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RESEARCH ARTICLE

Algal food identity affects morphological anti-predatory defense in *Daphnia pulex*

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Abstract

Microcrustaceans of the genus *Daphnia* have evolved various inducible anti-predator defenses; however, it is largely underexplored how the bottom-up factor food quality may affect the extent of these defenses. A well-studied example of an inducible defense is the deployment of neckteeth in *Daphnia pulex* in response to infochemicals from predatory larvae of *Chaoborus*, which prey on juvenile instars of *Daphnia*. In general, vulnerability depends on the body size in the respective juvenile *Daphnia* instars in relation to the mouth gape diameter of *Chaoborus* larvae. We hypothesized that algae that prolong the time in which juvenile animals remain in the vulnerable size also increase the predation risk and therefore increase the extent of induced neckteeth. To test this, we supplied three *D. pulex* genotypes with three food algae and quantified somatic growth rates, time in vulnerable instars, and neckteeth in response to *Chaoborus* infochemicals. Food algal identity affected the extent of induced neckteeth by up to 66%. However, this effect was not positively related to the time spent in vulnerable instars, indicating that the time that juveniles remain in a vulnerable size is not used to assess predation risk. We demonstrate that food identity may significantly affect the extent of induced neckteeth in *D. pulex* in a genotype-dependent way. This strongly suggests that in nature, changing phytoplankton composition may affect the degree of morphological defense and thus constitutes a bottom-up control of chemical predator–prey communication. This is another example of how environmental factors modulate the temporal dynamics of phenotypic plasticity.

In aquatic environments information transfer strongly relies on chemical cues, known as infochemicals (Pohnert et al. 2007; von Elert 2012). These infochemicals play a crucial role in conveying vital information about food sources, predators and potential mating partners and are particularly useful for most invertebrate prey, which cannot form visual images. Compared to visual cues, such infochemicals have the advantages of being persistent for a longer time, of reaching further in turbid aquatic environments, and of allowing prey to detect predators with much larger action radius than their own. Consequently, infochemicals form a network within aquatic food webs, mediating significant interactions both within and between species (Brönmark and Hansson 2012). In particular, the capability to remotely recognize predators enables the induction of adaptive changes in behavior, life history, or morphology in potential prey to reduce predation (Brönmark and Hansson 2012). The presence of predators is detected via kairomones, which are chemical substances emitted by the predators and which provide a benefit to the prey and a disadvantage to the predator (von Elert 2012). As, in general, inducible defenses are associated with costs, predators can strongly influence prey populations not only directly by consumptive but also indirectly by non-consumptive effects caused by induced defenses (Preisser et al. 2005; Zhang et al. 2022). Such non-consumptive effects are typically strong in aquatic food webs and may even exceed direct consumptive effects (Preisser et al. 2005; Zhang et al. 2022).

Microcrustaceans of the genus *Daphnia* play a key role in lakes and ponds, as they not only are major grazers of planktonic primary producers but also represent preferred prey of invertebrates and fish (Ebert 2022). In response to kairomones, various anti-predator defenses, such as changes in behavior

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(Ahlers et al. 2024), life history (von Elert and Stibor 2006) and morphology (Hahn and von Elert 2022; Tollrian 1994) are triggered in *Daphnia*. The strength of adaptive changes in behavior, life history, and morphology increases with predator abundance (Tollrian 1993; von Elert and Pohnert 2000; von Elert and Stibor 2006) and hence with predation risk. The respective kairomones have been identified in the case of behavioral predator avoidance in response to fish (Hahn et al. 2019; Ahlers et al. 2024) and in the case of induced morphological defenses in response to larvae of *Chaoborus* (Weiss et al. 2018) and fish (Hahn and von Elert 2022).

One intensively investigated model system for the induction of morphological defenses is the larvae of *Chaoborus* sp. preying on juvenile instars of *Daphnia pulex*. With increasing prey size, the encounter probability with *Chaoborus* larvae increases because larger prey swim faster, which leads to more encounters with this ambush predator. The strike efficiency of the larvae decreases with prey size (Pastorok 1981) because larger prey is more difficult to manipulate and ingest, and at some point they will be impossible to ingest. As a result, the probability of a successful capture is the highest for intermediate prey sizes, and with further instars, juvenile *D. pulex* outgrow this vulnerable prey size. However, *D. pulex* is able to reduce the rates of predation by deploying an inducible morphological defense (Krueger and Dodson 1981; Tollrian 1995). This morphological defense comprises the formation of small protuberances in the neck region of *D. pulex*, so-called neckteeth. In general, the timing and the extent of induced neckteeth across juvenile instars of *Daphnia* are related to the relative sizes of the *Daphnia* and *Chaoborus* species interacting (Riessen and Trevett-Smith 2009). In the case of *D. pulex* and *Chaoborus flavicans*, neckteeth are most pronounced during the 2nd juvenile instar (Tollrian 1993). The extent of induced neckteeth increases with the abundance of *Chaoborus* larvae and hence with predation risk and shows a Michaelis–Menten-like saturation curve (Tollrian 1993; Parejko and Dodson 1990). However, at a given predator abundance, the extent of induced neckteeth may also be affected by temperature (Klintworth and von Elert 2021a), UV (Eshun-Wilson et al. 2020) or food concentration: Parejko and Dodson (1990) found highest values for induced neckteeth at a low food concentration, and these findings were corroborated by Riessen (1992) who argued, based on a model, that for a given predator abundance, the extent of induced neckteeth would increase at lower food concentrations.

The increased extent of induced neckteeth at lower food concentrations might be because *Daphnia* grows more slowly at low food concentrations than at high ones (Giebelhausen and Lampert 2001; Lampert 2006). Such reduced somatic growth would extend the time span that juvenile *Daphnia* remain in the vulnerable size and thereby increase the risk of predation by larvae of *Chaoborus*, so that the overall predation risk would be higher for slow-growing *Daphnia*.

Except for the mid-season phytoplankton biomass minimum (clearwater phase, Sommer et al. 2012), *Daphnia* growth and reproduction are usually not limited by food quantity but rather by stoichiometric or biochemical food quality, which varies with season (e.g., Hartwich et al. 2012; Wacker and von Elert 2001). Here, we hypothesize that differences in algal identity would affect the time that *D. pulex* spends in vulnerable instars, which would translate into a higher degree of neckteeth formation in low-quality food algae. By this, food quality as a bottom-up factor would modulate the degree of neckteeth formation at a given predator abundance. As a basic test of the concept, we provided three different algae (*Acutodesmus obliquus*, *Chlamydomonas klinobasis*, and *Cryptomonas* sp.), which are known to result in different somatic growth rates (Ahlgren et al. 1990; Fink et al. 2011; Koch et al. 2012) as food for *D. pulex* and which we expected to tolerate the treatment with antibiotics according to Klintworth and von Elert (2021b). We determined the extent of induced neckteeth by scoring the neckteeth during the 2nd juvenile instar and body size during the juvenile instars. Furthermore, we investigated how the time spent in vulnerable instars would be related to the extent of induced neckteeth. We used three clones of *D. pulex* that differed in the extent of induced neckteeth to kairomones released from *Chaoborus* larvae to generalize our results.

Materials and methods

Study species

Daphnia pulex clones Gerstel (Koch et al. 2009), clone TCO (Colbourne et al. 2011), and clone SGus (pers. comm. S. Gustafsson, Sweden) were cultured in aged and aerated tap water at 20°C ± 1°C and a 16 : 8 light : dark cycle at a density of 15 ind. L^{-1}. The animals were transferred into fresh medium containing at least 1 mg C L^{-1} of *Chlamydomonas klinobasis* strain # 56 (Limnological Institute, University of Constance) every 2nd day.

Acutodesmus obliquus (formerly *Scenedesmus obliquus*) strain SAG 276-3a (Culture Collection of Algae, Göttingen University), *C. klinobasis*, and *Cryptomonas* sp. strain SAG 26.80 (Culture Collection of Algae, Göttingen University) were grown in 5 L semi-continuous batch cultures. *C. klinobasis* and *Cryptomonas* were grown in sterile cyanophycea medium (von Elert and Jüttner 1997) modified with vitamins, whereas *A. obliquus* was grown in sterile Z/4 medium modified with vitamins (Zehnder and Gorham 1960). One liter of the cultures was replaced every other day with fresh medium. The food suspensions were screened using a 30-μm gauze, and the needed food suspension volume was determined photometrically at a wavelength of 470 nm by using a respective calibration curve relating the carbon content to the optical density. The algae *A. obliquus*, *C. klinobasis*, and *Cryptomonas* sp. are subsequently referred to as *Acutodesmus*, *Chlamydomonas*, and *Cryptomonas*, respectively.

