

Ecological constraints on sensory systems: compound eye size in *Daphnia* is reduced by resource limitation

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Abstract Eye size is an indicator of visual capability, and macroevolutionary patterns reveal that taxa inhabiting dim environments have larger eyes than taxa from bright environments. This suggests that the light environment is a key driver of variation in eye size. Yet other factors not directly linked with visual tasks (i.e., non-sensory factors) may influence eye size. We sought to jointly investigate the roles of sensory (light) and non-sensory factors (food) in determining eye size and ask whether non-sensory factors could constrain visual capabilities. We tested environmental influences on eye size in four species of the freshwater crustacean *Daphnia*, crossing bright and dim light levels with high and low resource levels. We measured absolute eye size and eye size relative to body size in early and late adulthood. In general, *Daphnia* reared on low resources had smaller eyes, both absolutely and relatively. In contrast to the dominant macroevolutionary pattern, phenotypic plasticity in response to light was rarely significant. These patterns of phenotypic plasticity were true for overall diameter of the eye and the diameter of individual facets. We conclude that non-sensory environmental factors can influence sensory systems, and in particular, that resource availability may be an important constraint on visual capability.

Keywords Food level · Light environment · Phenotypic plasticity · Sensory ecology · Vision

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Introduction

Eye size is an important determinant of visual capabilities. In apposition-type compound eyes, differences in eye size are also reflected in the structural units that influence the principal elements of visual capabilities (Land 1997; Land and Nilsson 2012). One such element, sensitivity, which refers to the number of photons captured by an eye's receptor, can be enhanced by larger compound eye size. Apposition compound eyes are a composite of individual optical units called ommatidia, each of which is singularly capable of forming an image (Land and Nilsson 2012). An ommatidium contains a facet that collects and focuses light onto a set of photoreceptor cells. A bigger compound eye can accommodate wider facets, thus increasing aperture size, a critical aspect of improving sensitivity (Land and Nilsson 1990, 2012).

Comparative morphological studies across a broad range of taxa have demonstrated that the brightness of the light environment is a strong predictor of eye morphology. This evolutionary association is a robust pattern that has been demonstrated in mammals (Veilleux and Lewis 2011), bony fish (Schmitz and Wainwright 2011), sharks (Lisney and Collin 2007), birds (Hall and Ross 2007), lizards (Hall 2008), beetles (Bauer et al. 1998), bees (Somanathan et al. 2009), and crustaceans (Hiller-Adams and Case 1985). However, these studies focus on eye morphology as a fixed property of species, and ignore the potential for phenotypic plasticity of eye size.

Environmental factors that are directly tied to vision undoubtedly are key evolutionary drivers of visual systems (Nilsson 2009). However, factors that are not tied directly to vision may also affect visual systems. We refer to these factors as the “non-sensory environment.” The resource environment, for example, may constrain the size of eyes

because eyes are energetically expensive (Niven et al. 2007; Niven and Laughlin 2008), and their costs place limits on the net benefit of large eyes. In cavefish, eyes have regressed to near uselessness, but their close relatives that live above-ground have maintained fully functional eyes (Jeffery 2005; Borowsky 2008). Caves are resource-limited environments, and the loss of eyes in cavefish may be driven to some degree by the relatively high energetic costs of the visual system coupled with minimal benefit of vision (Niven and Laughlin 2008). The resource environment has also been implicated in variation of eye size in marine crustaceans (Hiller-Adams and Case 1985, 1988). Hiller-Adams and Case (1985) found that in benthic decapods eye size increases with decreasing ambient light levels (i.e., with increasing depth), in line with the expectation that larger eye size enhances photon capture and improves vision in dimmer environments. In contrast, they found the opposite trend in pelagic crustaceans (Hiller-Adams and Case 1984, 1988), and suggest the pattern is due to large eyes that become an energetic burden in the resource-limited pelagic zone. These correlative examples suggest that effects of light environment may depend on resource availability.

If the mechanism driving the macroevolutionary pattern reflects the balancing of costs and benefits of vision, we might expect to find a similar association when examining phenotypic variation within species. Larger eyes benefit an organism by increasing information acquisition, but at an energetic cost. Increasing the capacity to acquire information is only useful to an organism if it enhances some quality of fitness or survival. Developmental investment in eyes and the ability to acquire information beyond what is useful for an organism may needlessly siphon resources away from other somatic and reproductive tissue. Relevant data on fluctuating costs and benefits reflected in phenotypic plasticity of eyes are limited. In a selection experiment, Nijhout and Emlen (1998) found that allocation to horn development in beetles was negatively genetically correlated with eye size. Merry et al. (2011) found evidence of phenotypically plastic eye size in butterflies in response to resource availability. However, we know of no experimental study that has examined eye size plasticity in response to the light environment, nor of any study that has examined the combined effects of both sensory and non-sensory environments on eye size.

