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RESEARCH ARTICLE

Environmental impact on visual perception modulates behavioral responses of schooling fish to looming predators

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ABSTRACT

Aggregation in social fishes has evolved to improve safety from predators. The individual interaction mechanisms that govern collective behavior are determined by the sensory systems that translate environmental information into behavior. In dynamic environments, shifts in conditions impede effective visual sensory perception in fish schools, and may induce changes in the collective response. Here, we consider whether environmental conditions that affect visual contrast modulate the collective response of schools to looming predators. By using a virtual environment to simulate four contrast levels, we tested whether the collective state of minnow fish schools was modified in response to a looming optical stimulus. Our results indicate that fish swam slower and were less polarized in lower contrast conditions. Additionally, schooling metrics known to be regulated by non-visual sensory systems tended to correlate better when contrast decreased. Over the course of the escape response, schools remained tightly formed and retained the capability of transferring social information. We propose that when visual perception is compromised, the interaction rules governing collective behavior are likely to be modified to prioritize ancillary sensory information crucial to maximizing chance of escape. Our results imply that multiple sensory systems can integrate to control collective behavior in environments with unreliable visual information.

KEY WORDS: Collective behavior, Habitat effects on behavior, Predator–prey interactions, Minnow, *Pimephales promelas*

INTRODUCTION

Schooling in fish confers safety benefits that maximize probability of survival (Pitcher and Parrish, 1993). In social fish, this is likely to be motivated by the threat of predation (Ioannou, 2017), where schooling can improve survival by diluting risk (Hoare et al., 2004; Turner and Pitcher, 1986), reduce encounters with predators (Ioannou et al., 2011) and enhance group vigilance (Ward et al., 2011). When directly responding to predators, schools can exhibit different behaviors to evade predators (Magurran and Pitcher, 1987), including reducing inter-fish distance (Hoare et al., 2004) and initiating escape waves (Herbert-Read et al., 2015). However, the interactions between wild schooling fish and their aquatic predators occur in a wide range of environmental conditions, from turbulent coral reef to turbid estuarine waters, which may alter the ability for an effective anti-predator response (Higham et al., 2015).

Water turbidity (Abrahams and Kattenfeld, 1997) and light availability (Land, 1988) are well known to affect schooling tendency (Chamberlain and Ioannou, 2019; Ryer and Olla, 1998). In estuarine and riverine waters, turbidity impacts prey fish visual detection (Utne-Palm, 2002), plays a critical role in structuring predator–prey interactions (Abrahams and Kattenfeld, 1997; De Robertis et al., 2003) and decreases the ability for schools to perform successful antipredator responses (Figueiredo et al., 2016; Kimbell and Morrell, 2015). Previous studies have linked how sensory information and perception translates into escape responses in animals (Fotowat and Gabbiani, 2011), which supports the hypothesis that environmental factors (through changes in sensory perception) can directly influence antipredator responses. It has been demonstrated that the visual sensory system plays a key role in initiating and directing escape behaviors in both individual (Hein et al., 2018; Temizer et al., 2015) and schooling prey fish (Rosenthal et al., 2015). From a physiological perspective, the fish eye has largely evolved to maximize light-gathering power (Land, 2005); therefore, environmental changes that affect light availability (e.g. turbidity) may actively compromise visual perception and influence antipredator schooling responses. In light restricted or turbid environments, vigilant schooling fish under attack must respond to a decrease in perceived visual contrast, where the ability to distinguish a predator from the background environment becomes increasingly more difficult as contrast decreases (Land and Nilsson, 2012). However, it is currently unknown whether decreasing visual contrast impacts collective mechanisms that mediate group responses to predator attacks.

To a freely behaving prey fish, visual detection of a fast approaching predator is perceived by the visual system as a looming optical stimulus (Bhattacharyya et al., 2017; Cade et al., 2020; Temizer et al., 2015). These looming stimuli have been shown to reliably initiate escape maneuvers in fish both in laboratory settings (Cade et al., 2020) and in the wild (Hein et al., 2018). Escape behaviors to looming stimuli are also well conserved across many animal species (Peek and Card, 2016). Generally, a looming stimulus consists of an expanding image that simultaneously triggers both spatial and temporal motion detecting neurons (Bhattacharyya et al., 2017; Temizer et al., 2015) in the visual system. The optic flow on the retina by an expanding object can be affected by both the speed and rate of stimulus expansion, as well as the luminosity of the object and the contrast between object and background (Bhattacharyya et al., 2017). Temizer et al. (2015) demonstrated that luminance affects both escape and other responses to looming stimuli. A decrease in contrast of a looming stimulus may then affect the ability and magnitude of escape responses in fish schools.