Fatty acid analyses

Fatty acid measurements were performed according to Klintworth and von Elert (2020). Briefly, filters loaded with 0.5 mg C of each food alga suspension were extracted with dichloromethane–methanol (2 : 1, v : v) after the addition of 5 μg of C23:0 methyl ester as an internal standard. Fatty acids were then transesterified in 5 mL of methanolic HCl, and the fatty acid methyl esters were extracted with isohexane and finally dissolved in 100 μL of isohexane. One microliter per sample was injected splitlessly in a 6890-N gas chromatograph (GC) System (Agilent Technologies) equipped with a DB-225 capillary column (27.5 m, 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific). The GC conditions were as follows: injector and flame ionization detector (FID) temperatures 220°C; initial oven temperature 60°C for 1 min, followed by a 120°C min^{-1} temperature ramp to 180°C, then a ramp of 50°C min^{-1} to 200°C, followed by 10.5 min at 200°C, followed by a ramp of 120°C min^{-1} to 220°C; helium with a flow rate of 1.5 mL min^{-1} was used as a carrier gas. The fatty acid methyl esters were identified by comparison of retention times with those of reference compounds and quantified via the internal standard and previously established calibration curves.

Stoichiometric analyses

In order to determine particulate C and N, volumes of the algal suspensions equivalent to 0.3 mg C were filtered on precombusted glass fiber filters. The filters were dried at 60°C for at least 24 h and wrapped up in tin capsules for the measurements using a Flash EA 2000 elemental analyzer (Thermo Fisher). In order to determine P contents, glass fiber filters loaded with 0.5 mg C of the algal suspension were transferred into a solution of potassium peroxodisulfate and 1.5% sodium hydroxide, and autoclaved at 120°C for 1 h. Subsequently, the concentration of soluble reactive phosphorus was determined using the molybdate-ascorbic acid method and measured with a spectrophotometer at 885 nm. For each food alga, triplicate filters were prepared. The concentrations of the nutrients were used to calculate molar C : N : P ratios.

Preparation of *Chaoborus* incubation water extract

The extract of *Chaoborus* incubation water was prepared according to Klintworth and von Elert (2020). Approximately, 300–350 4th instar larvae (ordered from inter-aquaristik.de) of *Chaoborus flavicans* were fed with 1–2 neonates of *D. pulex* per larva. After 1–2 h of feeding, the larvae were transferred into 1 L of fresh aged and aerated tap water without food. After 24 h, the larvae were removed from the water using a 250-μm gauze, and the water was filtered through a glass fiber filter (Whatman, MN 85/220, 0.4 μm). Subsequently, the kairomone was enriched by solid phase extraction (VARIAN, Bond Elut-C18, 10 g of sorbent, volume 60 mL, Agilent Technologies) according to Christjani et al. (2016). All eluates were pooled and evaporated to dryness in a rotary evaporator and a vacuum centrifuge. The pooled and dried residues of 20 L were dissolved in 1 mL methanol and stored at −20°C until use. In previous experiments, a control extract, which was prepared the same way but without any animals in the water, did not induce neckteeth in *D. pulex* (Klintworth and von Elert 2020).

Dose–response experiments

Dose–response experiments were performed to estimate the concentration of *Chaoborus* incubation water extract needed for the induction of 50% of maximum neckteeth ($\text{Conc}_{0.5\text{max}}$) across the three *D. pulex* clones. In order to ensure that the resultant $\text{Conc}_{0.5\text{max}}$ would induce intermediate neckteeth on all three algal foods, these dose–response experiments were performed with *Chlamydomonas*, since, based on intermediate polyunsaturated fatty acids (PUFAs) content (Supporting Information Table S1), we expected intermediate somatic growth on this food alga. Animals that had released their 3rd or 4th clutch into the brood chamber were divided into the different treatments containing 0, 0.2, 0.5, 1, 2, 3, 5, 6, or 7 μL of *Chaoborus* incubation water extract in 100 mL of aged and aerated tap water. The treatments containing 0.2 and 6 μL of *Chaoborus* incubation water extract were only prepared for clone SGus; the treatment containing 7 μL of *Chaoborus* incubation water extract was only prepared for clone Gerstel. *Chlamydomonas* was provided at a concentration of 2 mg C L^{-1}. Animals were kept individually, and each treatment was replicated threefold. The animals were transferred daily to freshly prepared jars. After the neonates had hatched from the brood chambers, the mothers and surplus neonates were removed from the jars so that no more than six neonates remained in the jars. The extent of induced neckteeth was determined using the method developed by Tollrian (1993) with the slight modification that no differentiation was made between small and big teeth, and neckteeth scores were not expressed as percentages. During their 2nd juvenile instar, five randomly chosen animals per jar were used for scoring, and this slightly simplified scoring resulted in similar dose–response curves (*see* Fig. 1) as the scoring in Tollrian (1993).

Dose–response curves were fitted as Michaelis–Menten models on the data for each clone. From the model, the maximum induction and the concentration of *Chaoborus* incubation water extract needed for the induction of 50% of maximum neckteeth ($\text{Conc}_{0.5\text{max}}$) were derived for each clone. During subsequent experiments, a volume slightly higher than the volume of $\text{Conc}_{0.5\text{max}}$ was used to ensure that both increases and decreases in the extent of induced neckteeth would be well detectable within each clone. For clone Gerstel, TCO, and SGus, 1.1, 0.5, and 0.05 μL of the *Chaoborus* incubation water extract were used, respectively.

Extent of induced neckteeth and algal identity

In order to diminish any bacterial activity, the food algae were treated with antibiotics prior to being fed to *Daphnia*. To prepare the antibacterial medium, 500 mg of ampicillin and 50 mg of tetracycline were dissolved in 1 L of cyanophycea medium. A volume corresponding to 6–8 mg C of *C. klinobasis*, *A. obliquus*, or *Cryptomonas* sp. was centrifuged,

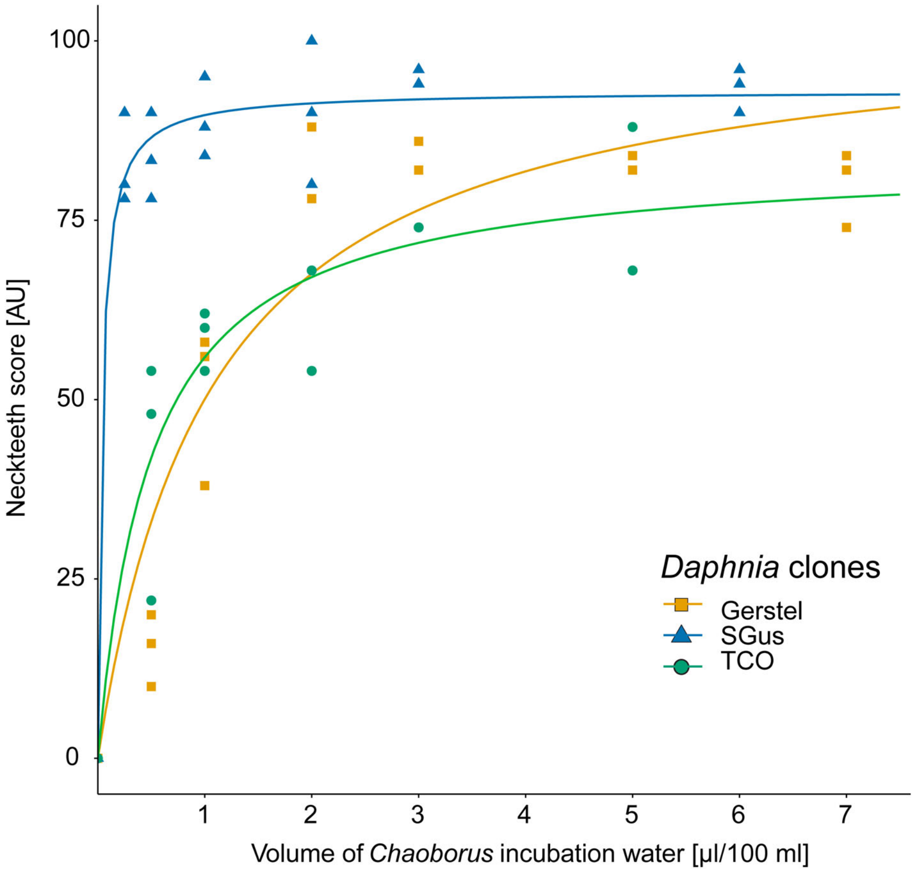

Fig. 1. Neckteeth induction of *Daphnia pulex* clones Gerstel (yellow), SGus (blue), and TCO (green), growing on *Chlamydomonas klinobasis* as a function of the volume of *Chaoborus* incubation water extract that was used in the respective treatments. The lines represent the results of Michael–Menten models fitted to the respective data sets. Details on the models are presented in Table 1. Details on the statistical analysis are given in the text.

