



Specific Turbulence- and *Chaoborus*-Induced Morphotypes Affect the Streamlining Properties of *Daphnia cucullata*

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Inducible defenses are a wide-spread defensive mechanism in *Daphnia*. For example, *D. cucullata* is known to form different adaptive morphologies under changing environmental conditions. In this species, predator presence elicits defensive helmets. Defended animals perform better and survive more frequently in predation attempts. Another peculiarity of *D. cucullata* is that they develop these defenses also in response to turbulence. The mechanisms that underly this defensive effect are so far unclear. Therefore, we investigated the three-dimensional morphology of the typical, the turbulence- and *Chaoborus*-exposed morph and analyzed their surface and volume alterations. We furthermore measured the swimming velocities of these morphs. With the determined shapes and the measured velocities, we simulated the drag force under different angles of attack and determined the energy necessary to move through the water. In the light of previous findings and hypotheses we here discuss biomechanical mechanisms that improve *D. cucullata*'s fitness in the investigated environments.

Keywords: inducible defense, streamline simulation, adaptive morphology, swimming velocity, drag force, phenotypic plasticity, *Daphnia cucullata*

INTRODUCTION

Predation is an almost omnipresent threat in ecosystems, but it varies in intensity (e.g., Volterra, 1926). Therefore, many species are phenotypically plastic and counter predators by inducible defenses (Tollrian and Harvell, 1999). Such inducible defenses are formed by many species of the genus *Daphnia*. Especially *Daphnia cucullata*, a common freshwater crustacean in European lakes, has been a textbook example of cyclomorphosis and phenotypic plasticity for decades (Woltereck, 1913; Jacobs, 1987; Lampert and Sommer, 1993; Laforsch and Tollrian, 2004c). Nowadays, the cyclomorphosis especially of helmets and crests in different *Daphnia* species is well-known and accepted as a phenotypically plastic trait in response to predators (Tollrian and Harvell, 1999; Weiss and Tollrian, 2018). Historically, the elicitor inducing the different morphs remained inconclusive for a long time (Wesenberg-Lund, 1900; Brooks, 1947, 1965; Jacobs, 1961, 1962). It had been hypothetically attributed to abiotic factors such as wind (and therefore water turbulence, Hrbáček, 1959) or temperature (Jacobs, 1961, 1967) and was hence thought to be a seasonal variation as

observed in other species, e.g., the lepidopteran *Araschnia levana* with its spring and summer brood (Shapiro, 1976; Windig and Lammar, 1999).

Later publications showed that a morphological alteration could be observed in response to different independent cues, i.e., turbulence and predation. Invertebrate predators, such as the phantom midge larvae *Chaoborus*, induce defensive helmets increasing survival chances during predator presence (Laforsch and Tollrian, 2004c). Helmeted individuals are more frequently attacked, but evasion and escape efficiency were found strongly increased, weighing up the increased attack rate (Laforsch and Tollrian, 2004c). The protective effect of the helmet enabling prey evasion is however still unknown.

In response to fish, *D. cucullata* changes its morphology to a smaller and transparent body (Hrbáček, 1962). A behavioral alteration such as diel vertical migration has not been observed yet (Hrbáček, 1962; Lampert and Wolf, 1986; Tollrian, 1990; Laforsch and Tollrian, 2004c; Tollrian and Laforsch, 2006; Karpowicz et al., 2019). Intriguingly, helmets are also induced by turbulence, and also here it is still unknown why (Hrbáček, 1959; Laforsch and Tollrian, 2004b). Turbulence is discussed as an indicator of vertebrate predator presence, but also of wind as a measure for season (Laforsch and Tollrian, 2004b,c).

Different authors hypothesized that seasonal morphology, which reaches its maximum expression strength in summer and disappears in winter (Lampert and Wolf, 1986; Laforsch and Tollrian, 2004c), could also be correlated with water viscosity, as viscosity is a function of temperature (Reynolds, 1886). Hebert suggested that in higher viscosities more muscle force and accordingly more energy is necessary for locomotion (Hebert, 1978). The observed morphologies would therefore be a proxy for altered muscle position and size. He also suggested the helmets of *Daphnia* could be involved in gas exchange (Hebert, 1978). Jacobs moreover showed that helmeted *Daphnia* are more efficient swimmers than unhelmeted morphs (Jacobs, 1964).

In order to reveal the underlying mechanisms and explain the function of the observed behavioral and morphological alterations in *D. cucullata*, we analyzed the shape of animals exposed to turbulence and *Chaoborus* predation and compared them using Procrustes distance (Horstmann et al., 2018, 2021). We also examined their swimming velocities. Combining morphology and behavior, we calculated drag forces, the “friction” of *D. cucullata* in water, enabling to quantify their streamline properties. We calculated drag force with water impinging in the typical forward-movement angle and from beneath, resembling the sinking phases. We furthermore tested the influence of water temperature (and therefore water viscosity) on drag force. By this we approximated the costs and benefits of these alterations, governing their ecological effect.

MATERIALS AND METHODS

Animal Culture

D. cucullata clone TSBR, originating from Lake Thalersee (near Munich, Germany) were cultured in the department for several generations under laboratory conditions (16:8-h day-night cycle,

20°C ± 1°C. Animals were reared in 1 L glass beakers (J. Weck GmbH und Co. KG, Wehr-Öflingen, Germany) containing charcoal filtered tap water and were fed *ad libitum* with the green algae *Tetradesmus obliquus*. Culture beakers and beakers of the experiments were cleaned every other day and water was exchanged monthly. *Chaoborus* sp. larvae of the 4th instar were caught in the Botanical Gardens of the Ruhr-University Bochum and kept in 1.5 L glass beakers at 4°C in charcoal-filtered tap water.

Chaoborus Induced *Daphnia cucullata*

Inductions were started with 5 female *D. cucullata* clone TSBR carrying offspring in the last embryonic stage, which is indicated by the occurrence of one black eye (Weiss et al., 2016), placed in 1 L glass beakers (J. Weck GmbH und Co. KG, Wehr-Öflingen, Germany). For predator exposure, we added 10 *Chaoborus* larvae and 100 juvenile *D. cucullata* TSBR and placed them into net cages prohibiting predation on the test animals (Laforsch and Tollrian, 2004b). After female mothers had released juveniles from the brood pouch, they were removed from the glass beakers.

Turbulence Induced *Daphnia cucullata*

D. cucullata was exposed to turbulence by placing individuals that bred the first clutch into 1 L glass beakers (J. Weck GmbH und Co., KG, Wehr-Öflingen, Germany). Turbulences were generated with the help of custom-made motor-driven rudders conducting three turns every 20 s (for reference see Laforsch and Tollrian, 2004b). After induction for 4 weeks, only animals that had just reached maturity were collected for analysis.

Swimming Velocities

When animals reached the egg bearing instar, we determined swimming velocities in stagnant, kairmone-free medium, using a self-developed Tracking-System (Langer et al., 2021). For each treatment, we determined swimming velocities of animals ($n_{\text{typical}} = 14$, $n_{\text{Chaoborus}} = 12$, $n_{\text{turbulence}} = 14$) over approximately 5 s at a frame rate of 30 fps (giving 150 measurements per individual) and a visual resolution of 1,920 × 1,080 pixel. The velocities per recording were pooled per animal, giving one average velocity per animal. Based on the parametric data set, we tested for statistical differences with an analysis of variance (ANOVA), and consecutively with a TukeyHSD-*Post hoc*-test in R (R Core Team, 2021).