Many studies have investigated the mechanisms of interaction in schooling fish (Conradt, 2012; Gautrais et al., 2012) and behavioral studies use motion-based metrics to quantify behavioral responses (Delcourt and Poncin, 2012). Common motion metrics to describe

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dynamic collective behavior include nearest neighbor distance (Parrish et al., 2002), average swimming speed (Berdahl et al., 2013; Kent et al., 2019; Zienkiewicz et al., 2018), orientational polarization (Cavagna et al., 2008; Viscido et al., 2004) and correlation length as a measure of information transfer rate (Cavagna et al., 2010; Handegard et al., 2012; Rodriguez-Pinto et al., 2020). Previous efforts have proposed simplified interaction models to simulate how an individual in the aggregation behaviorally responds to its immediate neighbors (Couzin, 2009; Couzin et al., 2002; Katz et al., 2011; Strandburg-Peshkin et al., 2013), which include positioning and speed within the school, as well as alignment between individuals. However, the underlying interaction mechanisms that control the behavioral state of a freely behaving fish school are much more complex and are influenced by variation in their local environment (Berdahl et al., 2013; Tunstrøm et al., 2013). Efforts at modeling individual interaction rules determined that the visual field is a large contributor to accurately replicating the behavioral patterns that control schooling state (Bastien and Romanczuk, 2020; Collignon et al., 2016; Strandburg-Peshkin et al., 2013). In freely behaving schools, fish utilize a combination of vision and mechanosensation to control schooling. Vision contributes to the control of velocity regulation and orientation (Partridge and Pitcher, 1980), while the lateral line system contributes to the control of inter-individual spacing (Faucher et al., 2010) and possesses the ability to detect small pressure changes in the local environment, which may contain cues about the movement of neighboring individuals (Mogdans and Bleckmann, 2012; Montgomery et al., 1995). The various contributions of both visual and mechanosensory systems to schooling behavior may become altered when schools are in an environment that limits visual contrast (Montgomery et al., 1995; Partridge and Pitcher, 1980). Therefore, determining whether contrast changes modulate interaction mechanisms of fish schools in response to looming predators will further inform the contribution of vision to the mechanisms of collective behavior.

The objective of this study is to determine whether abiotic factors affecting contrast – the extent to which the luminance of the stimulus differs from the environment (Land and Nilsson, 2012) – modulate the collective interaction mechanisms of schooling fish in response to looming predators. We hypothesize that schooling behavior (and their interaction mechanisms) may be affected by contrast decreases in three ways: (1) compromised vision results in changes in interaction mechanisms that elicit delayed, yet stronger overall responses over the behavioral response time interval, (2) low contrast conditions induce changes in interaction mechanisms to favor auxiliary sensory systems (such as the lateral line) that compensate for vision loss, or (3) contrast effects on vision are negligible and have no influence on schooling behavior. To determine how luminance contrast between stimuli and the background environment influences schooling behavior, we quantified the collective state of schools of fathead minnow [*Pimephales promelas* (Rafinesque 1820)], using five physical metrics, in response to looming optical stimuli at four decreasing contrast levels.

MATERIALS AND METHODS

Experimental setup and data collection

The experimental tank was a perspex cube, with 228 mm sides covered with back-projection screen material, filled to 100 mm depth. Two first-surface mirrors, angled at 45 deg to each side, allow a single projector (Lightspeed Designs DepthQ 360) to illuminate two sides of the tank, an additional two mirrors, orthogonal to each other, were angled at 45 deg to illuminate the rear side (Fig. 1). We used custom OpenGL software to animate the cube with three dimensional, perspective-corrected scenes at 360 frames s^{-1}. Within the cube, the virtual environment appears continuous, even as the looming stimulus transitions from one surface to another (Fig. 1). The four rendered scenes that reflected off the mirrors were mathematically reversed to account for the reflection (Cabrera and Theobald, 2013). The front face displayed 229×229 pixels (2.5 pixels deg^{-1}) and the side faces displayed 200×200 (2.2 pixels deg^{-1}). The small difference is due to the shorter path between the projector and the front surface, which displays an image of the same size but at a higher pixel density. During the course of the experiments, each side was illuminated uniformly with white light at a brightness of 18 cd m^{-2} and the looming stimulus was represented as a black disk for maximum contrast. The disk expanded at a constant rate, passed through the tank holding the schools, and contracted on the opposite surface, simulating a direct visual predator attack. Although the standard looming stimulus approach is purely expansion, here we sought to simulate a direct intrusion through the school, incorporating any potential response to the predator capturing a different member of the school, for example. In other words, we deviated from the traditional approach as this may be a common visual stimulus for a schooling fish, and we wanted to capture any responses of surviving fish, or responses if the approach turned out to be benign. The direction of the looming stimulus was randomized among the four sides of the cube, to prevent habituation. Contrast was manipulated by adding ‘visual turbidity’, consisting of 1 mm radius black dots at randomly determined positions on each surface, with each contrast level increasing in dot density according to an exponential growth scale. Dot densities were used instead of haze for contrast manipulation to keep the background light level constant. Haze required changing the luminance of both the dots and background; however, because of the small area of dots, the required brightening of our background would often be below the 255 luminance levels available for grayscale dots. In our study, the high contrast condition contained a single 1 mm dot, and the low contrast condition contained 10,000 dots across all surfaces (Fig. 1). This effectively results in backgrounds with 0.08, 1.7, 37 and 795 dots per steradian, with higher dot densities producing lower contrasts between the looming stimulus and the environment. An overhead camera captured video footage of behavioral responses recording at 180 frames s^{-1}, with an infrared illumination source below the tank for improved image visualization and ease of data processing in low contrast conditions.