the supernatant was discarded, and the pellet was resuspended in 100 mL of sterile cyanophycea medium in which 50 mg of ampicillin and 5 mg of tetracycline had been dissolved, and incubated in sterile Erlenmeyer flasks sealed with sterile plugs for 22 h on a rotary shaker (120 rpm) at constant light. After incubation, the suspensions were centrifuged, the pellets were resuspended in fresh cyanophycea medium without any antibiotics, centrifuged again, and the pellets were resuspended in 100 mL of fresh cyanophycea medium.

Experimental mothers were synchronized with newborns originating from 3rd clutches. When experimental mothers had released their 1st clutch into the brood chamber, they were distributed among the different food treatments, which consisted of 1 mg C of *Acutodesmus*, *Chlamydomonas*, or *Cryptomonas*, which had been treated with antibiotics and then had been suspended in 500 mL of water. These experimental mothers were transferred into freshly prepared food suspensions every other day until they released their 2nd clutch into the brood chamber. Then, the experimental mothers were placed individually into 100 mL of water, containing the same antibiotic-treated food alga as before, in either the control or the kairomone treatment. The kairomone treatment of each clone contained the previously calculated volume of the *Chaoborus* incubation water extract, that is, 1.1, 0.5, and 0.05 μL for clones Gerstel, TCO, and SGus. The control and kairomone treatments were replicated ninefold for each clone.

Once the neonates of the 2nd clutch had hatched, six neonates per replicate were transferred into freshly prepared glasses containing 100 mL of the respective treatment. Any remaining neonates were pooled, and their dry mass was measured in subsamples of three times 10 neonates per treatment. The six juveniles per treatment were transferred daily to freshly prepared jars. Throughout instars 2, 3, and 4, and at 1st reproduction, pictures of the animals were taken using a camera attached to a stereo microscope and with the software "ZEN" (Zeiss). The extent of induced neckteeth in instar 2 was determined as described above. Body sizes were recorded in instars 2, 3, and 4, and at 1st reproduction and size was measured from the top of the head to the base of the tail spine as according to Buikema (1973) and Riessen and Sprules (1990); all measurements were taken using the software ImageJ 1.50. Upon 1st reproduction, three individuals were taken at random for dry weight (w_t) determination. The somatic growth rate (g) was

Table 1. Maximum neckteeth induction and concentration of *Chaoborus* incubation water extract needed for induction of 50% of neckteeth induction maximum ($\text{Conc}_{0.5\text{max}}$). Both parameters were derived from the fit of a Michaelis–Menten model to the data obtained for *Daphnia pulex* clones Gerstel, SGus, and TCO (*see* Fig. 1). Presented are mean values ± SE and the respective *p*-values of the model fit. The values of the maximum neckteeth induction were not distinguishable among the clones while those for $\text{Conc}_{0.5\text{max}}$ were.

	Gerstel		SGus		TCO	
	Mean ± SE	*p*-value	Mean ± SE	*p*-value	Mean ± SE	*p*-value
Max. induction (AU)	103.7 ± 7.7	<0.001	93.0 ± 1.9	<0.001	83.8 ± 7.6	<0.001
$\text{Conc}_{0.5\text{max}}$ (μL 100 mL^{-1})	1.07 ± 0.3	<0.001	0.04 ± 0.0	0.011	0.50 ± 0.2	0.012

calculated according to the formula: $g = \frac{\ln(w_t) - \ln(w_0)}{t}$, with w_t being the individual weight at day t and w_0 being the individual weight at day 0 (Rothhaupt and Lampert 1992).

Statistics

All statistical analyses were performed using RStudio (RStudio Team 2020). The analysis of similarities (ANOSIM) on the fatty acid data was performed with 9999 permutations using Bray–Curtis distance. For the analysis of the data derived from the Michaelis–Menten models that were fitted to the dose–response data of each clone, a one-way ANOVA type 2 followed by Tukey's HSD test was performed. In the case of a parameter being determined on more than one animal per replicate, as was the case for the extent of induced neckteeth and body size, the average of those values per replicate was calculated and used for statistics. A Levene's test was performed on all measured data to test for homogeneity of variance. If given, an ANOVA followed by a Tukey's honest significance (HSD) test was performed. We were not interested in the comparison of the extent of induced neckteeth across clones, as different clones were exposed to different kairomone concentrations to compensate for differences in sensitivity. As a consequence, data analyses were performed for each clone independently.

Results

Fatty acids in food algae

The shares of single fatty acids of the three algae *Acutodesmus*, *Chlamydomonas*, and *Cryptomonas* can be found in Supporting Information Table S1. Using the categories of saturated fatty acids, monounsaturated fatty acids, and PUFAs fatty acids, these fatty acid profiles were dissimilar (ANOSIM: $R = 1$; $p = 0.0035$) with PUFAs being the most responsible for the dissimilarity. The shares of saturated fatty acids, monounsaturated fatty acids, and PUFAs were all significantly different among the three algae (Tukey's HSD after one-way ANOVA; for saturated fatty acids: $F = 761.01$, $p < 0.001$; for monounsaturated fatty acids: $F = 2033$, $p < 0.001$; for PUFAs: $F = 1819.5$, $p < 0.001$) with *Acutodesmus* showing the lowest share of PUFAs (Supporting Information Table S1). The C : N : P ratios of *Acutodesmus*, *Chlamydomonas*, and *Cryptomonas* were 298 : 25 : 1, 54 : 11 : 1, and 119 : 27 : 1, respectively.

Dose–response experiments

Dose–response experiments revealed that mean values of maximum neckteeth among the *D. pulex* clones ranged from 84 in clone TCO over 93 in clone SGus to 104 in clone Gerstel (Fig. 1, Table 1); however, the values for maximum neckteeth did not differ (one-way ANOVA, $F_{2;6} = 2.47$, $p = 0.17$) (Fig. 1). The concentration of *Chaoborus* incubation water extract needed for the induction of 50% of maximum neckteeth ranged from 0.04 μL in clone SGus over 0.5 μL in clone TCO to 1.1 μL in clone Gerstel (Fig. 1, Table 1). Accordingly, the concentration of *Chaoborus* incubation water extract needed for the induction of 50% of maximum neckteeth ($\text{Conc}_{0.5\text{max}}$) differed significantly (one-way ANOVA, $F_{2;6} = 8.28$, $p = 0.02$) (Fig. 1): For clone SGus, a significantly lower concentration than for clone Gerstel was needed (Tukey's HSD test, SGus vs. Gerstel, $p = 0.016$) while $\text{Conc}_{0.5\text{max}}$ for clone TCO was not significantly different from either of the other clones (Tukey's HSD test, TCO vs. Gerstel, $p = 0.14$, TCO vs. SGus, $p = 0.24$). The comparisons with clone Gerstel should be treated with caution because the Michaelis–Menten model did not produce a good fit of the data.

Extent of induced neckteeth and algal identity

The extent of induced neckteeth in the *Chaoborus* treatments on the different food algae ranged from 19 to 82 for all clones (Fig. 2). No neckteeth were observed in the control treatments. We wanted to test if food identity affects the extent of induced neckteeth within a clone. Independent one-way ANOVAs for each of the clones revealed that in two of the clones (TCO, SGus) neckteeth scores were affected by the type of food, but not in clone Gerstel (Fig. 2, Table 2). The extent to which food identity reduced induced neckteeth ranged from 56 down to 19 (−66%) in clone TCO to 82 down to 62 (−24%) in clone SGus. In clone TCO, the highest values for neckteeth occurred on *Chlamydomonas* and significantly lower values on *Cryptomonas* as food, while in clone SGus, the effects of *Cryptomonas* and *Chlamydomonas* were inverse.

