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Morphological defences and defence–cost trade-offs in *Daphnia* in response to two co-occurring invertebrate predators

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Abstract

1. Inducible morphological defences are crucial for understanding predator–prey interactions. Such defences have been mostly studied in a single-predator context, ignoring the fact that prey organisms are often exposed to multiple predators. In deep peri-Alpine European lakes, the keystone grazer *Daphnia* coexists with two cladoceran predators, *Bythotrephes longimanus* and *Leptodora kindtii*. Up to now, life history and morphological responses of *Daphnia* to these two predators have not been analysed systematically.
2. We studied the responses of two life history (age at first reproduction, and offspring production) and five morphological traits (body size, body width, head size, spina size, and eye diameter) of eight *Daphnia galeata* clones to the presence of *Bythotrephes* and *Leptodora* in a common garden experiment. We compared each response trait between treatments using linear mixed models, and investigated the covariation between defence traits and demographic costs (neonate production) for the two predators.
3. Our results show that the responses of *Daphnia* are predator- and trait-specific. *Daphnia* developed a typical helmet and a larger eye only in the presence of *Bythotrephes*, not in the presence of *Leptodora*. In contrast, both predators induced larger body sizes and longer spines. Age at first reproduction was latest and demographic costs were highest in the *Bythotrephes* treatment, suggesting that the development of a helmet exceeds the costs of spina elongation. The responses of *Daphnia* clones revealed a defence-cost trade-off for helmet formation in the *Bythotrephes*, and for spina elongation in the *Leptodora* treatment. Hence, despite *Bythotrephes* and *Leptodora* being closely related co-occurring predators, *Daphnia* responds with a unique combination of trait changes and defence-cost trade-offs to the two predators.
4. The presence of predator-specific clonal defence-cost trade-offs suggests that the presence of these invertebrate predators can drive different evolutionary processes in natural zooplankton communities. Disentangling the evolutionary

ecology of phenotypic responses of prey species to co-occurring predators will require multi-trait, multi-clone studies of induced antipredator defences.

KEYWORDS
allocation costs, *Bythotrephes*, clonal variability, *Daphnia galeata*, *Leptodora*, life history traits, morphological defences, multi-predator environment

1 | INTRODUCTION

Induced prey defences are important components of predator–prey interactions (Boeing & Ramcharan, 2010; Verschoor et al., 2004) and may have ecosystem consequences exceeding the direct effects of predation (Miner et al., 2005). Inducible prey defences include morphological (Sperfeld et al., 2020; Tanner & Branstrator, 2006), behavioural (Pangle & Peacor, 2006), and life history responses (Sakamoto et al., 2015; Weider & Pijanowska, 1993).

Prey species are typically confronted with various predators in their natural environment. Consequently, prey species evolved the ability to differentiate between predators and to show predator-specific defences (Crane et al., 2016; Diel et al., 2020; Dunn & Hovel, 2020). Species of the crustacean genus *Daphnia* respond differently to vertebrate and invertebrate predators (Boeing et al., 2006b; Engel et al., 2014; Gélinas et al., 2007) and can differentiate even between various invertebrate predators (Laforsch & Tollrian, 2004; Tanner & Branstrator, 2006). Most studies conducted so far did investigate predator-specific responses by comparing *Daphnia* from different habitats in which one type or species of predator dominates, e.g., ponds with and without fish or ponds with and without larvae of the phantom midge *Chaoborus* (Boersma et al., 1998; Reger et al., 2018). However, prey is likely to face co-occurring predators, and the response of *Daphnia* is proposed to depend on whether predators are functionally equivalent, functionally inverse, or functionally diverse (Herzog & Laforsch, 2013).

Predators can induce several morphological defences in *Daphnia*, including various forms of helmets (Bungartz & Branstrator, 2003), lengthened spina (Dzialowski et al., 2003), neck teeth (Lüning, 1992; Sperfeld et al., 2020), and thickened carapace (Rabus et al., 2013). These responses are *Daphnia* species-specific and partially also predator-specific (i.e., depend on predator modality). Hence, when faced simultaneously with functionally inverse or diverse predators, specific morphological traits or combinations of these traits might be selected.

In *Daphnia*, reproduction is characterised by an environmentally triggered shift between asexual and sexual reproduction (cyclical parthenogenesis). During most parts of the season, *Daphnia* reproduce parthenogenetically and *Daphnia* populations are thus mainly composed of clones of asexually produced individuals (Jankowski & Straile, 2004). These *Daphnia* clones can differ in their responses to abiotic (Barber et al., 1990; Connelly et al., 2016; Palaima & Spitze, 2004), and biotic pressures (Hairston et al., 2001; Isanta-Navarro et al., 2021). Likewise, there is evidence that *Daphnia* clones differ quantitatively and qualitatively in their anti-predator responses (Boeing et al., 2006b; Reger et al., 2018; Tams et al., 2018). Hence, when facing multiple co-occurring and functionally inverse or diverse predators, *Daphnia* may either evolve toward a capacity to respond to all predators (*jack-of-all-trades*, Palaima & Spitze, 2004), using varying combinations of different defensive traits, or toward a specific response to the most prevalent predator.

