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Food quantity and quality modulates inducible defenses in a common predator–prey system

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Abstract

Zooplankton displays different inducible defenses against invertebrate and vertebrate predators. The response pattern to gape-limited invertebrate predators involves increased somatic growth and offspring body size but delayed maturity and reduced offspring numbers. In contrast to this general pattern, the freshwater model organism *Daphnia magna* has been reported to exhibit a different response when encountering the gape-limited tadpole shrimp *Triops cancriformis*. Under laboratory conditions, *D. magna* showed increased somatic growth, earlier maturation, and an increase in both offspring number and size. We propose here that the discrepancy between the previously observed and the theory-based response patterns against invertebrate predators is due to differences in food availability in the applied laboratory settings and assessed whether the defensive response of *D. magna* against *T. cancriformis* is modulated differently by food quantity and quality. We found a strong impact of food quantity and quality on the defense response of *D. magna* to *T. cancriformis* kairomones. The prey seem to be able to overcome trade-offs between morphological defense traits and reproductive traits, but distinctly between high food quantity and high food quality. Thereby, reproductive traits were preferred over morphological defenses. Furthermore, the removal of particles from the *T. cancriformis*-conditioned water caused a defense pattern in *D. magna* that was consistent with the general response pattern known from other invertebrate predators, thus explaining the described discrepancy to previous studies with *T. cancriformis*. Our study highlights the importance of assessing food-related effects on predator–prey interactions to understand trophic relationships and food web processes.

Predator–prey interactions affect food web dynamics with consequences on the ecosystem scale (Sakamoto et al. 2015; Su et al. 2021; Twining et al. 2021). Phenotypic plasticity allows prey to develop defense mechanisms that reduce the impact of predation. Inducible defenses are expressed only if a reliable cue indicates an acute predation risk and plays a significant role in modulating predator–prey interactions (Vos et al. 2002). Induced defenses are expressed on the level of behavior, life history, physiology, and morphology (Lass and Spaak 2003; Diel et al. 2020). In aquatic ecosystems, defense responses in prey organisms are assumed to be specific to the predator guild (i.e., invertebrate vs. vertebrate) and to involve trade-offs in resource allocation (Lass and Spaak 2003; Kishida and Nishimura 2004; Bourdeau 2010). When confronted with visually hunting predators that exhibit a preference for larger prey, such as zooplanktivorous fish, zooplankton prey tend to allocate more resources to reproduction. This adaptive response manifests in earlier maturation and increased production of smaller offspring, which is thought to compensate for the potential losses caused by predation and reduce visibility. In contrast, in the presence of gape-limited invertebrate predators, zooplankton prey invests more in juvenile somatic growth to outgrow the most vulnerable size range, which is often associated with the development of special morphological features that impede the feeding process (Dodson 1974). Cladocerans of the genus *Daphnia* show a huge variety of inducible morphological defenses, including unspecific responses to entire predator guilds (as described above) as well as specific responses to single predator species, and the response patterns can differ among *Daphnia* species (Lass and Spaak 2003; Diel et al. 2020).

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The genus *Daphnia* plays a crucial role in lentic freshwater ecosystems by transferring energy and resources from primary producers to higher trophic levels. *Daphnia* are confronted with a multitude of different predators, occurring at different times and locations (Lass and Spaak 2003; Diel et al. 2020). A great variety of inducible defense mechanisms has been described among the numerous *Daphnia* species in response to various predators that are potentially shaped by the prevailing environmental conditions (Lass and Spaak 2003; Herzog and Laforsch 2013; Diel et al. 2020). The defense responses of *Daphnia* to invertebrate and vertebrate predators, documented in the literature, are largely consistent with the above-described general predator guild-specific defense patterns (O'Brien et al. 1979; Havel and Dodson 1987; Amundsen et al. 2009). However, there are exceptions that make it difficult to draw general conclusions. For example, when exposed to the tadpole shrimp, *Triops cancriformis*, the co-occurring *Daphnia magna* was found to express the expected increase in overall body size (increased bulkiness) and tail-spine length (Rabus and Laforsch 2011; Horstmann et al. 2021). However, despite this predator-induced investment in somatic growth, which is also reflected in an increase in offspring body size, *T. cancriformis*-exposed *D. magna* were found to mature earlier and to produce more offspring (Rabus and Laforsch 2011; Pietrzak et al. 2020), which contrasts the common theory on defense strategies against invertebrate predators.

The discrepancy between the previously observed and the expected response pattern in the *T. cancriformis–D. magna* predator–prey system is a paradox because higher somatic growth and increased reproduction should involve higher demands for resources. This extensive defense strategy, which includes increased production of prey biomass, is likely to increase energy and carbon consumption. In addition, the demand for essential nutrients, like sterols and long-chain polyunsaturated fatty acids (LC-PUFA), is likely to increase (Martin-Creuzburg and von Elert 2009; Martin-Creuzburg et al. 2010). Sterols and LC-PUFA are important membrane components (Hazel 1995; Martin-Creuzburg et al. 2012) and serve as precursors for a suite of other bioactive molecules (Stanley-Samuelson 1994; Heckmann et al. 2008; Schlotz et al. 2012). An adequate dietary sterol and LC-PUFA supply is crucial for the somatic and population growth of *Daphnia* (Müller-Navarra et al. 2000; von Elert 2002; Martin-Creuzburg et al. 2009). Food quantity-related effects on inducible defenses have been studied previously using other predator–prey systems (Tollrian 1995; Ślusarczyk 2001; Gliwicz and Maszczyk 2007; Pauwels et al. 2010; Hahn and von Elert 2020; Klintworth and von Elert 2020*a*,*b*). However, the results of these studies were not always clear, and no common pattern could be found. For instance, studies on the predator–prey relationship between *Daphnia pulex* and the phantom midge *Chaoborus* sp. did not reveal a clear relationship between the amount of food available to *D. pulex* and the induced defense responses (Ramcharan et al. 1992; Riessen 1992; Tollrian 1995; Klintworth and von Elert 2020*a*,*b*). In contrast to food quantity, the effects of food quality on inducible defenses have only recently been addressed in regard to diel vertical migration, an effective behavioral defense strategy (Klintworth and von Elert 2020*b*; Isanta-Navarro et al. 2021). Effects of food quality on morphological defense traits have not yet been studied, which is surprising given the strong impact of elemental and biochemical food quality on various life history traits of *Daphnia* (Müller-Navarra et al. 2000; Sterner and Elser 2002; Martin-Creuzburg et al. 2018). The strong impact of essential lipids on growth and reproduction (food quality), as well as the general need for dietary energy (food quantity), prompt questions about resource allocation and the expression of morphological defensive traits. In nature, *Daphnia* are confronted with varying food quantity and quality (Oliver and Ganf 2000; DeMott et al. 2001; Müller-Navarra et al. 2004; Sperfeld et al. 2012), and it seems likely that this will also distinctly affect the expression of morphological defenses. The above-described predator-induced trade-off in resource allocation between somatic growth and reproduction might be obsolete at high food quantity or quality because potentially limiting nutrients are provided in excess. Thus, is seems crucial to understand how prey animals are affected by surrounding conditions and how this affects predator–prey interactions.