Time remaining in vulnerable body size and somatic growth rate

Animals have outgrown the vulnerable size when their size exceeds 1.25 mm (Shei et al. 1988), which, except for one case, was the case from instar 4 on in all clones (Supporting Information Fig. S1). We therefore considered the time spent

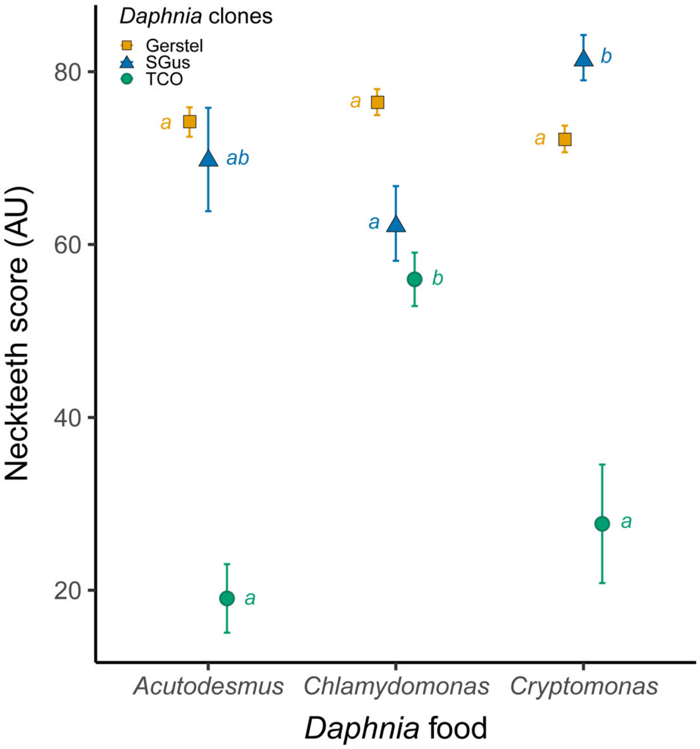

Fig. 2. Mean (± SE, $n = 9$) neckteeth scores in *Daphnia pulex* clones Gerstel (yellow), SGus (blue), and TCO (green) exposed to *Chaoborus* kairomone, growing either on *Acutodesmus*, *Chlamydomonas*, or *Cryptomonas* as a food source. Different letters (same color) indicate significant differences between different food sources within one *D. pulex* clone ($p < 0.05$, one-way ANOVA and Tukey's HSD test for each clone).

in instars 1–3 as the time spent in a vulnerable stage. The exception was clone SGus growing on *Acutodesmus*, of which the 4th instar size (1.16 ± 0.007 mm, mean ± SE) was below 1.25 mm; here we considered the time spent in instars 1–4 as the time spent in a vulnerable stage. The mean time spent in vulnerable instars ranged from 71 to 172 h across all clones (Fig. 3) exposed to *Chaoborus* kairomone. Independent one-way ANOVAs for each of the clones revealed that in all clones the time spent in vulnerable instars was affected by food identity (Table 3) with *Acutodesmus* causing significantly longer times to outgrow the vulnerable size in all three clones (Fig. 3).

It is reasonable to assume that the effects of food identity on developmental time are related to differences in somatic growth rates. Across all clones, somatic growth rates ranged from 0.17 to 0.66 d^{-1} (Supporting Information Fig. S2). Food identity affected juvenile somatic growth rates in all clones, with *Acutodesmus* causing significantly lower growth rates in all three clones, while higher growth on *Cryptomonas* than on the green alga *Chlamydomonas* was not observed across all clones (Supporting Information Fig. S2; Table S2).

Within each *D. pulex* clone, different food identities resulted in different extents of induced neckteeth and different times spent in vulnerable body sizes. When the extent of induced neckteeth was plotted against the time remaining in a vulnerable body size, for clones SGus and Gerstel no significant linear relation was detected, while for clone TCO a significantly negative relation was observed (Fig. 4).

Discussion

Our aim was to affect the growth of juvenile *D. pulex* such that the time that juveniles remain within the vulnerable body size for being preyed upon by larvae of *Chaoborus* would be affected. To achieve this, we supplied three *D. pulex* genotypes with three different food algae.

By creating dose–response curves and fitting a model to these curves, we could show that the three *D. pulex* genotypes did not differ in their possible maximum induced neckteeth, but differed in their sensitivity to *Chaoborus* kairomone. Such clonal variability in response to the *Chaoborus* kairomone is well known (Boeing et al. 2006; Hammill et al. 2008; Christjani et al. 2016). In order to account for these different sensitivities, each *D. pulex* genotype was exposed to a different kairomone concentration in the subsequent experiments testing the

Table 2. One-way ANOVA results on testing the effect of algal food identity on neckteeth induction scores (AU) in each of the three *Daphnia pulex* clones exposed to *Chaoborus* kairomone.

***Daphnia* clone TCO**	**df**	**Sums of squares**	**Mean squares**	***F*-value**	***p*-value**
Food	2	5773	2886.4	13.22	0.000192
Residuals	21	4584	218.3		

***Daphnia* clone Gerstel**	**df**	**Sums of squares**	**Mean squares**	***F*-value**	***p*-value**
Food	2	81.8	40.92	1.803	0.186
Residuals	24	544.6	22.69		

***Daphnia* clone SGus**	**df**	**Sums of squares**	**Mean squares**	***F*-value**	***p*-value**
Food	2	1518	759.1	4.324	0.0261
Residuals	22	3862	175.5		

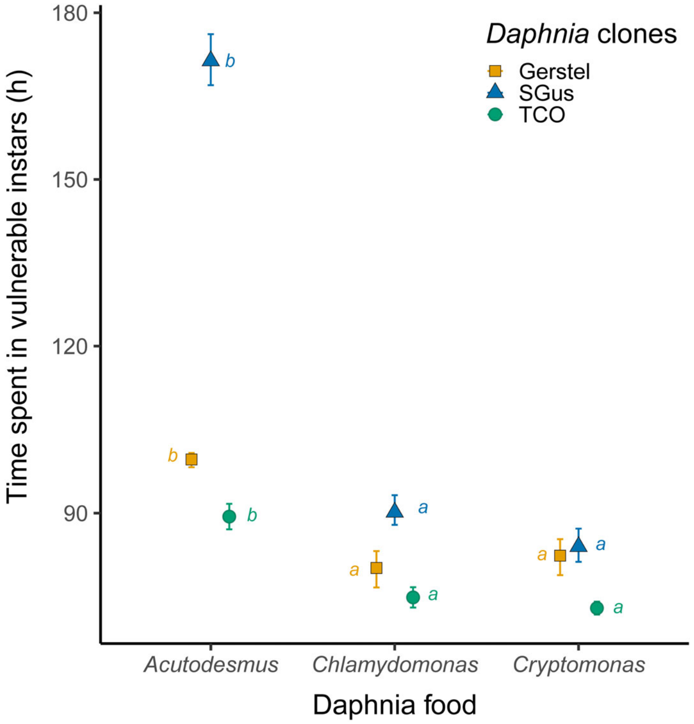

Fig. 3. Mean (± SE, $n = 9$) time spent in the vulnerable instars of *Daphnia pulex* clones Gerstel (yellow), SGus (blue), and TCO (green), exposed to *Chaoborus* kairomone and growing either on *Acutodesmus*, *Chlamydomonas*, or *Cryptomonas* as a food source. Instars, in which body size was ≤ 1.25 mm, were regarded as vulnerable. Different letters (same color) indicate significant differences between different food sources within one *Daphnia* clone ($p < 0.05$, one-way ANOVA and Tukey's HSD test for each clone).

effects of algal identity on the extent of induced neckteeth. This should guarantee that a higher extent of induced neckteeth would also be possible in response to different food algae. Since each *D. pulex* genotype was exposed to a different kairomone concentration, we did not compare the extent of induced neckteeth across genotypes.