Here, we test the hypothesis that resources and light jointly determine the plastic response of eye size in four species of *Daphnia*, a freshwater microcrustacean. *Daphnia* inhabit environments that vary in light and resource availability, and may therefore experience changes in the balance of costs and benefits of investment in vision. We consider the absolute eye size and eye size relative to body size to address both visual capabilities and energetic allocation.

Changes in absolute eye size may affect *Daphnia* visual performance through both sensitivity and resolution. *Daphnia* have relatively crude resolving capabilities due to the low number of ommatidia (22) present in their eye (Young and Downing 1976). We also measure facet lens diameter of ommatidia in conjunction with absolute eye size. Changes in relative eye size reflect shifts in the allocation of resources to the visual system and thus provide an index of the energetic investment an individual makes in vision.

We exposed *Daphnia* to a dim/bright environmental contrast and tested the prediction that (1) in dim light compound eyes would be larger, on average, in absolute (more light collection) and relative size (more resources allocated) than compound eyes of animals reared in a bright environment. We also examined *Daphnia* eye response under a high/low resource quantity contrast where we predicted that (2) animals reared in a low resource environment would exhibit smaller eyes on average, both in absolute and relative scale, than those reared in a high resource environment.

Methods

Experimental design

We manipulated *Daphnia* rearing environments by experimentally crossing high and low resource levels with bright and dim light levels. We conducted experiments in four species, allowing us to test whether eye size responses are robust across species that inhabit different light and resource environments. Since *Daphnia* have indeterminate growth, allocation patterns may change as animals grow older (Dudycha and Lynch 2005), and we therefore repeated the experiments at both early and late adulthood.

In the high resource treatments, animals were fed 20,000 cells/mL of the green alga *Ankistrodesmus falcatus* daily from birth, whereas in the low resource treatments animals were fed 5,000 cells/mL. Previous work has shown that this scale of resource availability induces substantial variation in *Daphnia* resource allocation (Tessier and Consolatti 1991; Dudycha 2003) and morphology (Lynch 1989).

Daphnia species and intra-specific populations inhabit a wide range of light environments that can fluctuate widely in terms of absolute light levels. We sought to impose a consistent environmental contrast of a relative order that multiple species of *Daphnia* experience. We used two lake species where light environments are best defined by the vertical distribution within a lake, and two pond species where light environments are best characterized by the amount of canopy cover. We, therefore, categorized light environments as bright versus dim based on similar magnitude differences found between light intensity in a lake

epilimnion and hypolimnion (Wetzel 2001), and ponds under sparse versus dense canopy (Cáceres et al. 2008).

Two environmental chambers (Percival Scientific, Inc., Iowa, USA) were set to subject the animals either to dim ($10 \mu\text{E m}^{-2} \text{s}^{-1}$) or bright light ($110 \mu\text{E m}^{-2} \text{s}^{-1}$) conditions. Light levels were measured using a 4π PAR radiometer (Biospherical Instruments Inc., California, USA). Each chamber had two shelves with two fluorescent lights above each shelf. We measured light on both shelves and found minimal differences (Fig. S1). Light attenuation was also measured between high resource and low resource treatments, and we found a difference equal to $\sim 6\%$ of the total difference between the dim and bright light treatments. Under the dim condition, lights were wrapped in three layers of neutral density screening (charcoal fiberglass screen wire; Phifer Inc. Alabama, USA), whereas the high light lamps were left unmanipulated. We randomized beaker locations and rotated them daily within chambers to control for minor variations of light within a chamber. To minimize chamber effects, the experimental lighting setup was switched between the two chambers on every third day during the experiment.

We assayed each ontogenetic stage in separate experimental cohorts (i.e., individual animals were only measured once). Early adulthood was defined as the instar after the release of the first clutch of offspring. Late adulthood was defined as the instar after the fourth clutch, where the animal is effectively past a point of adding to overall fitness (Taylor and Gabriel 1992).

Two species were isolated from permanent lakes (*D. parvula* Fordyce and *D. pulicaria* Forbes) and two were isolated from temporary ponds (*D. pulex* Leydig and *D. obtusa* Kurz). We conducted our experiment with a single clone from each of four species. *D. parvula* was isolated from McReynolds Lake ($30^{\circ}54'03''\text{N}$, $87^{\circ}55'47''\text{W}$) in southern Alabama, USA. *D. pulicaria* was isolated from Lake Sixteen ($42^{\circ}33'52''\text{N}$, $85^{\circ}36'47''\text{W}$), and *D. pulex* from Pond of the Village Idiot ($42^{\circ}43'10''\text{N}$, $85^{\circ}23'16''\text{W}$) in southwestern Michigan, USA. *D. obtusa* was isolated from Powerlines Pond ($33^{\circ}45'49''\text{N}$, $80^{\circ}38'30''\text{W}$) at Congaree National Park, South Carolina, USA.

Mothers of experimental animals were maintained at low density at 20°C on a 12:12 L:D photoperiod in filtered ($1 \mu\text{m}$) hypolimnetic lake water. Mothers were fed vitamin-enriched *A. falcatus* daily (Goulden et al. 1982).