Here, we used the well-established *T. cancriformis–D. magna* predator–prey model system to study the potential effects of food quantity and quality on the expression of defensive traits in *D. magna*. We hypothesized that the defense responses of *D. magna* to *T. cancriformis* are affected differently by food quantity and quality. In previous studies, *D. magna* was separated from *T. cancriformis* only by a net cage (Rabus and Laforsch 2011; Rabus et al. 2012, 2013; Ritschar et al. 2020*b*; Horstmann et al. 2021), a common practice in predator–prey studies. We additionally hypothesized here that the expression of defenses reported in previous studies was affected by additional food sources unintentionally supplied to the prey by excretions from the predators that may have been partially decomposed already by bacteria and passed through the net cages. Furthermore, we proposed that these additional food sources would supplement the commonly used standard *Daphnia* food with essential lipids, such as cholesterol, thus altering growth and reproduction.

Materials and methods

Cultivation of organisms

We used the green alga *Acutodesmus obliquus* (SAG 276-3a, Sammlung von Algenkulturen, Göttingen, Germany) as food for maintaining the *Daphnia* stock culture and for conducting the experiments. *A. obliquus* is of moderate food quality for *Daphnia* due to its lipid content (von Elert 2002; Martin-Creuzburg and Merkel 2016). In addition, we used the eustigmatophyte *Nannochloropsis limnetica* (SAG 18.99) as high-quality food in the experiments. *N. limnetica* contains high amounts of physiologically important lipids, such as cholesterol and the LC-PUFA eicosapentaenoic acid (EPA, 20:5n-3; Martin-Creuzburg and von Elert 2009). Both food algae were grown at a day–night cycle of 20 : 4 h at 20 ± 0.5°C in batch cultures with Z-medium (Zehnder and Gorham 1960).

For the experiments, we used the *D. magna* clone K34J, which originates from a former fishpond in Ismaning, Germany (Rabus and Laforsch 2011). This clone was cultured in 1.5-liter jars filled with M4 medium (Elendt and Bias 1990) with 20 animals per jar, and ad libitum food, at 20 ± 0.5°C and a day–night cycle of 15 h : 9 h with a transition time in the beginning and the end of these periods of 30 min, representing dusk and dawn. The predator used for defense induction was the invertebrate tadpole shrimp *T. cancriformis* originating from a laboratory-cultured clonal line, kindly provided to us by Dr. E. Eder of the University of Vienna. This predator has previously been shown to induce distinct morphological changes in the *D. magna* clone used here (Rabus and Laforsch 2011; Rabus et al. 2012, 2013; Ritschar et al. 2020*b*; Horstmann et al. 2021). This predator is globally co-occurring with *D. magna*. For the experiments, we used predators of a body length between 1.5 and 2 cm, which are known from previous experiments to be able to prey upon *D. magna*, but also experience handling issues when the prey expresses morphological defenses. They quickly acclimate to experimental conditions and start foraging already after a few minutes.

Experimental setup

We aimed to test whether *D. magna* distinctly responds to food quantity and quality. Since we expect the predator to supply nutrients to the experimental animals, it was necessary to exclude the predator from the experimental vessels. Therefore, the experiment was conducted in small beakers containing 100 mL of 0.2 μm-filtered (PVDF-membrane filter, Merck Millipore, USA), 24 h *T. cancriformis*-conditioned M4 medium (hereafter predator-conditioned water). For the incubation, the predators were fed with 10 adult *D. magna* and exchanged daily to reduce the stress for the animals. After exchange, the same predators have not been used for at least two consecutive days. The predator-conditioned water was filtered to remove all predator-derived particles, while allowing the predator cues (kairomones) to pass through. As a control, we used untreated M4 medium (hereafter control water). To obtain the maximum possible defense response in *D. magna*, we already exposed the mothers of the experimental animals to (unfiltered) *T. cancriformis*-conditioned water starting 1 d after oviposition of the second clutch into the brood chambers to ensure exposure already early during ontogenesis. Mothers of the control animals were not pre-exposed to *T. cancriformis*-conditioned water. Each experimental beaker of 10 replicates per treatment was stocked with one randomly chosen third-clutch neonate *D. magna* (age < 12 h). The *D. magna* were provided with two different concentrations of *A. obliquus* (0.7 and 2 mg C L^{-1}) to test for food quantity effects, and a 50% : 50% mixture of *A. obliquus* and *N. limnetica* (0.35 mg C L^{-1} each, for a total of 0.7 mg C L^{-1}; Fig. 1) to test for food quality effects. The *D. magna* were transferred daily into freshly prepared beakers to ensure constant food conditions and kairomone concentration.

