometry differ in cyclomorphosis patterns, while laboratory experiments show that there is no difference in the ability to form helmets in the clones of each lake. Although helmet formation in *D. cucullata* is inducible with predator kairomones, helmet elongation is not as strong as that induced by turbulence. We discuss the possible role of helmets under turbulent conditions.*

INTRODUCTION

Cyclomorphosis, which means seasonal variation in morphological traits, has attracted the attention of scientists since the beginning of the last century (Wesenberg-Lund, 1900). A variety of *Daphnia* species are reported to show these polymorphisms. For example *Daphnia pulex* generates neck teeth, *Daphnia carinata* forms huge crests, and *Daphnia galeata* and *Daphnia retrocurva* both develop helmets [for a review, see (Jacobs, 1987)]. In early studies abiotic factors were predominantly discussed as proximate cues for cyclomorphosis in Cladocera. In this context temperature (Ostwald, 1904; Wesenberg-Lund, 1908; Coker and Addlestone, 1938; Jacobs, 1967), water turbulence (Brooks, 1947; Hrbáček, 1959), light (Hazelwood, 1966), food conditions (Jacobs, 1967) and the combined effects of these parameters were tested in field studies or laboratory experiments. However, only sparse induction effects were obtained in these studies. At that time, a parachute effect to reduce sinking speed (Wesenberg-Lund, 1908, 1939) or a balance function to facilitate horizontal swimming (Woltereck, 1913, 1921) of the inducible traits was speculated. Since Gilbert (Gilbert, 1966) showed that cyclomorphic changes in rotifers are inducible with chemical cues released by predators,

research has focused on biotic interactions. In recent years many organisms have been reported to be inducible by predator kairomones and a defensive effect of the inducible traits could be demonstrated for several of these examples [for a review, see (Tollrian and Harvell, 1999)].

Likewise, laboratory experiments with *Daphnia cucullata*, a very common species in eutrophic lakes across Europe (Flößner, 1972), showed that elongated helmets could be induced with kairomones and act as inducible defences against several invertebrate predators (Tollrian, 1990; Agrawal *et al.*, 1999; Laforsch and Tollrian, 2004). These results are in accordance with Pijanowska's (Pijanowska, 1991) suggestion that high helmets in *D. cucullata* in two Polish lakes are linked to the abundance of invertebrate predators. However, cyclomorphic changes of the magnitude observed in the field have not previously been induced in the laboratory. Results of Hrbáček (Hrbáček, 1959) imply that helmets of *D. cucullata* are slightly inducible with water turbulence.

In our work we return to this old idea experimentally, using two different methods for generating small-scale turbulence. Additionally, we monitored cyclomorphosis in two lakes from the same area with similar morphometries, tested for clonal differences concerning turbulence

induction and compared laboratory experiments with field data. To test for induction strength we compared predator kairomone and turbulence as inducing agents for these plastic traits of *D. cucullata*. Furthermore, we tested inducibility of *D. cucullata* with small-scale turbulence under cold conditions.

METHOD

Clones of *D. cucullata* were isolated from Lake Thalersee and Lake Bansee, Germany. The animals were reared in artificial medium (Laforsch and Tollrian, 2004) and were fed daily with *Scenedesmus obliquus* [1.5 mg carbon (C) L⁻¹]. The experiments were conducted in the laboratory under constant conditions in 1.5 L glass beakers at 20°C and under fluorescent light. Twenty primipar age-synchronized non-helmeted animals with freshly deposited eggs were randomly introduced into each beaker. The medium was changed weekly in each vessel.

Turbulence-1 set-up (Figure 1a)

In this set-up, a PVC disk driven by an electric motor revolves permanently. A piece of silicone tube is attached to the front of the disk. The silicone tube alternately drives three paddles made of acrylic (10 cm/3 cm). The paddles are installed in a rack. A smooth motion is guaranteed by ball bearings. The length of the silicone tube determines the degree and the frequency of the spin of the paddles. In our experiment, the paddle made a half-revolution every 5 s. A strong small-scale turbulence was generated with this method, which the animals apparently survived well. The daphnids obviously had a high reproductive success and no dead animals were found during the weekly medium exchange inside the beaker. This pulse-generated turbulence was optically verified in a prior test with stained water (KMnO₄). Three glass beakers could be placed into this device in accordance with the number of paddles. Three vessels without turbulence served as controls.

