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Original Article

Exposure to fungal infection decreases eye size in the zooplankton, *Daphnia*

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ABSTRACT

Immune responses can be energetically expensive and subject to trade-offs. Prior work on the freshwater zooplankton, *Ceriodaphnia cornuta*, demonstrated an association between eye size and infection, leading to questions about whether investment in eyes trades off against investment in immunity. We used the crustacean host, *Daphnia dentifera,* and its fungal parasite, *Metschnikowia bicuspidata*, to investigate the relationships between eye size, parasite resistance and infection. In the field, we found a negative correlation between size-corrected eye area (SCEA) and *Metschnikowia* infection, suggesting that either SCEA decreases infection (thereby indicating resistance) or that infection decreases SCEA. Controlled laboratory experiments reinforced the latter result: exposure to the fungal parasite decreased a host's SCEA, regardless of the parasite dose or host genotype. We also uncovered significant plasticity in this trait—both host age and resource level increased SCEA. Identifying causality in physiological correlations is challenging. Our results suggest that negative associations between parasitism and energetically-expensive traits can arise through plasticity.

KEYWORDS: disease; fungus; immunity; zooplankton

INTRODUCTION

The constant danger posed by parasites and pathogens suggests that hosts should invest maximally in immune function. However, the frequent documentation in wild populations of underperforming immune responses and severe infections indicates a high cost to immunity and a potential trade-off between immune function and other traits (Kraaijeveld and Godfray, 1997; Sandland and Minchella, 2003; Graham *et al.*, 2011; Auld *et al.*, 2013; Weber *et al.*, 2017). The lack of suitable methods to measure immune responses in wild populations has made these potential trade-offs difficult to measure, especially in non-model invertebrate species (Graham *et al.*, 2011; Hawley and Altizer, 2011). Moreover, much of what we know about the potential costs of mounting an effective immune response result from studies using vertebrate animals or model invertebrates such as *Drosophila* (Tzou *et al.*, 2002). As a result, even though the drivers of variable immunity have been a research subject since the late 1800s, much remains assumed rather than known in many invertebrate species (Metschnikoff, 1884; Whitten and Coates, 2017; Coates *et al.*, 2022).

Within arthropods, one of the most well-studied immune responses is melanization. Melanization is an outcome of the prophenoloxidase (proPO) cascade, where phenoloxidase (PO) converts phenols into quinones, which are then polymerized into melanin (Söderhäll and Cerenius, 1998). Melanin is deposited around the foreign body in the hemolymph or cuticle to entrap and kill the invader (Söderhäll and Cerenius, 1998; Ebert, 2005). This process is known as encapsulation and is often used to defend against larger parasites, such as fungi (González-Santoyo & Córdoba-Aguilar, 2011). The cascade that causes melanization is also involved in other functions such as sexual signaling, wound healing, cuticle hardening and pigmentation (Siva-Jothy, 2000; Schmid-Hempel, 2005), and the association between darker cuticles and enhanced immunity has been the focus of a rich area of research in arthropods (Robb *et al.*, 2003; Lee *et al.*, 2015; Ehrlich and Zuk, 2019; Wang *et al.*, 2021). This array of functions illuminates why melanin production, which is energetically expensive, is highly conserved throughout invertebrates (Blois, 1978; True, 2003; Støehr, 2006; Lee *et al.*, 2008; González-Santoyo & Córdoba-Aguilar, 2011). Threats that require the upregulation of melanin synthesis may then divert energy away from other energetically expensive traits.

One trait that may be subject to these energetic trade-offs is eye size. Increased eye size is associated with better vision, but the energetic cost of eyes often limits their size (Niven *et al.*, 2007; Niven and Laughlin, 2008; Brandon and Dudycha, 2014). A possible connection between eye size and parasite defense is intriguing, given the relationship of these traits with their energetic costs.

In a field study investigating differences in infection status (a result of a failed immune response) among different morphs of the freshwater crustacean *Ceriodaphnia cornuta,* Stewart *et al.* (2018) found that smaller-eyed morphs were more likely to be infected than larger-eyed morphs. They suggested that this

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observation might arise if large-eyed individuals are better at synthesizing melanin than small-eyed individuals (i.e. if eye size is correlated with a morph's capacity to allocate simultaneously to both immune function and eye size). However, those data were correlational and did not address other potential drivers of that relationship. For example, if an infected host has sufficient energy to allocate toward both visual systems and immunological defenses, one might observe a negative relationship between eye size and infection (where those individuals with the largest eyes are the least likely to be infected). It is also possible that a trade-off between visual systems and immune function could result in a positive relationship between eye size and infection, where large-eyed individuals have greater levels of infection, because those large-eyed individuals have invested in eyes at the expense of immune responses. In each of these possibilities, it is eye size and immunity at the time of exposure that determine the outcome of infection. However, the converse is also possible that infection determines eye size.