Our fatty acid analyses corroborate earlier findings that the algae differ in their fatty acid profiles (Ahlgren et al. 1990; von Elert and Stampfl 2000; von Elert 2002). We can further exclude any stoichiometric nutrient limitation as the C : N and C : P ratios, which we have measured here, have been shown to be non-limiting to *Daphnia* growth (Lürling and van Donk 1997). *Acutodesmus* contained the lowest share of PUFAs and resulted in significantly lower somatic growth than *Chlamydomonas* and *Cryptomonas* in all three *D. pulex* clones, which suggests that the different fatty acid compositions of the food algae may have caused the differences in somatic growth. However, we did not attempt to confirm this by PUFA supplementation experiments, since we aimed to test the effect of food identity on the extent of induced neckteeth more generally. Accordingly, our data demonstrate that algal identity affects somatic growth in each of the three *D. pulex* clones exposed to *Chaoborus* kairomone. The somatic growth rates that we determined during this study are comparable with somatic growth rates determined during other studies using the same algal and *Daphnia* species (Fink et al. 2011; Koch et al. 2012; Klintworth and von Elert 2020). Furthermore, the identity of the *D. pulex* clone affected the somatic growth response to the different food algae, which is in line with earlier reports of clonal variability in response to biochemical food quality (Brzezinski et al. 2010; Werner et al. 2021).

Here we assumed that juvenile *D. pulex* have outgrown the vulnerable size when the size exceeds 1.25 mm (Shei et al. 1988). However, this is only true for *Chaoborus flavicans*, as larger *Chaoborus* species like *C. americanus* and *C. trivittatus* can ingest *D. pulex* to at least 1.5 mm in body length, which expands the time and the number of instars that *D. pulex* are vulnerable (Riessen and Trevett-Smith 2009). Hence, our assumption of a vulnerable body length of 1.25 mm (usually instars 1–3) can be regarded as a decent approximation of the time spent in vulnerable stages for the purpose of this present study.

Algal identity affected the time that *D. pulex* spent in the vulnerable instars with the common pattern across all three

Table 3. One-way ANOVA results on testing the effect of algal food identity on time spent in vulnerable instars in each of the three *Daphnia pulex* clones exposed to *Chaoborus* kairomone.

***Daphnia* cloneTCO**	**df**	**Sums of squares**	**Mean squares**	***F*-value**	***p*-value**
Food	2	1314	657	23.64	3.31E-06
Residuals	23	611.4	27.8		

***Daphnia* clone Gerstel**	**df**	**Sums of squares**	**Mean squares**	***F*-value**	***p*-value**
Food	2	2178	1089.1	15.9	4.00E-05
Residuals	24	1644	68.5		

***Daphnia* clone SGus**	**df**	**Sums of squares**	**Mean squares**	***F*-value**	***p*-value**
Food	2	42,039	21,019	188.9	5.31E-15
Residuals	23	2559	111		

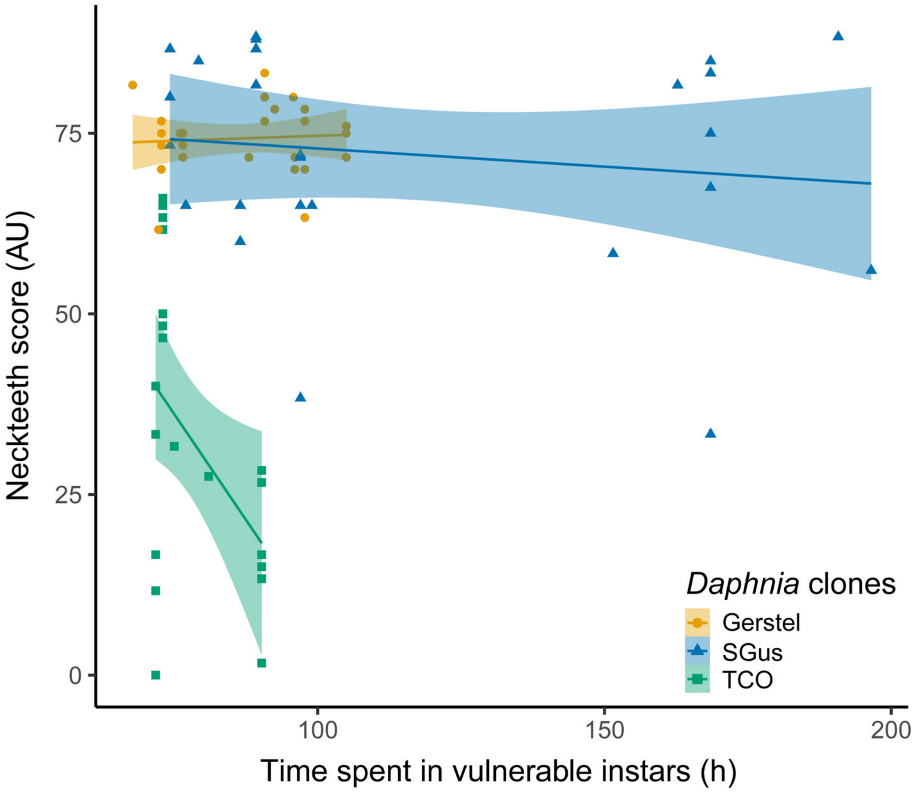

Fig. 4. Neckteeth scores as a function of the time spent in the vulnerable instars. Depicted are *Daphnia pulex* clones Gerstel (yellow circles), SGus (blue triangles), and TCO (green squares) exposed to *Chaoborus* kairomone. Solid lines indicate linear regressions with 95% confidence intervals for the effect of time spent in vulnerable instars on neckteeth induction. This regression is significant for clone TCO ($p = 0.029$, $R^2 = 0.17$) and not significant for clone SGus ($p = 0.494$, $R^2 = -0.02$) and clone Gerstel ($p = 0.734$, $R^2 = -0.04$).

D. pulex clones that *Acutodesmus* caused significantly longer times of *D. pulex* remaining in the vulnerable size. In none of the *D. pulex* genotypes a difference between *Chlamydomonas* and *Cryptomonas* as food was detectable. In conclusion, the fact that *Acutodesmus* caused the lowest somatic growth and the longest time spent in vulnerable instars is consistent across all three *D. pulex* clones.

Furthermore, food algal identity affected the extent of induced neckteeth. Theoretically, such effects of algal identity may be caused by differences in kairomone degradation that result from differences in bacteria accompanying different algae cultures. However, here we have pre-incubated food algae with concentrations of two antibiotics that previously have been shown to significantly reduce bacterial counts (Klintworth and von Elert 2021b) and thus minimize the probability that accompanying bacteria differ among algae; therefore, it seems reasonable to attribute the reported effects on the extent of induced neckteeth within a *Daphnia* genotype to algal identity. In this study, based on three different food identities, we found up to 66% less induced neckteeth. These results demonstrate that, for a given kairomone concentration, algal identity affects the extent of induced neckteeth, and it remains to be tested if including further food algae taxa would lead to a similar or even stronger reduction of induced neckteeth. The effects of algal identity on neckteeth expression suggest that seasonal variations of phytoplankton composition may function as a bottom-up factor that modulates the extent of induced neckteeth of a *D. pulex* genotype in response to the neckteeth-inducing kairomone.

However, these effects of algal identity on the extent of induced neckteeth were not consistent across *D. pulex* clones: there was no effect in clone Gerstel though algal identity had affected growth and time spent in vulnerable instars. Already from this it may be concluded that algal identity factors that constrain somatic growth in *D. pulex* must be different from those that constrain the extent of induced neckteeth. This reasoning is corroborated by the finding that *Acutodesmus* caused the lowest somatic growth in all three clones (Supporting Information Fig. S2), but neither caused consistently the highest nor the lowest neckteeth across the clones (Fig. 2).

The respective alga, which allowed for maximum extent of induced neckteeth, was different across the three *D. pulex* clones, which demonstrates that the unknown factors that constrained maximum induced neckteeth were different across the three *D. pulex* clones. This suggests that during seasonal phytoplankton succession and for a given concentration of *Chaoborus* kairomone, these clones would deploy maximum neckteeth at different times of the succession.

The induction of more neckteeth and a larger neckteeth pedestal in response to *Chaoborus* larvae has recently been reported for *Daphnia longispina* (Maurone et al. 2018; Sperfeld et al. 2020), and it remains to be tested if food identity has comparably strong effects on these morphological changes in *D. longispina* as reported here for *D. pulex*. A kairomone from fish induces morphological defenses in *Daphnia lumholtzi* (Hahn and von Elert 2022), which represents another case in which the effect of algal identity on the extent of morphological defenses remains to be investigated.