Animal Fixation, Staining and Scanning

Animals were fixed, stained and processed as described previously (Horstmann et al., 2018, 2021). In short, we fixed 10 animals per treatment with 4% formaldehyde diluted in PBS (pH 7.4, 0.1 M), dissected the antennae as they can be problematic during the confocal scanning, stained them with Congo red (Carl Roth GmbH + Co., KG, Karlsruhe, Germany) and scanned them with a confocal microscope (Leica TCS SP5II (Leica Microsystems, Wetzlar, Germany). We stitched image stacks of the specimens as they were often overarching the visual field of the microscope.

Model Generation

Three-dimensional meshes were extracted from the scans using MorphoGraphX (de Reuille et al., 2015). After projecting a grid onto the meshes using Blender (Blender, version 2.73, Blender Foundation, Blender Institute Amsterdam,¹ 2015), the resulting individual models consisting of comparable vertices were averaged to a single model per treatment, using an automated Matlab Script (Horstmann et al., 2018; Matlab R2014b, The Mathworks Inc., Natick, MA, 2015). With this script, we also determined displacement vectors between averaged models per treatment, i.e., vectors between respective vertices in the typical and altered morph models. We projected their length with color gradients onto the 3D surface reconstructions. Also, Wilcoxon-tests were calculated for the position of vertices in all three dimensions of space between specimens of the three treatments. To correct for error based on multiple testing with the FDR (false discovery rate)-analysis, we calculated q -values, which give a value that allows to evaluate the trustworthiness of each derived p -value (Storey and Tibshirani, 2003). These q -values are based on the false discovery rate, which is estimated based on the distribution of p -values of the conducted Wilcoxon tests. We define p -values smaller than 0.05 and q -values smaller than 0.01 at the same time as significant alteration, if q -values are larger than 0.01, we define it as statistical tendency. If p -values are larger than 0.05, we reject any statistical relevance.

The average 3D-models were used to calculate partial and full average Procrustes-distances between the treatments. Average Procrustes-distances give the sum of the distances of respective vertices after a Procrustes analysis normalized on the number of vertices. While partial Procrustes allows comparisons of form, including size effects and therefore allowing the alteration of form, full Procrustes compares shapes without influence by rotation, translation and scaling, enabling pure shape comparisons (Horstmann et al., 2021). These measures therefore enable to evaluate the degree of shape and form alterations between the morphs.

The reconstructed average body halves were then mirrored using Blender (Blender, version 2.73, Blender Foundation, Blender Institute Amsterdam, 2015; see text footnote 1) to create a full model of three morphs of the investigated species. Using GOM Inspect (GOM Software 2016, Hotfix 2, Rev. 96633, Build 2016-09-01, GOM GmbH, Braunschweig, Germany), the transitions remaining after the merging of the body halves were smoothed and holes closed, taking care not to alter the shape unnaturally.

Alterations in Surface, Volume and “Shadow Area”

Based on the full 3D models we also measured the surface area and the volume of the models of the respective morphs. These values can be measured in Blender using the “3D printing toolbox,”² respectively, its “3D printing” functionality with the “Statistics” option.

In addition to this we calculated the alteration of the cross-sectional area in swimming direction, which we call the “shadow area,” measured with Photoshop (Photoshop CS6, Adobe Systems Software Ireland Limited, Dublin, Ireland) as pixel values on virtual photographs of the models taken at the same scale and at an angle of 40° (compare Scourfield, 1900). The so-called “angle of attack” is defined from 0° (water current from directly above) over 40° (from obliquely upwards and frontally) to 180° (from directly beneath), which resembles the angles during hop and sink movement.

Alterations in Drag Force According to Velocity, Water Viscosity and Water Current Direction

The full models, excluding antennas, were exported as stl-files and imported in ANSYS ICEM CFD (ANSYS 2020 R1, ANSYS Inc., Canonsburg, PA, United States) to prepare a model of the animals in a virtual stream channel. For these models, a virtual fluid space was created and adjusted to the morphs’ size, making sure that it is large enough to observe water currents in front of the animals, around them and in their trailing (8 mm × 8 mm × 3 mm). Water temperature and viscosity was changed as needed. As in this approach it was only possible to simulate static objects, we did not virtually move the models through the water but rather let the water flow around them. The swimming velocities determined with the help of the 3D-Tracking System (Langer et al., 2021) were then applied on the different morphs with different angles. As angle for comparison we chose 40° (Scourfield, 1900), as this mimics a typical hop and sink movement, in which an upward and at the same time forward movement is conducted, with 5° prevalence for the upward movement. That allowed to calculate the drag in all spatial dimensions for the whole model automatically by the software.

We followed two approaches. First, we applied the velocity determined for the morph not exposed to turbulence or *Chaoborus*-larvae on all morphs. In a second comparison we applied the velocities determined for both treatments, respectively.

In addition to the comparison at a water temperature of 25°C, we calculated the simulations of the typical and turbulence exposed morph with a water temperature of 5°C and an accordingly higher water viscosity. In our analysis we altered the dynamic viscosity value from 0.0008899 Pa*s (25°C) to 0.0015187Pa*s (5°C). We both simulated with the swimming velocities of unexposed as well as turbulence exposed animals.

As the shape alterations of *Daphnia* have long been discussed as “Schwebefortsätze” (floating extensions, Woltereck, 1913; Jacobs, 1987), probably slowing down the sinking, we additionally measured the drag force while the water current impinges on the model directly from beneath (180°). For this comparison we used the velocity measured for the typical morph, as sinking is a passive movement and different velocities would render the results inconclusive.

¹<https://www.blender.org/>

²https://docs.blender.org/manual/en/latest/addons/mesh/3d_print_toolbox.html

Based on these parameters, we calculate the drag coefficient with a typical velocity as

$$C_D = \frac{2 * F_D}{\rho A v^2} \quad (1)$$

with the drag force F_D , the density of the fluid ρ , the cross-sectional area A and the squared movement velocity v^2 .