To test our hypothesis that the net cage setup of previous studies on this predator–prey system supplied the prey with additional food, we also performed a net cage setup in parallel (Ritschar et al. 2020*a*; Horstmann et al. 2021). For this, we used 2-liter beakers containing 1.5 liters of M4 medium that were stocked with 15 randomly selected third-clutch neonate *D. magna* per beaker (age < 12 h) and acryl net cages (height 13.5 cm, diameter 7.5 cm, material thickness 3 mm) inserted into the beakers to expose *D. magna* to the predators without direct contact (Fig. 1). The cages had a nylon gauze (mesh size 120 μm) covered opening at the bottom and three nylon gauze-covered openings at the sides (5 × 6.5 cm each) to ensure water and kairomone exchange. The top of the cages was kept above water level. In one treatment, each cage was stocked with one *T. cancriformis* provided with live adult *D. magna* ad libitum. The predators have been exchanged every other day, likewise previous studies, to reduce the stress for the animals. The medium in the beakers was exchanged every 4 d, similar to previous studies (Rabus and Laforsch 2011; Ritschar et al. 2020*b*; Diel et al. 2021; Horstmann et al. 2021). In a second treatment, however, the animals were transferred daily into freshly prepared beakers with cleaned net cages to see whether the proposed accumulation of food and *T. cancriformis*' kairomones has an effect on the defense responses of *D. magna* in this common experimental setup (Fig. 1). Both treatments, that is, net cage refreshed every 4 d (hereafter: accumulated treatment) and net cage refreshed daily (hereafter: refreshed treatment), were replicated three times.

Measurements

During the experiments, we recorded the following parameters of the animals: age of first reproduction, day of death, number of viable offspring, body length at the age of first reproduction, defined as the distance between the upper edge of the compound eye and the base of the tail-spine, body width at the age of first reproduction, defined as the orthogonal distance between the tangents of the dorsal and the ventral edge of the carapace, and tail-spine length at the age of first reproduction, defined as the distance between the base and the tip of the tail-spine, as well as neonate body length and neonate tail-spine length (Rabus and Laforsch 2011). For analysis, we calculated the relative tail-spine length, relative body width and the neonate's relative spine length by dividing the respective value by body length.

GC-MS analyses

We expected that the food introduced by the predator increases the food quality through resources supplied by

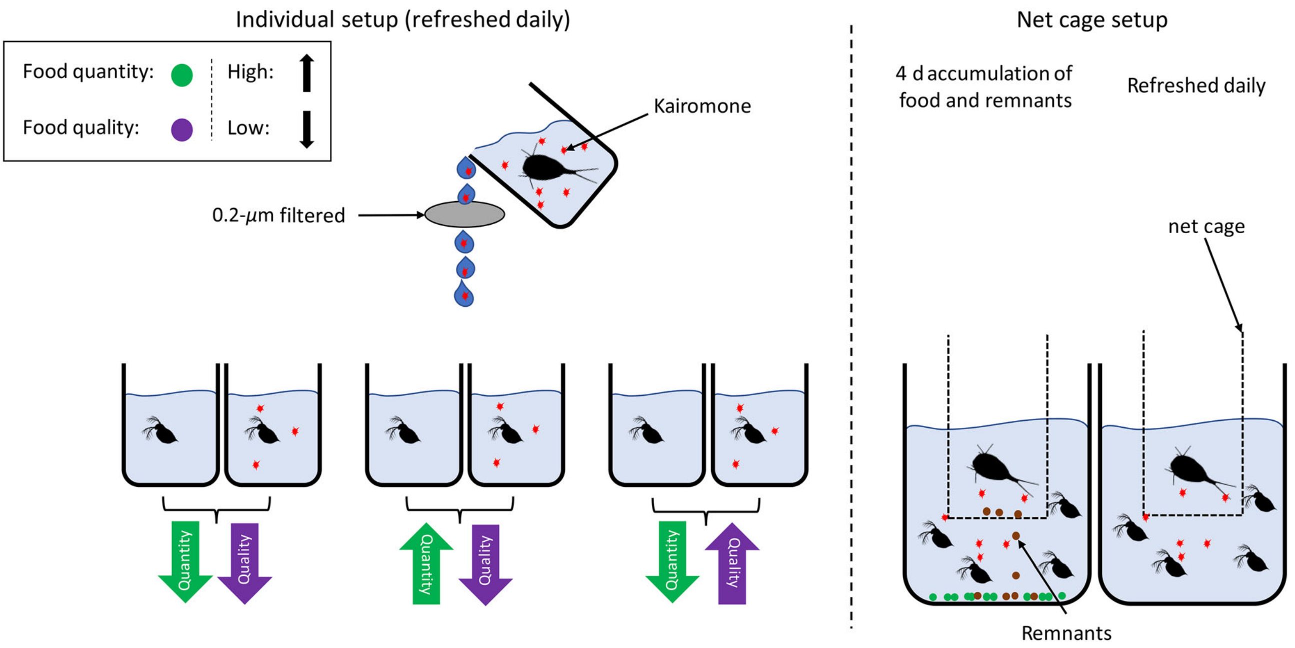

Fig. 1. Experimental setup. Two experimental designs were used: an individual setup for full control over food and a net cage setup for comparison with previous studies. For the individual setup, the predator-conditioned water was filtered particle-free and different food treatments were applied. In the net cage setup, the predator was placed in a gauze-covered net cage to ensure the exchange of predator cues. Water was exchanged daily in the individual setup. In the net-cage setup, water was either refreshed daily or every 4 d in two treatments with 2-liter beakers.

particulate food remnants and predator feces. Cholesterol is absent from the green alga *A. obliquus* but present in *N. limnetica* and contributes to food quality differences between the two algae (Martin-Creuzburg and Merkel 2016). Cholesterol is a crucial lipid for the growth and reproduction of *Daphnia* (Martin-Creuzburg et al. 2009; Martin-Creuzburg and von Elert 2009). In a targeted approach, we, therefore, specifically assessed the presence of cholesterol in the particulate matter of the predator incubation water. We explored whether a simple filtration of the predator-conditioned water would reduce the proposed nutrient input via the predators. We expected to find high amounts of sterols in the predator-conditioned water. In a semi-quantitative assessment, we explored the proposed dietary provisioning of cholesterol through the predators. Providing a detailed assessment of the nutrient composition of the predator's feces was beyond the scope of this study and remains to be explored in future studies.