Experiments were conducted over a period of 3 months, using a new population of ∼100 fathead minnow for each day of data collection. Each population was given 24 h to acclimate in a large holding tank before experiments were run. Prior to each experiment, we randomly selected schools of 8–12 fish from the larger population located in the holding tank and placed them in the experimental tank to acclimate for 10 min. In a given experiment, each school was exposed to four simulated predator attack trials at a particular contrast level condition. For each trial, the predator stimulus was initiated sequentially from each of the four sides of the tank, presented in a randomized order. The time span of each attack was 6 s, and the behavioral response interval was determined from the first frame exhibiting individual fish motion to the last frame showing movement, ranging between ∼1 and 2 s. Once the trials were completed within an experiment, each school was returned to the large holding tank and given a minimum of 30 min to reacclimate with the larger population. Subsequent experiments used random sampling from the larger population to minimize the

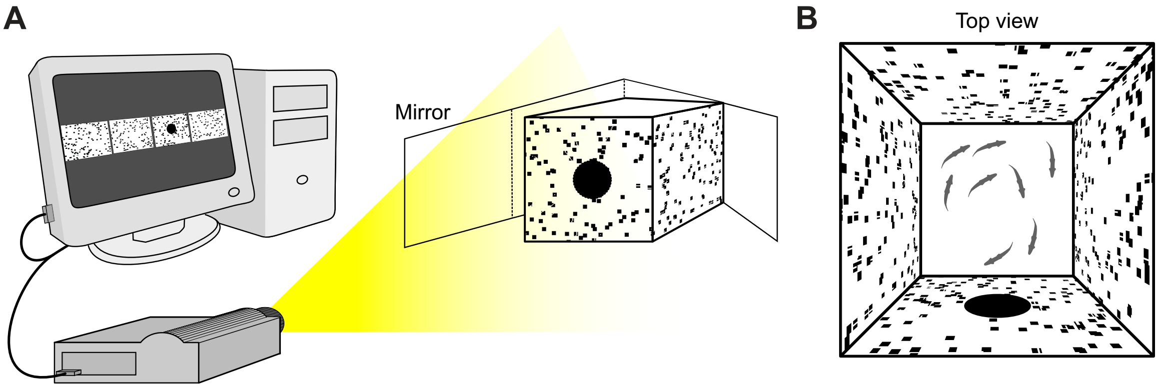

Fig. 1. Schematic of experimental apparatus (Holocube) during the data collection process. (A) A computer running custom software projects virtual environment onto cube via reflecting mirrors to cover each side face. (B) A high speed camera is fixed above the holocube, viewing down, recording images of each school. Contrast is changed by manipulating dot density against the pure white background. The programmed stimulus expands across one face, passes through the arena, and contracts on the opposite face onto a point.

probability that no two schools recorded were identical, even though single fish may have been tested more than once. All experiments were conducted at similar times (late morning/early afternoon) for each day of data collection, with a single feeding upon placement in the larger holding tank to ensure consistent hunger states during the experiments. The 10 min acclimation time before an experiment was given to minimize stress levels, and the 30 min acclimation time in the holding tank after an experiment served to minimize the effect of habituation in individual fish. Experiments were then repeated for each of the four contrast level conditions. Data were collected on seven different dates over the recording period. For each date, the larger population was replaced to minimize potential bias, resulting in seven different larger populations used in the study. In total, we observed 161 different shoals across the four contrast levels, with observations recorded from a total of 1457 individual fish.