Turbulence-2 set-up (Figure 1b)

A gel-dyer (KS 501; IKA Labortechnik) was used for the second method. Three glass beakers were placed on the dyer on a 2 cm polystyrene sheet to avoid heat transfer between the dyer and the beakers. A rod made of acrylic (2 cm wide) was fixed from above over the beakers. The gel-dyer ran at 30 r.p.m. The rod would have generated only a laminar flow due to the permanent spin of the dyer (tested with stained water), so a plate made of acrylic (18 cm/4 cm) was also attached at the edge of the glass beaker to break the laminar flow and to generate small-scale turbulence.

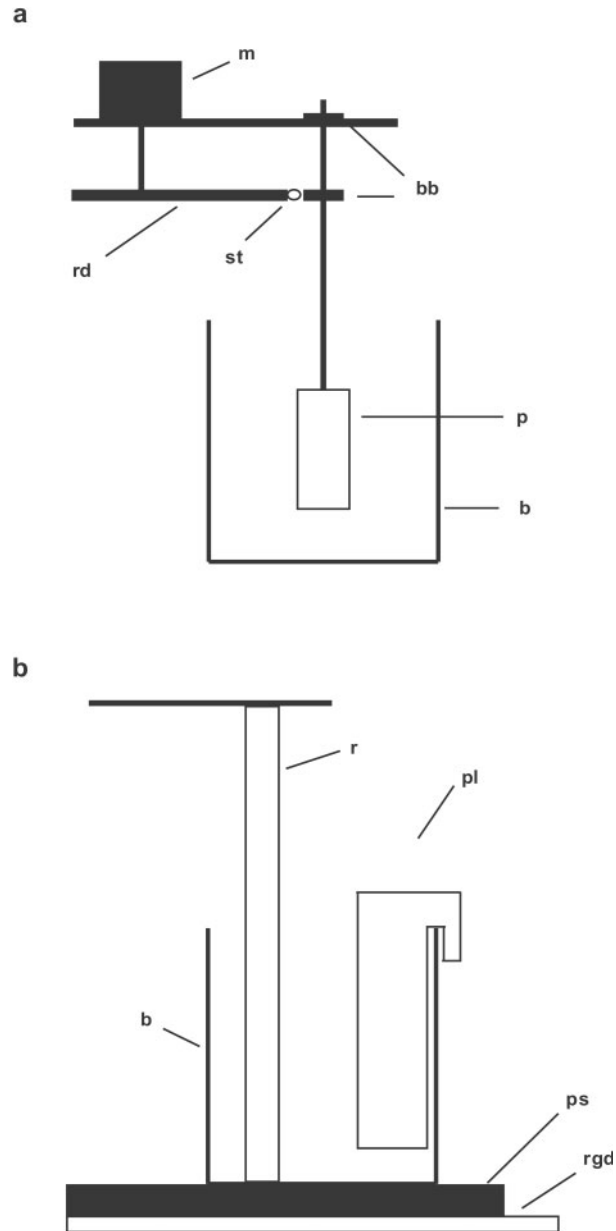


Fig. 1. Schematic line drawings of both turbulence generating set-ups. **(a)** Turbulence-1 set-up. Abbreviations: m, electric motor; rd, permanently rotating PVC disk driven by the motor; st, piece of silicone tube attached to the front side of the PVC disk; bb, ball bearings; p, paddle; b, beaker. **(b)** Turbulence-2 set-up. Abbreviations: rgd, permanently rotating gel dyer; ps, polystyrene sheet; r, acrylic rod; pl, acrylic plate; b, beaker.

Both turbulence experiments were conducted simultaneously with *D. cucullata* originating from Lake Thalersee.

Field samples

Field samples were collected from mid-May to mid-August of 1999 from Lake Thalersee (3.8 ha; z_{\max} 7.0 m)

and Lake Bansee (3.3 ha; z_{\max} 4.1 m) in southern Germany. Both lakes are eutrophic and located in the same area and do not differ much in their topography. Lake Thalersee is completely surrounded by forest, but Lake Bansee is only ~60% surrounded, and therefore Lake Thalersee is slightly better protected from wind impact. *Daphnia cucullata* was reported to show distinct cyclomorphosis every year in Lake Thalersee (Pawlowski, 2000), whereas no cyclomorphic change was observed in Lake Bansee (Siebeck, personal communication). Two net hauls (250 μm mesh size; 25 cm diameter; 50 cm height; Hydrobios, Germany) were drawn from the deepest point of each lake on each sampling date. The samples were conserved in sugar-formalin (4%). Fifty randomly selected *D. cucullata*, with body sizes between 600 and 800 μm body length, were used for analysis at each sample date.