The cladocerans *Bythotrephes longimanus* and *Leptodora kindtii* are important predators of zooplankton, including *Daphnia* spp. (Manca et al., 2008; Wojtal et al., 2004). They co-occur in many lakes in central and northern Europe (Hessen et al., 2011; Horváth et al., 2017) and more recently, since the invasion of *Bythotrephes*, also in North America (Cavaletto et al., 2010). Both are typical members of the zooplankton communities of deep pre-Alpine lakes in Europe (Horváth et al., 2017; Manca et al., 2008; Molinero et al., 2007; Straile, 2015). In these lakes, both predators show similar spatio-temporal patterns, that is, they occur at rather similar water depths and show similar seasonal dynamics with peak abundances around the same time of the year (Molinero et al., 2007; Seebens et al., 2012).

Bythotrephes and *Leptodora* belong to sister suborders within the order Diplostraca and are closely related (Richter et al., 2001). Both species are cruising predators of roughly similar size, suggesting that they select for a similar suite of defensive traits in their prey. However, both species differ in how they locate and catch their prey (Manca et al., 2008). *Bythotrephes* locates its prey either via mechanoreceptors or visually and then captures its prey using long feeding appendages (Manca et al., 2008; Schulz & Yurista, 1998). In contrast, *Leptodora* requires direct contact with its prey to initiate an attack (Branstrator, 1998; Manca et al., 2008) and employs a feeding basket to catch its prey. The latter limits the maximum prey size for *Leptodora*. Hence, *Leptodora* feeding might be more size limited compared to *Bythotrephes* feeding, and defences aimed at increasing body size might be more efficient against *Leptodora* predation. In contrast, *Daphnia* defences aimed at reducing visibility are expected to be successful only against the visual predator *Bythotrephes*. Taken together, this implies that both predators are functionally diverse (sensu Herzog & Laforsch, 2013), but this has not yet been studied experimentally.

Here, we explored life history (age at first reproduction [AFR] and number of offspring) and morphological responses (head size, spina size, body width, and eye diameter, see Figure 1) of eight clones of *Daphnia galeata* to the presence of *Bythotrephes* and *Leptodora*. We analysed whether both predators are functionally diverse, that is whether *Daphnia* responded differently to the two predators, and whether the expression of defences to both predators did entail demographic costs, i.e., reduced offspring production. Finally, we proposed that there is clonal variability in response to predators, as well as in the demographic costs of the responses.

2 | METHODS

2.1 | Study design

We tested the response of eight *D. galeata* clones (A–H) to predator kairomones. The clones were hatched from ephippia, which were isolated from the upper 20 cm of a sediment core taken from Lake Constance. All clones were already cultured in the laboratory for several months prior to the experiment. The experiment consisted of three treatments, namely control (C), *Bythotrephes* (B), and *Leptodora* (L) with four replicates each containing six daphnids. Mortality of *Daphnia* (38%) caused the loss of four experimental units (two in the L treatment [clones G and H], one in B [clone H] and one in the C treatment [clone C]) resulting in a total of (8 × 3 × 4) − 4 = 92 experimental units. Each experimental unit was composed of a 200-mL beaker filled with 180 mL filtered (<0.2 μm) lake water. All beakers contained a cylindrical plastic cage (4 cm diameter and 6 cm height) with a 140-μm nylon mesh that ensured separation between predators and *D. galeata* while guaranteeing the exchange of kairomones. While we cannot exclude the possibility that daphnids might also be able to see the predators using this design, we consider this highly unlikely. Furthermore, other experiments (Baludo et al. personal communication) have shown that *D. galeata* will also respond to kairomones of these two predators without the possible presence of visual cues. On the first day of the experiment, five individuals of *Bythotrephes* and *Leptodora* were placed in the plastic cages (B and L treatment), respectively; control (C) cages were left empty. Daphnids were fed daily with 1 mg C/L of the green alga *Scenedesmus obliquus* (Culture Collection of Algae, University of Göttingen, Germany, SAG 276-3a), which was grown semi-continuously at 20°C in 5-L batch cultures in Cyano medium (Jüttner et al., 1983) and harvest in the late-exponential growth phase (illumination at 120 μmol quanta m^{-2} s^{-1}). The animals were transferred into new jars containing freshly prepared food and cages with freshly collected living predators every other day. Prior to each transfer, predators were isolated from Lake Constance zooplankton samples taken with net hauls towed behind a boat.