This research was conducted in accordance with the guidelines set forth in the Florida International University IACUC Animal Care and Use Protocol #19-054.

Data processing

After data collection, each experiment was partitioned into its respective behavioral response trials. Owing to the variable nature of the time span of behavioral responses, individual frames for analysis were subsampled at 10 Hz across the response interval for comparison. Points at the head and base of the tail that generated a line segment along the sagittal axis were manually digitized for each fish in the school at every subsampled image frame. The midpoint between the identified points, approximating the center of mass, was computed and utilized to provide a position and orientation angle for each fish in the school (Fig. S1). Using the position and angle values, we quantified five parameters of the collective response: (1) nearest neighbor distance, (2) individual velocity, (3) angle to nearest neighbor, (4) school polarization and (5) correlation length. To estimate nearest neighbor distance, we performed a k-nearest neighbors (k-NN) search, based on Euclidean distance between positions, for each individual fish in each frame. Velocity was calculated by initially tracking individual fish positions using k-NN search between successive frames for each fish across the entire time interval. The resulting velocities were estimated via the gradient of position and time interval, respectively. Angle to nearest neighbor was calculated from the angular displacement between the direction of a focal fish and its nearest neighbor, for each fish in the school across the time interval (Rieucau et al., 2018). We computed school polarization as the sum of fish directions (velocity normalized by magnitude) divided by the total number of fish in the school (Attanasi et al., 2014; Tunstrøm et al., 2013). Finally, we calculated correlation length, the distance with which information about an individuals' behavior can be socially transmitted, in the same manner as Cavagna et al. (2010) and Handegard et al. (2012).

Each of the five collective response parameters measured are independent and describe the collective response in a different manner. Nearest neighbor distance, angle, and velocity describe the schooling response from the perspective of a focal fish; polarization is a global metric summing over individual heading information from all members of the school and is not directly dependent on the angle between an individual and its nearest conspecific. Similarly, the correlation length function is not dependent on the velocity of individuals, but rather the fluctuation of velocity deviations from the mean velocity of the school itself. To quantify the time to collision of the looming stimulus and the time of first response, we used a half size to approach speed ratio (r/v) of 133 ms, which corresponds to an object with a cross sectional radius of 20 cm approaching at ~150 cm s^{-1}. The angular size of the object was: $\theta=2\times\arctan(r/vt)$, and estimated time to collision, assuming constant velocity towards the school, was $\theta/\ (\mathrm{d}\theta/\mathrm{d}t)$. This approach appears the same for any object with the same r/v ratio, such as an animal twice as large approaching at twice the speed. With these parameters, the disk appeared at 4.5 deg, then expanded at a rate of $-2(r/v)\ /\ \left(\left(\frac{r}{v}\right)^2+t^2\right)$, with a collision 3.3 s later, then contracted on the opposite side for 5 s.

Statistical analysis

We treated each school as an independent sample, with measured values for each individual fish aggregated with all members of each school. Residual analysis via QQ plot and the Shapiro–Wilk test were performed at each contrast level on the mean values of each schooling metric over both the time of response and all members of the school. Nearest neighbor distance, nearest neighbor angle, and polarization pertaining to each observed school maintained the assumption of normality; a base-10 logarithm transformation was applied to velocity and correlation length metrics to obtain normality in the residuals. To determine whether behavioral responses to contrast level were different, we conducted univariate ANOVAs on each schooling metric averaged over the entire response interval, as well as Tukey HSD *post hoc* comparisons (at 95% significance level), Bonferroni corrected for multiple comparisons. When comparing schooling behaviors over the time of the behavioral response, we fit generalized linear mixed models (GLMMs) to incorporate the larger population as a random effect to control for possible pseudo replication. GLMMs were used since the data falls under a hierarchical structure, where each larger population may possess unique features and influence results. The GLMM fit was found to significantly explain more of the observed variance for all schooling parameters. Comparison of fixed effects were performed by ANOVA using Satterthwaite's method for *F*-statistic computation. Pairwise comparisons (corrected with Holm's Sequential Bonferroni Procedure) between contrast levels and response intervals were performed to identify differences. Finally, a principal component analysis (PCA) was conducted at each contrast level to visualize correlations in schooling metrics. Each variable was scaled to unit variance, with no rotation. For interpretability of results, all transformed data was back-transformed to their original scale prior to visualization. All analyses were done using CRAN R statistical software (2018-07-02, https://www.r-project.org/; Feather Spray).

