

RESEARCH ARTICLE

Predator-specific inducible morphological defenses of a water flea against two freshwater predators

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Abstract

The expression of inducible morphological defenses in *Daphnia* in response to a single predator is a well-known phenomenon. However, predator-specific modifications of the same defensive traits as an adaption to different predator regimes is so far only described for *Daphnia barbata*. It is unknown if this accounts only for this species or if it is a more widespread, general adaptive response in the genus *Daphnia*. In the present study, we therefore investigated whether a clone of the pond-dwelling species *Daphnia similis* responds to different predatory invertebrates (*Triops cancriformis*; *Notonecta maculata*) with the expression of predator-specific modifications of the same defensive traits. We showed that *Triops*-exposed individuals express a significantly longer tail-spine, while body width decreased in comparison to control individuals. Additionally, they also expressed inconspicuous defenses, that is, significantly longer spinules on the dorsal ridge. The *Notonecta*-exposed *D. similis* showed a significantly longer tail-spine, longer spinules and a larger spinules bearing area on the dorsal ridge than control individuals as well. However, a geometric morphometric analysis of the head shape revealed significant, predator-specific changes. *Triops*-exposed individuals expressed a flattened head shape with a pronounced dorsal edge, while *Notonecta*-exposed individuals developed a high and strongly rounded head. Our study describes so far unrecognized inducible defenses of *D. similis* against two predators in temporary waters. Furthermore, the predator-dependent change in head shape is in concordance with the 'concept of modality', which highlights the qualitative aspect of natural selection caused by predators.

KEYWORDS

head shape, morphometry, phenotypic plasticity, predator–prey interactions

1 | INTRODUCTION

The structure and composition of freshwater communities is highly dependent on interspecific interactions (Holt, 1977), with predation as a major selective agent. In response to predation, prey species have evolved numerous defensive strategies. These defenses can be either

constitutive or inducible: while constitutive defenses, such as the quills of an hedgehog or shells of snails or clams, are permanently expressed, inducible defenses are only expressed under acute predation pressure and allow the prey to save the costs associated with the defense when the predator is absent (Harvell & Tollrian, 1999). Inducible defenses are a ubiquitous phenomenon in many taxa, ranging

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from protists to vertebrates (Altwegg, Marchinko, Duquette, & Anholt, 2004; Frost, 1999), and are especially well studied in the model organism *Daphnia* (Lass & Spaak, 2003; Seda & Petrusek, 2011). Since they are prey for many different aquatic predators, *Daphnia* have evolved a broad range of inducible defenses, including changes in behaviour (Loose & Dawidowicz, 1994; Vetti Kvam & Kleiven, 1995), shifts in life history (Stibor, 1992), physiological responses (Weiss, Leese, Laforsch, & Tollrian, 2015) and changes in morphology (Tollrian, 1995b).

Inducible morphological defenses in *Daphnia* are considered to act as post-contact defenses, which prevent or impair handling and consumption by the predator, by changing the shape and size of their body (Dodson, 1974). Well studied examples are the expression of pointed helmets and an elongation of the tail spine, for example, in *D. cucullata* exposed to *Chaoborus* spp., *Leptodora kindtii* and *Cyclops* spp. (Laforsch & Tollrian, 2004) or in *D. lumholtzi* exposed to fish (Engel & Tollrian, 2009). Furthermore, the formation of specialized defensive structures has been described, for example, the expression of neckteeth in *D. pulex* as a response to *Chaoborus* larvae (Krueger & Dodson, 1981), or the large crest in species of the *D. carinata* complex induced by backswimmers (*Anisops* sp., Notonectidae; Grant & Bayly, 1981). Although inducible defenses in *Daphnia* have been intensively studied throughout the last decades, so far unrecognized defenses as well as novel defense mechanisms and adaptations are still revealed on a regular basis (Herzog & Laforsch, 2013; Herzog, Rabus, Wolfschoon Ribeiro, & Laforsch, 2016; Herzog, Tittgen, & Laforsch, 2016; Maurone, Suppa, & Rossi, 2018). Many of these recently discovered defenses have been found in *Daphnia* inhabiting pools and temporary ponds. For instance, *D. magna* exposed to *T. cancriformis*, expresses an increased bulkiness, that is, a larger and wider body, an elongated tail spine and a fortified exoskeleton (Rabus & Laforsch, 2011; Rabus, Söllradl, Clausen-Schaumann, & Laforsch, 2013; Rabus, Waterkeyn, Van Pottelbergh, Brendonck, & Laforsch, 2012; Riessen et al., 2012). *Daphnia barbata* not only responds to predatory invertebrates in general but in fact modulates its defensive traits, specifically adapted against the different hunting strategies of *T. cancriformis* or the backswimmer *N. glauca* (Herzog & Laforsch, 2013; Herzog, Rabus, et al., 2016; Herzog, Tittgen, & Laforsch, 2016).