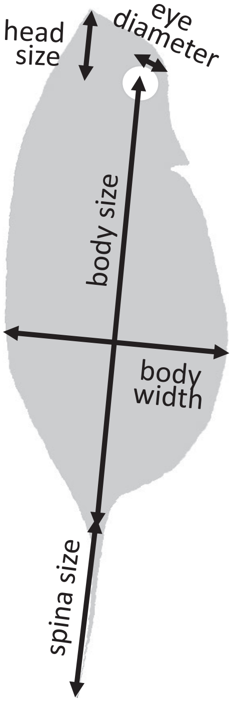

FIGURE 1 Body dimensions of *Daphnia galeata*. Body size was measured from the base of the spina until the mid of the eye and described the body axis of the *Daphnia*. Spina size was measured from the top until the base of the spina. Body width was measured perpendicular to the body axis at the midpoint of body size. Head size was measured in parallel to the body axis from the top of the head (or top of the helmet) until the line perpendicular to the body axis crossing the midpoint of the eye. Eye diameter was measured at the largest dimension of the eye

The experiment started with 1-day-old daphnids and was terminated when the daphnids reached age 10 days. At this age, daphnids of all clones and in all treatments had produced neonates in at least one replicate. For logistical reasons, the experiment was split into two blocks (hereafter: *temporal blocks*) that were run separately (4 weeks apart), with four clones in each block. Block I contained clones A, D, E, and F, and block II contained clones B, C, G, and H.

2.1.1 | Life history and body size measurements

Neonates were observed in 91 out of 92 replicates (beakers) when the experiment was terminated (i.e., at day 10 of the experiment). Beakers were daily checked for neonates and the day neonates were first observed in a beaker was noted as AFR of daphnids in this beaker. For statistical analyses, AFR was assumed to be 11 days in the replicate without neonates, as embryos were already present in the brood chambers of the daphnids. To account for mortality during the experiment, reproductive output during the 10 days of the experiment was calculated as per capita total number of neonates. At the end of the experiment, daphnids were stored in 70% ethanol (Black & Dodson, 1990) and subsequently examined using a Stemi 2000-C binocular equipped with a camera and an image analysing software. Body size, body width, head size, spina size, and eye diameter were measured as shown and described in Figure 1.

2.2 | Data analysis

Only *Daphnia* were used for morphological measurements, which allowed quantification of all morphological traits. This required the exclusion of some *Daphnia* that were damaged and with broken spina (i.e., for which spina size was less than the 95% prediction interval based on their body size). This reduced the data set from 359 to 337 daphnids; on average 3.5 daphnids per replicate were measured. Statistical analyses were run with trait averages within each replicate. Variation in body size, head size, spina size, body width, eye diameter, AFR, and per capita number of neonates was analysed using linear mixed-effect models (lmer function in lmerTest package) (Kuznetsova et al., 2017). Body size (except for models with body size and AFR as dependent variables) and treatment (*Bythotrephes*, Control, and *Leptodora*) were considered as fixed effects and clonal identity nested in temporal block as random effect. We used the step function in lmerTest to eliminate non-significant fixed and random effects from the full model, considering interaction between the fixed effects, as well as random intercepts (mean clonal effects) and random slopes (mean clonal responses to treatments). Likelihood ratio tests were used to test for the significance of fixed and random effects (ANOVA and RANOVA functions) (Kuznetsova et al., 2017). If significant treatment effects were observed, we used the emmeans package (Lenth, 2020) to perform pairwise post hoc comparisons (Tukey test). In case models proposed by the step function resulted in singular fits, that is random effects explained zero or small variance, models were further simplified via removing temporal block as a random factor (Matuschek et al., 2017). In those cases, predictions of models without temporal block yielded identical predictions to models that included temporal block as a random factor. Residuals were checked for normality and heteroscedasticity. Relationships between trait expressions and offspring per *Daphnia* were analysed using clonal means and linear regression. All statistical analyses were performed in R v 4.02.0 (R Core Team, 2020).

3 | RESULTS

Daphnia in the *Bythotrephes* treatment reproduced later than in the control (t = 3.1, $p < 0.001$) whereas *Daphnia* in the *Leptodora* treatment differed neither from the *Bythotrephes* treatment (t = 2.2, ns) nor from the control (Figure 2a). Both predator treatments resulted in a larger *Daphnia* body size in comparison to the control (Tukey test, $p < 0.005$), but body sizes did not differ between predators (Tukey test, $p > 0.05$, Figure 2b). Body sizes were larger in block I than in block II ($p < 0.005$) and showed a tendency for clonal variability nested in blocks ($p < 0.1$, Table 1). Across all treatments later AFR was associated with larger body size at the age of 10 days (fixed effect of age, t = 2.2, $p < 0.05$).

Trait specific analyses showed that all traits except head size were significantly influenced by body size (Figure 3), although no significant treatment × body size interaction was observed for any traits. Per capita number of offspring at the age of 10 days was lower in the *Bythotrephes* treatment as compared to the *Leptodora* (t = −6.7, $p < 0.0001$) and control (t = −5.8, $p < 0.0001$) treatments (Figures 3, 4). Head size was significantly larger in the *Bythotrephes* treatment than in the *Leptodora* treatment and in the control (Figures 3, 4). In contrast body width was related to body size (Figure 3) but did not differ between treatments (Figures 3, 4). Spina size and eye diameter increased with increasing body size (Figures 2, 3) and differed between treatments (Table 1). Spina size in both predator treatments was larger than in the control treatment (Tukey test, $p < 0.01$). Eye diameter in the *Bythotrephes* treatment was larger than in the control treatment (Tukey test, $p < 0.01$) and tended to be larger in the *Bythotrephes* treatment than in the *Leptodora* treatment (Tukey test, $p < 0.1$).