We combined a field survey spanning six lakes over 6 months with laboratory experiments to test hypotheses that explore the potential bidirectional relationship between infection and size-corrected eye area (SCEA): eye size (corrected for body size) affects susceptibility, and infection shapes SCEA. If baseline SCEA (before parasite exposure) is correlated with resistance to infection, then large-eyed individuals should be less likely to become infected. If individuals allocate resources to screening pigmentation in eyes over immune function (a trade-off), then large-eyed individuals should be more likely to become infected. If exposure to parasites shapes SCEA and there are among-genotype differences in screening pigmentation synthesis, then SCEA should be largest in unexposed hosts and smallest in infected hosts. If ocular screening pigments and immunological melanin are produced via a shared pathway, then we would also expect to observe that unexposed individuals have large eyes (individuals have optimal allocation to SCEA) and exposed individuals have small SCEA (resources have been allocated away from eyes and instead to immunity). Although the outcome is the same in these last two predictions, the underlying mechanisms could be different.

METHODS

We combined field data collected from Stewart Merrill *et al.* (2021a) with new laboratory experiments to test for associations between SCEA and parasite infection and to disentangle the directionality of this potential relationship (i.e. that SCEA drives susceptibility to infection or that SCEA changes as an outcome of infection). We began by measuring the body sizes and eye lengths of *Daphnia dentifera* from photographs that had been taken by Stewart Merrill *et al.* (2021). In that study, the authors tracked naturally occurring *Metschnikowia bicuspidata* infections before and during epidemics in six lakes in Central Indiana (coordinates included in original publication). The resulting dataset ("Parasite_data.xls" from doi: 10.5061/dryad.v15dv41ts) included *Metschnikowia* infection data for 2229 *D. dentifera*, which allowed us to explore correlations between SCEA and infection. Images were captured using a Leica DMLB (or Digital Microscope Laboratory Binocular) compound microscope at 40× magnification. We used ImageJ (Schneider *et al.*, 2012) to measure the body length (center of the eye to base of the tail spine) and maximum eye length for the pigmented portion of the eye. Eye area (surface area of a sphere) and SCEA were then calculated using a method modified from Brandon and Dudycha (2014) where eye area = $4\pi\,(^1/_2$ maximum eye length$)^2$ and SCEA = eye area/body size.

To test the hypothesis that SCEA affects susceptibility more directly, we used additional data collected by Stewart Merrill *et al.* (2021a; "Immune_data.xls" from doi: 10.5061/dryad.v15dv41ts). In this dataset, susceptibility of wild-caught *Daphnia* from the same six lakes was quantified by exposing hosts to *Metschnikowia* under controlled laboratory conditions and at a standard dose of 200 spores/mL and then assessing them for late-stage (terminal) infections 9 days later. Photographs had been taken of all individuals at the time of exposure, and we again used ImageJ to measure body lengths and eye lengths to calculate their eye area and SCEA.

We conducted two laboratory experiments to determine the directionality of the relationship between eye size and immunity. In both experiments, *Daphnia* were obtained from mothers raised at low densities under high resource conditions (2 mg C/L of *Ankistrodesmus falcatus*) for at least three generations to standardize maternal effects (Lynch and Walsh, 1998). We exposed *Daphnia* to *Metschnikowia* from birth by placing individual neonates (<24 hours old) into 50 mL centrifuge tubes with 45 mL of filtered lake water and infective *Metschnikowia* spores (doses varied by experiment). All *Daphnia* hosts were incubated at 20°C under a 14:10 light:dark photoperiod.

In the first laboratory experiment, we established 90 *Daphnia* from three genotypes (30 per genotype) known to differ in susceptibility to *Metschnikowia* (A45, Standard [ST] and W2; Hall *et al.*, 2010) in three dose treatments (0 [unexposed], 100 or 200 spores/mL). *Metschnikowia* spores were added on Day 1 of the experiment (when *Daphnia* were neonates) and *Daphnia* were maintained in their treatments for 15 days. Water was changed at least weekly, at which point newly produced offspring were removed and tubes were re-inoculated with spores at their designated dose. To keep the infective spores suspended, water was agitated on the days that tubes were not being mixed from feeding or removing neonates. All tubes were fed 2 mg C/L of *A. falcatus* every other day. On the final day of the experiment (Day 15), photographs of the eye (400×) and body (40×) were taken using Leica software (LAS v4.13) on a compound microscope for measurements and calculations of eye area and SCEA. We also recorded each host's final infection status as "uninfected" or "infected," designated by the presence of late-stage conidia or asci in the body cavity (Stewart Merrill and Cáceres, 2018).