So far, little is known about inducible morphological defenses in a species complex globally labelled as *D. similis*, which is currently separated into two species complexes (*D. similis* and *D. exilis*, respectively) in the Old and New World (Adamowicz, Petrusek, Colbourne, Hebert, & Witt, 2009; Popova et al., 2016). Though it has to be mentioned, that this is just the use of the same name and both species are not even in a sister relationship. A recent study by Santangelo et al. (2018) probably performed with a species from the New World complex showed that *D. similis* responds to phantom midge larvae and fish with changes in life history. Further, morphological changes, such as an elongation of the tail spine, of hatchlings from asexually and sexually produced eggs against fish kairomones, has been shown in individuals labelled as *D. similis* from Korea (La, Chang, Jang, Joo, & Kim, 2014). However, the origin of the daphnids used by La et al. may imply that not *D. similis* but *D. sinensis* (Ma et al., 2016; Popova

et al., 2016), a species related to *D. similis* was used for the study. *Daphnia similis* sensu stricto is alike representatives of the *D. atkinsoni* species complex a pond-dwelling member of the Cladocera. For the *D. atkinsoni* species complex a unique morphological defense, the so called 'crown of thorn', an enlargement of two spine bearing, heart-shaped lobes in the dorsal region of the head, has been shown in response to this predator (Laforsch et al., 2009; Petrusek, Tollrian, Schwenk, Haas, & Laforsch, 2009). Further, both species complexes show an overlapping distribution and therefore share the same habitat and predators such as the tadpole shrimp *T. cancriformis* or *N. maculata* (Alonso, 1985). Hence, we hypothesize, that *D. similis* which likely encounters both invertebrate predators in its habitat, responds with the expression of different phenotypic traits since the hunting modes of both predators differ as well.

In the present study, we investigate therefore whether a clone of *D. similis* originating from Spain expresses inducible morphological defenses in response to two invertebrate predators, *T. cancriformis* and *N. maculata*. Moreover, we assessed if the expression of the defensive traits shows a predator-specific modality, comparable to the response of *D. barbata* exposed to the same predators as used in the study by Herzog and Laforsch (2013), because different populations of *D. similis* sampled in the field occasionally may differ in head shapes (Adam Petrusek, personal communication). We apply a geometric morphometric approach, comparable to the approach applied for species variation by Dlouhá et al. (2010), for the analysis of defensive traits in *D. similis*.

2 | MATERIALS AND METHODS

2.1 | Induction experiment (exposure to predator kairomones)

For the induction experiment, we used a laboratory-cultured clone of *Daphnia similis* Claus, 1876. The animals were cultured in a climate chamber with a 15 hr-day and 9 hr-night rhythm on semi-artificial medium following Rabus and Laforsch (2011). The clone originates from the Doñana national park, 30 km south of Sevilla, Spain, and was provided by Joachim Mergeay (KU Leuven). It most certainly belongs to *D. similis* s.str (Popova et al., 2016). Two predators were used: the tadpole shrimp *T. cancriformis*, originating from a laboratory cultured strain originally provided by Erich Eder (University of Vienna), and the backswimmer *N. maculata*, which were caught in ponds at the University of Bayreuth.

The experiment was conducted in a climate chamber at $20 \pm 1^\circ\text{C}$ with a constant photoperiod (15 hr light; 9 hr darkness). 2 L-glass beakers, filled with 2 L semi-artificial medium based on tap water, phosphate buffer, and trace elements (Rabus & Laforsch, 2011), were used as experimental vessels. A pinch of cetylalcohol (Tokyo Chemical Industry Co., Nihonbashi-honcho, Japan) was added on the water surface to lower surface tension. Each beaker contained a mesh cage made of acrylic glass and gauze (mesh width: 180 μm).

Three treatments ('Control', '*Triops*', '*Notonecta*') were established, each replicated 10 times. At the start of the experiment, each beaker