Models for all traits revealed significant clonal variability (random intercepts) either nested in temporal block (body size, body width, and offspring per *Daphnia*) or un-nested (AFR, head size, spina size and eye diameter; Table 1). Random block effects contributed to the best model for body size, offspring per *Daphnia* and body width only (Table 1), but in the case of offspring per *Daphnia* the temporal block effect was not significant. Random slope effects (i.e., clone specific treatment effects), did not significantly contribute to models of any trait variable.

Treatment responses of individual clones were most homogenous in respect to head size (Figure 4a). For other traits, treatment effects were evident only after adjusting for body size differences between replicates (Figure 4, middle panel), or after adjusting for

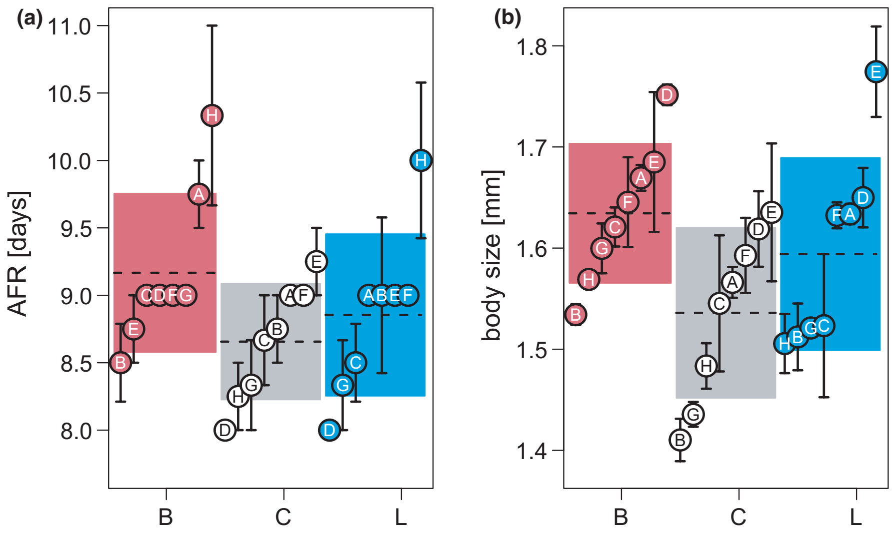

FIGURE 2 (a) Age at first reproduction (AFR) and (b) body size at the age of 10 days (means ± 1 SE) of eight *Daphnia* clones grown in the *Bythotrephes* (B, red), control (C, grey), and *Leptodora* (L, blue) treatments. Clones F, A, E, and D were grown in block I and clones B, G, H, and C were grown in block II. Clones are arranged with increasing AFR or body size, respectively, within each treatment

TABLE 1 Statistics of fixed effects (type II ANOVA with Satterthwaite's method) and random effects (Likelihood ratio tests) in models relating age at first reproduction (AFR), body size, per capita number of offspring, helmet size, spina size, body width and eye diameter to the fixed effects of body size and treatment (T), and the random intercept effects of clone nested in temporal block (1|clone:block), temporal block (1|block), and clone (1|clone)

	Fixed effects		**Random effects**		
	Body size	**T**	**1|clone:block**	**1|block**	**1|clone**
AFR	------	**$F_{2,84} = 5.1$** **			**$LRT_1 = 9.6$** **
Body size	------	**$F_{2,81.4} = 14.65$** ***	**$LRT_1 = 3.74$** .	**$LRT_1 = 7.89$** **	
Number of offspring	**$F_{1,73.6} = 13.8$** ***	**$F_{2,81} = 26.4$** ***	**$LRT_1 = 20.9$** ***	$LRT_1 = 0.26$ ns	
Helmet size		**$F_{2,81} = 292$** ***			**$LRT_1 = 44.6$** ***
Body width	**$F_{1,85.5} = 16.7$** ***		**$LRT_1 = 17.35$** ***	**$LRT_1 = 5.4$** *	
Spina size	**$F_{1,87} = 37.2$** ***	**$F_{2,81} = 9.1$** ***			**$LRT_1 = 34.3$** ***
Eye diameter	**$F_{1,83.7} = 7.2$** **	**$F_{1,82.4} = 4.9$** **			**$LRT_1 = 22.0$** ***

Note: Only fixed and random effects contributing to the best non-singular model as identified by backward elimination of factors are shown.
***$p < 0.001$. **$p < 0.01$. *$p < 0.05$, .$p < 0.1$.

body size and clonal differences (Figure 4, lower panel). The larger head size of *Daphnia* in the *Bythotrephes* treatment (Figure 4a) resulted from the formation of helmets in this treatment, whereas no helmets were formed in the two other treatments.