Our second laboratory experiment focused on a single *Daphnia* genotype from the previous experiment (ST). We manipulated parasite exposure (exposed versus unexposed) and resource availability (0.5, 1.0 or 2.0 mg C/L) for a 2 × 3 factorial design. Knowing that the death rate for exposed individuals would be higher than for the unexposed individuals and that not all exposed individuals would become infected, the number of replicates varied by treatment: for unexposed *Daphnia*, there were five individuals per resource level, whereas for exposed *Daphnia*, there were 20 individuals per resource level (total $N = 75$). Parasite-exposed *Daphnia* received 10 spores/mL

each week (a lower level of spore exposure than in our first experiment). All *Daphnia* were fed *A. falcatus* at their designated resource level every other day. Due to a calculation error, all individuals were fed one-tenth of the normal food treatment on Day 1 of the experiment but were fed the full amount on the second day. As with the first experiment, tubes were agitated to keep spores suspended in the water. Water changes were done once every week. Photographs of the eye (400×) and body (40×) of all individuals were taken on Days 10, 15 and 20.

Statistical analyses

Effects of SCEA on infection

With the previously published field data (containing naturally occurring infections), we ran a generalized linear mixed model that evaluated the effects of SCEA on infection. In this data set, we had measurements of each individual's eye, as well as its infection status at the time of capture. This analysis could therefore detect an association between SCEA and susceptibility, but we stress that the direction of causality is unknown. In this model, the response variable was infection status (a binomial factor, where "1" denotes infected and "0" denotes uninfected), and the predictor variable was SCEA. We modeled the residuals with a binomial distribution (logit-link). We ran the model with the function "glmmTMB" in the "glmmTMB" package (Brooks *et al.*, 2017) and included random intercepts for site, date and date nested within site to account for potential sources of autocorrelation. Stewart Merrill *et al.* (2021a) had scored each individual as having no *Metschnikowia* infection, early-stage infection (spores, hyphae or sporocysts in the body cavity) or late-stage infection (conidia or asci filling the body cavity; all stages described in Stewart Merrill and Cáceres, 2018). Early-stage infections can be cleared by the host, while late-stage infections will almost certainly result in host death (Stewart Merrill *et al.*, 2019; Stewart Merrill *et al.*, 2021a). We restricted our analysis to uninfected individuals and individuals with late-stage infections because the fate of early-stage infections is uncertain. Further, we excluded individuals with any co-infecting parasites or pathogens. This resulted in us excluding 53.4% of the data, for a final sample size of 1039 *Daphnia*.

We ran a similar generalized linear mixed model with the wild-caught *Daphnia* that had been experimentally exposed to *Metschnikowia* in the lab. By measuring SCEA at the time of exposure and then determining late-stage infection status 9 days later, we could incorporate some causality into our assessment. In this model, we also incorporated the number of spores that entered the body cavity as a covariate. The number of spores that successfully penetrate the gut and invade the host haemocoel is a strong predictor of late-stage infection. Thus, by allowing newly infecting spores to interact with SCEA, we could assess how eyes shape the likelihood of infection given exposure. Our model contained infection status at Day 9 as the response variable (binomial), with infecting spores, SCEA and their interaction as fixed effects. Again, we incorporated random intercepts of site, date and date nested within the site.

Effects of parasite exposure on SCEA

Using data from our first new experiment, which manipulated host genotype and spore dose, we examined how exposure to the *Metschnikowia* parasite influenced *Daphnia* SCEA. We ran a two-way analysis of variance (ANOVA) with SCEA at Day 15 as the response variable. We included fixed effects of spore concentration (0 [unexposed], 100 or 200 spores, coded as a factor), host genotype and their interaction. Planned contrasts between the spore doses were included in the model. It is possible that the effects of exposure on SCEA depend on the outcome of infection. That is, an infection that progresses to a late-stage infection may have different consequences than an infection that is immunologically cleared. To explore this possibility, we ran a second ANOVA on the experimental data and incorporated information on the outcome of infection. *Daphnia* were categorized into three infection classes: unexposed individuals, exposed-uninfected individuals and exposed-infected individuals. Given the high spore doses and repeated inoculations, we assume that all exposed-uninfected individuals cleared their early infections with an immune response. For the ANOVA, our response variable was SCEA, measured at Day 15, and our predictor was infection class. All three genotypes were pooled, and we did not differentiate between spore doses (i.e. individuals exposed to 100 versus 200 spores per milliliter were both pooled as "exposed").