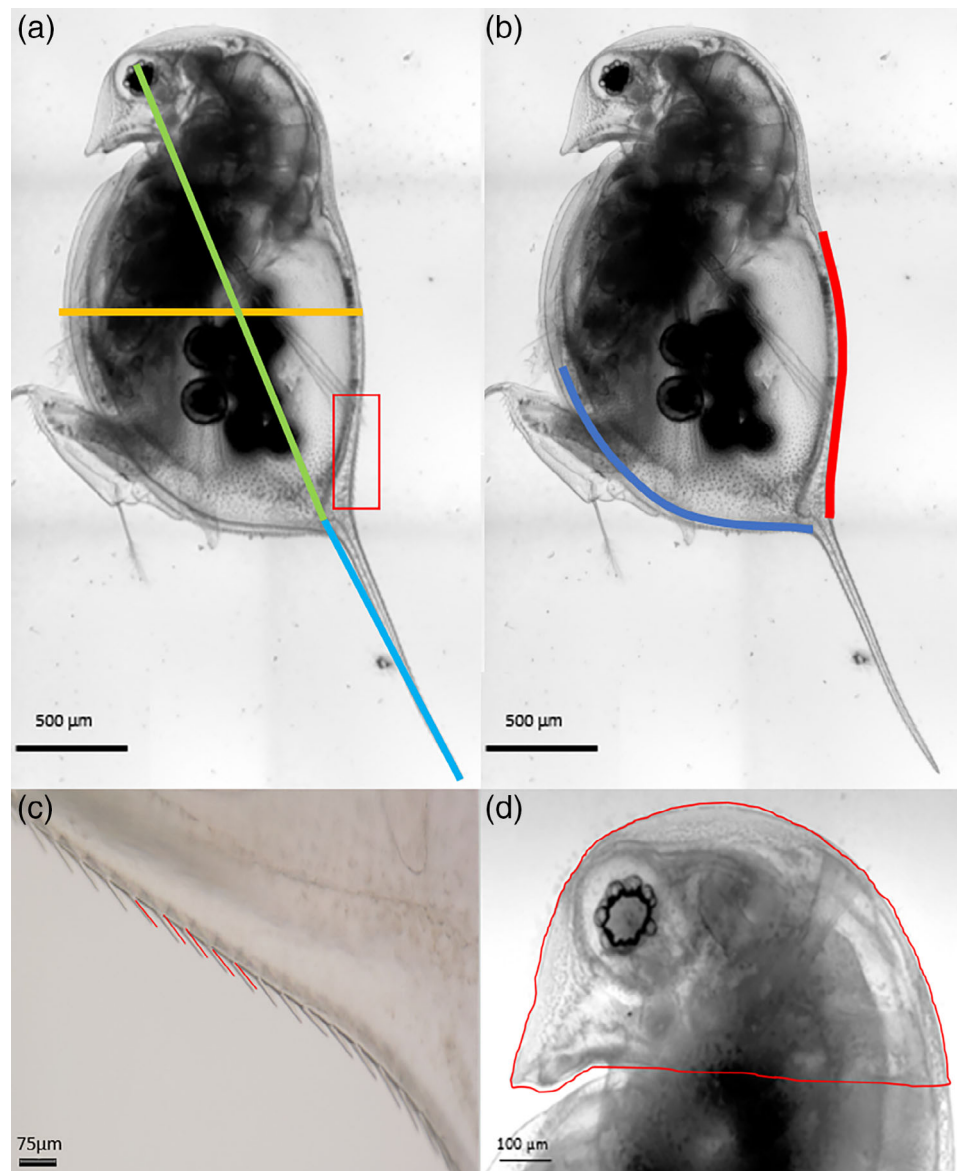
was stocked with 15 randomly chosen, age-synchronized neonate *D. similis*. A single predator, that is, either a *T. cancriformis* with a body length of 15–20 mm, or a juvenile *N. maculata* (2–3 instar), was placed into each of the mesh cages. In the control treatment, no predator was introduced into the mesh cages. The daphnids were fed 0.083 mM C L⁻¹ of the green algae *Acutodesmus obliquus*, every day. Every day, the predators were fed 10 individuals of *D. similis* and 5 chironomid larvae (*Chironomus aprilius*) (purchased from 'Bachflohkrebs.de', Stuttgart, Germany). The same amount of food organisms was also added to the mesh cages in the control treatment, to exclude any food effects on the expression of the defensive traits. During the experiment, the cages were cleaned every other day with a brush, to remove algae or bacterial film from the gauze as well as to ensure the exchange of medium, and with it kairomones, between the cage and the surrounding medium. Every 4 days, a complete exchange of the medium was performed. When the daphnids released their first clutch, 20 randomly picked neonates per replicate were transferred to

fresh beakers and the experiment was continued until they reached primiparity. Measurements at this developmental state are common for experiments on inducible defenses in *Daphnia* (Repka & Pihlajamaa, 1996; Tollrian, Duggen, Weiss, Laforsch, & Kopp, 2015).

Animals were preserved in 70% ethanol and measured using a stereo microscope (M50, Leica Microsystems GmbH, Wetzlar, Germany) equipped with a digital camera (DP26, Olympus Deutschland GmbH, Hamburg, Germany) and a digital image-analysis system (CellSens Dimension v.1.11, Olympus Deutschland GmbH, Hamburg, Germany). The following traits were analysed: body length, defined as the distance between the upper edge of the compound eye and the ventral basis of the tail spine; body width, defined as the maximal length between the dorsal and ventral edge of the carapace; and the tail spine length, defined as the distance between the ventral base and the tip of the tail spine (Figure 1a). We measured inconspicuous inducible defensive traits: length of the ventral spinules bearing area (SBA), that is, the distance between the first visible

FIGURE 1 Measured body parameters of *Daphnia similis*.

(a) Green: Body length, defined as length of the upper end of the compound eye to the basis of the tail spine. Yellow: Body width, defined as length from the dorsal to the ventral carapace margin. Light blue: Length of the tail spine, defined as the length from the basis of the tail spine to its tip. (b) Dark blue: Length of the ventral spinules bearing area (ventral SBA), defined as the length from the first ventral spinule to the basis of the tail spine. Red: Length of the dorsal spinules bearing area (dorsal SBA), defined as the length from the first dorsal spinule to the basis of the tail spine. (c) Zoom-in on the dorsal SBA (red bracket in a). Length of the dorsal spinules, defined as length from the basis of the spinule to its tip. For data acquisition, five spinules per animal were measured. (d) Exemplary outline of the helmet, that was considered for the shape analysis. The red line indicates the manually selected outline of the helmet shape. Data was acquired with the help of the Photoshop lasso tool



cranial spinule and the last spinule, at the base of the tail spine (Figure 1b) and spinule length, defined as the distance between the caudal base of the spinule and its tip (spinule length was measured for five spinules located in the middle of each SBA) (Figure 1c). We also analysed the shape of the helmet, as an inducible defense figure (Dlouhá et al., 2010) (Figure 1d).