We conducted an incubation experiment involving three different treatments. The first treatment consisted of 1 liter of M4 medium as a control, the second treatment consisted of 1 liter of M4 medium containing 10 *D. magna* (exchanged after 24 h) that were squashed that their body fluids leached out (referred to as the *Daphnia* control), and the third treatment consisted of 1 liter of M4 medium stocked with a single *T. cancriformis*, which was fed 10 *D. magna* per day, but not exchanged for 2 d (likewise the nested setup). Both the *D. magna* squashed and those fed to *T. cancriformis* were fed with *A. obliquus* prior to this experiment. The incubation lasted for 48 h. The remains of the squashed *D. magna* and food remains were removed after 24 h. Each treatment was replicated three times. For the next step, all tubes and filters were preconditioned with cyclohexane for 2 h and rinsed with ultra-pure water to wash out potential additives of the material. After incubation, the particles were filtered on a 0.2 μm PVDF membrane. After filtration of the medium, the filter was washed with 200 mL ultra-pure water and then transferred into a 5 mL reaction vessel (Eppendorf, Germany). The filter was placed in 5 mL cyclohexane, an internal standard was added (C17:0 with 10 ng μL^{-1} end concentration), and incubated for 24 h to extract all soluble compounds. At the beginning and the end of the incubation phase, the vessels were sonicated for 5 min. After incubation, the vessels, including the filters were centrifuged at 5500 rpm to sediment the particles. The supernatants were transferred into new reaction vessels. The supernatants were then evaporated under a gentle nitrogen stream to 1 mL, transferred into 1.5 mL glass vials (Carl Roth GmbH + Co KG), and then evaporated under nitrogen to ~ 100 μL (a ~ 50-fold concentration in total). This extract was used for GC-MS analysis on a GC-MS (Shimadzu QP2020 NX, Shimadzu, Japan) equipped with a DB-1 capillary column (Agilent, 30 m × 0.25 mm ID × 0.25 mm film). For the analysis, the following configuration was used: oven, starting at 150°C, 1 min hold, then to 320°C at 10°C/min; carrier gas, helium (flow, 1.5 mL/min; velocity, 38 cm/s); ion source, 230°C; injector, 280°C (total run time 23 min per sample). Temperature-programmed Kovats retention indices were calculated with an alkane series measured before the extracts. We calculated a correction factor using the mean peak area of the internal standard across all replicates of each treatment. With that, the amount of cholesterol was calculated semi-quantitatively.

Statistical analysis

The analysis was carried out using R version 4.0.3 (R Core Development Team 2020). The low-quantity, low-quality food treatment with and without predator exposure were used as controls. These controls were applied separately, for the comparison with the high-quantity and the high-quality treatments, with and without predator, to indicate whether *D. magna* responds differently to these factors (*see* Figs. 2–4). These data underwent two-way analyses of covariance (ANCOVA) with predator (none and *T. cancriformis*) and food (low and high quality or quantity) as predictors, utilizing linear regression. A subsequent Tukey's HSD posthoc test was performed with Holm's correction method for multiple comparisons using the R package "emmeans" version 1.5.3 (Lenth et al. 2018). However, a Kruskal–Wallis *H* test was performed if assumptions for normal distribution and/or homogeneity of variance of the residuals were violated. A subsequent Dunn's posthoc test from the R package "FSA" version 0.8.32 (Ogle et al. 2018) was performed, again with Holm's correction method for multiple comparisons. In the results section, we report the F or t values if an ANCOVA and Tukey HSD were performed, and a chi-squared (χ^2) or a z value in case a Kruskal–Wallis *H* and Dunn's post hoc was performed.

The two treatments with net cages (i.e., accumulated and refreshed) from the second experiment were compared to each other using nested linear regression in case of met assumptions of normal distribution and homogeneity of variance of the residuals. If assumptions were violated, we tested the mean values of all animals of one replicate with the Kruskal–Wallis *H* test. However, we did not statistically compare the results of the individual setup with the net cage setup, as the setups deviated too strongly, and statistical analysis would not provide meaningful results. In all statistical methods, the significance level was set to 0.05.

Results

Basic antipredator response under low food conditions

In previous studies, the predator evoked an increase in body length, body width and relative tail-spine length, offspring number and size, as well as a reduction in the age of first reproduction. In contrast, in our study, the only response evoked by predator cues under low food conditions (i.e., quantity and quality) was a significant increase in the relative tail-spine length ($t = -4.187$, $p = 0.001$) compared to control animals without predator (Fig. 2). In all other measured traits, no significant change could be observed (Figs. 2–4).

Response to the predator under high food quantity

In the absence of predator cues, the increase in food quantity evoked no significant changes (Figs. 2–4). However, the age of first reproduction was significantly increased when exposed to predator cues, compared to the high-quantity food without predator ($z = -2.707$, $p = 0.034$), but not compared to the low quantity without predator ($z = -0.894$, $p = 0.371$; Fig. 3). The offspring number was significantly increased when predator cues were present compared to both high-quantity ($z = -2.647$, $p = 0.033$), and low-quantity food without predator ($z = -3.577$, $p = 0.002$; Fig. 3). Furthermore, the body length was significantly increased compared to high-quantity food ($t = -3.044$, $p = 0.018$), and low food control ($t = 4.269$, $p < 0.001$; Fig. 2). In contrast to the other treatments with predator cues, we did not find a significant increase in the relative tail-spine length when exposed to predator cues and high-quantity food compared to high-quantity food without predator cues ($t = -0.366$, $p = 0.717$; Fig. 2). When exposed to high-quantity food and predator cues in combination, the neonate body length was significantly decreased compared to high quantity food ($t = 5.466$, $p < 0.001$), and compared to low quantity food without predator cues ($t = -5.407$, $p < 0.001$; Fig. 4). The neonate relative tail-spine length was significantly increased compared to high quantity food ($t = 5.640$, $p < 0.001$), and low quantity food without predator cues ($t = -8.120$, $p < 0.001$ respectively; Fig. 4).