The mean number of offspring produced per clone across treatments was significantly negatively related to AFR ($t = -4.7$, $p < 0.0001$), head size ($t = -4.3$, $p < 0.0001$), and spina size ($t = -2.7$, $p < 0.05$), but not to eye diameter ($t = -1.1$, ns; Figure 5). Furthermore, there was a significant negative relationship between neonates per *Daphnia* and head size within the *Bythotrephes* treatment ($t = -2.8$, $p < 0.05$, Figure 5b) and a negative relationship between neonates per *Daphnia* spina size in the *Leptodora* treatment ($t = -2.5$, $p < 0.05$, Figure 5c). Within the *Bythotrephes* treatment, AFR was not significantly related to neonates per *Daphnia* ($t = -1.96$, ns), whereas within the *Leptodora* treatment AFR was significantly related to neonates per *Daphnia* ($t = -2.6$, $p < 0.05$).

4 | DISCUSSION

Our experiments revealed that *D. galeata* is responsive to kairomones from both predators, *B. longimanus* and *L. kindtii*. However, the responses differed in the traits that were affected and the overall demographic costs of defences. *Daphnia galeata* clones developed larger body sizes and spines when exposed to both predators, whereas increased head sizes, due to helmet formation, and increased eye diameters were induced only by *Bythotrephes*. Demographic costs (i.e., a reduction in per capita number of offspring), were observed only in response to *Bythotrephes*, not in response to *Leptodora*. This implies that the two predators induce different responses in *Daphnia* and thus should be considered distinct predators when assessing inducible defences in zooplankton.

Kairomones from both predators induced a larger body size in *D. galeata*, which is in line with the typical response pattern of *Daphnia* to invertebrate predators (Diel et al., 2020). As all morphological traits, except head size, scaled positively with body size, predator treatments increased all body dimensions. However, body size-adjusted responses varied between predator treatments and between traits considered. Typical helmets and larger eyes were observed only in the *Bythotrephes* treatment, whereas longer spines were found in both predator treatments.

The most obvious difference in *D. galeata* antipredator response was the formation of a typical helmet, which was observed only in the *Bythotrephes* treatment. *Bythotrephes* as well as *Leptodora* have been shown previously to induce helmets or larger heads in other *Daphnia* species: *Bythotrephes* has been shown to induce helmets in *Daphnia mendotae* (Bungartz & Branstrator, 2003) and *Leptodora* has been shown to induce larger heads in *D. mendotae* (Tanner & Branstrator, 2006) and *D. cucullata* (Laforsch & Tollrian, 2004). However, no previous study has compared the responses to both predators using a single *Daphnia* species. The differences in helmet induction in *D. galeata* between *Leptodora* and *Bythotrephes* that were shown here can potentially be explained by *Leptodora* being able to prey upon a lower maximum prey size, which is determined by the size of its feeding basket. Hence, growing a helmet may be more effective against *Bythotrephes* predation than against *Leptodora* predation. We cannot exclude that *Leptodora* exposure would have resulted in a stronger head/helmet size response in subsequent *D. galeata* generations (transgenerational effects) that have not been explored here experimentally. However, morphological