Clonal comparison

Because we found that daphnids from Lake Thalersee showed a distinct cyclomorphosis during the course of the year, whereas daphnids from Lake Bansee did not, we compared two clones from both lakes regarding turbulence induction.

The turbulence-1 set-up was used for the clonal comparison experiment. The experiment was replicated three times.

Comparison of predator kairomone and turbulence

Ten individuals of fourth-instar larvae of *Chaoborus flavicans* were placed in a cylindrical cage made of acrylic (10 cm diameter and 12 cm length) and 100 μm nylon mesh located inside the glass beakers. This method was used to ensure spatial separation between predators and *D. cucullata* originating from Lake Thalersee while still guaranteeing the exchange of chemical cues. The predators were fed daily with different kinds of prey. Pupate phantom midge larvae and dead predators were replaced and the cages were cleaned daily. The experiment was replicated three times. Beakers without predators served as controls.

Temperature experiment

Coker and Addlestone (Coker and Addlestone, 1938) reported an upper threshold value of 11°C for cyclomorphosis in *Daphnia longispina*. Therefore, an additional experiment with *D. cucullata* originating from Lake Thalersee was conducted in a climate chamber at a constant level of 8°C using the turbulence-1 set-up to test whether *D. cucullata* is able to form helmets in cold environments.

Data analysis

All experiments lasted 3–5 weeks. After that time period, ~100 daphnids were randomly selected from each beaker. These animals were measured under a dissecting microscope with a digital image-analysis system (Soft Imaging System; Analysis Pro, Münster, Germany). The morphological parameters recorded from *D. cucullata* were helmet length (defined as the length between the tip of the helmet and the upper edge of the compound eye) and body length (defined as the length between the upper edge of the compound eye and the base of the tail spine). For analysis, the daphnids were divided into four body-length size classes (size class 1, <600 μm ; size class 2, 600–799 μm ; size class 3, 800–1000 μm ; size class 4, >1000 μm). In the predator kairomone induction experiment only body-length size class 2 was used for analysis, because cyclomorphic traits are most pronounced during this life stage.

To compensate for small size-dependent changes in helmet length within the classes, we calculated the relative helmet length (helmet length/body length \times 100) of the animals in all experiments. The three replicates per treatment could be pooled for analysis in all cases because they showed no significant within-treatment difference in Kruskal–Wallis tests. Conservative non-parametric Mann–Whitney *U*-tests were then used to compare the two independent groups: treatment and control. The significance level in multicomparisons was adjusted to $P \leq 0.017$ for three treatments according to Rice (Rice, 1989). The relative values were arcsin-square-root transformed prior to analysis (Sokal and Rohlf, 1995) in the predator kairomone experiment. A one-way ANOVA was then performed to test for treatment effects.

RESULTS

Helmet elongation of *D. cucullata* originating from Lake Thalersee was distinctly stronger in the turbulence-1 set-up than in the turbulence-2 set-up (Figure 2a; Mann–Whitney *U*-test; size class 1–3, all $P < 0.001$; size class 4, $P < 0.01$). Additionally, the maximum helmet length induced with the turbulence-1 set-up (56.00% relative helmet length in body-length size class 600–800 μm) was longer than the maximum helmet length (44.18% relative helmet length in body length size class 600–800 μm) observed in the field in this study. Furthermore, field data show that cyclomorphosis in *D. cucullata* differs between each lake. Daphnids from Lake Thalersee showed the typical cyclomorphic change of their plastic traits, while animals from Lake Bansee showed almost no difference in helmet formation (Table I). Laboratory