We used the software package 'SHAPE ver. 1.3' (Iwata & Ukai, 2002) for the morphometric analysis of head shape, following the procedure and picture pre-processing described by Gore, Nawrocki, Langdon, and Bouzar (2011). Briefly, the software generates a chain code (Freeman, 1974) based on a binary picture (area of the helmet coloured in black; background coloured in white) of a helmet. Then Elliptic Fourier descriptors (EFDs; Kuhl & Giardina, 1982) of a defined number of harmonics (we used five harmonics) are calculated based on

a ellipse. The coefficients of the EFDs are subsequently normalized to be invariant with respect to the size, rotation and starting point. The results are then summarized by a principal component analysis (PCA) and could be further analysed with an ANOVA. It is important to mention that the software SHAPE allows a step-by-step processing of the images following the described workflow above (for detailed explanation see Iwata & Ukai, 2002). This shape analysis takes the complete shape of the head (exemplary pictures see Figures 1d and 4) into account in contrast to the previously used approach of describing modifications of the helmet by using only landmarks.

2.2 | Statistical analysis

Statistical analysis was performed using the software package IBM SPSS v.21 (IBM SPSS Statistics Version 21, IBM Deutschland GmbH, Ehningen, Germany). For every morphological parameter, except body length and length of the dorsal spinules, relative values were calculated, by dividing the respective trait length by body length to compensate for size-dependent differences. These relative values were then arc-sin-squareroot-transformed for analysis using an ANOVA (Rohlf & Sokal, 1995). The replicate-means were calculated for each parameter. The means were tested for homogeneity of variances, with the Levene's test, and normality, with the Shapiro-Wilk test. When the assumptions were met, a one-way ANOVA, followed by a Tukey HSD post hoc test was performed, to test for differences between the treatments. For non-parametric data, a Welch test, followed by a Tamhane-T2 post hoc test was performed.

For the head shape analysis, the values of the first two principal components were further processed using a ANOVA or, if the data was not normally distributed, a Kruskal-Wallis test was applied.

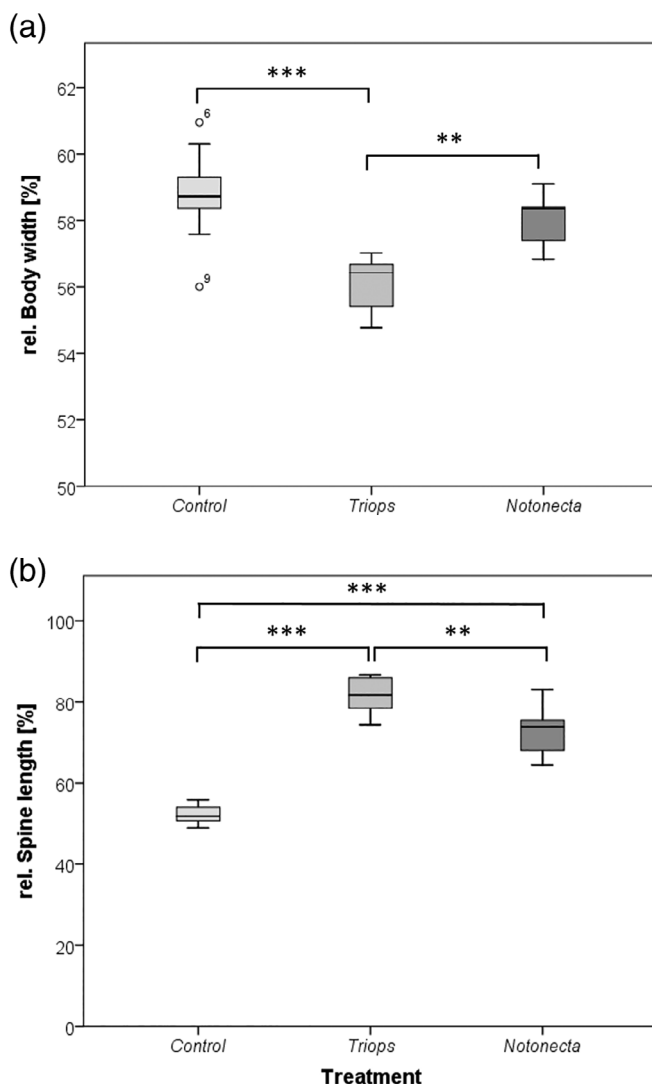


FIGURE 2 Comparison of the conspicuous traits of *Triops*-exposed (*Triops*), *Notonecta*-exposed (*Notonecta*) and control *Daphnia similis*. (a) Comparison of the relative body width (%). (b) Comparison of the relative tail spine length (%). Asterisks indicate statistical significance: *: $p < .05$; **: $p < .01$; ***: $p < .001$

3 | RESULTS

3.1 | Conspicuous defensive traits

Body length did not differ significantly between the treatments (mean body length of the control-group: $2226.9 \pm 113.99 \mu\text{m}$; *Triops*-treatment: $2314.98 \pm 83.93 \mu\text{m}$; *Notonecta*-treatment: $2221.78 \pm 73.97 \mu\text{m}$) (ANOVA; $F_{2,25} = 3.134$; $p = .061$). Relative body width differed significantly between the treatments (ANOVA; $F_{2,25} = 16.187$; $p < .001$; Figure 2a). Thereby, *Triops*-exposed *D. similis* showed a significantly smaller relative body width than control individuals (Tukey HSD; $p < .001$) and *Notonecta*-exposed individuals (Tukey HSD; $p = .002$). Control and *Notonecta*-exposed individuals did not differ significantly in relative body width (Tukey HSD; $p = .318$). Relative tail spine length differed significantly between the treatments (ANOVA; $F_{2,25} = 88.491$; $p < .001$; Figure 2b). In both predator treatments, relative tail spine length was significantly larger compared to the control treatment (Tamahane; $p < .001$). Additionally, it was significantly larger in *Triops*-exposed compared to *Notonecta*-exposed daphnids (Tamahane; $p = .004$).