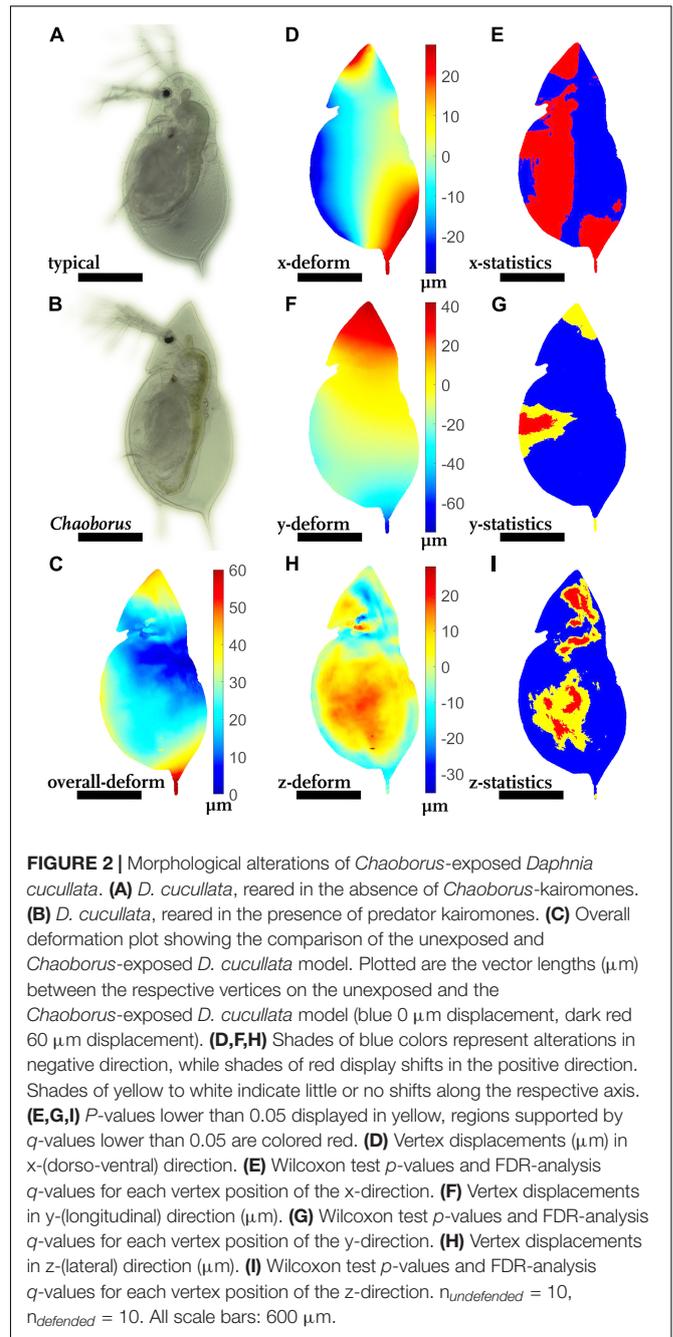
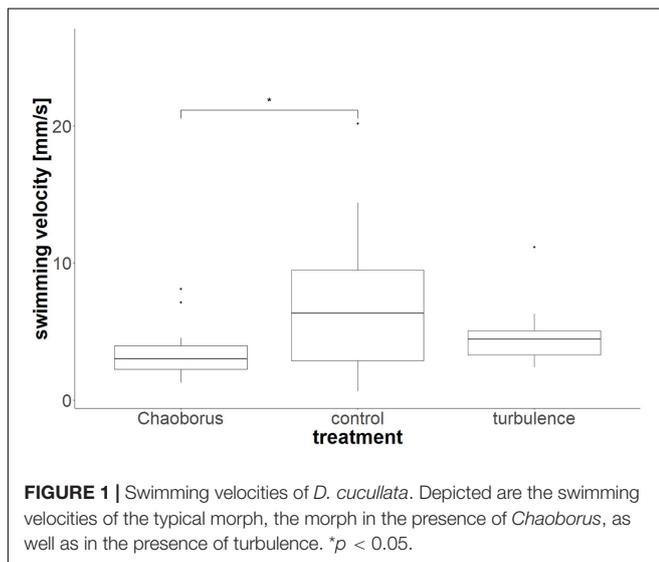
RESULTS

Swimming Velocities

We determined average velocities of 7.2 ± 5.6 mm/s for the typical morph, 3.6 ± 2.1 mm/s for the *Chaoborus*-defended morph and 4.7 ± 2.2 mm/s for the turbulence-exposed morph. The velocity of the *Chaoborus*-exposed morph was therefore reduced by 50%, the velocity of the turbulence-exposed morph reduced by 35%. We found significant differences between the swimming velocities of *D. cucullata* in the typical morph and the *Chaoborus*-exposed morph [ANOVA, $n_{ctrl} = 12$, $n_{turbulence} = 14$, $n_{Chaoborus} = 14$, $F(2, 37) = 3.269$, $p = 0.049$, pairwise t -test, $p = 0.047$, **Figure 1**]. No significant differences were found between the typical and turbulence exposed morph [ANOVA, $n_{ctrl} = 12$, $n_{turbulence} = 14$, $n_{Chaoborus} = 14$, $F(2, 37) = 3.269$, $p = 0.049$, pairwise t -test, $p = 0.192$], as well as between the two exposed morphs [ANOVA, $n_{ctrl} = 12$, $n_{turbulence} = 14$, $n_{Chaoborus} = 14$, $F(2, 37) = 3.269$, $p = 0.049$, pairwise t -test, $p = 0.729$].

Chaoborus Induced Morphological Defenses in *Daphnia cucullata*

Typically-shaped and *Chaoborus*-exposed animals of the species *D. cucullata* appear rather indifferent in most regions (**Figures 2A,B**), but the overall deformation plot shows differences in the helmet as well as the tail spine (**Figure 2C**, shades of red, $> 50 \mu\text{m}$).



In general, animals are slightly bulkier in dorso-ventral direction (**Figure 2D**, shades of red at dorsal body parts, shades of blue at ventral regions), which is supported with a tendency mainly for the ventral body parts (**Figure 2E**, $p < 0.05$, $q > 0.01$).

In longitudinal direction, the defended morph is elongated by up to $40 \mu\text{m}$ (**Figure 2F**, shades of red at the head, shades of blue in the tail spine region), but statistically only supported by a tendency (**Figure 2G**, $p < 0.05$, $q > 0.01$). Shifts at the central ventral margin are statistically supported with a tendency as well ($p < 0.05$, $q > 0.01$).

In lateral dimension the head capsule is 10–20 μm slimmer (Figure 2H, shades of blue at the head), while the carapace is laterally 10–20 μm wider (Figure 2I, shades of red on the carapace). Both alterations of lateral width are rather diffuse instead of strong local variations and were statistically supported with a tendency ($p < 0.05$, $q > 0.01$).

In terms of Procrustes distance, the models differed on average 26.4 μm (12.6 μm , Table 1).

Turbulence Induced Morphological Alterations in *Daphnia cucullata*

Turbulence-exposed *D. cucullata* display an elongated helmet (Figures 3A,B). The most strongly altered region is the helmet and the rostrum (Figure 3C, shades of red, $> 120 \mu\text{m}$).

Turbulence-exposed animals are significantly more slender by 40–50 μm at each side (Figure 3D, shades of red at the ventral body margin, shades of blue at the dorsal margin), reducing the total dorso-ventral width ($> 100 \mu\text{m}$, Figure 3E, $p < 0.05$, $q < 0.01$).

In longitudinal direction turbulence-exposed *D. cucullata* are significantly elongated (Figures 3F,G shades of red at the tip of the helmet, $> 100 \mu\text{m}$, $p < 0.05$, $q < 0.01$).

In lateral dimension, parts of the head capsule are laterally about 20 μm more slender than the typical morph (Figure 3H, shades of blue on the head), while other parts of the head are almost unaltered or enhanced in lateral width, e.g., the rostrum region (Figure 3I, shades of red at the animals head). Similarly, carapace regions at the ventral and dorsal margin are significantly wider in the lateral dimension (Figure 3G, shades of red on the carapace, Figure 3I, $p < 0.05$, $q < 0.01$).

In terms of Procrustes distance, the unexposed and turbulence-exposed model differed on average by 49.0 μm (average partial Procrustes distance, average full Procrustes distance 46.3 μm , Table 1).