Studies on the morphological defense in *D. pulex* against predation by *Chaoborus* larvae have largely focused on changes in the number of neckteeth and in pedestal size. However, the kairomone further induces changes in resource allocation (Klintworth and von Elert 2020), and the whole-organism response in shape and ultrastructure is more complex (Laforsch et al. 2004; Paplauskas et al. 2024). It remains to be tested if these accompanying effects of *Chaoborus* kairomone are also affected by food algae identity.

In line with the fact that the extent of induced neckteeth increases with predator abundance and hence with predation risk (Parejko and Dodson 1990; Tollrian et al. 2015), we had hypothesized that increased predation risk caused by extended time spent in vulnerable instars would as well go along with a higher extent of increased neckteeth. However, in none of the three clones neckteeth scores increased with the time spent in vulnerable instars, which demonstrates that the assessment of predation risk in *D. pulex* does not seem to include differences in developmental time. A remarkable exception was observed in clone TCO, in which neckteeth decreased with time spent in vulnerable instars. Among the three clones, TCO seems unique in such that *Acutodesmus* caused the smallest increase in the time spent in vulnerable instars (90 h) and the strongest negative effects on somatic growth and on induced neckteeth. It is largely these strong constraining effects on neckteeth that cause the significantly negative correlation of neckteeth and time spent in vulnerable instars. As somatic growth on *Acutodesmus* was fairly high, the strong decrease of induced neckteeth in this treatment indicates a specific constrain, perhaps a limitation by an unknown compound fully absent or present in low concentrations in *Acutodesmus* but essential for the deployment of neckteeth. In TCO the same pronounced decrease in induced neckteeth occurs on *Cryptomonas* which supports the reasoning that clone TCO is unusually sensitive to a putative absence or low availability of an unknown compound that is essential for the deployment of neckteeth. These strong differences to the two other *D. pulex* clones may be due to the fact that the latter two originate from European lakes, whereas TCO originated from North America and belongs to the "Panarctic" clade of the *D. pulex* species complex, which is distinct from the European *D. pulex* s.s. (Taylor and Hebert 1993; Molinier et al. 2023). Clone TCO originates from a natural population in Oregon with a strongly reduced genetic diversity and is thus less heterozygous than most other *Daphnia* c.f. *pulex* clones (Lynch et al. 2017).

Neckteeth induction in *D. pulex* is frequently accompanied by other, apparently costlier, traits such as an elongated tail spine, increased pedestal size, and increased carapace thickness, strength, or stiffness (Laforsch et al. 2004; Riessen et al. 2012; Rabus et al. 2013; Kruppert et al. 2017; Riessen and Gilbert 2018), which cannot be experimentally separated from those for the deployment of neckteeth. Here, for the first time, we demonstrate that the induction of neckteeth may be constrained by inadequate biochemical food quality with differences in clonal sensitivity to a putative absence or low availability of an unknown essential compound.

Because of the formation of neckteeth and of further additional allocation of material into other obvious morphological changes, which accompany the deployment of neckteeth, costs of neckteeth formation are to be expected. It follows from these considerations that these costs for a particular juvenile stage should be independent of how long this stage lasts, since no additional material needs to be allocated into morphological changes if, for example, instar two lasts longer. Still, in clone TCO, the increased time spent in vulnerable instars, which is due to an extended duration of instar two, goes along with a decrease in neckteeth induction. This unexpected negative relation suggests that the maintenance of a changed phenotype within a given instar is associated with costs due to a limitation by an unknown compound. Clone TCO seems to be most sensitive to a limitation by this unknown compound, which is corroborated by the finding that this clone shows the lowest maximum increase in time spent in vulnerable instars.

In an earlier study, we used increased shares of a non-toxic cyanobacterium to create a food quality gradient for *D. pulex*. Similar to the different food alga identities in the present study, the gradient of increasing cyanobacterial shares affected developmental time in *D. pulex*, and developmental time was not related to the extent of induced neckteeth (Klintworth and von Elert 2021a). However, although this earlier study corroborates the present study's findings that differences in developmental time do not affect the extent of induced neckteeth, it was based on a single *D. pulex* clone and included one cyanobacterial strain only, and we here generalize this observation by crossing three *D. pulex* genotypes and three algal species.

We conclude that the predator–prey system of gape-limited larvae of *Chaoborus* and *D. pulex* represents a system, in which prey may outgrow vulnerable body size. Therefore, slower developmental progression will elongate the time that *D. pulex* remains within the vulnerable body size and increase predation risk. However, differences in developmental time, both those triggered by different algae (this study) and those triggered by increasing proportions of a cyanobacterium (Klintworth and von Elert 2021a), did not correlate with the extent of induced neckteeth. This shows that in *D. pulex* developmental progression is not included in the assessment of the predation risk by larvae of *Chaoborus*. However, the extent of induced neckteeth was affected by algal identity and different cyanobacterial shares in the diet, which demonstrates pronounced dietary effects on the extent of induced neckteeth in *D. pulex* in response to *Chaoborus* kairomone.

Other factors that may affect developmental time and thus the time window of being vulnerable to predation by *Chaoborus* larvae would be temperature or toxic cyanobacteria. For increases in temperature, Klintworth and von Elert (2021a) have found decreased neckteeth induction, which is in favor of the idea that developmental speed affects the degree of neckteeth induction. However, the authors urge caution when evaluating the results as their setup does not allow to exclude indirect effects of temperature on bacterial degradation of the kairomone.

It further may be interesting to investigate if food quality constrains for one inducible defense may foster the onset of another inducible defense. Such inverse coupling of two inducible defenses has been demonstrated with respect to diel vertical migration (DVM) and life history changes (Effertz and von Elert 2014, 2017), but not yet for morphological changes and DVM, as would be possible, since *D. pulex* is known not only to deploy morphological changes in response to *Chaoborus* larvae but also to perform DVM in response to fish (Beklioglu et al. 2008).

Since phytoplankton composition changes with season (Sommer et al. 2012; Sommer 2023), such dietary bottom-up effects in *Daphnia* will modulate the extent of induced neckteeth in response to *Chaoborus* kairomone and by this strongly affect chemical communication in this predator–prey system. Brzezinski and von Elert (2015) have reported a conceptually similar bottom-up effect modulating *Daphnia*'s behavioral avoidance of fish where a diet low in polyunsaturated fatty acids constrained the response by DVM to the chemical presence of fish. These food quality constrains were as well reported for *D. longispina*, a species which has recently been reported to deploy, similar to *D. pulex*, morphological changes in response to *Chaoborus* larvae (Sperfeld et al. 2020). These findings that food identity strongly modulates both behavioral and morphological inducible defenses in *Daphnia* demonstrate that inducible defenses against predators may, to varying degrees, be under top-down and bottom-up control.

Author Contributions

Eric von Elert: conceptualization, methodology, formal analysis, data curation, writing – original draft, supervision. **Sandra Klintworth:** investigation, methodology, writing and review. **Yannick Hill:** investigation. **Carlos Sánchez Arcos:** methodology, visualization, writing and review.

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Conflicts of Interest

The authors declare no conflicts of interest.

References

Ahlers, J., M. Hahn, M. Stockenreiter, H. Stibor, and E. von Elert. 2024. "Mesocosm Experiments Validate Induction of *Daphnia* Vertical Migration by the Fish-Derived Kairomone 5α-Cyprinol Sulfate." *Limnology and Oceanography Letters* 9: 307–315. https://doi.org/10.1002/lol2.10375.

Ahlgren, G., L. Lundstedt, M. T. Brett, and C. Forsberg. 1990. "Lipid Composition and Food Quality of some Freshwater Phytoplankton for Cladoceran Zooplankters." *Journal of Plankton Research* 12: 809–818. https://doi.org/10.1093/plankt/12.4.809.

Beklioglu, M., A. G. Gozen, F. Yildirim, P. Zorlu, and S. Onde. 2008. "Impact of Food Concentration on Diel Vertical Migration Behaviour of *Daphnia pulex* under Fish Predation Risk." *Hydrobiologia* 614: 321–327. https://doi.org/10.1007/s10750-008-9516-8.

Boeing, W. J., C. W. Ramcharan, and H. P. Riessen. 2006. "Multiple Predator Defence Strategies in *Daphnia pulex* and their Relation to Native Habitat." *Journal of Plankton Research* 28: 571–584. https://doi.org/10.1093/plankt/fbi142.