To start each experiment, neonates (<15 h old) were placed individually into 100 mL of filtered lake water, and randomly assigned a treatment. A light dusting of cetyl alcohol prevented surface film entrapment (Desmarais 1997). We began each experiment with approximately 40 replicate individuals per treatment per ontogenetic stage (see Table S1 for sample sizes).

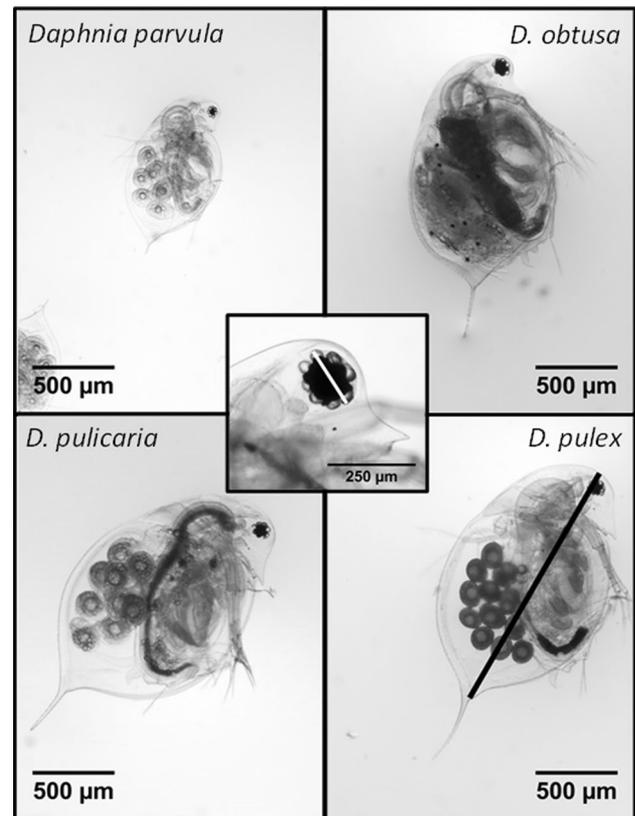


Fig. 1 A photomicrograph collage of the *Daphnia* species used in this study. The white line represents eye diameter measurements, and the black line represents body length measurements

Experimental animals were moved into fresh filtered lake water every other day. We performed feeding and water changes under dim red light during the dark cycle of the photoperiod to prevent disruptions to the brightness of light during the day phase.

Measurements

Animals were sacrificed in droplets of 0.25 M KCl and photographed within 5 min. Lateral photographs were taken through a Nikon 1500 SMZ dissecting scope at $30\times$ magnification to include the entire body, and at $112.5\times$ to maximize precision in measuring eye size, then analyzed in ImageJ (Schneider et al. 2012). Body length was measured from the top of the head just above the eye to the base of the tail-spine (Fig. 1). Although *Daphnia* eyes are approximately spherical, most individuals deviate somewhat. Eye diameter was, therefore, taken at the widest diameter.

We measured the diameter of ommatidial facets to verify that the actual light collecting units varied in tandem with eye size. Measurements of ommatidia were taken at $112.5\times$ magnification. *Daphnia* ommatidia are large and bulbous, but the pigmentation of the *Daphnia* compound

Table 1 Results of ANOVA on the effects of different environmental treatments on *Daphnia* spp. absolute eye diameter, and the percent increase in the compound eye surface area (total light collection ability) in high resource and dim light levels (see “Methods” for details of calculation)

Species	Stage	df	Resource		Light		Resource × light		Percent increase	
			F	p	F	p	F	p	High resource	Dim light
<i>D. parvula</i>	Early	1, 100	3.84	0.0527	1.58	0.2121	4.16	0.044	5.9	3.6
	Late	1, 88	11.98	0.0008	1.23	0.2705	0.04	0.8507	13.1	−4.6
<i>D. obtusa</i>	Early	1, 76	83.42	<0.0001	4.38	0.0398	0.31	0.5814	21.5	4.3
	Late	1, 76	10.81	0.0015	1.15	0.2879	7.12	0.0093	6.6	−2.8
<i>D. pulex</i>	Early	1, 140	106.58	<0.0001	1.55	0.216	12.44	0.0006	20.2	−1.9
	Late	1, 50	33.86	<0.0001	6.25	0.0158	0.01	0.9276	33.8	−12.4
<i>D. pulicaria</i>	Early	1, 144	157.82	<0.0001	11.46	0.0009	4.19	0.0424	18.5	−4.3
	Late	1, 132	55.92	<0.0001	1.36	0.2456	0.97	0.327	14.9	−1.9

Means were tested at $\alpha = 0.05$. Significant differences between means are noted in bold

eye makes it impossible to see all the facets clearly. For each individual, we therefore measured three ommatidial facets (of 22) based on the clarity of the facet, and not with regard to the regional position of the ommatidium within the eye.