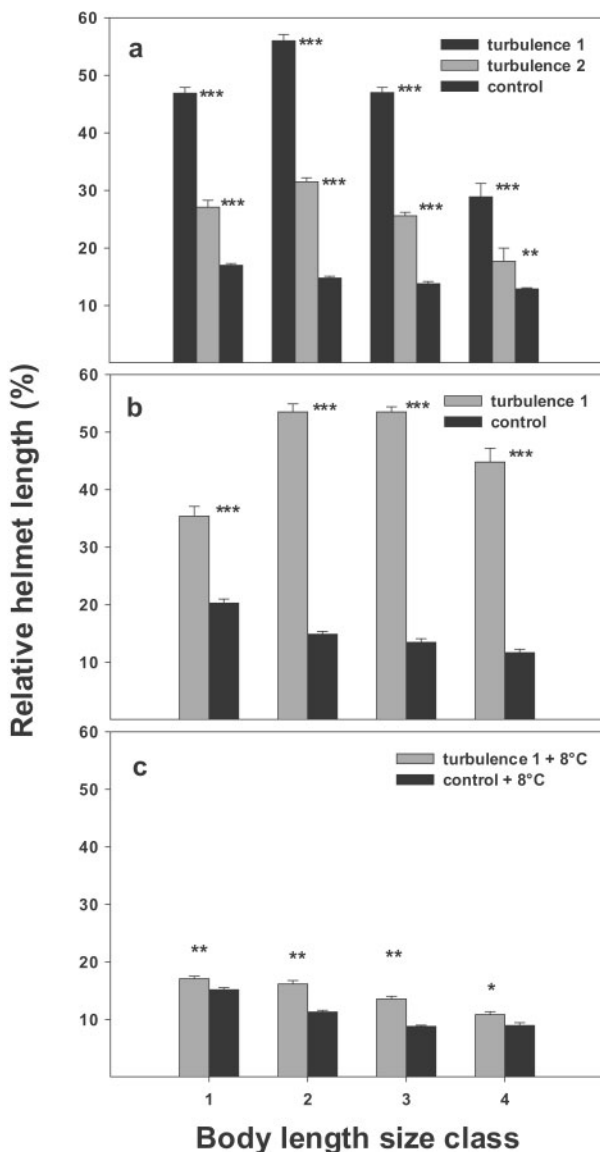


Fig. 2. Relative helmet length of *D. cucullata* from two lakes [(a) clone from Lake Thalersee; (b) clone from Lake Bansee] in the control and in the induction treatment with small-scale turbulence. Turbulence was generated using two different methods (turbulence 1 and turbulence 2). (c) Helmet induction under cold (8°C) conditions with turbulence-1 set-up and a clone from Lake Thalersee. Asterisks indicate significant differences from the control (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$).

experiments with different clones of *D. cucullata* from both lakes revealed that daphnids showed significantly longer helmets in induction experiments with turbulence than did the controls (Figure 2a and b). Furthermore, daphnids from Lake Bansee show an almost identical inductive pattern as *D. cucullata* from Lake Thalersee (Figure 2b).

Daphnids induced with predator kairomones showed significantly longer helmets than control *Daphnia*. However, helmet length was significantly longer in the

turbulence-1 treatment as compared with the predator-kairomone induction (Table II). Although helmet length of *D. cucullata* differed significantly in the 8°C experiment between turbulence treatment and control (Mann-Whitney *U*-test; size class 1–4, all $P < 0.05$; Figure 2c), helmet growth of the daphnids was approximately three times lower under cold turbulent conditions as compared with warm turbulent environments (Figure 2a–c).

DISCUSSION

During its cyclomorphosis, *D. cucullata* exhibits a maximum shaping of 55% relative helmet length (Hrbáček, 1959) in nature. We have shown for the first time that such extreme helmet formation can be induced by small-scale turbulence in the laboratory. Hereby, the method used to generate these turbulences seems to be crucial because different modes of small-scale turbulences induce different levels of helmet formation in *D. cucullata* (Figure 2a). Although our study confirms Hrbáček's idea that turbulence acts as an inducing agent, our results that different kinds of turbulence vary in effectiveness may also explain why he failed to induce high helmets in *D. cucullata*. He speculated that cyclomorphosis is linked to water circulation caused by wind stress on the surface. But the strongest wind effect moving the water column would be expected during lake circulation in spring and autumn, exactly at the time when *D. cucullata* usually shows its non-helmeted morphs. Even our field data indicate that water circulation caused by wind exposure seems not to be the reason for helmet formation in *D. cucullata*. The two neighbouring lakes in our study show an almost identical morphometry. Furthermore, Lake Thalersee is surrounded by forest and is therefore less exposed to wind impact. Nevertheless, animals from Lake Thalersee have higher helmets and the population in Lake Bansee showed no cyclomorphic pattern. Our laboratory induction with water turbulence revealed an almost identical helmet elongation of different clones originating from both lakes, suggesting that the patterns of cyclomorphosis in the field are not caused by clonal differences in phenotypic plasticity. Thus, large-scale turbulence caused by wind impact may not act as inducer, but rather small-scale turbulence, possibly generated by shear between stratified microlayers. Although we can induce maximum helmet size with our turbulence-1 set-up, this turbulence seems to be rather strong compared with natural conditions. On the other hand, we may not conclude that we found the most effective way of generating turbulence which caused cyclomorphosis.