Our second new experiment also investigated the effects of parasite exposure on SCEA but incorporated resource availability. The inclusion of resources allowed us to more directly assess whether SCEA is influenced by parasite exposure via an energetic cost. If so, we would expect that any negative effects of parasite exposure on SCEA would be smallest in the high-resource environments (where *Daphnia* are not resource-limited). We conducted a three-way repeated-measures ANOVA with host age, resource level, spore exposure and their interaction as fixed factors and SCEA as the response variable. As before, we also ran a second ANOVA that evaluated the effects of infection class (unexposed, exposed-uninfected, exposed-infected), resource availability and their interaction on SCEA. This second ANOVA used measurements taken on Day 15 when terminal infections could be detected and before infected *Daphnia* had died. Tukey *post hoc* contrasts between infection class and resource levels were also conducted using the package "emmeans" (Lenth, 2022). Another binomial model was done to analyze the effect of SCEA and resource levels on infection class (terminal infection or cleared infection), excluding unexposed individuals. This was done to test if resource levels influence any potential trade-off between SCEA and parasite resistance.

SCEA can vary based on a change in body size, eye area or both, and we provide analyses for all three metrics in the supplementary material. All statistical models were conducted in R (version 4.3.1; R Core Team, 2023).

RESULTS

Effects of SCEA on infection

For the field-collected *Daphnia*, there was a negative association between SCEA and *Metschnikowia* infection ($N = 1\ 039$; Est $= -0.15$; Std Err $= 0.06$; $z = -2.5$; $P = 0.015$). As the size of the eye increased (controlling for body size), individuals were less likely to be infected (Fig. 1a). This pattern was similar, but non-significant, in the field-collected *Daphnia* that