Percent increase

We used mean values of eye diameter calculated for each treatment and stage level to calculate percent increase in eye area (Table 1). We calculated *Daphnia* eye area for each mean value of absolute eye diameter for each treatment level, developmental stage, and species (Table S1). We used the surface area equation for a sphere to calculate eye area:

$$\text{eye area} = 4\pi \left(\frac{1}{2} \text{mean eye diameter} \right)^2$$

We present percent increase in eye area as the percent difference in eye area in the high food treatment versus low food treatment, and the difference in dim light versus high light.

Statistical analysis

Our main objective was to examine the plasticity of eye size within species and developmental stages. We used ANOVA to examine the fixed effects of resource environment, light environment, and their interaction on absolute eye size and body size, running the analysis separately on each species at each ontogenetic stage. We were also interested in the treatment effects on eye size relative to body size, since this reflects resource allocation trade-offs. We, therefore, ran an analysis of covariance (ANCOVA) on eye diameter (response) and body length (predictor) variables for each species at each ontogenetic stage, considering resource and light as fixed factors. These analyses were performed in SPSS v. 21 (IBM Corp., New York, USA).

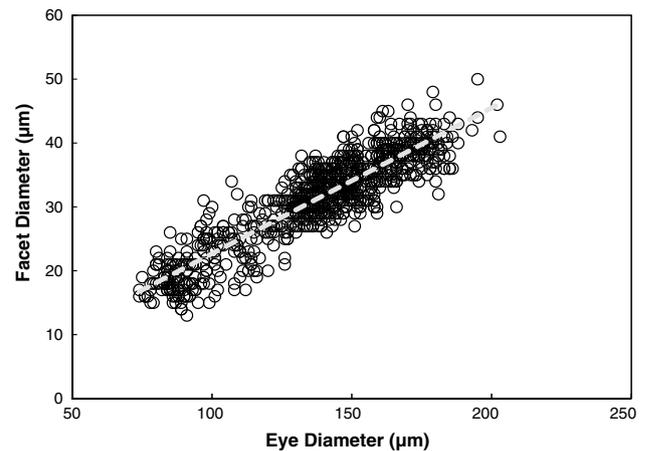


Fig. 2 *Daphnia* facet diameter in relation to eye diameter. Ordinary least squares regression reveals a strong positive relationship (slope = 0.228, adj. $R^2 = 0.81$, $p < 0.0001$)

To test the assumption that sensitivity increases with increasing eye size, we used ordinary least squares regression to analyze the relationship between ommatidial facet (the light collecting unit) diameter and eye diameter. For this analysis, we used R (R Foundation for Statistical Computing, Vienna, Austria). We were interested in the global relationship, thus we performed our analysis on all experimental observations, pooling all measurements from all species, ages, and treatments. We measured three facets per individual eye, regressing mean facet diameter value against eye diameter.

Results

Facet lens and eye size

Regression analysis revealed a strong positive relationship between facet lens diameter and eye diameter (Fig. 2; slope = 0.228, adj. $R^2 = 0.81$, $p < 0.0001$), supporting the assumption that facet lens diameter increases with eye diameter.

Table 2 Results of ANOVA on the effects of different environmental treatments on *Daphnia* spp. body length

Species	Stage	df	Resource		Light		Resource × light	
			F	p	F	p	F	p
<i>D. parvula</i>	Early	1, 100	0.702	0.404	0.141	0.708	7.433	0.008
	Late	1, 88	8.987	0.004	1.036	0.312	0.003	0.959
<i>D. obtusa</i>	Early	1, 76	54.767	<0.001	3.569	0.063	0.062	0.804
	Late	1, 76	31.766	<0.001	0.206	0.651	4.314	0.041
<i>D. pulex</i>	Early	1, 140	181.345	<0.001	12.06	0.001	8.123	0.005
	Late	1, 50	47.589	<0.001	8.137	0.006	0.32	0.574
<i>D. pulicaria</i>	Early	1, 144	216.461	<0.001	24.172	<0.001	3.131	0.079
	Later	1, 132	45.287	<0.001	20.598	<0.001	0.446	0.505

Means were tested at $\alpha = 0.05$. Significant differences between means are noted in bold

Absolute eye size and body size

High resources consistently led to larger absolute eye diameter than did low resources (Table 1). This reflected the pattern for body size, where individuals raised in a high resource environment were larger (Table 2). The only exception was *D. parvula* at early adulthood, where neither body size nor eye size was affected by resource level. Depending on species and ontogenetic stage, high resources increased eye area, a strong determinant of light sensitivity, by 7–34 % (Table 1).

Effects of light intensity were inconsistent across species and ontogenetic stage. Both *D. pulex* and *D. pulicaria* exhibited larger body sizes in bright light than in dim light by 3–5 % (Table 2). However, absolute eye size was larger in bright light than in the dim light only in late adulthood for *D. pulex* (14 % increase) and only in early adulthood for *D. pulicaria* (4 % increase). Both observations directly contradict the predicted effect of light intensity. Light intensity did not affect body size or absolute eye size in *D. parvula*. In *D. obtusa*, the only significant difference was that absolute eye size was ~4 % larger in dim light at early adulthood (Table 1).