RESULTS

Changes in contrast level were found to influence velocity-based components of the escape responses of schooling fish. With increased contrast the average speed over the entire behavioral response for all schools changed from 2.1 to 4 cm s^{-1} and polarization from 0.37 to 0.46, and the changes were significant for speed (ANOVA: $F_{3,157}$=13.495, P<0.001) and polarization (ANOVA: $F_{3,157}$=2.818, P=0.041) (Fig. 2), respectively. Fish were more polarized at high contrast (P=0.029) and swam faster in each increasing contrast level: ∼15% faster in medium–low contrast (P<0.001), ∼25% faster in medium–high contrast (P<0.001) and ∼30% faster in high contrast (P<0.001) compared with low contrast conditions (Fig. 2), supporting the hypothesis that speed regulation is visually mediated. Change in contrast did not explain variability in nearest neighbor distance (ANOVA: $F_{3,157}$=0.452, P=0.716), nearest neighbor angle (ANOVA: $F_{3,157}$=0.817, P=0.486) or correlation length (ANOVA: $F_{3,157}$=1.093, P=0.353).

Over the time span of the behavioral response, contrast level had a significant effect on inter-individual spacing (GLMM: $F_{3,\ 14526}$=14.2007, P<0.001). Schools in the high contrast conditions exhibited a faster increase in nearest neighbor distance over the course of the response and remained loosely shoaled at the end of the behavioral response, whereas schools in the low contrast condition maintained consistent spacing over the entire behavioral response (Fig. 3). Individual fish were also more closely aligned to their nearest neighbor in the high contrast condition at the beginning of the behavioral response; alignment angle to their nearest neighbor was greater at lower contrast levels compared to the high contrast condition, with a significant difference between the high and medium-high condition (1st response interval, $t_{14,523}$=−3.311, P=0.005). Within the first half of the response, schools in the high contrast condition maintained a closer alignment angle than those in the low (4th response interval, $t_{14,523}$=−3.234, P=0.006), medium–low (4th response interval, $t_{14,523}$=−3.003, P=0.014) and medium–high (4th response interval, $t_{14,523}$=−2.990, P=0.015) conditions. By the end of the response all schools converged to similar angle values. Average speed for higher contrast levels began high, with a sinusoidal increase/decrease pattern over the course of the response that is consistent with the response of individual fish (Fig. S3). At the beginning of the response in the low contrast condition, the average speed was lower than at high (1st response interval, $t_{14,530}$=4.010, P<0.001), medium-high (1st response interval, $t_{14,530}$=5.667, P<0.001), and medium-low (1st response interval, $t_{14,530}$=3.718, P=0.001) contrasts, and increased to a smaller peak speed at a slower rate (Fig. 3). Fish speed in the low contrast condition remained slower than in higher contrast condition over the course of the response. School polarization demonstrated an increase at all contrast levels over the course of the escape responses, with the low contrast condition exhibiting a slower rate of polarization change relative to the other contrast levels. Correlation length, the distance in which the behavior of one fish affects another, at all contrast levels, decreased over the course of the response interval and no significant effect due to contrast level was found (GLMM: $F_{3,\ 1513}$=1.4067, P=0.24) (Fig. 3).

Contrast level also influenced the correlation between schooling state metrics. At high contrast, polarization, angle, and nearest neighbor distance were loaded strongly on the first principal component, while speed loaded strongly on the second and correlation length on the third principal component (Table 1, Fig. S2). Schooling state metrics in high contrast conditions were not highly correlated, and no groupings of correlated metrics occurred. In the medium–high contrast condition, polarization and angle maintain their strong loadings on the first principal component, with speed loading similarly between components 1 and 2. Correlation length and nearest neighbor distance loaded similarly on the primary and secondary principal component and were highly correlated. In the medium–low contrast condition, nearest neighbor distance was no longer highly correlated with correlation length but correlated with nearest neighbor distance. At low contrast, correlation length, nearest neighbor distance and speed loaded strongly on the first principal component, while polarization and nearest neighbor angle loaded strongly on the second. At this lowest contrast level, speed, correlation length and nearest neighbor distance were all highly correlated (Table 1).