According to the average Procrustes-distances, the unexposed and *Chaoborus*-exposed morph are not as different as the typical and the turbulence-exposed morph. The average partial Procrustes-distances between the two altered morphs are greater than the distances between the unexposed morph and each altered morph. At the same time, the average full Procrustes distance between the changed morphs is larger than the value between unexposed and *Chaoborus*-exposed models, but smaller than the value between the unexposed and turbulence-exposed morph.

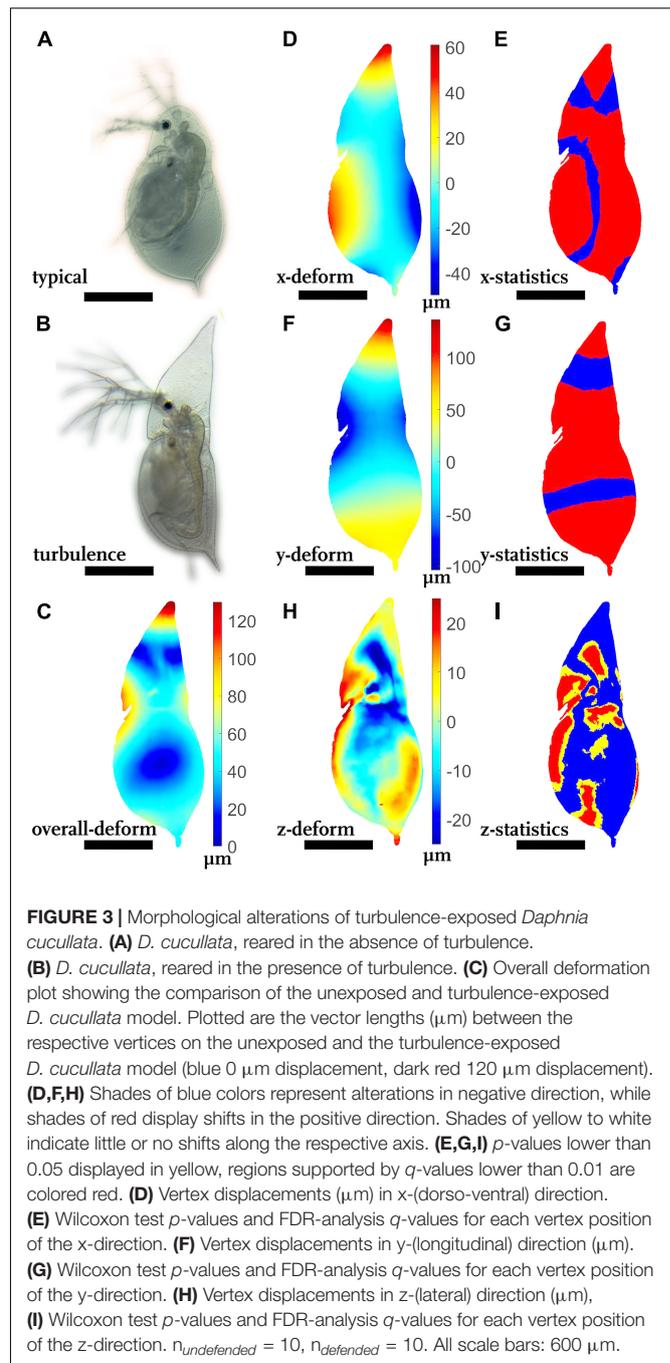


TABLE 1 | Average partial and full Procrustes-distances between the different morphs of *Daphnia cucullata*.

Morph 1	Morph 2	Partial procrustes distance	Full procrustes distance
Unexposed	<i>Chaoborus</i> -exposed	26.4	16.6
Unexposed	Turbulence-exposed	49.0	46.3
<i>Chaoborus</i> -exposed	Turbulence-exposed	77.5	35.3

Alterations in Surface, Volume and “Shadow Area”

Animals exposed to *Chaoborus*-larvae or turbulence changed their shape and form and accordingly show alterations of body surface, i.e., “shadow area” and volume (Table 2).

The body surface of turbulence exposed animals decreases by 16%, as well as the volume, which decreases about 32% in relation to the typical morph. The shadow area decreases more than the body surface area (22%).

TABLE 2 | Alterations in surface, volume and “shadow area”.

	Surface (mm ²)	Surface in (%) to typical	Volume (mm ³)	Volume in (%) to typical	Shadow area in (%) to typical
Typical	1.03	100	0.067	100	100
Chaoborus	1.15	111.8	0.080	119.3	113.1
Turbulence	0.86	84.0	0.046	68.3	77.7

Alterations are given in absolute values and percentage in comparison to the typical morph.

The body surface of *Chaoborus*-exposed animals increases by 12%, the volume is increased about 19% in comparison to the typical morph. The shadow area is larger by 13%.

Alterations in Drag Force According to Velocity, Water Viscosity and Water Current Direction

Drag force depends partly on the “shadow area” and is altered in both morphs. The drag force under an angle of attack of 40° is reduced by 11.2% (11.6% at 5°C, **Table 3**) in the turbulence-exposed morph with the typical morph's velocity. The drag force reduction calculated in a simulation with the respective velocity is reduced even stronger (-41.5%). The drag force of the *Chaoborus*-exposed morph stays almost constant with a decrease of just 0.5% simulated with the typical morph's velocity, simulating the drag force with the treatment-respective velocity gives a drag force decrease of even 51% (**Table 3** and **Figures 4A,B**). The drag coefficient C_D for the typical morph is 23.82, for the *Chaoborus*-morph 20.83 and 24.81 for the turbulence induced morph.

Streamline simulations with different water temperatures at an angle of attack of 40° lead to altered drag forces in the typical and turbulence-exposed morph. The increase of viscosity with decreasing water temperature results in drag force alteration of 74.2% (typical), respectively 76.4% (turbulence), each compared to the drag force at 25°C (**Table 4**). Independent of the water

temperature, the turbulence-exposed morph causes less drag based on its body shape.

Comparing the drag force at an angle of attack of 180°, the turbulence exposed morph shows lowest drag forces (**Figure 4C**). In comparison to the typical morph, drag during sinking is reduced by 26%. In the *Chaoborus*-exposed morph drag force is slightly reduced (4.5%). Based on the body morphology, the turbulence-exposed morph sinks the fastest, the unexposed morph sinks the slowest.

DISCUSSION

Swimming Velocities

Among the morphs we investigated in this study, *Chaoborus*-exposed *D. cucullata* swam significantly slower. According to the calculation of Gerritsen and Strickler, swimming slower mathematically reduces the chances of encounter between predator and prey (Gerritsen and Strickler, 1977). A defense mechanism in the presence of *Chaoborus* based on swimming velocity reduction, as e.g., observed in *D. magna* is therefore likely (Langer et al., 2019). A behavioral defense can also manifest e.g., in swimming type or depth selection, and needs to be investigated in future studies (De Meester et al., 1999; Wickramaratna et al., 2014). Also, turbulence-exposed animals did not show a significant alteration in swimming velocity in our sample. Nevertheless, it is important to note that we here measured the swimming velocity during the typical hop and sink movement in stagnant medium, not in turbulence or during e.g., flight behavior. Under such conditions, swimming velocities may differ.