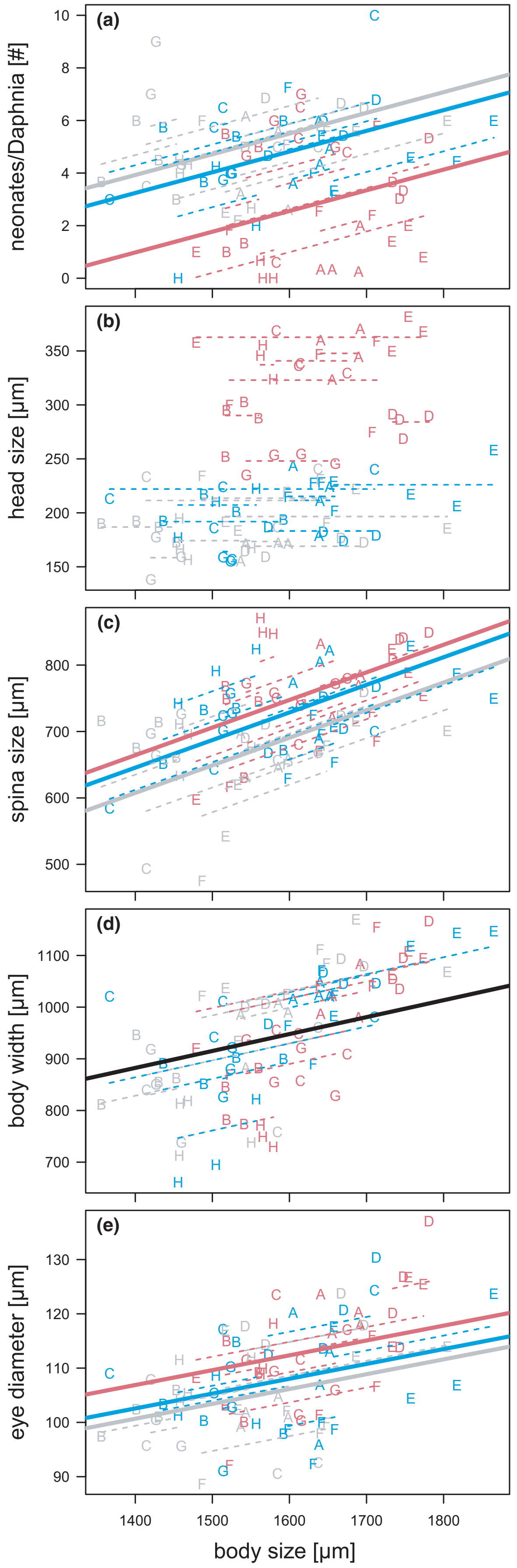

FIGURE 3 Relationships between body size and (a) neonates per *Daphnia*, (b) head size, (c) spina size, (d) body width, and (e) eye diameter for eight *D. galeata* clones. Solid lines show fixed effects of treatments (control: grey, *Bythotrephes*: red, *Leptodora*: blue) and dashed lines show random intercepts for clones. As there was no relationship of head size with body size, only random intercepts are shown in (b). As no significant treatment effects for body width were found (Table 1), only the body size relationship across all treatments is shown in (d)

defence responses are typically rather quick (Weiss et al., 2015), and experiments with *D. mendotae* have shown that a larger helmet can be induced as early as in the first generation after kairomone exposure (Tanner & Branstrator, 2006).

Kairomones of a large variety of predators, including fish (Spaak & Boersma, 1997), *Chaoborus* (Lüning, 1995; Sell, 2000; Sperfeld et al., 2020), copepods (Caramujo & Boavida, 2000), and *Notonecta* (Kruppert et al., 2017), have been shown to induce larger spines in *Daphnia*. Likewise, *Leptodora* kairomones have been shown to induce larger spines in *D. cucullata* (Laforsch & Tollrian, 2004), whereas spina enlargement in response to *Bythotrephes* kairomones had not been shown previously.

Visual predators may preferentially target individuals with large and dark pigmented eyes (Zaret & Kerfoot, 1975). We proposed that *Bythotrephes*, but not *Leptodora* kairomones, would induce a reduction in eye diameter in *Daphnia*, because only the former is considered a visual predator (Jokela et al., 2013). In contrast to our expectation, *Bythotrephes* kairomones induced an increase in eye diameter in *D. galeata*. As larger eyes are costly to build (Brandon & Dudycha, 2014) and increase the detectability to predators (Zaret & Kerfoot, 1975), benefits of large eyes need to outweigh these costs for selection to occur. Potential benefits of larger eyes in zooplankton are not well understood. In *Daphnia*, larger eyes have been suggested to increase the ability to detect and thus escape fish predators (Beston et al., 2019). Likewise, *D. galeata* might use larger eyes to detect and avoid predation by *Bythotrephes*. Alternatively, larger eyes might benefit *Daphnia* in deeper water layers. *D. mendotae* has been shown to migrate to deeper water layers in the presence of *Bythotrephes* (Pangle & Peacor, 2006). Whether the vertical migration behaviour of *D. galeata* in Lake Constance is influenced by *Bythotrephes* has not yet been studied.

Theory predicts that induced antipredator responses should bear costs (Diel et al., 2020; Tollrian & Dodson, 1999). However, these costs are difficult to quantify (Tollrian & Dodson, 1999; Diel et al., 2020; but see Riessen & Sprules, 1990; Riessen, 2012). Moreover, possible demographic costs of morphological defences are difficult to separate from demographic costs of life history responses (Tollrian & Dodson, 1999).

In our study, significant demographic costs (i.e., a reduced number of offspring), were only observed for the *Bythotrephes* treatment. This suggests that the combined morphological responses to *Leptodora* kairomones (i.e., larger body size and spina elongation), were less costly than the combined responses (larger body size, later AFR, elongated spina, helmet formation, and larger eyes) to *Bythotrephes*