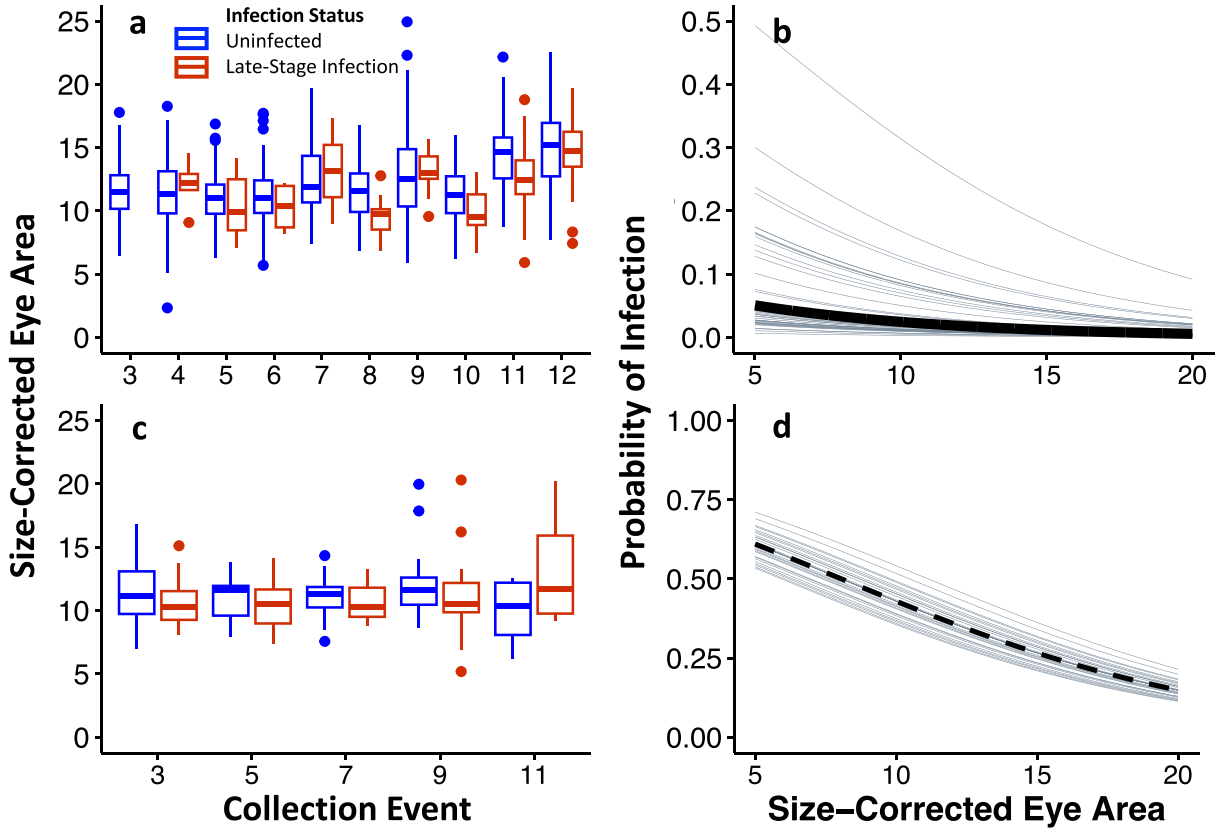

Fig. 1. (**a**) In *Daphnia* collected from six lakes in the field from July to November, there is a lot of variation in both infected and uninfected SCEA during each collection event. (**b**) SCEA was negatively associated with infection. Variation in the strength of this relationship may have emerged from natural variation in the presence and prevalence of *Metschnikowia*. (**c**, **d**) When field-collected *Daphnia* were challenged with *Metschnikowia* in the laboratory, we observed less variation and a non-significant effect of SCEA on susceptibility to infection. We note that the infection outcomes in this relationship are most strongly affected by the number of infecting spores (those that breached the gut barrier and entered the body cavity), and, for simplicity, we visually depict the relationship between infection and SCEA with infecting spores held at a constant value of 1. In both (b) and (d), the black lines depict the overall main effect of SCEA (solid for significant and dashed for non-significant) and the gray lines represent the random effects. Probabilities are predicted from generalized linear mixed models.

were challenged experimentally (Fig. 1b). For the challenged *Daphnia*, there was a trend for a negative effect of SCEA on probability of infection ($N = 178$; Est $= -0.48$; Std Err $= 0.28$; $z = -1.69$; $P = 0.091$) with no significant interaction between infecting spores (number that penetrated the gut and entered the body cavity) and SCEA (Est $= 0.12$; Std Err $= 0.08$; $z = 1.48$; $P = 0.14$). As expected, the probability of infection increased with the number of infecting spores (Est $= 0.38$; Std Err $= 0.09$; $z = 4.19$; $P < 0.001$). Once we were able to control for exposure (both experimentally and by statistically accounting for spores infecting the body cavity), SCEA was weaker as a predictor of infection. This suggests that the field pattern we detected may be more indicative of infection-shaping SCEA.

Effects of parasite exposure on SCEA

In the laboratory experiment that manipulated genotype and spore dose, parasite exposure decreased SCEA ($F_{2,48} = 34.1$, $P < < 0.0001$; Fig. 2a). SCEA was larger in unexposed individuals compared to *Daphnia* exposed to spores (100 or 200 spores/mL; $t_{48} = -8.1$, $P < < 0.0001$), but there was no difference between spore treatment groups ($t_{48} = -0.7$, $P = 0.47$; Fig. 2a). Body size and eye area both decreased significantly due to exposure, indicating that this decrease in SCEA was caused by the decrease in both factors (Supplemental Fig. 1). Genotype had no significant effect on SCEA ($F_{2,48} = 2.4$, $P = 0.1$), nor did the genotype∗dose interaction ($F_{4,48} = 0.8$, $P = 0.52$).

For infection outcomes, 57 of 90 *Daphnia* survived to day 15 (Supplemental Table I). Of these, 12 of 17 *Daphnia* in the 100 spores/mL treatment became infected (70.5%), and 7 of 17 *Daphnia* in the 200 spores/mL treatment became infected (41%). We detected a significant effect of infection class on SCEA ($F_{2,54} = 32.5$, $P < < 0.0001$, Fig. 2b). As before, *Daphnia* that had been exposed to the parasite had a smaller SCEA than unexposed individuals ($t_{54} = -8.1$, $P < < 0.0001$). This was caused by a decrease in both eye area and body size (Supplemental Fig. 2). There was no difference in SCEA between exposed-infected and exposed-uninfected individuals ($t_{54} = -0.3$, $P = 0.75$), suggesting that exposure alone, regardless of whether the infection was immunologically cleared, decreased SCEA.

In the final laboratory experiment, both the age of the host and resource availability influenced SCEA (Fig. 3). As individuals