Morphological Alterations of *Daphnia cucullata* in the Presence of *Chaoborus*

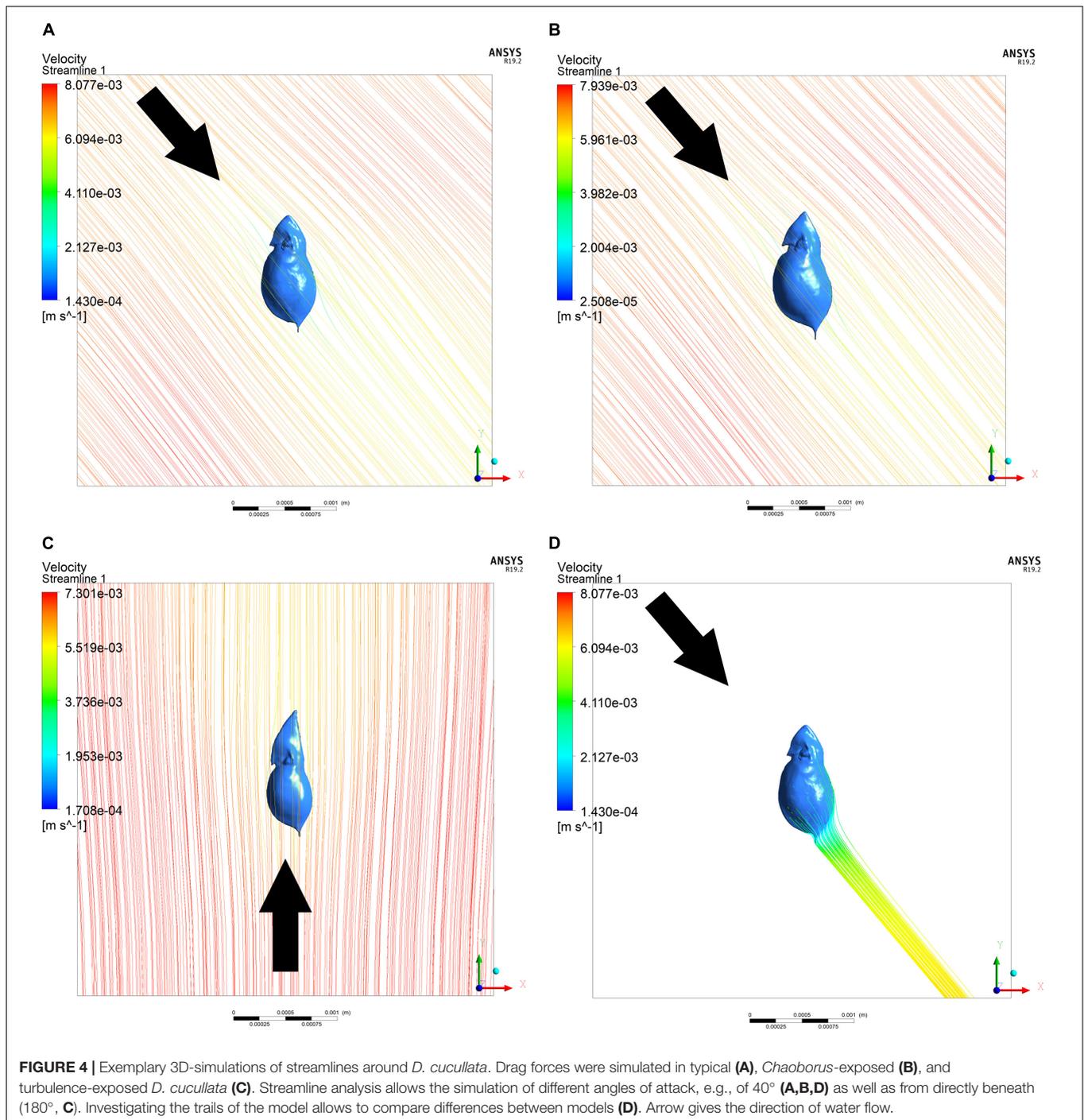
Chaoborus-defended *D. cucullata* are characterized by an elongated helmet and a slightly slender body in dorso-ventral direction. The head to tail spine distance is increased. Our results confirm the observations made in X and Y direction previously (Jacobs, 1987; Tollrian, 1990; Laforsch and Tollrian, 2004a). The deformation in z direction was yet unknown. We observe that the fold located in anterior-posterior direction on the head is strongly reduced laterally in defended animals. In combination with the slightly altered head morphology, this may support an anti-lock-and-key mechanism, especially as the head morphology gets more edgy (Dodson, 1974). In *D. cucullata* the defense is effective at the post attack level (Laforsch and Tollrian, 2004c), so that these alterations could provide protection through an anti-lock-and-key-system (Jeschke et al., 2008; Weiss et al., 2012). These alterations may be advantageous as *D. cucullata*

TABLE 3 | Drag force alteration (%) of the turbulence and *Chaoborus*-exposed morph of *D. cucullata* in comparison to the typical morph at typical and respective velocities.

Treatment	Drag force (typical-velocity) (%)	Drag force (respective velocity) (%)
Turbulence (40°)	-11.2	-41.5
<i>Chaoborus</i> (40°)	-0.5	-50.9
Turbulence (180°)	-25.6	-
<i>Chaoborus</i> (180°)	-4.4	-

TABLE 4 | Absolute drag forces and drag force alterations.

Treatment	Temperature (°C)	Drag force (N)	Difference (% to 25°C)
Typical	5	1.82E-07	74.2
Typical	25	1.04E-07	
Turbulence	5	1.08E-07	76.4
Turbulence	25	6.10E-08	



thereby outgrows *Chaoborus*' mouth gape (Riessen and Young, 2005) or by reducing handling efficiency, as in *D. pulex* (Krueger and Dodson, 1981).

Furthermore, a reduction of the wake vortices eliciting *Chaoborus*' catching movement by morphological alteration seems to be part of *D. cucullata*'s strategy. Our streamline analysis shows the trail to be wider in defended animals and therefore potentially less intense (Figure 4D). That way, *D. cucullata* may swim in a kind of 'stealth mode'. The head capsule's lateral width

at the fornices is reduced and the carapace is laterally wider, as previously described as defense of *D. pulex* against *Chaoborus* (Horstmann et al., 2021).

Morphological Alterations of *Daphnia cucullata* in the Presence of Turbulence

The morphological reaction of *D. cucullata* to turbulence is not a direct reaction to a predator, but to movements in the water,

speculated to be an indicator of predator presence (Hrbáček, 1959). We found that the lateral width of the head including the fornices is even stronger reduced in comparison to the *Chaoborus* defended morph. A more pronounced defense feature may indicate a defense against a different predator or at least a different function of the defense. Concerning the origin of the inducing effect of turbulences, it is hypothesized that the time of the year at which the turbulences naturally occur by winds (Hrbáček, 1959) correlates with high predation pressure by invertebrate or vertebrate predators. Moreover, the predators itself could not only emit chemical signals (kairomones) but also the turbulence (Lampert and Wolf, 1986; Laforsch and Tollrian, 2004b). Nevertheless, an additive effect of predator and turbulence appears unlikely at least in the case of *Chaoborus*, as during our induction microturbulences were present due to movements of the predators in the experimental beakers. Though, we did not observe strong defense expression. Especially as helmets were shown to be an effective protection against smaller fish (Kolar and Wahl, 1998), swarms of young fish, which elicit turbulences by swimming may be the natural cue for the observed alterations in this treatment (Lampert and Wolf, 1986; Laforsch and Tollrian, 2004b).