In some cases, there were resource–light interactions, but the form of these interactions was not consistent across species. In *D. parvula* and *D. pulex*, there were interactive effects in body size (Table 2) and absolute eye size (Table 1) in early adulthood. In *D. obtusa*, there was a significant interaction in late adulthood in body size (Table 2) and absolute eye size (Table 1). The only resource–light interaction in *D. pulicaria* was in absolute eye size during early adulthood (Table 1).

Relative eye size

Daphnia generally showed significantly larger eyes relative to body size when raised in a high resource environment versus a low resource environment (Table 3; Fig. 3). There were two exceptions in late adulthood. In *D. parvula*, the

increase was only marginally significant, and in *D. obtusa* there was no effect.

The light environment generally had no effect on relative eye size in *Daphnia*, with exceptions in two cases. In *D. pulicaria*, relative eye size was slightly, but significantly, larger in bright environments at late adulthood (Fig. 4). *D. parvula*, in contrast, had larger relative eye size in dim environments at early adulthood (Fig. 4).

The effects of treatment × body length interactions were few and inconsistent across species and ages. The light environment affected the relationship of eye size to body length in *D. pulicaria* at early adulthood, and in late adulthood in *D. parvula* (Table 3). In *D. obtusa*, an interaction of resource environment × body length was observed in early adulthood (Table 3).

Discussion

We found that resources have a more substantial influence on eye size than light intensity does. We consistently observed larger eyes in higher resource environments across species and ontogeny. In contrast, we observed few and inconsistent effects of light environments on eye size. This was a surprise because studies that examine eye size across species often find that dim environments are associated with the evolution of large eyes.

We also found a strong positive relationship between facet diameter and eye diameter in *Daphnia*. Facet diameter—or aperture size—is a prominent factor in determining a compound eye’s sensitivity, where larger facets lead to increased sensitivity (Land and Nilsson 1990). *Daphnia* have few ommatidia and limited resolving abilities (Young and Downing 1976), and likely the most relevant visual capability affected by changes in eye size is sensitivity. Optical sensitivity in apposition compound eyes can be described by:

$$S = 0.62D^2 \Delta\rho^2 P_{\text{abs}}$$

Table 3 Results of an ANCOVA on the effects of different environmental treatments on *Daphnia* spp. eye size using body length as a covariate

Source of variation	<i>D. parvula</i>		<i>D. obtusa</i>		<i>D. pulicaria</i>		<i>D. pulex</i>	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Early adulthood								
Resource (<i>R</i>)	$F_{(1,99)} = 4.14$	0.0445	$F_{(1,75)} = 19.15$	<0.0001	$F_{(1,143)} = 35.00$	<0.0001	$F_{(1,139)} = 8.03$	0.0053
Light (<i>L</i>)	$F_{(1,99)} = 5.71$	0.0188	$F_{(1,75)} = 1.29$	0.2601	$F_{(1,143)} = 4.17$	0.0431	$F_{(1,139)} = 0.24$	0.6239
<i>R</i> × <i>L</i>	$F_{(1,99)} = 0.01$	0.9418	$F_{(1,75)} = 0.26$	0.6141	$F_{(1,143)} = 2.91$	0.0900	$F_{(1,139)} = 5.62$	0.0190
Body length (bl)	$F_{(1,99)} = 140.77$	<0.0001	$F_{(1,75)} = 47.80$	<0.0001	$F_{(1,143)} = 8.18$	0.0049	$F_{(1,139)} = 43.25$	<0.0001
<i>R</i> × bl	$F_{(1,96)} = 3.04$	0.0845	$F_{(1,72)} = 4.74$	0.0327	$F_{(1,140)} = 0.01$	0.9093	$F_{(1,137)} = 0.08$	0.7817
<i>L</i> × bl	$F_{(1,96)} = 0.30$	0.5842	$F_{(1,72)} = 0.31$	0.5786	$F_{(1,140)} = 4.87$	0.0290	$F_{(1,137)} = 2.31$	0.1308
<i>R</i> × <i>L</i> × bl	$F_{(1,96)} = 1.57$	0.2126	$F_{(1,72)} = 2.92$	0.0917	$F_{(1,140)} = 0.32$	0.8593	$F_{(1,137)} = 1.23$	0.2688
Late adulthood								
Resource (<i>R</i>)	$F_{(1,87)} = 2.73$	0.1023	$F_{(1,75)} < 0.01$	0.9875	$F_{(1,131)} = 25.06$	<0.0001	$F_{(1,49)} = 5.37$	0.0247
Light (<i>L</i>)	$F_{(1,87)} = 0.22$	0.6408	$F_{(1,75)} = 2.68$	0.1060	$F_{(1,131)} = 0.01$	0.9177	$F_{(1,49)} = 1.78$	0.1880
<i>R</i> × <i>L</i>	$F_{(1,87)} = 0.07$	0.7953	$F_{(1,75)} = 2.99$	0.0877	$F_{(1,131)} = 0.68$	0.4108	$F_{(1,49)} = 0.12$	0.7271
Body length (bl)	$F_{(1,87)} = 192.94$	<0.0001	$F_{(1,75)} = 39.24$	<0.0001	$F_{(1,131)} = 10.59$	0.0014	$F_{(1,49)} = 9.99$	0.0027
<i>R</i> × bl	$F_{(1,84)} = 0.55$	0.4621	$F_{(1,72)} < 0.01$	0.9888	$F_{(1,129)} = 1.28$	0.2594	$F_{(1,47)} = 0.37$	0.5439
<i>L</i> × bl	$F_{(1,84)} = 6.34$	0.0137	$F_{(1,72)} = 0.49$	0.4851	$F_{(1,129)} = 0.59$	0.4441	$F_{(1,47)} = 0.18$	0.6710
<i>R</i> × <i>L</i> × bl	$F_{(1,84)} = 0.02$	0.8981	$F_{(1,72)} = 0.65$	0.4220	$F_{(1,129)} = 4.40$	0.0378	$F_{(1,47)} = 0.10$	0.7518