3.2 | Inconspicuous defensive traits

The length of the dorsal spinules bearing area (SBA) differed significantly between the three treatments (Welch test; $F_{2,25} = 14.261$; $p = .004$; Figure 3a). The relative dorsal SBA of *Triops*-exposed individuals was not significantly longer than that measured for individuals of the control treatment (Tamhane; $p = .087$), though this might be the result of a vastly higher variance within the *Triops* treatment. Between the *Notonecta*-exposed *Daphnia* and those of the control treatment a significant difference in relative length of the dorsal SBA was measured (Tamhane; $p = .004$). The *Notonecta*-exposed *D. similis* expressed a larger dorsal SBA. The length of the dorsal SBA between *Notonecta*- and *Triops*-exposed *D. similis* did not differ (Tamhane; $p = .904$).

The length of the ventral SBA differed significantly between the groups (ANOVA; $F_{2,25} = 18.855$; $p < .001$; Figure 3b). The relative length of the ventral SBA of *Triops*-exposed *Daphnia* was significantly shorter compared to the relative length of the ventral SBA of *D. similis* of the control (Tukey HSD; $p < .001$) and the *Notonecta*-exposed individuals (Tukey HSD; $p < .001$). Between the *D. similis* of the control treatment and the *Notonecta*-exposed individuals, no difference in length of the ventral SBA could be detected (Tukey HSD; $p = .958$).

The mean length of the dorsal spinules differed significantly between *Daphnia* from the three treatments (ANOVA; $F_{2,25} = 4.973$; $p = .015$; Figure 3c). The spinules in both, *Triops*-exposed (Tukey HSD; $p = .031$) and *Notonecta*-exposed daphnids (Tukey HSD; $p = .027$), were significantly longer than the dorsal spinules in control individuals. Dorsal spinule length did not differ significantly between the predator-treatments (Tukey HSD; $p = .988$).

3.3 | Predator-specific changes in head shape

Daphnia similis exposed to *T. cancriformis* and *N. maculata* expressed predator-specific changes in head shape (Figure 4). Figure 5a illustrates, that the head shapes resulting from the three treatments can be separated in a morphospace, even though they overlap in a certain proportion. All three treatments, control to *Triops* (Kruskal-Wallis; $H = -41.530$; $p = .001$), control to *Notonecta* (Kruskal-Wallis; $H = -105.438$; $p < .001$) and *Triops* to *Notonecta* (Kruskal-Wallis; $H = -63.908$; $p < .001$), differed significantly from each other when comparing PC1 (47% of variance). Thereby, PC1 describes the major change in shape of the overall rounding of the helmet (see Figure 5a, b). For PC2 (29% of variance) the pairwise comparison of the treatments showed significant differences between the control and *Triops*-Treatment (Kruskal-Wallis; $H = -104.570$; $p < .001$) and the *Notonecta*- and *Triops*-Treatment (Kruskal-Wallis; $H = 72.986$; $p < .001$) but not between the control and *Notonecta*-Treatment (Kruskal-Wallis; $H = -31.584$; $p = .066$). PC2 described minor changes of the rostral and dorsal part of the helmet (see Figure 5a,b).