Brönmark, C., and L. A. Hansson, eds. 2012. Chemical Ecology in Aquatic Systems. New York: Oxford University Press.

Brzezinski, T., P. Dawidowicz, and E. von Elert. 2010. "The Role of Food Quality in Clonal Succession in *Daphnia*: An Experimental Test." *Oecologia* 164: 379–388. https://doi.org/10.1007/s00442-010-1662-9.

Brzezinski, T., and E. von Elert. 2015. "Predator Evasion in Zooplankton Is Suppressed by Polyunsaturated Fatty Acid Limitation." *Oecologia* 179: 687–697. https://doi.org/10.1007/s00442-015-3405-4.

Buikema, A. L. 1973. "Filtering Rate of the Cladoceran, *Daphnia pulex* as a Function of Body Size, Light and Acclimation." *Hydrobiologia* 41: 515–527. https://doi.org/10.1007/BF00016470.

Christjani, M., P. Fink, and E. von Elert. 2016. "Phenotypic Plasticity in Three *Daphnia* Genotypes in Response to Predator Kairomone: Evidence for an Involvement of Chitin Deacetylases." *Journal of Experimental Biology* 219: 1697–1704. https://doi.org/10.1242/jeb.133504.

Colbourne, J. K., M. E. Pfrender, D. Gilbert, et al. 2011. "The Ecoresponsive Genome of *Daphnia pulex*." *Science* 331: 555–561. https://doi.org/10.1126/science.1197761.

Ebert, D. 2022. "*Daphnia* as a Versatile Model System in Ecology and Evolution." *EvoDevo* 13: 16. https://doi.org/10.1186/s13227-022-00199-0.

Effertz, C., and E. von Elert. 2014. "Light Intensity Controls Anti-Predator Defences in *Daphnia*—The Suppression of Life-History Changes." *Proceedings of the Royal Society of London B* 281: 20133250. https://doi.org/10.1098/rspb.2013.3250.

Effertz, C., and E. von Elert. 2017. "Coupling of Anti-Predator Defences in *Daphnia*: The Importance of Light." *Hydrobiologia* 798: 5–13. https://doi.org/10.1007/s10750-015-2387-x.

Eshun-Wilson, F., R. Wolf, T. Andersen, D. O. Hessen, and E. Sperfeld. 2020. "UV Radiation Affects Antipredatory Defense Traits in *Daphnia Pulex*." *Ecology and Evolution* 10: 14082–14097. https://doi.org/10.1002/ece3.6999.

Fink, P., C. Pflitsch, and K. Marin. 2011. "Dietary Essential Amino Acids Affect the Reproduction of the Keystone Herbivore *Daphnia Pulex*." *PLoS One* 6: e28498. https://doi.org/10.1371/annotation/6d71b282-8e08-43ba-bef7-7ad45cd48784.

Giebelhausen, B., and W. Lampert. 2001. "Temperature Reaction Norms of *Daphnia magna*: The Effect of Food Concentration." *Freshwater Biology* 46: 281–289. https://doi.org/10.1046/j.1365-2427.2001.00630.x.

Hahn, M., and E. von Elert. 2022. "One Kairomone and Multiple Effects in *Daphnia* Species—5α-Cyprinol Sulfate Induces Morphological Defenses in the Invasive Species *Daphnia lumholtzi*." *Frontiers in Ecology and Evolution* 10: 804521. https://doi.org/10.3389/fevo.2022.804521.

Hahn, M. A., C. Effertz, L. Bigler, and E. von Elert. 2019. "5α-Cyprinol Sulfate, a Bile Salt from Fish, Induces Diel Vertical Migration in *Daphnia*." *eLife* 8: e44791. https://doi.org/10.7554/eLife.44791.

Hammill, E., A. Rogers, and A. P. Beckerman. 2008. "Costs, Benefits and the Evolution of Inducible Defences: A Case Study With *Daphnia pulex*." *Journal of Evolutionary Biology* 21: 705–715. https://doi.org/10.1111/j.1420-9101.2008.01520.x.

Hartwich, M., D. Martin-Creuzburg, K. O. Rothhaupt, and A. Wacker. 2012. "Oligotrophication of a Large, Deep Lake Alters Food Quantity and Quality Constraints at the Primary Producer-Consumer Interface." *Oikos* 121: 1702–1712. https://doi.org/10.1111/j.1600-0706.2011.20461.x.

Klintworth, S., and E. von Elert. 2020. "Risk of Predation Alters Resource Allocation in *Daphnia* Under Food Limitation." *Journal of Plankton Research* 42: 45–56. https://doi.org/10.1093/plankt/fbz074.

Klintworth, S., and E. von Elert. 2021*a*. "Effect of Developmental Time on *Chaoborus*-Induced Phenotypic Plasticity." *International Review of Hydrobiology* 106: 1–9. https://doi.org/10.1002/iroh.202002055.

Klintworth, S., and E. von Elert. 2021*b*. "Inducible Morphological Defense in *Daphnia pulex*: Food Quantity Effects Revised." *Aquatic Ecology* 55: 47–57. https://doi.org/10.1007/s10452-020-09809-2.

Koch, U., D. Martin-Creuzburg, H. P. Grossart, and D. Straile. 2012. "Differences in the Amino Acid Content of Four Green Algae and Their Impact on the Reproductive Mode of *Daphnia pulex*." *Fundamental and Applied Limnology* 181: 327–336. https://doi.org/10.1127/1863-9135/2012/0396.

Koch, U., E. von Elert, and D. Straile. 2009. "Food Quality Triggers the Reproductive Mode in the Cyclical Parthenogen *Daphnia* (Cladocera)." *Oecologia* 159: 317–324. https://doi.org/10.1007/s00442-008-1216-6.

Krueger, D. A., and S. I. Dodson. 1981. "Embryological Induction and Predation Ecology in *Daphnia pulex*." *Limnology and Oceanography* 26: 219–223. https://doi.org/10.4319/lo.1981.26.2.0219.

Kruppert, S., M. Horstmann, L. C. Weiss, et al. 2017. "Biomechanical Properties of Predator-Induced Body Armour in the Freshwater Crustacean *Daphnia*." *Scientific Reports* 7: 9750. https://doi.org/10.1038/s41598-017-09649-5.

Laforsch, C., W. Ngwa, W. Grill, and R. Tollrian. 2004. "An Acoustic Microscopy Technique Reveals Hidden Morphological Defenses in *Daphnia*." *Proceedings of the National Academy of Sciences of the United States of America* 101: 15911–15914. https://doi.org/10.1073/pnas.0404860101.

Lampert, W. 2006. "*Daphnia*: Model Herbivore, Predator and Prey." *Polish Journal of Ecology* 54: 607–620.

Lürling, M., and E. van Donk. 1997. "Life History Consequences for *Daphnia pulex* Feeding on Nutrient-Limited Phytoplankton." *Freshwater Biology* 38: 693–709. https://doi.org/10.1046/j.1365-2427.1997.00242.x.

Lynch, M., R. Gutenkunst, M. Ackerman, et al. 2017. "Population Genomics of *Daphnia pulex*." *Genetics* 206: 315–332. https://doi.org/10.1534/genetics.116.190611.

Maurone, C., A. Suppa, and V. Rossi. 2018. "Polymorphisms in Predator Induced Defences of Coexisting *Daphnia pulex* and *D. longispina*." *Hydrobiologia* 823: 121–133. https://doi.org/10.1007/s10750-018-3701-1.

Molinier, C., T. Lenormand, and C. R. Haag. 2023. "No Recombination Suppression in Asexually Produced Males of *Daphnia pulex*." *Evolution: International Journal of Organic Evolution* 77: 1987–1999. https://doi.org/10.1093/evolut/qpad114.

Paplauskas, S., O. Morton, M. Hunt, et al. 2024. "Predator-Induced Shape Plasticity in *Daphnia pulex*." *Ecology and Evolution* 14: e10913. https://doi.org/10.1002/ece3.10913.

Parejko, K., and S. Dodson. 1990. "Progress towards Characterization of a Predator Prey Kairomone: *Daphnia pulex* and *Chaoborus americanus*." *Hydrobiologia* 198: 51–59. https://doi.org/10.1007/BF00048622.