Treatment effects were tested at $\alpha = 0.05$. Significant values are noted in bold

where D is the facet diameter, $\Delta\rho$ is the rhabdom acceptance angle, and P_{abs} is the proportion of photons absorbed (Land and Nilsson 2012). All other things being equal, changes in facet diameter will change the values in S . We show that changes in facet diameter show a strong correlation with changes in eye diameter, thus larger eye diameters increase facet diameters and ultimately enhance sensitivity. It seems unlikely that changes in the other parameters would change in an opposite fashion as to negate increases in sensitivity. Therefore, abundant resources allow for greater relative investment in eyes and lead to improved *Daphnia* visual capabilities.

Eye size scales positively with body size in *Daphnia*, and thus effects on body size may in part drive differences in absolute eye size. Nonetheless, absolute differences in eye size necessarily change optical characteristics. Body size constrains absolute eye size (Wehner 1981; Rutowski 2000), such that the optimal eye size in *Daphnia* may actually lie beyond what its body plan can accommodate. *Daphnia* may therefore benefit visually as a consequence of a larger body size whereby the added space is exploited to expand the size of the eye. Indeed, *Daphnia* grow indeterminately and continue to add size to the eye with no apparent plateau well after reproductive maturity (C. S. Brandon, unpublished).

Our results generally refute the hypothesis that phenotypic plasticity within species follows a pattern similar to the macroevolutionary pattern. Furthermore, our study highlights that a non-sensory factor can have strong effects

on eye size, potentially large enough to have a major impact on visually mediated ecological interactions. Together, these results indicate that the mechanisms driving within-species phenotypic variation in visual capability differ from those driving macroevolutionary divergence.

Eye size and the light environment

We were surprised that our results showed no consistent response of compound eye size with respect to the light intensity. *Daphnia* possess an apposition-type compound eye, which is common among diurnal arthropods. Many comparative studies have documented differences of apposition eye size in closely related taxa that have diurnal, nocturnal or crepuscular members, where they have shown that dim light environments tend to harbor animals with comparably larger eyes than their cousins in brighter environments (Bauer et al. 1998; Land et al. 1999; Greiner 2006; Somanathan et al. 2009). If plasticity is adaptive within generations, it should match adaptively evolved differences between generations. Thus, we predicted that *Daphnia* eye size would be larger in dim environments. That prediction failed in seven of our eight experiments. In fact, in two situations with a significant light effect, the direction was opposite to the prediction, with larger absolute eyes in bright light for late adult *D. pulex* and early adult *D. pulicaria*. Our prediction was supported only in early adult *D. obtusa*, and there it was merely a 4 % increase of eye area in dim light.