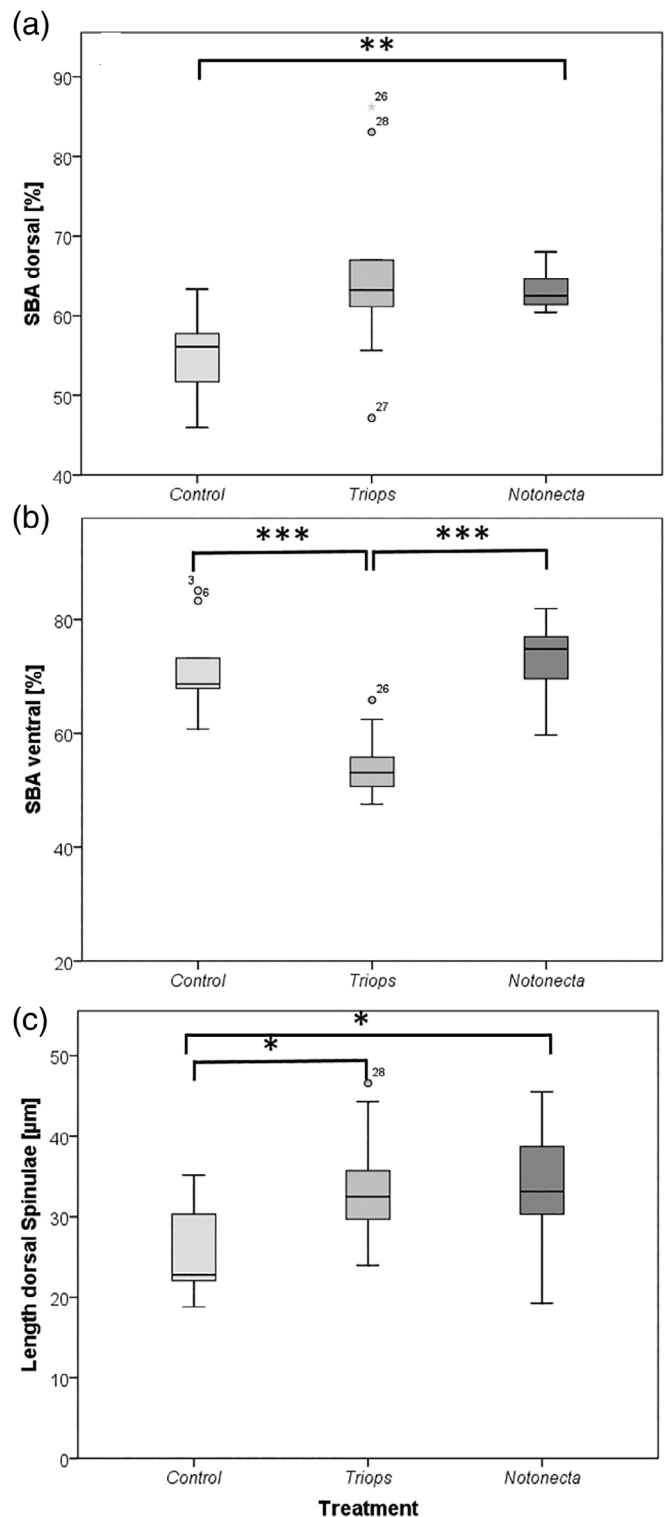


FIGURE 3 Comparison of the inconspicuous traits in *Triops*-exposed (*Triops*), *Notonecta*-exposed (*Notonecta*) and control *Daphnia similis*. (a) Comparison of the relative dorsal SBA length (%). (b) Comparison of the relative ventral SBA length (%). (c) Comparison of the mean length of the dorsal spinulae. Asterisks indicate statistical significance: *: $p < .05$; **: $p < .01$; ***: $p < .001$

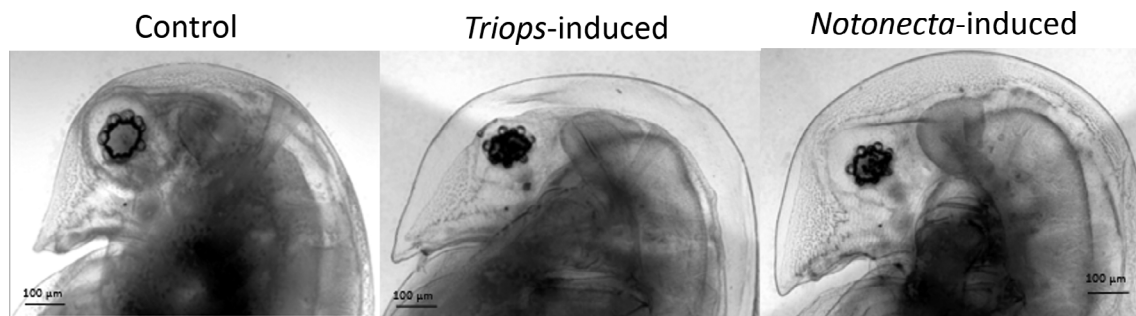


FIGURE 4 Exemplary shape variations between *Daphnia similis* of the control-, *Triops*- and *Notonecta*-treatment