Pastorok, R. A. 1981. "Prey Vulnerability and Size Selection by *Chaoborus* Larvae." *Ecology* 62: 1311–1324. https://doi.org/10.2307/1937295.

Pohnert, G., M. Steinke, and R. Tollrian. 2007. "Chemical Cues, Defence Metabolites and the Shaping of Pelagic Interspecific Interactions." *Trends in Ecology & Evolution* 22: 198–204. https://doi.org/10.1016/j.tree.2007.01.005.

Preisser, E. L., D. I. Bolnick, and M. F. Benard. 2005. "Scared to Death? The Effects of Intimidation and Consumption in

Predator–Prey Interactions." *Ecology* 86: 501–509. https://doi.org/10.1890/04-0719.

Rabus, M., T. Sollradl, H. Clausen-Schaumann, and C. Laforsch. 2013. "Uncovering Ultrastructural Defences in *Daphnia magna*—An Interdisciplinary Approach to Assess the Predator-Induced Fortification of the Carapace." *PLoS One* 8: e67856. https://doi.org/10.1371/journal.pone.0067856.

Riessen, H. P. 1992. "Cost–Benefit Model for the Induction of an Antipredator Defence." *American Naturalist* 140: 349–362. https://doi.org/10.1086/285416.

Riessen, H. P., and J. J. Gilbert. 2018. "Divergent Developmental Patterns of Induced Morphological Defenses in Rotifers and Daphnia: Ecological and Evolutionary Context." *Limnology and Oceanography* 401: 60–557. https://doi.org/10.1002/lno.11058.

Riessen, H. P., R. D. Linley, I. Altshuler, M. Rabus, et al. 2012. "Changes in Water Chemistry Can Disable Plankton Prey Defenses." *Proceedings of the National Academy of Sciences of the United States of America* 109: 15377–15382. https://doi.org/10.1073/pnas.1209938109.

Riessen, H. P., and W. G. Sprules. 1990. "Demographic Costs of Antipredator Defenses in *Daphnia pulex*." *Ecology* 71: 1536–1546. https://doi.org/10.2307/1938290.

Riessen, H. P., and J. B. Trevett-Smith. 2009. "Turning Inducible Defenses On and Off: Adaptive Responses of *Daphnia* to a Gape-Limited Predator." *Ecology* 90: 3455–3469. https://doi.org/10.1890/08-1652.1.

Rothhaupt, K. O., and W. Lampert. 1992. "Growth-Rate Dependent Feeding Rates in *Daphnia pulicaria* and *Brachionus rubens*: Adaptation to Intermediate Time-Scale Variations in Food Abundance." *Journal of Plankton Research* 14: 737–751. https://doi.org/10.1093/plankt/14.5.737.

RStudio Team. 2020. "RStudio." In RStudio: Integrated Development for R. Boston, MA: RStudio, PBC.

Shei, P., T. Iwakuma, and K. Fujii. 1988. "Feeding of *Chaoborus flavicans* Larvae (Diptera, Chaoboridae) on *Ceratium hirundinella* and *Daphnia rosea* in a Eutrophic Pon." *Japanese Journal of Limnology* 49: 227–236. https://doi.org/10.3739/rikusui.49.227.

Sommer, U. 2023. Freshwater and Marine Ecology. Cham: Springer.

Sommer, U., R. Adrian, L. D. Domis, et al. 2012. "Beyond the Plankton Ecology Group (PEG) Model: Mechanisms Driving Plankton Succession." *Annual Review of Ecology, Evolution, and Systematics* 43: 429–448. https://doi.org/10.1146/annurev-ecolsys-110411-160251.

Sperfeld, E., J. P. Nilssen, S. Rinehart, K. Schwenk, and D. O. Hessen. 2020. "Ecology of Predator-Induced Morphological Defense Traits in *Daphnia longispina* (Cladocera, Arthropoda)." *Oecologia* 192: 687–698. https://doi.org/10.1007/s00442-019-04588-6.

Taylor, D. J., and P. D. N. Hebert. 1993. "Cryptic Intercontinental Hybridization in *Daphnia* (Crustacea): The Ghost of Introductions Past." *Proceedings of the Royal Society B: Biological Sciences* 254: 163–168. https://doi.org/10.1098/rspb.1993.0141.

Tollrian, R. 1993. "Neckteeth Formation in *Daphnia pulex* as an Example of Continuous Phenotypic Plasticity: Morphological Effects of *Chaoborus* Kairomone Concentration and their Quantification." *Journal of Plankton Research* 15, no. 11: 1309–1318. https://doi.org/10.1093/plankt/15.11.1309.

Tollrian, R. 1994. "Fish-Kairomone Induced Morphological Changes in *Daphnia lumholtzi* (SARS)." *Archiv Fur Hydrobiologie* 130: 69–75.

Tollrian, R. 1995. "*Chaoborus crystallinus* Predation on *Daphnia pulex*: Can Induced Morphological Changes Balance Effects of Body Size on Vulnerability?" *Oecologia* 101: 151–155. https://doi.org/10.1007/BF00317278.

Tollrian, R., S. Duggen, L. C. Weiss, C. Laforsch, and M. Kopp. 2015. "Density-Dependent Adjustment of Inducible Defenses." *Scientific Reports* 5: 12736. https://doi.org/10.1038/srep12736.

von Elert, E. 2002. "Determination of Limiting Polyunsaturated Fatty Acids in *Daphnia galeata* Using a New Method to Enrich Food Algae With Single Fatty Acids." *Limnology and Oceanography* 47: 1764–1773. https://doi.org/10.4319/lo.2002.47.6.1764.

von Elert, E. 2012. "Information Conveyed by Chemical Cues." In Chemical Ecology in Aquatic Systems, edited by C. Brönmark and L. A. Hansson, 19–38. New York: Oxford University Press.

von Elert, E., and F. Jüttner. 1997. "Phosphorus Limitation and Not Light Controls the Extracellular Release of Allelopathic Compounds by *Trichormus Doliolum* (Cyanobacteria)." *Limnology and Oceanography* 42: 1796–1802. https://doi.org/10.4319/lo.1997.42.8.1796.

von Elert, E., and G. Pohnert. 2000. "Predator Specificity of Kairomones in Diel Vertical Migration of *Daphnia*: A Chemical Approach." *Oikos* 88: 119–128. https://doi.org/10.1034/j.1600-0706.2000.880114.x.

von Elert, E., and P. Stampfl. 2000. "Food Quality for *Eudiaptomus Gracilis*: The Importance of Particular Highly Unsaturated Fatty Acids." *Freshwater Biology* 45: 189–200. https://doi.org/10.1046/j.1365-2427.2000.00671.x.

von Elert, E., and H. Stibor. 2006. "Predator-Mediated Life History Shifts in *Daphnia*: Enrichment and Preliminary Chemical Characterisation of a Kairomone Exuded by Fish." *Archiv für Hydrobiologie* 167: 21–35. https://doi.org/10.1127/0003-9136/2006/0167-0021.

Wacker, A., and E. von Elert. 2001. "Polyunsaturated Fatty Acids: Evidence for Non-Substitutable Biochemical Resources in *Daphnia galeata*." *Ecology* 82: 2507–2520. https://doi.org/10.1890/0012-9658(2001)082[2507:PFAEFN]2.0.CO;2.

Weiss, L. C., B. Albada, S. M. Becker, et al. 2018. "Identification of *Chaoborus* Kairomone Chemicals That Induce Defences in *Daphnia*." *Nature Chemical Biology* 14: 1133–1139. https://doi.org/10.1038/s41589-018-0164-7.

Werner, C., K. A. Otte, and E. von Elert. 2021. "Phenotypic Convergence in a Natural *Daphnia* Population Acclimated to Low Temperature." *Ecology and Evolution* 11: 15312–15324. https://doi.org/10.1002/ece3.8217.

Zehnder, A., and P. R. Gorham. 1960. "Factors Influencing the Growth of *Microcystis aeruginosa* Kutz, Emend, Elenkin." *Canadian Journal of Microbiology* 6: 645–660. https://doi.org/10.1139/m60-077.

Zhang, C., E. Goitom, K. Brans, L. de Meester, and R. Stoks. 2022. "Scared to Evolve? Non-Consumptive Effects Drive Rapid Adaptive Evolution in a Natural Prey Population." *Proceedings of the Royal Society B: Biological Sciences* 289: 20220188. https://doi.org/10.1098/rspb.2022.0188.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

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