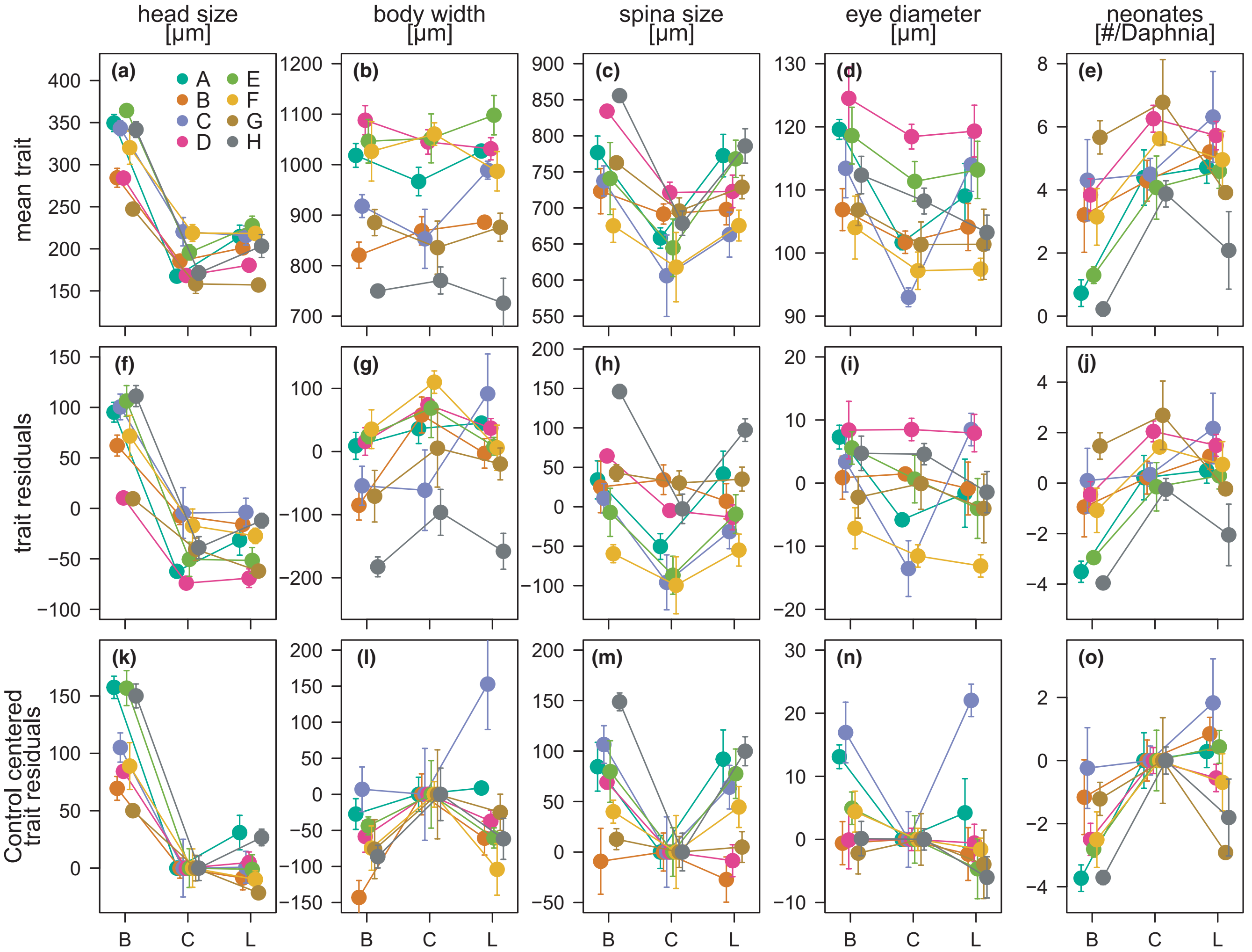

FIGURE 4 *Daphnia* trait measurements in *Bythotrephes* (B), *Leptodora* (L) and control (C) treatments (mean ± *SE*) for eight individual clones (A–H). Head size, body width, spina size, eye diameter and neonates per *Daphnia* are shown as mean values for each trait (upper panel, a–e), as mean values for traits corrected for body sizes (middle panel, f–j), and traits corrected for body size and centred around the control values of each clone (lower panel, k–o)

kairomones. As *Bythotrephes* kairomones induced various responses, it is difficult to quantify the contributions of each response to overall costs. However, as daphnids grew larger and developed larger spines in response to both predators, and did not differ significantly regarding their AFR responses, it seems likely that these three traits did not contribute a major part of the costs in the *Bythotrephes* treatment. Furthermore, the significant negative correlation of offspring numbers with head size, but not with eye diameter within the *Bythotrephes* treatment, suggests that helmet formation contributes strongly to overall costs in this treatment, although developing larger eyes has also been shown to be costly (Brandon & Dudycha, 2014).

Although offspring production in the *Leptodora* treatment was not reduced compared to the control, there was a significant negative correlation between spina size and offspring production within the *Leptodora* treatment, suggesting that spina elongation is also costly. However, as these costs seem to be rather small, spina elongation costs might have been outweighed by other costs, such as those associated with helmet formation, in the *Bythotrephes* treatment. In response to predators, *Daphnia* will have to weigh costs of individual responses against their benefits in respect to mortality reduction. Hence, the absence of helmet induction in response to *Leptodora* kairomones might reflect an alternative fitness optimum in which higher mortality rates, due to the absence of helmet formation, are outweighed by enhanced birth rates.