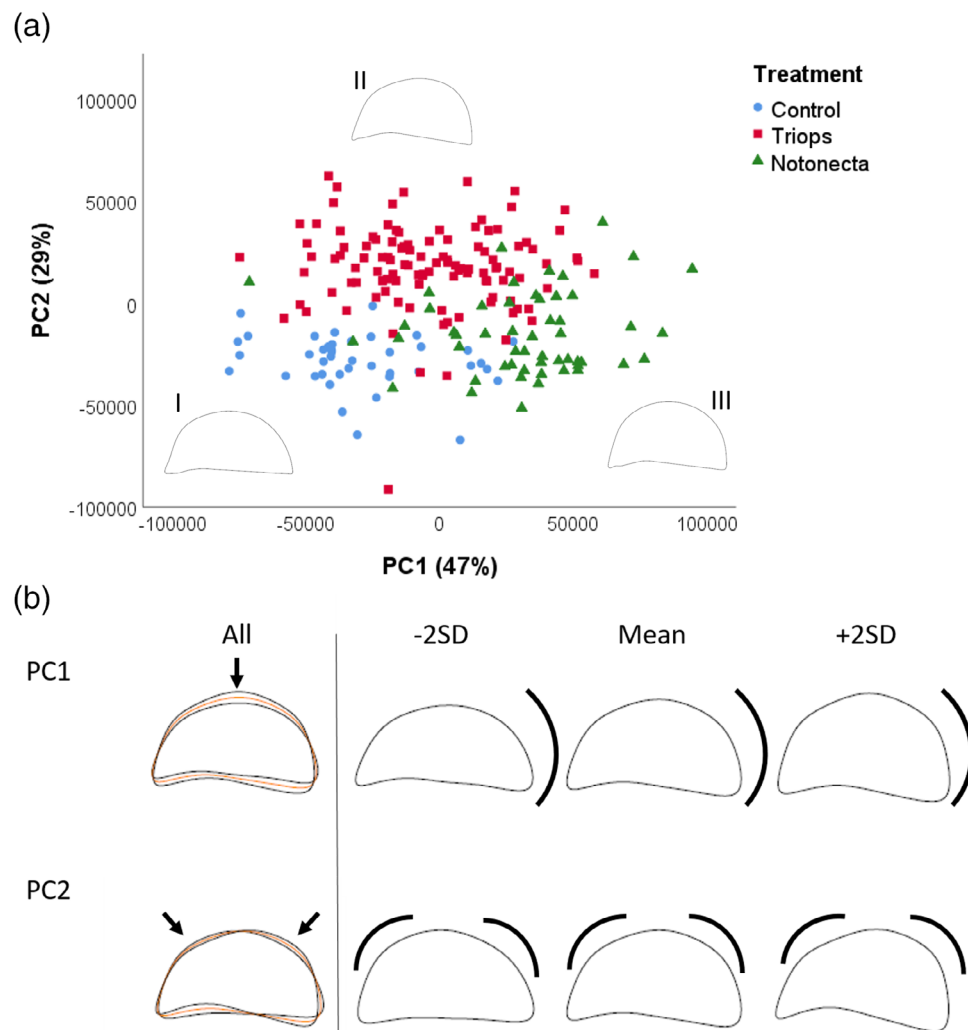


FIGURE 5 (a) Results of the PCA analysis of Fourier descriptors of the *Daphnia similis* headshape. X-axis: Principal component 1 (PC1) (Eigenvalue: 0.0012) accounting for 47% of the overall variance in shape within the data set. Y-axis: Principal component 2 (PC2) (Eigenvalue: 0.0007) accounting for 29% of the overall variance in shape within the data set. Blue circles: *D. similis* from the control treatment. Red square: *D. similis* from the *Triops* treatment. Green triangle: *D. similis* from the *Notonecta* treatment. (I) Mean head shape of the *D. similis* from the control treatment; (II) Mean head shape of the *D. similis* of the *Triops* treatment; (III) Mean head shape of the *D. similis* of the *Notonecta* treatment. (b) Shape variation in the analysed dataset. Each row represents a shape variation described by a specific principle component. Mean: The mean head shape of the analysed shape difference in the data set, next to it are the shapes $-2SD$ and $+2SD$ representing the broadness of shape variance within the data sample. All: Overlay of the three outputs on the right. The mean is highlighted in orange. Black lines indicate the described shape variance. Black arrows: Indicate the area where the PC describes the shape variation. PC1 describes the differences of the shape regarding the higher rounding of the helmet. PC2 covers minimal differences of shape variation of the rostral and dorsal part of the head

4 | DISCUSSION

The presence of different predators may require the prey to express distinct morphological defenses, especially if the predators differ in prey preference, hunting strategy or handling of the prey. In *Daphnia*, such a predator-dependent modality, that is, a differential rather than a gradual expression of specific defensive traits, has so far only been shown in *D. barbata* (Herzog & Laforsch, 2013). In this study, we reveal not only that *D. similis* expresses an array of distinct morphological defenses against predatory invertebrates, but also provide evidence for a predator-specific modality in defensive traits in *D. similis*, for example, distinctly different head shapes in response to *T. cancriformis* and *N. maculata* (Figures 4 and 5a,b).

Compared to control individuals, *Triops*-exposed *D. similis* show a slimmer body, a longer tail spine and an elongated dorsal SBA bearing significantly longer spinules. Regarding head shape, *Triops*-exposed *Daphnia* had a more angular head, which is relatively flattened with a pronounced dorsal edge (Figures 4 and 5a,b). Given the way *Triops* is catching and handling its prey, these morphological changes can be assumed to act as an effective defense. After catching the prey by engaging it with the anterior limbs, it is then taken into the midventral food groove built by the endites of the legs (Fryer, 1988). Especially at this point, the elongated tail spine, which is also expressed in *Triops*-exposed *D. magna* (Rabus & Laforsch, 2011), is likely to hinder the handling of the prey, that is, prevent the uptake into the food groove. The SBA, spinulae length and the altered head shape might act as defensive structures when the *Daphnia* has already been placed in the food groove. *Triops* frequently positions *Daphnia* with the dorsal ridge facing the food groove, with either the head or the tail spine directed towards the mouth and transports the prey towards the mandibles with movements of the gnathobases, which bear numerous spines and bristles (Fryer, 1988). We suggest that the elongated dorsal spinules, that also tend to cover a larger area of the dorsal ridge, might interfere with these spines and bristles by getting stuck or even causing damage to these fine structures. The straightened dorsal ridge may thereby serve to increase the contact area between the SBA and the food groove. The angular shape of the head may further complicate the transport of the prey since it may get stuck within the food groove. In combination, the spinules and the altered head shape may increase handling time, and ultimately the chance to escape the predator. Nevertheless, we cannot rule out that inconspicuous changes are the result of a modification of the more prominent conspicuous traits. For instance, the elongated dorsal ridge of *Triops*-exposed *D. similis* might be a result of the increased body length.