Response to the predator under high food quality

An improvement in the food quality evoked a significantly reduced age of first reproduction ($z = 2.971$, $p = 0.019$) and a significant increase in the offspring number compared to low food control ($z = -4.213$, $p < 0.001$; Fig. 3). Furthermore, the body length was significantly increased compared to low quality without predator cues ($t = 3.406$, $p = 0.008$; Fig. 2). But contrasting the response under high food quanity, this response was reversed when the animals were exposed to predator cues ($t = 3.825$, $p = 0.003$; Fig. 2). The relative tail-spine length was significantly increased with high-quality food only under exposure to predator cues ($t = -3.244$, $p = 0.011$; Fig. 2). Similar to the response with high food quantity, the neonate body length was significantly reduced ($t = 8.357$, $p < 0.001$), but the neonate relative tail-spine length was significantly increased ($t = 8.359$, $p < 0.001$) under exposure to high-quality food and predator cues compared to the absence of predator cues (Fig. 4).

Differences caused by accumulation of food in the net cage setup

By refreshing the beakers and net cages daily, we prevented the accumulation of food and other substances like kairomones. This led to a significant increase in the age of first reproduction ($\chi^2 = 3.86$, $p = 0.05$) and a significantly lower offspring number compared to the accumulated treatment

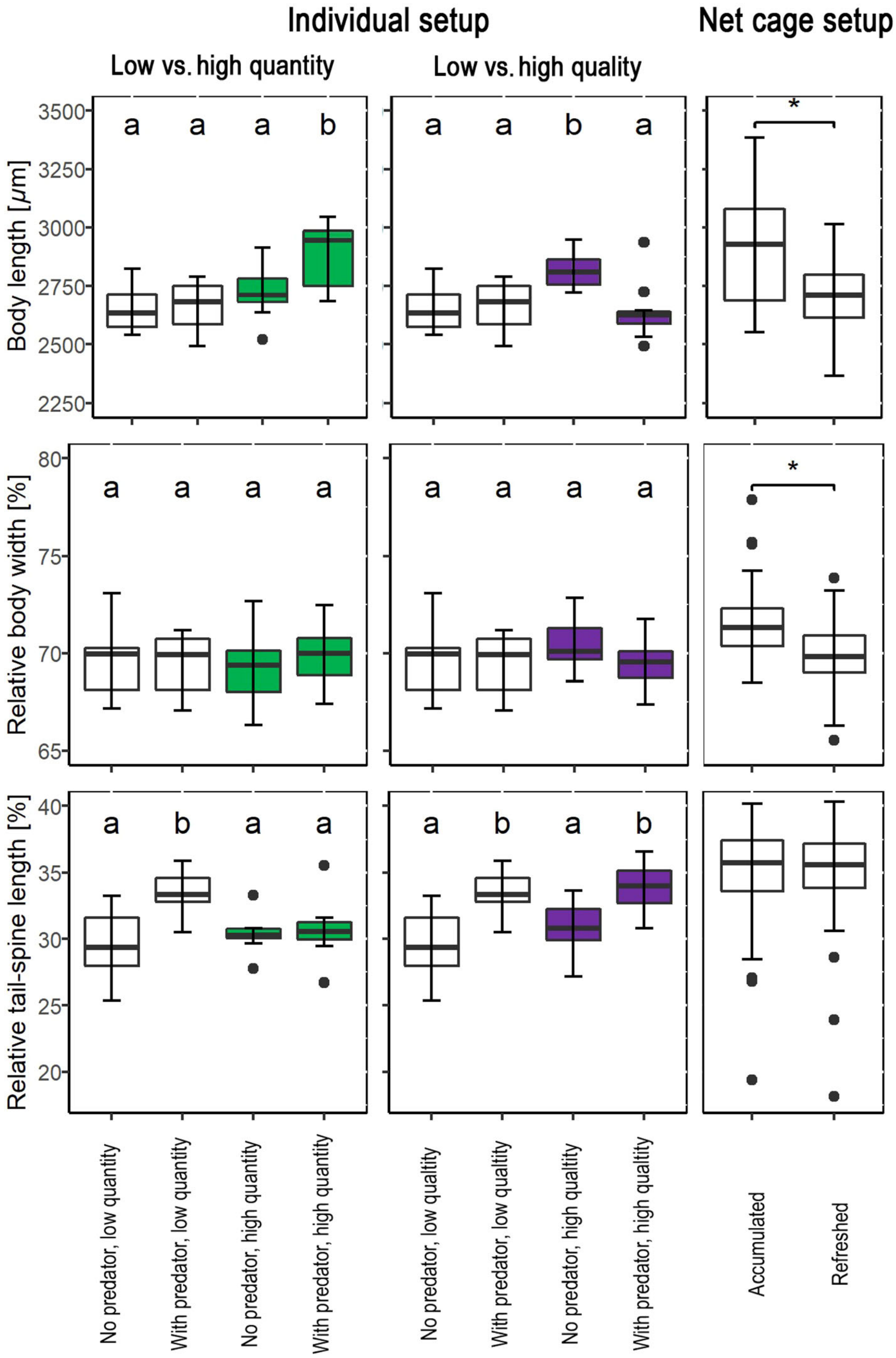

Fig. 2. Morphological parameters of *Daphnia magna* in response to *Triops cancriformis* under low food quantity and low quality (i.e., control with white boxes) compared to either high food quantity (green) or high food quality (purple) of the individual setup ($n = 10$, left and middle box). The controls with and without predator (white boxes) were used for comparison with both high quality and high quantity. In addition, the results of the nested setup ($n = 3$ beakers with 15 animals each, right box) show the response to direct predator contact in a beaker. The accumulated treatment represents the setup commonly used when investigating this predator–prey system, refreshed only every 4 d. The refreshed treatment indicates how the defense response changes when the water is exchanged daily to remove predator feces and food remnants. The dotted horizontal line indicates the median of the low quantity/quality control without predator, for better comparability of the other treatments. The horizontal lines indicate the median of the respective treatment. The boxes indicate the 50% and 75% quantiles, and the whiskers indicate the 1.5-fold of these quantiles. Letters indicate significant differences between groups for the individual setup, and the bracket with an asterisk indicates statistical significance for the nested setup. For statistical details, *see* the main text.