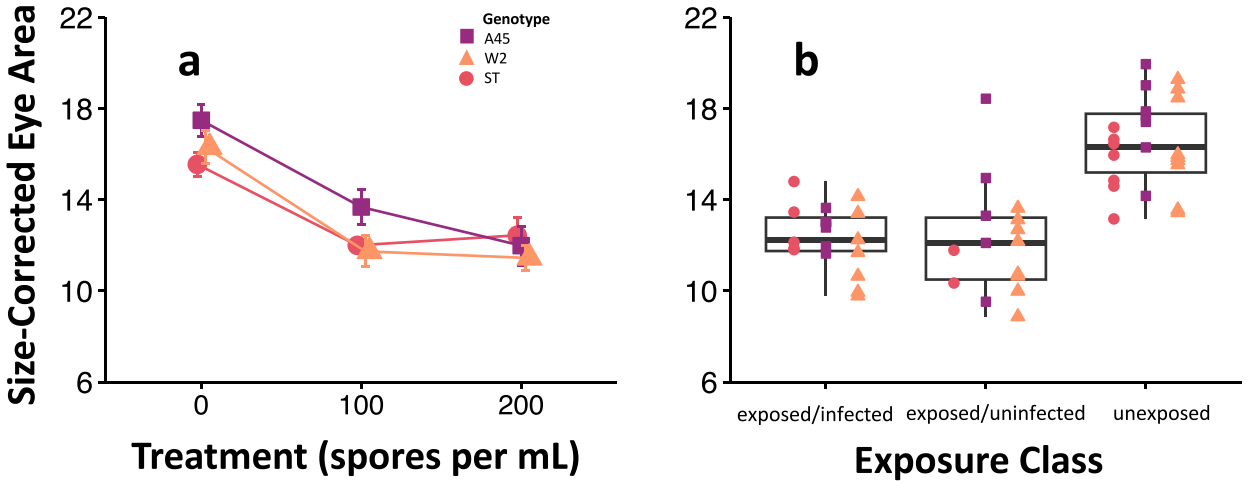

Fig. 2. (**a**) The mean SCEA (with standard error bars) was smaller for individuals exposed to *Metschnikowia* (100 and 200 spores per milliliter) relative to unexposed individuals (0 spores per mL). The responses of the three different genotypes, A45 (square symbols), W2 (orange triangle symbols) and ST (circle symbols), did not differ. (**b**) SCEA of unexposed individuals was greater than those exposed to spores, regardless of ultimate infection status. Doses and genotypes were pooled to create the exposed group, with infection status designated by presence or absence of late-stage *Metschnikowia*. Sample sizes for the three infection classes were: 23 unexposed, 15 exposed/uninfected and 19 exposed/infected.

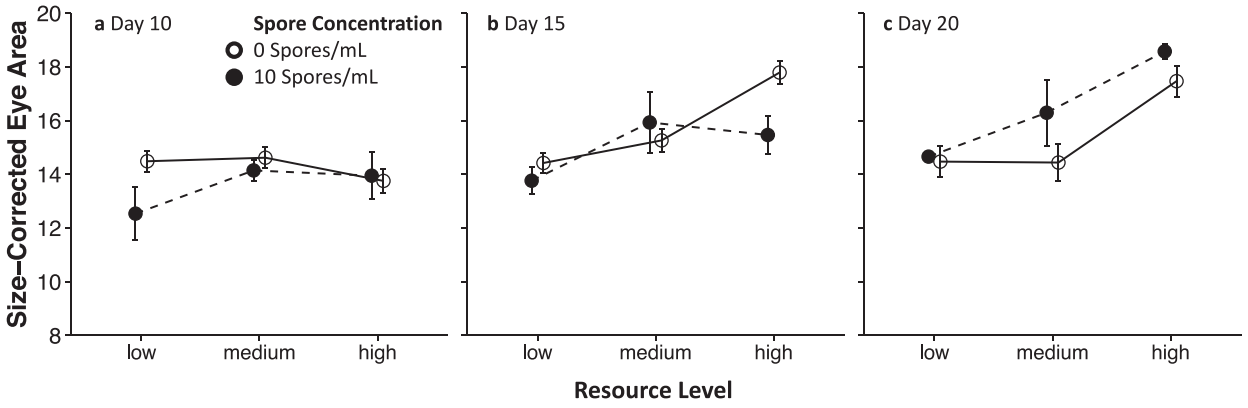

Fig. 3. The SCEA increases with resource levels at later ages. The symbol fill represents spore concentrations: open for individuals not exposed to spores and filled for individuals exposed to 10 spores/mL. Each panel shows the relationship for a different time point (**a**) Day 10, (**b**) Day 15 and (c) Day 20. SCEA increased as individuals got older, but the effects of resources and parasite exposure are not seen until Day 15/Day 20.

got older, SCEA increased ($F_{2,87} = 15.1$, $P << 0.0001$), and this increase resulted from both an increase in body size and absolute eye area (Supplemental Material). There was also a significant positive effect of resources on SCEA ($F_{2,67} = 11.4$, $P = 0.0001$), which again resulted from an increase in both absolute eye area and body size (Supplemental Material). A significant interaction between resources and age ($F_{4,87} = 4.8$, $P < 0.005$) on SCEA was driven by a significant increase in absolute eye area and body size (Supplemental Material). There was no effect of parasite exposure on SCEA ($F_{1,67} = 0.3$, $P = 0.62$), nor was there a significant interaction between host age and parasite exposure ($F_{2,87} = 2.4$, $P = 0.1$). There was no significant three-way interaction ($F_{4,87} = 2.0$, $P = 0.1$). This experiment had less initial death than the first, but the sample size decreased as the experiment progressed (Supplemental Table II).