While we identified a clear defensive effect of the induced traits in *D. cucullata* against various invertebrate

Table I: Seasonal course of mean relative helmet length (%) and standard error (SE) of *D. cucullata* (body-length size class: 600–800 μm) sampled in two different lakes with similar morphometry and similar wind effects

Date	Bansee			Thalersee		
	Mean	SE	<i>n</i>	Mean	SE	<i>n</i>
27/5	14.08	0.62	22	23.09	0.74	50
18/6	13.05	0.54	49	44.18	0.90	50
30/7	8.76	0.31	48	24.62	0.58	49
19/8	11.00	0.34	48	28.01	0.67	49

Table II: Mean relative helmet length of *D. cucullata* (body-length size class: 600–800 μm) originating from Lake Thalersee exposed to turbulence (set-up 1), raised with (kairomone) and without (control) chemical cues released from *C. flavicans*

Treatment	Mean	SE	<i>n</i>	H	ANOVA
Turbulence	55.94	1.10	51	A	$F_{2, 318} = 1363.3$
<i>Chaoborus</i>	30.61	0.29	135	B	$P < 0.001$
Control	18.86	0.24	135	C	

Data were tested using one-way ANOVA. The respective ANOVA values and probabilities are given. Letters (H) indicate homogeneous groups based on Tamhane post hoc tests. All groups differ significantly from both other treatments (all $P < 0.001$).

predators (Laforsch and Tollrian, 2004), *Chaoborus*-released chemicals failed to induce maximum head shape in *D. cucullata* in this and in previous studies, suggesting that predator cues themselves could not exclusively explain extreme cyclomorphosis in nature. Perhaps helmets alter hydrodynamics, leading to an as yet unknown benefit. Hrbáček (Hrbáček, 1958) revealed that *D. cucullata* only occurs in lakes and ponds containing high densities of fish. He therefore concluded (Hrbáček, 1959) that helmets provide the capability of staying in deeper layers of a circulating water column, thereby allowing daphnids to avoid fish predation. But *D. cucullata* is a very small cladoceran species which occupies the entire water column, even when fish are present (Pawlowski, 2000) because *D. cucullata* does not fit into the prey-size spectrum of adult fish. Therefore, even an elongated helmet that is completely transparent should not increase vulnerability to adult fish predation, but vice versa cyclomorphic traits could possibly be a defence against young fish. For example, the long helmets of *Daphnia lumholtzi* have been shown to act as protection against fish (Kolar and Wahl, 1998). In this context, it seems possible that helmet formation in *D. cucullata* is somehow connected to the swimming activity

of fish as also suggested by Lampert and Wolf (Lampert and Wolf, 1986), because the cyclomorphosis peak of the plastic traits is concordant with the abundance and activity of young-of-the-year fish (0+). These young fish can aggregate to dense swarms of several thousand individuals (Wanzenböck, personal communication). Thus, it is possible that fish or invertebrate predators cause an effectively inducing small-scale turbulence pattern through their movements.

Our result that helmet elongation is just barely inducible in cold environments may suggest that in natural habitats the defence is not required, because even predators are less active in cold conditions. Relating to this, we can hypothesize that high helmets additionally restrict the manoeuvrability of daphnids while moving in a more viscous medium. For the evolution of phenotypic plasticity, benefits in one environment have to be offset by costs in the other environments (Tollrian and Harvell, 1999), otherwise a selection on constitutive defences would be favoured. Trade-offs have not yet been identified in this system. The direct allocation of energy to helmet development is relatively small and costs are not measurable (Tollrian, 1991). Therefore, it might be speculated that other costs, e.g. an increased



Fig. 3. Scanning electron micrograph [for method see (Laforsch and Tollrian, 2000)] showing frontal and dorsal view of induced individuals of *D. cucullata*.

hydrodynamic drag when the water temperature is colder, could be relevant.

To summarize, the role of small-scale turbulence regarding cyclomorphosis in *D. cucullata* still remains unclear. It has been shown that helmets of *D. cucullata* are inducible both with biotic and with abiotic cues. Therefore it seems

possible that helmets provide additional benefits beside the proven defensive effect against invertebrate predators. Hence, synergistic effects of different parameters could be responsible for extreme helmet formation in nature, indicating that inducible helmets have evolved as a multi-tool for different environmental conditions.

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