Furthermore, it was so far unclear, whether the morphological alterations change the hydrodynamic properties. Previously it was hypothesized that the morphological alterations lead to so far unknown benefits, e.g., through shifts in the buoyancy properties (Hrbáček, 1959; Jacobs, 1967; Laforsch and Tollrian, 2004b).

Comparison of All Three Morphs

At a first glance, the morphs of *D. cucullata* exposed to *Chaoborus* and turbulence seem quite different. Considering Procrustes distance, the average partial Procrustes-values are 26 μm , respectively 49 μm , the difference between the two altered morphs is 78 μm . Therefore, the altered morphs are more different to each other than each of them is different to the typical morph, suggesting a completely different defense mechanism, confirming the hypothesis of different defenses. Though, correction for size reduces these values to 17 μm , respectively, 46 μm , the difference between the altered morphs is reduced to 35 μm (50%). The latter difference is smaller compared to the distance between the unexposed and turbulence-exposed morph. This suggests a striking effect of size. We therefore find that the turbulence morph may just be the extreme formation of the *Chaoborus* morph. Both morphs were already discussed as different expression strengths of a single morphological defense previously (Laforsch and Tollrian, 2004b). Though, in our experiment, we did not observe a turbulence morph in *Chaoborus*-exposed animals, especially not developed that fast as observed in the turbulence-exposed treatment. The different expression strengths of this alteration support the theory of an invertebrate-specific defense, as *D. cucullata* were naturally often found with broken tail spines, which were assessed relics of invertebrate predator attacks (Murtaugh, 1981; Pijanowska, 1990). Additionally, other studies assessed this species as not vulnerable to fish predation, due to their low conspicuity (Pijanowska, 1992). Despite these results, we assume that vulnerability is dependent on fish size. Especially

swarms of young fish may indeed be a threat to *D. cucullata*. Nevertheless, our results support a single, adjusted defense in different size classes of *D. cucullata*. Such a gradual alteration is contrary to e.g., *D. barbata*'s defense, which is different or even oppositional confronted with its two respective predators (Herzog and Laforsch, 2013).

Alterations in Surface, Volume and "Shadow Area"

According to the drag equation for Reynold's numbers > 1 , drag coefficient and reference area are important factors for drag calculation (Nakayama, 1999; Batchelor, 2000).

$$F_D = \frac{1}{2} \rho C_D A v^2$$

In this term, ρ represents the fluid density, C_D the drag coefficient, A the cross-sectional area and v the relative velocity between the fluid and the body.

The reference area is defined by the cross-sectional area in direction of the objects motion, while shape of the object is included in the drag coefficient. Therefore, the drag force is coupled with surface and volume via the shape. Based on our results, we calculated the drag coefficients for the three morphs. Interestingly, the *Chaoborus*-morph has the smallest drag coefficient (20.83), while the turbulence exposed morph has the highest value (24.81), suggesting that the small cross-sectional area is governing the low drag found in this morph, which we see in our data. In *Chaoborus*-exposed animals, alterations of surface and "shadow area" are less pronounced (12 and 13%), suggesting that under predation pressure the surface exposed to the water current is not under a selection toward a reduction or an increase. In comparison, "shadow area" in turbulence-exposed *D. cucullata* is decreased by 22% in comparison to the typical morph, while the overall surface is decreased by 15%. It can be argued, that the decrease in surface area is especially favoring the direction of movement more as the overall *Daphnia* shape, which is optimized for a forward movement (Zaret and Kerfoot, 1980). This is even more impressive as the volume decreases by 32%, and the accompanying surface reduction is focused mainly on the "shadow area".

Alterations in Drag Force According to Velocity, Water Viscosity, and Water Current Direction

The drag force under an angle of attack of 40° and with velocity of the typical morph (7.2 mm/s), is reduced by only 11% in turbulence-exposed animals, compared to a 22% decrease in shadow area. This is a clear indication that also the body shape of *D. cucullata* and not only "shadow area" has, as a further parameter in the drag equation, a strong influence on the energy efficiency of swimming. As the treatments' swimming velocities were not significantly different, these comparisons with standard velocity are most reliable. In addition, the drag force reduction with the treatment specific velocity is 42% in the turbulence-exposed treatment, as not only the shape is more streamlined, but also swimming velocity is considerably reduced (4.7 mm/s).

In general, turbulence-exposed *D. cucullata* experience less drag forces and are therefore more energy-saving swimmers. This could help to withstand turbulences or keep position in the water column despite turbulences are present, similar to the hypotheses of Woltereck (1913).

The drag force under an angle of attack of 40° and with the velocity of the typical morph decreased by only 0.5% in the *Chaoborus*-exposed morph. Using the velocity measured for this treatment in our tracking setup (Langer et al., 2021), we found the drag force decreased by 51% compared to the typical morph. The velocity of *Chaoborus*-defended *D. cucullata* was 50% lower (3.6 mm/s), which explains this clear decrease in drag force using the respective velocity. Therefore, the defense against *Chaoborus* does not seem to alter the body in favor of a more streamlined shape. Moreover, as more streamlined morphs of *D. cucullata* do not swim faster but rather slower, swimming velocity seems to be adjusted independently of morphological alteration.

As the turbulence-exposed morph was discussed to alter swimming abilities at different water temperatures (Laforsch and Tollrian, 2004b), we modeled the typical and the turbulence-exposed morph at 5°C with, respectively, altered water viscosity. In colder water (5°C), drag is highly increased, especially at low Reynold's numbers (Reynolds, 1886; Wickramaratna et al., 2014). We observed an increase of drag force, compared to 25°C warm water, of 74% in the typical morph and an increase of 76% in the turbulence-exposed morph. In both treatments, the increase of drag force between these two treatments at 5°C compared to the simulation at 25°C was approximately 11%. Therefore, no morph has an additional advantage of shape at colder temperatures. This contradicts the assumption that high helmets are not expressed at colder temperatures to save energy (Laforsch and Tollrian, 2004b). Moreover, at 25°C the turbulence morph shows a 11.2% reduced drag force compared to the typical morph, at 5°C an even 11.6% reduced drag force, which suggests a slightly drag reducing effect of the turbulence-exposed morphology at colder temperatures.

Based on body shape, turbulence-exposed animals sink fastest and un-exposed animals slowest. Jacobs showed that additionally antennae have an important effect on sinking (Jacobs, 1967), so that morphology cannot solely predict sinking properties. He found different antennae parachutes in the winter and summer morph (Jacobs, 1967). Similarly, the *Chaoborus*- and turbulence morph may also have altered antennae parachutes. Nevertheless, it can be hypothesized that the typical and *Chaoborus*-exposed morph with its relatively high drag forces in sinking enable the maintenance of a vertical position, avoiding antennae strokes to be energetically more efficient. In winter, when food is limited and physiology slowed, the typical morphology could serve as an energy saving mechanism. The morphology in the presence of the tactile predator *Chaoborus* shows a 4% decreased drag force during sinking, which just slightly increases this morph's sinking velocity. Therefore, also the *Chaoborus*-morph needs just very few antennae strokes to maintain vertical position, avoiding movements suspicious to this predator.