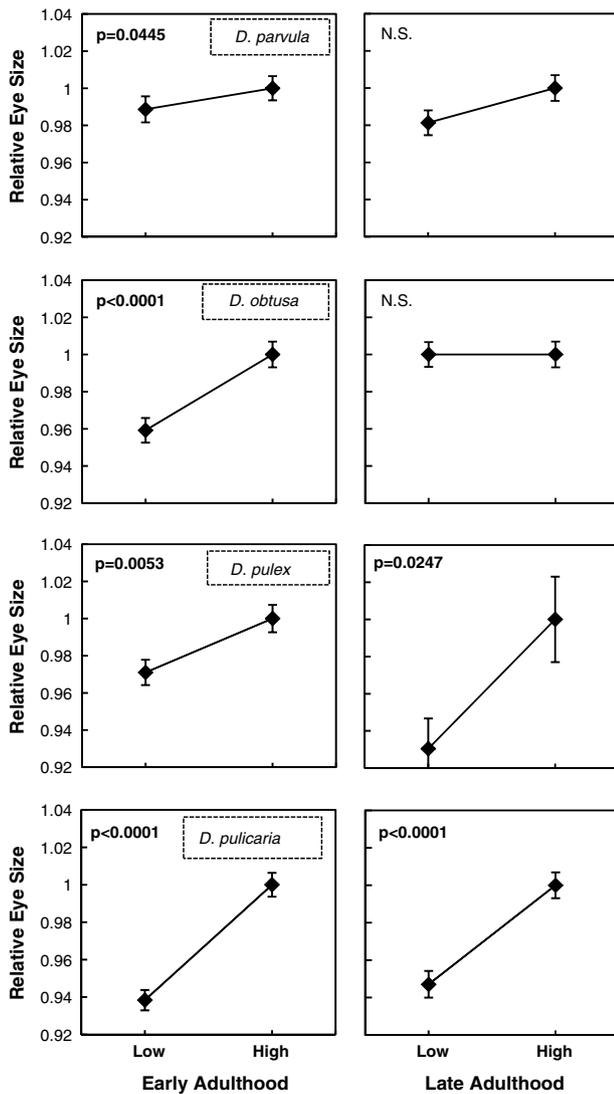


Fig. 3 The effect of resource environment on relative eye size in *Daphnia*. Relative eye size values are based on body size covariate adjusted means from ANCOVA where eye diameter was the response variable and body length set as the covariate (see “Methods” for details) separately for each species and stage. To present data on the same scale, means were normalized to the high resource environment within each species and stage (i.e., high resource is always set to 1.0). Means were tested at $\alpha = 0.05$. Significant differences between means are noted with a *p* value in bold. *N.S.* not significant. *Error bars* are $\pm 95\%$ confidence intervals

There are other parameters that enhance a compound eye’s sensitivity, which were not measured in this study, but could have changed in *Daphnia* as a consequence of the light environment. We focus on facet width in this study, a parameter that can be reasonably measured in an experiment at the scale presented here. Another prominent factor which affects sensitivity is the photoreceptor width, where an increase in photoreceptor width increases sensitivity (Land and Nilsson 1990). This alternative strategy to

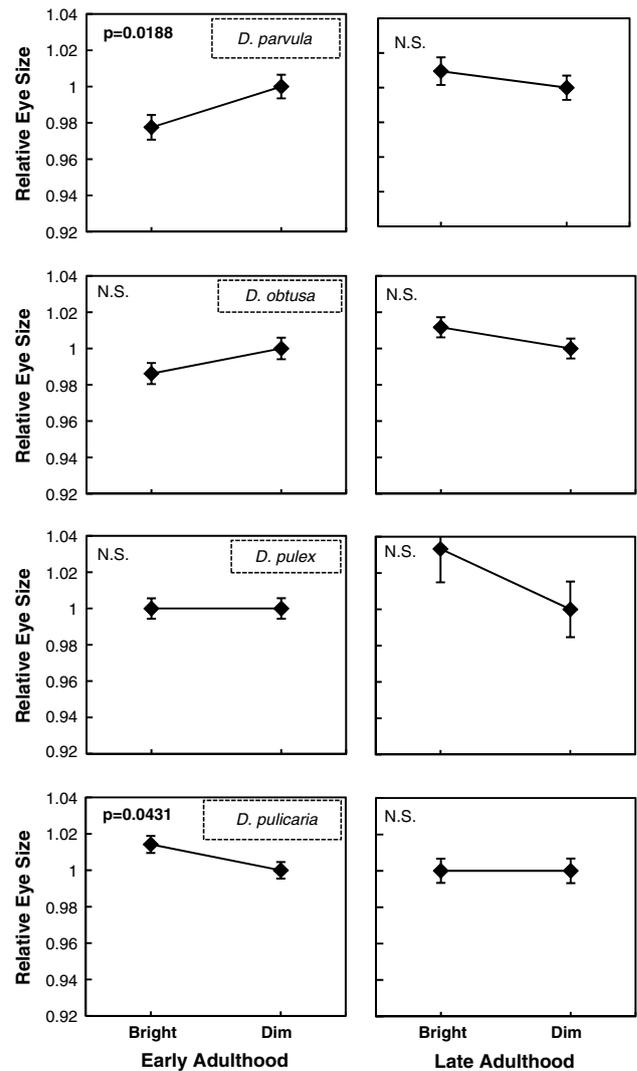


Fig. 4 The effect of light environment on relative eye size in *Daphnia*. Relative eye size values are based on body size covariate adjusted means from ANCOVA where eye diameter was the response variable and body length set as the covariate (see “Methods” for details) separately for each species and stage. To present data on the same scale, means were normalized to the dim light environment within each species and stage (i.e., dim light is always set to 1.0). Means were tested at $\alpha = 0.05$. Significant differences between means are noted with a *p* value. *N.S.* not significant. *Error bars* are $\pm 95\%$ confidence intervals

enhance sensitivity comes with a cost for resolving abilities. It seems unusual that *Daphnia* would opt to increase the width of photoreceptors at the expense of resolution, when they are capable of changing investment in eye size and facet width, which enhance sensitivity without sacrificing resolution. Increases in the time over which photoreceptors collect and process light signals (temporal summation) remain another option (Land and Nilsson 2012), however, longer sampling times can lead to blurring of the image especially in actively moving organisms such as *Daphnia*.