However, even if some morphological alterations appear relatively small, these small-scale changes, such as neckteeth in *D. pulex*, are already proven to have a strong protective effect against predatory invertebrates (Havel & Dodson, 1984; Pastorok, 1981; Tollrian, 1995a) which might also be connected with an increased rigidity of the overall carapace itself. The analysed small-scale defense structures, for example, head shape and spinules, are not expressed uniformly when facing different predators. Instead they tend to be predator specific (Figures 2–4 and 5a,b). *Notonecta*-exposed *D. similis*,

on the other hand, show no change in body width and length of the ventral SBA compared to the control individuals, but an increase in dorsal SBA length, length of the spinules on the dorsal ridge, and tail spine length. Additionally, *Notonecta*-exposed individuals express a slightly higher and more rounded head shape (Figures 4 and 5a,b). The potential mode of action of these defenses can again be deduced from the predator's way of feeding: *Notonecta* catch their prey with the two anterior leg pairs, which are also used to position the prey in order to pierce it with the proboscis and inject digestive fluids (Dahm, 1972). In this context, the elongated tail spine can be assumed to render the *Daphnia* bulkier, which interferes with handling during positioning of the prey. Additionally, longer spinulae and larger SBA's may further impede handling as they likely interfere with the short hair located on the inner side of the predator's legs, similar to protective effects anticipated for the small neckteeth in *D. pulex*. Given that the higher and more rounded head of the *Notonecta*-exposed *D. similis* is far less pronounced than the crest in *D. longicephala* exposed to notonectid predators (Grant & Bayly, 1981) it can be assumed that the protective effect of this trait does not increase escape efficiency as shown for *D. longicephala*. However, changes in head shape may increase the chance to evade the predator's attack due to an increased manoeuvrability, as also shown in *D. longicephala* (Barry & Bayly, 1985), *D. cucullata* (Laforsch & Tollrian, 2004) or *D. ambigua* (Hebert & Grewe, 1985).

The comparison of *Triops*- and *Notonecta*-exposed individuals shows that *D. similis* responded differentially to the two predators. While some traits did not differ between the two predator-induced morphotypes, for example, dorsal spinule length and size of the dorsal SBA, other traits, for example, tail spine length and body width, differed in the magnitude of expression, indicating a gradual response. Regarding head shape, however, a predator-specific modality, that is, a differential expression of the same trait, could be observed (Figures 4 and 5a,b). While gradual responses are commonly observed in *Daphnia* exposed to different predators (Barry, 2000; Laforsch & Tollrian, 2004; Riessen & Trevett-Smith, 2009) the phenomenon of a modal expression of defensive traits has so far only been reported in *D. barbata* exposed to *Triops* and *Notonecta* (Herzog & Laforsch, 2013). Analogous to *D. barbata*, the evolution of such a specialized defensive trait in *D. similis* may have been favoured by the different feeding modes of both predators and maybe also by differences in their temporal and spatial occurrence (Herzog & Laforsch, 2013). However, it has to be mentioned, that we analysed a single clone, that most likely co-occurs with both predators. Therefore, another clone of *D. similis* from another region, where one predator is much rarer or more frequent, could possibly differ in its reaction norm.

The functionality of rather small-scale changes of the head shape in comparison to major changes of the helmet like, for example, the crest in *D. longicephala*, remains speculative. The separation of the head shapes in a specific direction in the morphospace (Figure 5a) renders them of value for the overall defense of *D. similis* against these predators, since the reaction norm is not uniform but rather predator specific. In predation experiments, it may be difficult to disentangle

the protective effect of a single trait. Nevertheless, it is likely that these predator-specific changes may play a major role in the defense mechanisms of *D. similis*. For other species, like *D. pulex*, it has already been shown, that even small-scale defensive traits, that is, neckteeth, act as an effective defense against *C. crystallinus* (Tollrian, 1995a). Further, the defense mechanisms should not be discussed for effectiveness of single traits. Respectively, the interplay between a variety of morphological changes (conspicuous and inconspicuous), where also minor changes may have a considerable impact, may constitute the overall defense mechanism.

5 | CONCLUSION

We showed that a *Daphnia similis* clone expresses an array of inducible morphological defenses when exposed to the invertebrate predators *T. cancriformis* and *N. maculata*. The application of a morphometric analysis based on Elliptic Fourier descriptors, further allowed a quantitative evaluation and comparison of the *Daphnia* head shape, which would hardly be possible using the common landmark-based approach. Thereby, we were able to reveal a new example of predator-specific modality in phenotypically plastic defenses by applying morphometric analysis which is to our knowledge rarely used in plankton research (e.g. onostracodes; Kaesler).

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AUTHOR CONTRIBUTIONS

Sven Ritschar: Data curation; formal analysis; investigation; methodology; visualization; writing-original draft; writing-review and editing. **Max Rabus:** Data curation; formal analysis; methodology; resources; supervision; validation; visualization; writing-original draft. **Christian Laforsch:** Conceptualization; project administration; resources; supervision; validation; visualization; writing-original draft; writing-review and editing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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