As vertebrate predators were discussed to be the natural origin of turbulence (Lampert and Wolf, 1986; Laforsch and Tollrian, 2004b), the more streamlined morphology of the

turbulence-morph could help the animals to sink faster in deeper water layers in the presence of e.g., fish, especially if antennae are kept tight to the body. This migration into deeper water layers is known as diel vertical migration to avoid fish predation (Bayly, 1986; Lampert, 1993).

Summarizing these findings, the turbulence-exposed morph is slightly more efficient in the active swimming but sinks faster based on our experimental framework, in which we assumed nearly laminar flow based on previous publications on the body Reynolds number of *Daphnia* (Lord et al., 2006; Wickramaratna et al., 2014). The material costs of the helmet are not determined yet, but assumed to be low (Tollrian, 1991). Therefore, the fast sinking of the turbulence exposed morph combined with the necessity of more antennae strokes to keep the vertical position is possibly the only, but very relevant cost, as it increases energy investment.

In reaction to phantom-midge larvae, the assumed sinking rate based on simulated drag forces, is close to typical *D. cucullata*. This keeps the antennae beat frequency low, which in turn may avoid *Chaoborus* attacks, as they are tactile predators and sensitive for such kind of water movements. We also found the *Chaoborus*-morph to swim slower than the typical, reducing encounter probability (Gerritsen and Strickler, 1977). This is contradictory to previous findings, which showed helmeted morphs to be attacked more frequently than the typical morph (Laforsch and Tollrian, 2004c).

CONCLUSION

Based on our geometric morphometric analysis, the two morphs appear as different expression strengths of a single multitool response (Laforsch and Tollrian, 2004b). The swimming velocity seems independent of the morphs, as it was not increased despite drag forces were decreased, which would allow higher swimming velocities at the same energy investment.

The function of the morphological alterations remains somewhat inconclusive. Nevertheless, we can now e.g., exclude that the turbulence-exposed morph reduces the streamlining maleficently in cold water (Laforsch and Tollrian, 2004b). We can furthermore rule out the hypothesis that the helmeted morph serves as support in maintaining the vertical position in the water column, as the opposite is the case, it sinks faster. Though, the turbulence-exposed morph shows an advantage at more turbulent conditions, potentially being more maneuverable, due to lower drag. Thus, this is an advantage besides the pure defensive effect. This is in agreement with Woltereck's hypothesis. He stated that the helmet of *D. cucullata* mainly serves to determine swimming direction (Woltereck, 1913). Further (simulated) investigations on the swimming with morphological alterations and potentially altered antennae parachutes in combination with predation experiments will reveal further beneficial effects of the observed alterations. In the light of the previous and current investigations it appears likely that not a single positive effect, but an accumulation of favorable effects causes the distinct changes in morphology and swimming velocity.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

MH, LW, and RT contributed to conception and design of the study and interpreted the data. MH conducted model generation, determination of swimming velocities and streamline simulations and wrote the first draft of the manuscript. MH and LW performed the statistical analysis. RT provided lab facilities. All authors read and approved the submitted version.

REFERENCES

- Batchelor, G. K. (2000). *An Introduction to Fluid Dynamics*. Cambridge: Cambridge University Press.
- Bayly, I. A. E. (1986). "Aspects of diel vertical migration in zooplankton, and its enigma variations," in *Limnology in Australia*, eds P. De Deckker and W. D. Williams (Dordrecht: Springer), 61.
- Brooks, J. L. (1947). Turbulence as an environmental determinant of relative growth in *Daphnia*. *Proc. Natl. Acad. Sci. U.S.A.* 33, 141–148. doi: 10.1073/pnas.33.5.141
- Brooks, J. L. (1965). Predation and relative helmet size in cyclomorphic *Daphnia*. *Proc. Natl. Acad. Sci. U.S.A.* 53, 119–126. doi: 10.1073/pnas.53.1.119
- De Meester, L., Dawidowicz, P., Van Gool, E., and Loose, C. J. (1999). "Ecology and evolution of predator-induced behaviour of zooplankton: depth selection behaviour and diel vertical migration," in *The Ecology and Evolution of Inducible Defenses*, eds R. Tollrian and C. D. Harvell (Princeton, NJ: Princeton University Press), 160–176.
- de Reuille, P. B., Routier-Kierzkowska, A. L., Kierzkowski, D., Bassel, G. W., Schüpbach, T., Tauriello, G., et al. (2015). MorphoGraphX: a platform for quantifying morphogenesis in 4D. *eLife* 4:e05864. doi: 10.7554/eLife.05864
- Dodson, S. I. (1974). Adaptive change in plankton morphology in response to size-selective predation: a new hypothesis of cyclomorphosis. *Limnol. Oceanogr.* 19, 721–729.
- Gerritsen, J., and Strickler, J. R. (1977). Encounter probabilities and community structure in zooplankton: a mathematical model. *J. Fish. Res. Board Can.* 34, 73–82.
- Hebert, P. D. N. (1978). The adaptive significance of cyclomorphosis in *Daphnia*: more possibilities. *Freshw. Biol.* 8, 313–320.
- Herzog, Q., and Laforsch, C. (2013). Modality matters for the expression of inducible defenses: introducing a concept of predator modality. *BMC Biol.* 11:113. doi: 10.1186/1741-7007-11-113
- Horstmann, M., Ralph, T., and Weiss, L. C. (2021). Thwarting predators? A three-dimensional perspective of morphological alterations in the freshwater crustacean *Daphnia*. *PLoS One* 16:e0254263. doi: 10.1371/journal.pone.0254263
- Horstmann, M., Topham, A. T., Stamm, P., Colbourne, J. K., Tollrian, R., and Weiss, L. C. (2018). Scan, extract, wrap, compute – a 3D-method to analyse morphological shape differences. *PeerJ* 6:e4861. doi: 10.7717/peerj.4861
- Hrbáček, J. (1959). Circulation of water as a main factor influencing the development of helmets in *Daphnia cucullata* sars. *Hydrobiologia* 13, 170–185.
- Hrbáček, J. (1962). Species composition and the amount of zooplankton in relation to fish stock. *Rozpr. Ces. Akad. Ved Rada Mat. Prir. Ved* 72, 1–117.
- Jacobs, J. (1961). Cyclomorphosis in *Daphnia galeata mendotae* birge, a case of environmentally controlled allometry. *Arch. Hydrobiol.* 58, 7–71.
- Jacobs, J. (1962). Light and turbulence as co-determinants of relative growth rates in cyclomorphic *Daphnia*. *Int. Rev. Gesamten Hydrobiol.* 47, 146–156.
- Jacobs, J. (1964). Hat der hohe Sommerhelm zyklomorpher *Daphnien* einen Anpassungswert? *SIL Proc.* 15, 676–683. doi: 10.1080/03680770.1962.11895590