Pigment migration is also a common strategy used in compound eyes (De Bruin and Crisp 1957), and possibly employed by *Daphnia* (Cellier-Michel et al. 2000).

The canalization of the compound eye size and facet width with respect to the light environment may have arisen from the variant light environments that *Daphnia* inhabit. There is no systematic information on the light environment experienced by different species of *Daphnia*, but all of our species occupy a range of habitats that expose them to large differences in light environments. The light environment can vary from waterbody to waterbody (Wetzel 2001). For example, ponds can vary in amount of canopy cover leading to a range of dim to bright ponds within a small geographic locale. The light environment also changes within a waterbody, especially in its vertical distribution. Even in shallow ponds, the dissolved and particulate matter can absorb light so rapidly as to practically extinguish light within the first half meter. In these environments, an individual may thus experience a large jump in available light within decimeters. Furthermore, spatial partitioning of lakes and ponds either through diel vertical migration and non-migration behaviors is highly variable within lakes and across water bodies (Weider 1984; Tessier and Leibold 1997), and among species (Tappa 1965). *Daphnia* species may, therefore, experience highly divergent light environments on very short timescales, and the compound eye may have evolved to operate in a broad range of light environments.

One limitation of our study is that in real lakes and ponds, changes in light availability are often accompanied by changes in spectrum (Hutchinson 1975; Wetzel 2001). For example, the hypolimnion of relatively clear waters is dominated by blue light, but waters containing calcium or dissolved organic substances shift the light field to the green or orange-red, respectively. *Daphnia* can inhabit the range of these environments, thus dim light in a white light field does not necessarily represent dim light conditions for all *Daphnia*. The change in environmental spectrum may elicit changes in other physiological features such as in the composition of visual pigments (Cronin and Caldwell 2001; Fuller et al. 2005). However, the strategy to deal with sustained differences in bright versus dim light across broad taxonomic scales has been to increase aperture and eye size.

Eye size and resource environment

In general, *Daphnia* raised on high resources had larger eyes, both in absolute and relative dimensions, than those raised on low resources. This shows that a major aspect of the non-sensory environment can substantially influence visual capability and the investment organisms make in vision.

One important outcome of our data is that relative, and not simply absolute eye size, responds to resource environment. If *Daphnia* eyes were locked into a fixed allometric relationship with body size, only absolute eye size would have responded to resources. In contrast, our results demonstrate that these animals have the ability to modulate their allocation of resources to visual systems in response to a non-sensory aspect of the environment. One previous report has also demonstrated resource-driven eye size plasticity, but the direction of eye response to low nutrition was opposite from our results. Merry et al. (2011) showed that the butterfly *Colias eurytheme* had relatively larger eyes when raised on a poor quality diet. The authors reasoned that animals raised on a poor quality diet invested relatively more in eye development to compensate for visual performance lost as a function of overall smaller size. This makes sense for an animal that requires high visual performance as an essential tool for foraging, oviposition, and mate detection. *Daphnia* are filter-feeding grazers, however, and the marginal gain from increasing investment in visual performance under poor resource environments may not offset the costs of resources re-allocated from other functions.

Species differences

The response of eye size to resources was robust across species and ages, suggesting that it has deep evolutionary origins that may be maintained because it is generally adaptive for *Daphnia*. However, the consistent responses highlight that there were no obvious differences due to the environments in which these species evolved, i.e., lake versus pond. *D. pulex* and *D. pulicaria* had relative eye sizes that were larger in high resources at both ontogenetic stages. The parallel response may be explained by phylogeny as these are probably ecotypes of a single species (Pfrender et al. 2000; Heier and Dudycha 2009). The distantly related *D. parvula* also showed this pattern, although the differences between high and low resources were not as pronounced. *D. parvula* have the smallest absolute eye size and may be on the lower range of what is a functional eye for *Daphnia*, and small sacrifices in investment of the eye may severely hinder its relevant visual capabilities. Lastly, *D. obtusa* displayed a relative eye size response only at early adulthood, showing that, at least for this species, investment in visual systems development can vary through ontogeny.

Conclusion

We found that resources, an aspect of the environment not directly tied to vision, strongly influenced eye size in

Daphnia, whereas light intensity, typically an important determinant of macroevolutionary divergence of eye morphology, had little effect. Our results show that environmental factors outside of those that directly mediate visually guided behaviors have likely influenced the evolution of visual systems in *Daphnia*. The sensory environment has certainly been a major driver of variation in eye size across multiple taxa, but our findings show that phenotypic variation in eye size cannot be understood solely in the context of the sensory environment.

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