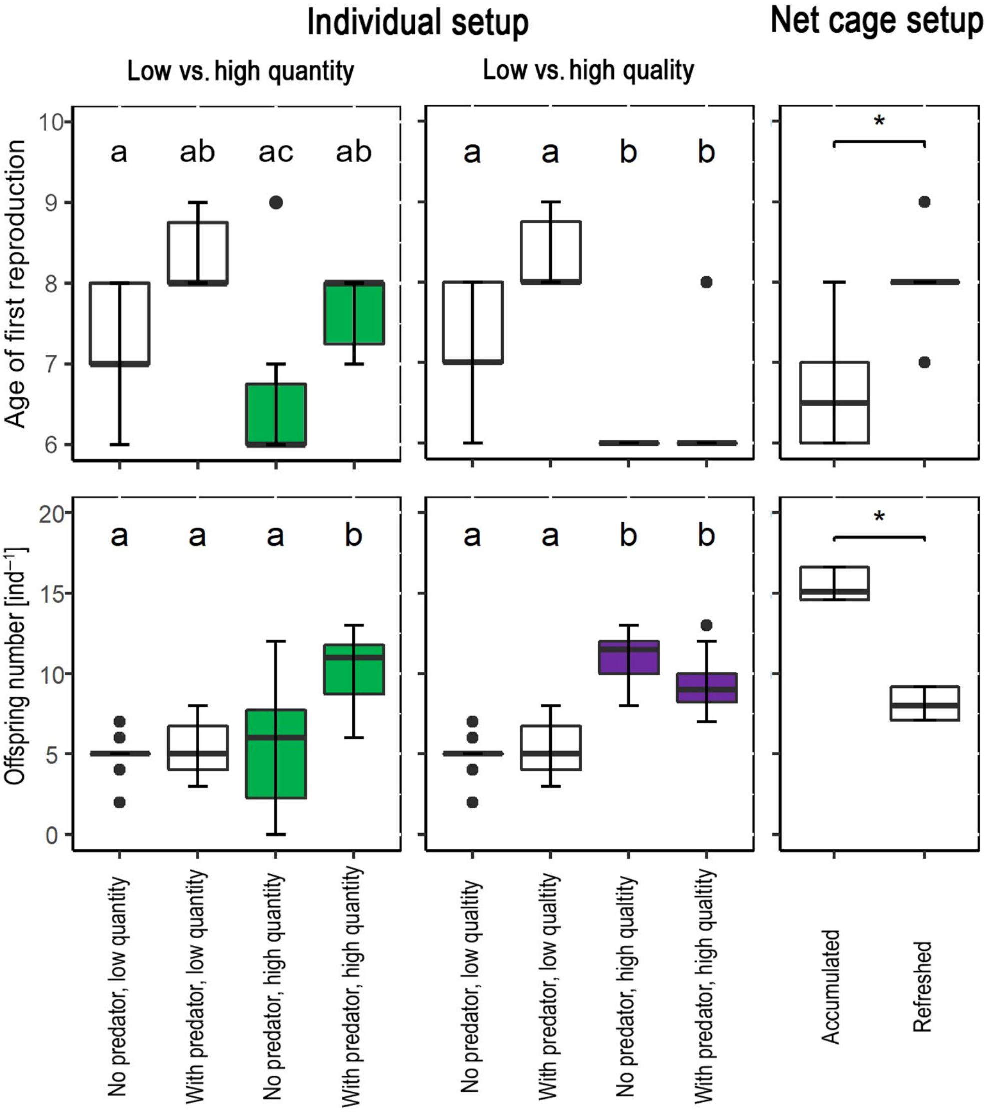

Fig. 3. Reproductive parameters of *Daphnia magna* in response to *Triops cancriformis* under low food quality and low quantity (i.e., control with white boxes) compared to either high food quantity (green) or high food quality (purple) of the individual setup ($n = 10$, left and middle box). The controls with and without predator (white boxes) were used for comparison with both high quality and high quantity. In addition, the results of the nested setup ($n = 3$ beakers with 15 animals each, right box) show the response to direct predator contact in a beaker. In this setup, for the offspring number, the values had to be used as the mean per beaker since it could not be determined at an individual level. Note that for the age of first reproduction there are actually 45 data points, but with almost no variance. The accumulated treatment represents the setup commonly used when investigating this predator–prey system, refreshed only every 4 d. The refreshed treatment indicates how the defense response changed when the water was exchanged daily to remove predator feces and food remnants. The dotted horizontal line indicates the median of the low quantity/quality control without predator, for better comparability of the other treatments. The horizontal lines indicate the median of the respective treatment. The boxes indicate the 50% and 75% quantiles, and the whiskers indicate the 1.5-fold of these quantiles. Letters indicate significant differences between groups for the individual setup, and the bracket with an asterisk indicates statistical significance for the nested setup. For statistical details, *see* the main text.

($\chi^2 = 3.86$, $p = 0.05$; Fig. 3). Furthermore, the body length was significantly reduced ($\chi^2 = 3.86$, $p = 0.05$) as well as the relative body width ($F_{(1,84)} = 18.02$, $p < 0.001$; Fig. 2). In contrast, there was no significant difference in the relative tail-spine length between the two net cage treatments ($F_{(1,75)} = 0.01$, $p = 0.93$; Fig. 2). In the refreshed treatment, the neonate body length was significantly increased ($F_{(1,58)} = 26.26$, $p < 0.001$), but the neonate relative tail-spine was significantly decreased ($F_{(1,58)} = 38.79$, $p < 0.001$; Fig. 4) compared to the accumulated treatment.