DISCUSSION

Overall, our results demonstrate that fish schools, with no physiological changes to the contrast sensitivity of their visual system, exhibit weaker antipredator responses in lower contrast environments, suggesting sensory driven re-weighing of the individual interaction rules that drive collective state in dynamic environments. Consistent with studies exploring the effect of turbidity (Kimbell and Morrell, 2015), we found that school polarization, as well as speed, decreased in lower contrast environmental conditions; a result supporting the conclusion that abiotic environmental drivers have a direct effect on the schooling responses to predator attacks. The environments with decreased contrast appear to reproduce the effect of turbidity on predator detection, reducing a school's ability to adequately recognize the magnitude of predator threat (Ferrari et al., 2010), which results in a weaker behavioral response. The ability for schooling fish to adjust escape patterns relative to the perceived threat level, or 'threat-sensitive response' (Brown et al., 2006; Ferrari et al., 2008; Rieucau et al., 2014), may be diminished when visual predator information is confounded, leading to decreased evasion success. In natural dynamic conditions schooling fish face the ceaseless challenge of fine-tuning their aggregative tendencies to minimize their vulnerability to predation while optimizing other fitness gains. It is now well understood that schooling fish can make behavioral and structural adjustments of their collective to modulate

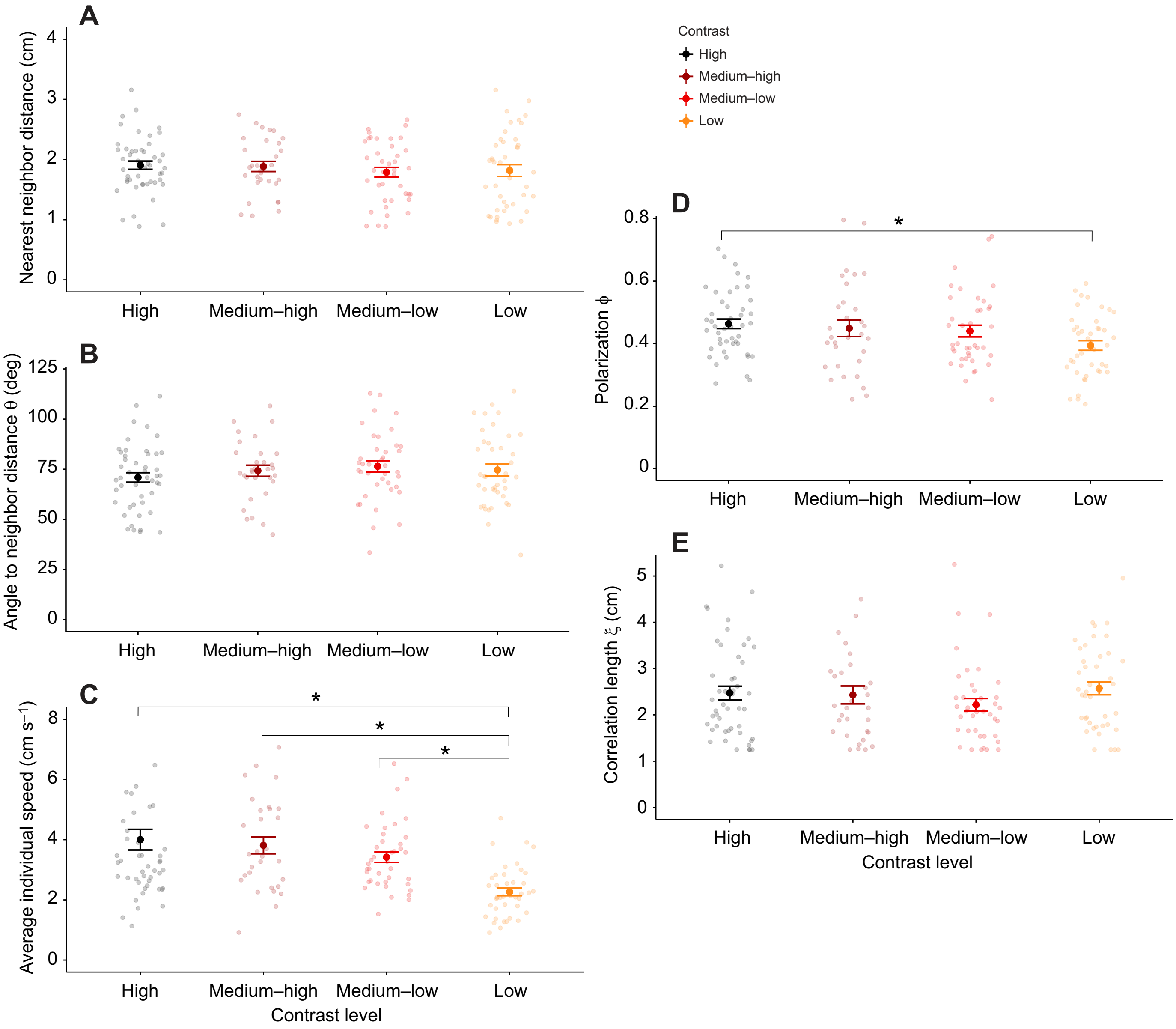

Fig. 2. Mean values of five behavioral parameters in schooling minnow (*Pimephales promelas*) across four contrast levels. Error bars represent standard error and jitter points represent the raw values. Nearest neighbor distance (A), nearest neighbor alignment angle (B) and swimming speed (C) were calculated from each fish in the school across the entire behavioral response time and averaged (mean) to provide one measurement per school tested. Polarization (D) and correlation length (E) were calculated per school, averaging using the mean across the entire time span of each behavioral response, providing one measurement per school tested (N_{total}=161; high contrast, N=48; medium–high contrast, N=31; medium–low contrast, N=40; low contrast, N=42). Univariate ANOVAs on each schooling metric averaged over the entire response interval were conducted, as well as Tukey HSD *post hoc* comparisons (at 95% significance level), Bonferroni corrected for multiple comparisons.

information transfer and collective responsiveness during predator attacks in order to reduce their risk of capture (Rieucau et al., 2016). Consequently, environmental conditions that impair those mechanisms can have profound effects on the outcome of predator–prey interactions with cascading effects on both prey populations and communities.

Additionally, our results provide supporting evidence to previous studies that demonstrate the role of vision and mechanosensation on schooling behavior, with vision particularly playing a pivotal role in determining the rules of interaction (Strandburg-Peshkin et al., 2013) via control of velocity and orientation (Partridge and Pitcher, 1980). Our study also shows that vision affects the ability of a school to adequately respond to a sudden predator attack. During a predator attack, both the predator and other members of the school act as sources of visual sensory information, which provide threat information and collective information, respectively. In our experiment, the environmental conditions influence the fishes' perception of the predator, which affects threat information, and not perception of other school members. This retention of collective information, in conjunction with the absence of manipulation of the lateral line system, suggests that the modified interaction rules resulting in decreases in polarization and speed are induced via the visual response to the perceived predator, rather than perception of the socially transmitted reaction wave. The lack of differences in