On Day 15, there was a marginally significant difference in SCEA between infection classes ($F_{2,57} = 3.1$, $P = 0.055$; Fig. 4). These differences were driven by a significant difference in body size and a marginally significant difference in eye area (Supplemental Material). Increasing resource availability also significantly increased SCEA ($F_{2,57} = 12.7$, $P << 0.0001$; Fig. 4). This was influenced by a significant increase in both body size and eye area due to resource availability (Supplemental Material). However, the interaction between infection class and resource levels was not significant ($F_{4,57} = 1.9$, $P = 0.1$) for SCEA.

Tukey's post-hoc contrasts revealed that within each resource level, the only difference was at high food concentrations where the eyes of unexposed hosts were smaller than that of hosts that were exposed but had cleared their infection ($t_{57} = -4.9$, $P << 0.0001$). Within the exposed/uninfected class (i.e. hosts that had cleared their infection), SCEA increased with increasing resources (low to high: $t_{57} = -5.7$, $P << 0.0001$; medium to high: $t_{57} = 4.0$, $P = 0.005$). The exposed/infected class trended in the same direction, but there was no significant

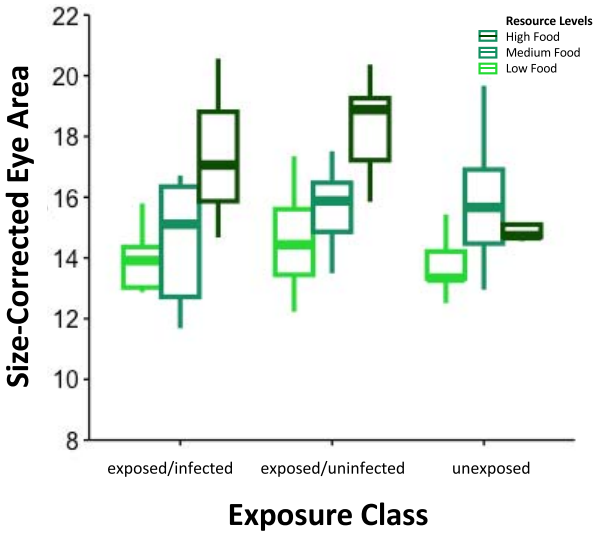

Fig. 4. Boxplots of SCEA with standard error bars for one genotype (ST). SCEA increases with resource levels in each infection class: exposed/infected, exposed/uninfected and unexposed. Infection is designated by the presence or absence of late *Metschnikowia* stages (conidia or asci) on Day 15.

difference among resource treatments (low to high: $t_{57} = -2.8$, $P = 0.15$; medium to high: $t_{57} = -2.4$, $P = 0.31$). There was no significant effect of resource levels ($\chi 2_2 = 0.09$, $P = 0.9$), SCEA ($\chi 2_1 = 2.7$, $P = 0.10$) or their interaction ($\chi 2_2 = 0.06$, $P = 0.97$) on infection class (terminal infection or cleared infection) of exposed individuals on Day 15.

DISCUSSION

We examined two hypotheses testing the potential bidirectional relationship between visual systems and parasitism: SCEA affects susceptibility, or parasite exposure shapes SCEA. Results from two assays using field-collected *D. dentifera* supported the second hypothesis, parasite exposure decreased SCEA. Not surprisingly, we found considerable variation in that relationship, which motivated us to explore other factors (parasite dose, host genotype, resource level, host age) influencing SCEA in controlled laboratory experiments. In the first laboratory experiment, exposure to the parasite decreased SCEA, regardless of the parasite dose or host genotype, reinforcing the field results. In the second laboratory experiment, as host age and resource level increased, so did SCEA. This demonstrates plasticity in SCEA. Teasing apart cause and effect in physiological correlations is challenging and requires experiments testing both directions.