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- Jacobs, J. (1967). Untersuchungen zur funktion und evolution der Zyklomorphose bei *Daphnia*, mit besonderer Berücksichtigung der Selektion durch Fische. *Arch. Hydrobiol.* 62, 467–541.
- Jacobs, J. (1987). "Cyclomorphosis in *Daphnia*," in *Daphnia*, Vol. 45, eds R. H. Peters and R. de Bernardi (Verbania Palanza: Memorie dell'Istituto Italiano di Idrobiologia), 325–352.
- Jeschke, J. M., Laforsch, C., and Tollrian, R. (2008). "Animal prey defenses," in *General Ecology*, eds S. E. Jorgensen and B. D. Fath (Oxford: Elsevier), 189–194.
- Karpowicz, M., Ejsmont-Karabin, J., Więcko, A., Górniak, A., and Cudowski, A. (2019). A place in space—the horizontal vs vertical factors that influence zooplankton (Rotifera, crustacea) communities in a mesotrophic lake. *J. Limnol.* 78, 243–258.
- Kolar, C. S., and Wahl, D. H. (1998). *Daphnid* morphology deters fish predators. *Oecologia* 116, 556–564. doi: 10.1007/s004420050621
- Krueger, D. A., and Dodson, S. I. (1981). Embryological induction and predation ecology in *Daphnia pulex*. *Limnol. Oceanogr.* 26, 219–223.
- Laforsch, C., and Tollrian, R. (2004c). Inducible defenses in multipredator environments: cyclomorphosis in *Daphnia cucullata*. *Ecology* 85, 2302–2311.
- Laforsch, C., and Tollrian, R. (2004a). Embryological aspects of inducible morphological defenses in *Daphnia*. *J. Morphol.* 262, 701–707. doi: 10.1002/jmor.10270
- Laforsch, C., and Tollrian, R. (2004b). Extreme helmet formation in *Daphnia cucullata* induced by small-scale turbulence. *J. Plankton Res.* 26, 81–87.
- Lampert, W. (1993). Ultimate causes of diel vertical migration of zooplankton: new evidence for the predator-avoidance hypothesis. *Arch. Hydrobiol. Beih. Ergebn. Limnol.* 39, 79–88.
- Lampert, W., and Sommer, U. (1993). *Limnoolökologie*. Oxford: Oxford University Press.
- Lampert, W., and Wolf, H. G. (1986). Cyclomorphosis in *Daphnia cucullata*: morphometric and population genetic analyses. *J. Plankton Res.* 8, 289–303. doi: 10.1093/plankt/8.2.289
- Langer, S. M., Horstmann, M., Weiss, L. C., Junke, W., Herlitze, S., and Tollrian, R. (2021). On *Daphnia*'s path: a semi-automatic system to analyze three-dimensional movement patterns. *Ecosphere* 12:e03847. doi: 10.1002/ecs2.3847
- Langer, S. M., Weiss, L. C., Ekvall, M. T., Bianco, G., Hansson, L.-A., and Tollrian, R. (2019). A three-dimensional perspective of *Daphnia*'s swimming behavior with and without predator cues. *Limnol. Oceanogr.* 64, 1515–1525. doi: 10.1002/lno.11132
- Lord, H., Lagergren, R., Svensson, J. E., and Lundqvist, N. (2006). Sexual dimorphism in *Bosmina*: the role of morphology, drag, and swimming. *Ecology* 87, 788–795. doi: 10.1890/05-0082
- Murtaugh, P. A. (1981). Inferring properties of mysid predation from injuries to *Daphnia*. *Limnol. Oceanogr.* 26, 811–821.
- Nakayama, Y. (1999). *Introduction to Fluid Mechanics*, ed. R. F. Boucher (Oxford: Butterworth-Heinemann).
- Pijanowska, J. (1990). Cyclomorphosis in *Daphnia*: an adaptation to avoid invertebrate predation. *Hydrobiologia* 198, 41–50.
- Pijanowska, J. (1992). Anti-predator defence in three *Daphnia* species. *Int. Rev. Gesamten Hydrobiol.* 77, 153–163.

- R Core Team (2021). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.
- Reynolds, O. (1886). On the theory of lubrication and its application to Mr. Beauchamp tower's experiments. *Proc. R. Soc. London* 40, 191–203.
- Riessen, H. P., and Young, J. D. (2005). *Daphnia* defense strategies in fishless lakes and ponds: one size does not fit all. *J. Plankton Res.* 27, 531–544.
- Scourfield, D. J. (1900). The swimming peculiarities of *Daphnia* and its allies. *J. Queckett. Microsc. Club* 2, 217–244.
- Shapiro, A. M. (1976). Seasonal polyphenism. *Evol. Biol.* 9, 259–333.
- Storey, J. D., and Tibshirani, R. (2003). Statistical significance for genomewide studies. *Proc. Natl. Acad. Sci. U.S.A.* 100, 9440–9445. doi: 10.1073/pnas.1530509100
- Tollrian, R. (1990). Predator-induced helmet formation in *Daphnia cucullata* (Sars). *Arch. Hydrobiol.* 119, 191–196.
- Tollrian, R. (1991). Some aspects in the costs of cyclomorphosis in *Daphnia cucullata*. *SIL Proc.* 24, 2802–2803. doi: 10.1080/03680770.1989.11899165
- Tollrian, R., and Harvell, C. D. (1999). *The Ecology and Evolution of Inducible Defenses*. Princeton, NJ: Princeton University Press.
- Tollrian, R., and Laforsch, C. (2006). Linking predator kairomones and turbulence: synergistic effects and ultimate reasons for phenotypic plasticity in *Daphnia cucullata*. *Arch. Hydrobiol.* 167, 135–146.
- Volterra, V. (1926). Fluctuations in the abundance of a species considered mathematically. *Nature* 118, 558–560.
- Weiss, L. C., and Tollrian, R. (2018). “Inducible defenses in Crustacea,” in *The Natural History of the Crustacea, Life Histories*, Vol. 5, eds T. Martin and G. A. Wellborn (Oxford: Oxford University Press).
- Weiss, L. C., Heilgenberg, E., Deussen, L., Becker, S. M., Kruppert, S., and Tollrian, R. (2016). Onset of kairomone sensitivity and the development of inducible morphological defenses in *Daphnia pulex*. *Hydrobiologia* 779, 135–145.
- Weiss, L. C., Laforsch, C., and Tollrian, R. (2012). “The taste of predation and the defenses of prey,” in *Chemical Ecology in Aquatic Systems*, ed. C. Brönmark (Oxford: Oxford University Press), 111–126.
- Wesenberg-Lund, C. J. (1900). Von dem Abhängigkeitsverhältnis zwischen dem Bau der Planktonorganismen und dem spezifischen Gewicht des Süßwassers. *Biol. Zbl.* 20, 606–619.
- Wickramaratna, L. N., Noss, C., and Lorke, A. (2014). Hydrodynamic trails produced by *Daphnia*: size and energetics. *PLoS One* 9:e92383. doi: 10.1371/journal.pone.0092383
- Windig, J. J., and Lammar, P. (1999). Evolutionary genetics of seasonal polyphenism in the map butterfly *Araschnia levana*. *Evol. Ecol. Res.* 1, 875–894.
- Woltreck, R. (1913). Über Funktion, Herkunft und Entstehungsursachen der sogen. Schwebefortsätze pelagischer Cladoceren. *Z. Indukt. Abstamm. Vererbungsl.* 10, 475–550.
- Zaret, R. E., and Kerfoot, C. W. (1980). The shape and swimming of *Bosmina longirostris*. *Limnol. Oceanogr.* 25, 126–133.
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