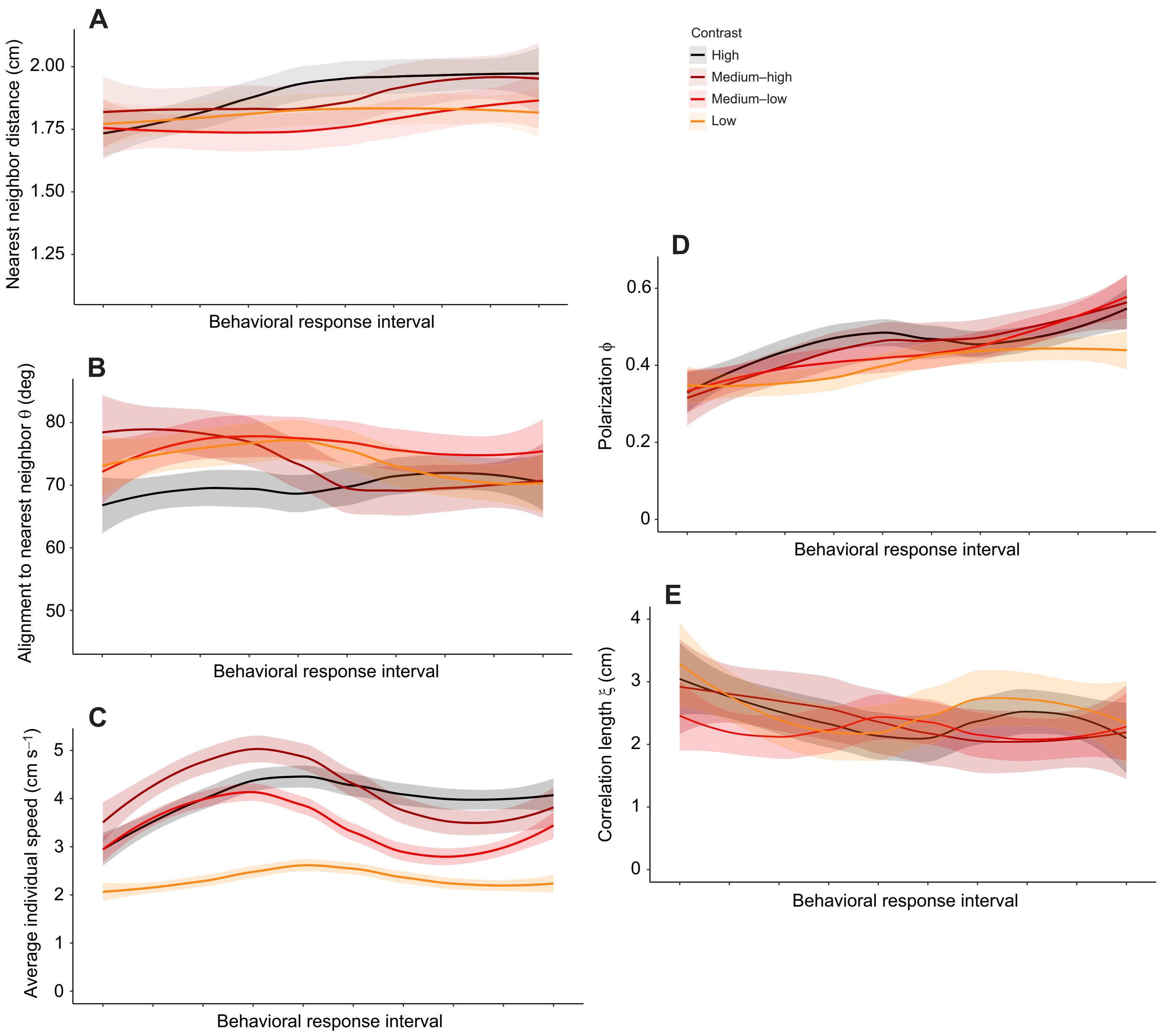

Fig. 3. Five behavioral parameters in schooling minnow along the time span of response to a looming stimulus across four contrast levels. (A) Nearest neighbor distance, (B) nearest neighbor alignment angle, (C) swimming speed, (D) polarization and (E) correlation length. The time span of the behavioral response varied per trial and was standardized by partitioning each behavioral response time span into 10 equidistant time intervals. Solid lines represent mean values of parameters, with contrast denoted by increasingly lighter color; standard error is represented by a similarly hued region bounding the mean value line. Generalized linear models (GLMs) with identity link functions were fitted to each schooling metric with contrast level and each time step in the discretized response interval set as fixed effects. Pairwise comparisons (corrected with Holm's sequential Bonferroni procedure) between contrast levels and response intervals were performed to identify differences (N_{total}=14,570; high contrast, *N*=4460; medium–high contrast, *N*=2660; medium–low contrast, *N*=3600; low contrast, *N*=3850).

other metrics of schooling tendency with decreasing visual perception indicates that the interaction rules are also regulated not just by vision, but by the lateral line system, which is used to maintain cohesion to neighbors once the escape response has been initiated (Partridge and Pitcher, 1980). The presence of other external sensory stimuli, in which food and alarm cues have been shown to affect collective dynamics in schools (Schaerf et al., 2017), may further influence the interaction mechanisms that control the magnitude of the antipredator response and potentially explain the persistence of prey success in vision compromised environments.