Cholesterol in the predator-conditioned water

We found cholesterol in the particulate matter of both squashed *D. magna* and *T. cancriformis*-conditioned water,

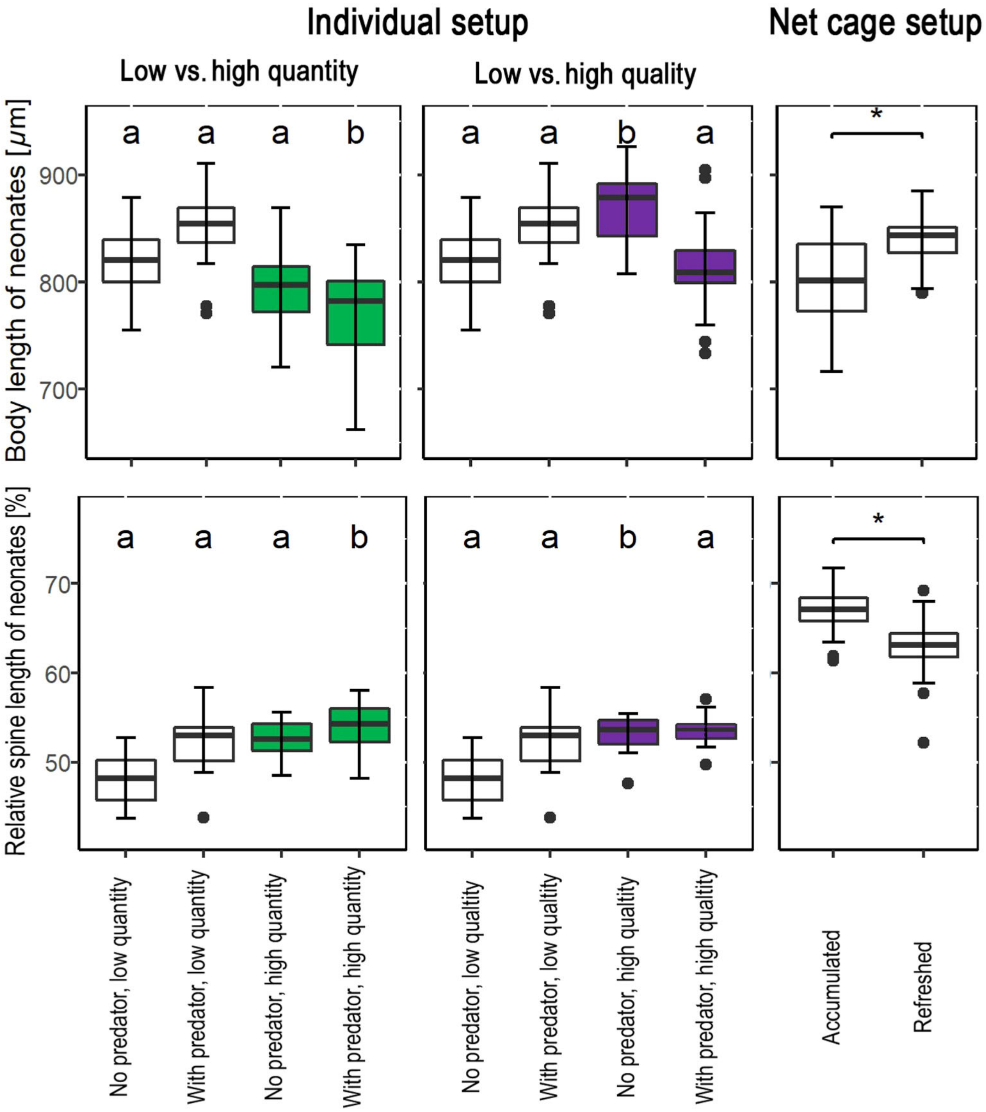

Fig. 4. Morphological parameters of the offspring (neonates) of the experimental animals in response to *Triops cancriformis* under low food quantity and low quality (i.e., control with white boxes) compared to either high food quantity (green) or high food quality (purple) of the individual setup ($n = 10$, left and middle box). The controls with and without predator (white boxes) were used for comparison with both high quality and high quantity. In addition, the results of the nested setup ($n = 3$ beakers with 15 animals each, right box) show the response to direct predator contact in a beaker. The accumulated treatment represents the setup commonly used when investigating this predator–prey system, refreshed only every 4 d. The refreshed treatment indicates how the defense response changes when the water is exchanged daily to remove predator feces and food remnants. The dotted horizontal line indicates the median of the low quantity/quality control without predator, for better comparability of the other treatments. The horizontal lines indicate the median of the respective treatment. The boxes indicate the 50% and 75% quantiles, and the whiskers indicate the 1.5-fold of these quantiles. Letters indicate significant differences between groups for the individual setup, and the bracket with an asterisk indicates statistical significance for the nested setup. For statistical details, *see* the main text.

but not in the control medium. Following the semi-quantitative evaluation, the cholesterol in the filtrate of the predator-conditioned water was approximately 1.4-fold higher than the squashed *D. magna*-conditioned water (area under the curve for *Daphnia* medium: 3,174,707 ± 305,614; for predator medium: 4,712,754 ± 838,322). However, this difference was not significant between the treatments ($t = -1.67$, $p = 0.172$).

Discussion

Our results indicate that the availability of nutrients can change the defense strategy of *D. magna*. We show that *D. magna* can overcome the trade-offs assumed in the theory of defense strategies against invertebrate predators (i.e., increased somatic growth, but delayed and decreased reproduction) at high resource supply. While increasing the food quantity resulted in earlier maturation, this response was counteracted by the exposure to the predator. We found an increase in body length and higher offspring numbers in response to *T. cancriformis* exposure, but only at high food quantity. Therefore, *D. magna* may increase its brood chamber capacity by increased investment in somatic growth when exposed to *T. cancriformis*, which requires higher amounts of food, eventually leading to postponed maturity. On the other hand, an increase in food quality also resulted in earlier maturation in the absence of the predator but the predator-induced increase in age at maturity observed at low food quality was not evident at high food quality. Thus, the delayed maturation under predator exposure in the control and high food quantity treatments was offset by high food quality.