Previous studies have suggested that animals with higher melanin levels (often measured as cuticular, wing-spot or plumage-based) exhibit greater resistance to pathogens (Siva-Jothy, 2000; Cotter *et al.*, 2004; de Souza *et al.*, 2018). We also assumed this relationship based on previous work in *Ceriodaphnia* where it was suggested that smaller-eyed individuals were more susceptible to parasites than larger-eyed individuals because larger-eyed individuals were better at synthesizing melanin (Stewart *et al.*, 2018). Contrary to this prediction, we found that parasite exposure resulted in decreased SCEA. Although we only found support for parasites impacting eye size (and not eye size impacting parasite susceptibility), prior work in the mealworm beetle, *Tenebrio molitor*, provides support for both possibilities. Individual beetles with darker-pigmented cuticles were less susceptible to parasite challenge (Barnes and Siva-Jothy, 2000), and larvae that were challenged had lighter pigmented cuticles (Kangassalo *et al.*, 2016). An additional example of parasites influencing pigment is provided by Freitak *et al.* (2005), who found that parasite challenge during the pupal stage leads to darker forewing tips in large white butterflies, *Pieris brassicae*. Clearly, parasites and pigments are closely intertwined, and the nature of the bidirectional relationship varies across systems.

Predictably, we found more variation in the relationship between eye size and susceptibility in natural populations relative to the laboratory-challenged, field-collected animals. This increased variation in the natural populations could result from the presence of larger-eyed individuals in the field occurring at times of both high and low parasite exposure. During periods of low exposure (i.e. they were unchallenged or challenged by smaller parasite doses), multiple factors likely influenced the variation in eye size. Previous work has found that both abiotic (e.g. light) and biotic (e.g. fish predator kairomones and resource levels) factors can influence SCEA (Hiller-Adams and Case, 1985, 1988; Brandon and Dudycha, 2014; Beston *et al.*, 2019). As a result, it is important to exhibit caution when attempting to assign causation from brief periods of field sampling, especially when parasite exposure is unquantified and variable in space and time.

Our laboratory experiments demonstrate that exposure alone, even when it does not result in a late-stage infection, can lead to decreased SCEA, provided there is a high-enough spore dose. This suggests that early-life parasite exposure may divert resources to the immune system at the expense of other traits. This is consistent with other research showing investment into immunity, specifically melanin, can decrease an organisms' ability to invest in other important traits (Cotter *et al.*, 2004; Schulenburg *et al.*, 2008; Busso *et al.*, 2017). The amino acid tyrosine has been proposed as the limiting shared resource between melanin in the immune system and other traits driving this interaction (Siva-Jothy, 2000; Støehr, 2006; González-Santoyo and Córdoba-Aguilar, 2012; Kangassalo *et al.*, 2016; Wang *et al.*, 2021). Not surprisingly, this trade-off is most apparent when the host is resource limited, highlighting the need to manipulate resources when investigating potential interactions between immune system melanin and other traits (Sandland and Minchella, 2003; Freitak *et al.*, 2005).

Hence, our second laboratory experiment varied resource levels, allowing us to assess whether SCEA is influenced by parasite exposure via an energetic cost. We also measured changes throughout development to gain insight into when these costs manifest. In general, SCEA increased with both increasing resource level and host age. Neither result is surprising and supports previous work that shows increasing resource levels increases SCEA in *Daphnia* (Brandon and Dudycha, 2014) and other aquatic invertebrates (Hiller-Adams and Case, 1985). Although SCEA was decreased in parasite challenged individuals

in the earlier experiment, here, at lower spore doses, we did not capture as strong of an effect, likely because the effect of resources overwhelmed any effect of exposure. Interestingly, host age interacted significantly with both resource level and parasite exposure in determining SCEA (Fig. 3). As hosts grow, investment into immunity can change. For example, in the mosquito, *Anopheles gambiae*, females' ability to mount a sufficient melanin response to an immune challenge drops substantially over the course of a week after eclosion (Chun *et al.*, 1995). Another explanation for the significant age∗exposure interaction is that the manifestation of the costs of parasite challenge can be delayed (Sandland and Minchella, 2003).

CONCLUSIONS

Daphnia populations are regularly exposed to *Metschnikowia*, but considerable work remains before we can fully link the mechanisms driving the physiological traits to the ecological consequences of disease (Hall *et al.*, 2011; Stewart Merrill *et al.*, 2021b). By studying the relationship between SCEA and parasite exposure/susceptibility, we found that parasite exposure influences SCEA, but there was little support for SCEA influencing susceptibility. Disease-induced reduction to SCEA could impair the ability of individual *Daphnia* to forage for food and detect predators, as has been hypothesized for other mechanisms that decrease eye-size (Hathaway and Dudycha, 2018). A more complete understanding of how parasite exposure shapes host traits will broaden our understanding of the direct and indirect effects of disease on host populations.

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SUPPLEMENTARY DATA

Supplementary data can be found at *Journal of Plankton Research* online.

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DATA AVAILABILITY

Data has been deposited in Dryad (doi: 10.5061/dryad.31zcrjdvq).

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