Several studies have shown that there is clonal variability in antipredator defences of *Daphnia* (e.g., Boeing et al., 2006a; Carter et al., 2017; Dennis et al., 2011; Hammill et al., 2008; Reger et al., 2018; Sperfeld et al., 2020; Weiss et al., 2016). In our study, clonal differences were evident in head sizes, spina sizes, and in eye diameters (significant random intercepts), but we did not detect clone- specific treatment responses (significant random slopes). Presumably, the number of replicates per clone (three or four) was not sufficient to identify treatment specific responses. However, the observed negative correlation between head sizes and neonate production indicates that individual clones differed regarding their position in the defence–costs trade-off. Changing abundances of *Bythotrephes*

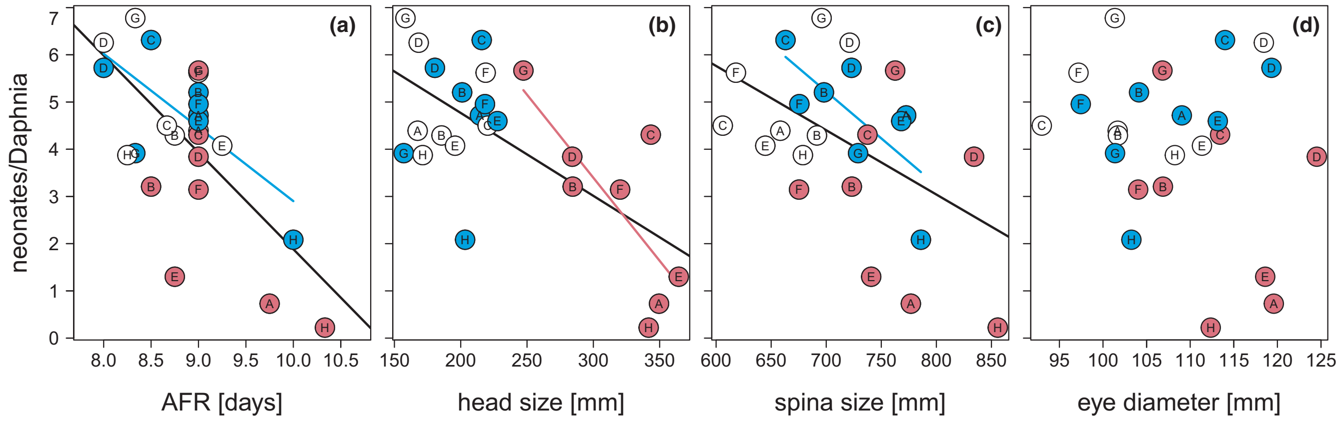

FIGURE 5 Relationship between clonal means of (a) age at first reproduction (AFR), (b) head size, (c) spina size, (d) eye diameter and clonal means of neonates per *Daphnia* in the control (white circles), *Leptodora* (blue circles), and *Bythotrephes* (red circles) treatment. Letters within circles indicate clone notations (A–H). Black lines indicate regression lines across all treatments, blue and red lines show the regression lines for the *Leptodora* and *Bythotrephes* treatments, respectively. Only regression lines significant at $p < 0.05$ are shown

versus *Leptodora* might hence result in changing selection regimes and consequently changes in the fitness of individual clones.

While we showed that *Bythotrephes* and *Leptodora* kairomones induced morphological changes in *D. galeata* clones, an important next step will be to study whether these changes indeed reduce predation by one or both predators. The mode of action of inducible defences and how they protect against certain predators is often unclear. For example, a longer spina may increase the escape ability because of a faster swimming speed (Mort, 1986) or may decrease the catchability due to a suboptimal attack distance (Laforsch & Tollrian, 2004). Investigating the role of helmets and elongated spines in the defence against *Leptodora* versus *Bythotrephes* predation is a difficult task, because these two predators are difficult to handle in the laboratory.

In conclusion, we showed that the antipredator responses of *D. galeata* differ between two closely related invertebrate predators, *Bythotrephes* and *Leptodora*. Hence, these two predators should be regarded separately in studies assessing the impact of invertebrate predation on natural zooplankton communities. Functional diversity might also partially explain the strong impact of *Bythotrephes* invasion on zooplankton communities in North American lakes (Foster & Gary, 2009; Lehman & Cáceres, 1993). The successful establishment of *Bythotrephes* in these lakes probably added a new invertebrate predator type to zooplankton communities and did not simply replace a functionally similar predator (i.e., *Leptodora*). Moreover, our results revealed predator-specific defence–demographic cost trade-offs, suggesting that the presence of these invertebrate predators can drive different evolutionary processes in natural zooplankton communities. Multi-trait, multi-clone studies are needed to disentangle the phenotypic responses of prey species to co-occurring predators.

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CONFLICTS OF INTEREST

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

D.S. conceived the ideas and experimental design; A.B. performed the experiment and collected the data; P.O. and D.S. analysed the data; P.O. led the writing of the manuscript. P.O., A.B., D.M.C., and D.S. made substantial contributions to the interpretation of the data, contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

All data will be made available at the KonDATA Repository of the University Konstanz after manuscript acceptance (Octorina et al., 2022).

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