Offspring numbers increased only at high food quantity and predator exposure. This suggests an increased resource requirement when coping with the predator and that the capacity to express defense responses depends on food quantity. These findings are in line with the findings of previous studies where increasing reproduction, but decreasing expression of morphological defenses, with increasing food quantity, was found in *D.* magna exposed to fish (Pauwels et al. 2010), as well as in *D. pulex* exposed to *Chaoborus* (Klintworth and von Elert 2020*b*). In both studies the investment into morphological features of the prey was favored under low food quantity. Adult body length was only enhanced with high food quantity under exposure to predator cues, indicating an adaptive response. However, the simultaneous earlier maturation under high food quantity, but delayed maturation under predator exposure points toward a higher resource demand for the increase in the body size, which was observed in previous studies (e.g., Rabus and Laforsch 2011; Horstmann et al. 2021). Our findings support the hypothesis that the defense responses in reproductive and morphological traits result from the increased food supply and imply that elongation of the tail-spine length is the minimal defensive response into which *D. magna* invests against *T. cancriformis*. Thus, it is likely, that the tail-spine can be elongated and maintained even under food-limiting conditions.

Our observations point toward a trade-off between higher reproductive output and morphological defenses, which can be offset under sufficient food and/or lipid supply. It is known that the offspring size and number are adjusted to the available food quantity and quality (e.g., Glazier 1992; Gliwicz and Guisande 1992; Martin-Creuzburg and von Elert 2009). At high food quantity and predator exposure, the relative tail-spine length was not increased. Instead, we observed an increased body length in this treatment. An increase in body size is likely preferred over an overproportioned elongation in tail-spine length under sufficient food supply to increase the capacity of the brood chamber. In addition, *T. cancriformis* is a gape-limited predator, meaning it has a prey size limitation due to handling issues (Höxter 1999). We observed a reduced neonate body length but an increase in neonate relative tail-spine length in the same treatments, where the adult body size and reproduction were increased. The increase in adult body length might compensate for the smaller tail-spine length in adults, while the increase in neonate tail-spine-length compensates for the reduced neonate body length. The tail-spine is considered a putative start-up defense for neonate daphnids (Rabus et al. 2012) with low spatial (i.e., in terms of space in the brood chamber) and resource demand, which potentially loses importance with increasing body size. The observed changes possibly allow for an increase in offspring number due to bigger brood chambers (due to increased adult body length) and smaller offspring size (Glazier 1992; Lampert 1993). At the same time, the predator handling is impaired by increased body size or bulkiness in adults (Rabus and Laforsch 2011; Rabus et al. 2012) and longer tail spines in neonates. This further supports our hypothesis that *D. magnas'* phenotypic response is optimized toward reproductive output. Hence, under low food conditions, an elongation of the tail spine is resource-conserving, while under high food conditions, the investment in more costly somatic tissue and, after maturity, into reproduction would be a beneficial strategy. Therefore, this might be a trade-off between somatic investment to escape the predator's preferred prey size and increased reproduction to overcome predation losses. These changes, especially the increase in reproduction, are likely to increase the resource demand, again pointing toward the modulation of the defensive response by both food quantity and quality.

Food availability in aquatic ecosystems changes seasonally but also due to anthropogenic impacts, like nutrient input, pollution, and climate change-related constraints (Sommer et al. 1986; Tadonléké 2010; Hartwich et al. 2012; von Elert and Fink 2018; Wentzky et al. 2020). Eutrophication is often associated with the occurrence of cyanobacterial blooms. Cyanobacteria are nutritionally inadequate for most aquatic consumers due to poor ingestibility, the production of harmful secondary metabolites, and the lack of essential lipids (Carmichael 1994; DeMott et al. 2001; von Elert et al. 2003; Wilson and Hay 2007; Sanchez et al. 2024). Conditions of sufficient carbon but limited lipid supply (i.e., food quality) may occur regularly in nature, especially in eutrophic waters (Müller-Navarra et al. 2004; Taipale et al. 2019). Therefore, it seems important that *Daphnia* is able to adjust its defense response to differences in food availability to optimize growth and reproduction but at the same time to defend itself against predators if necessary. However, even though sufficient carbon supply is mandatory for increased reproduction, essential lipids may constrain the reproductive output or the viability of offspring (Martin-Creuzburg and von Elert 2009; Martin-Creuzburg et al. 2012), potentially leading to a dilemma at low dietary lipid supply under the circumstance of enhanced and more regularly occurring cyanobacterial blooms: *Daphnia* might abandon parts of their morphological defenses, like the tail-spine, to favor reproductive output. However, they cannot produce more or viable offspring due to a lack of essential lipids. It remains to be investigated how fine-tuned these mechanisms are, and how precise *Daphnia* responds to food quantity and quality as distinct factors.

We show here that the previously reported strong responses of *D. magna* to *T. cancriformis* exposure, is caused by an increase in food quantity and quality due to keeping the predator in net cages within the experimental beakers. Thereby, food and predator-derived organic, particulate matter (feces and *Daphnia* remnants) accumulate over time, which may supply *Daphnia* with additional resources. The analysis of particulate matter from the filters of the conditioned water further indicates that the food quality for *D. magna* is improved by an increase in cholesterol that has been made available through the predators' activities. In addition, we generally observed a weaker response in the individual setup, compared to the net cage treatments. The reduced relative tail-spine length of the neonates in the refreshed net cage treatment could indicate differences in bacterial abundance after filtration of the predator-conditioned water. The bacteria may change the concentration or quality of the kairomone. Evidence has been found that bacteria introduced by "contaminated" food supply reduced kairomone concentration (Klintworth and von Elert 2020*a*). Thus, the animals in the net cage setup might have been exposed to higher kairomone concentrations compared to the individual setup, indicating a concentration dependency of the defense response of *D. magna* against *T. cancriformis*.

We show here that the defense pattern of *D. magna* is modulated according to food quality and quantity and found a trade-off between maternal and neonate body length and offspring number. This highlights the importance of assessing food effects in predator–prey studies. However, further research is required to investigate prey responses to different food and predator regimes. Differences in resource requirements might indicate direct costs for defense development, rendering potential constraints under naturally occurring resource limitations. This will contribute to a deeper understanding of how *Daphnia* is affected by changes in food availability and predator exposure and how predator–prey interactions shape ecosystems.

Data availability statement

Data are available at the data repository Zenodo at 10.5281/zenodo.7996937.

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Conflict of Interest

None declared.

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