Variation in behavior over the course of the entire escape response further illustrate differences in schooling behavior at different contrast levels. In addition to the lower speeds and polarization with decreasing contrast, schools maintained tighter schooling at lower contrast levels across the entire behavioral response, which suggests that mechanosensory information becomes prioritized when visual perception is compromised. Similarly, fish were less locally aligned prior to attack at lower contrasts, which may reflect the need to maximize the collective visual field to enhance individual responses to neighbors and maximize escape success. The consistency in social information

Table 1. Principal component variable loadings for each schooling metric, evaluated at each contrast level

	PC1	PC2	PC3	PC4	PC5
High contrast					
Nearest neighbor distance	0.7431607	0.39184375	−0.06255103	−0.480746	0.2431899
Nearest neighbor angle alignment	0.6281712	0.04331195	−0.61491323	0.4566845	0.1297919
Average speed	0.1280408	0.90657781	0.22740564	0.1868462	−0.274039
Polarization	−0.7809628	0.43790675	−0.02569501	0.1144974	0.4296102
Correlation length	0.496784	−0.18619814	0.77211068	0.2735389	0.2180768
Medium–high contrast					
Nearest neighbor distance	0.4330552	0.57396491	0.62600662	−0.29266947	0.07407915
Nearest neighbor angle alignment	0.7856829	0.002839977	0.18215015	0.58026011	0.11319821
Average speed	−0.5999326	0.675872129	0.01361152	0.29352611	−0.31134376
Polarization	−0.8586388	0.108471405	0.15080096	0.138883	0.45710388
Correlation length	0.4217174	0.587658	−0.65579023	−0.08017737	0.20080652
Medium–low contrast					
Nearest neighbor distance	0.7519345	0.2455167	0.1188536	−0.43764956	0.410673461
Nearest neighbor angle alignment	0.3968071	0.6805326	−0.488485	0.37522094	0.003350618
Average speed	0.7873217	0.1373564	0.3464902	−0.05865161	−0.4876088
Polarization	−0.4676196	0.5711667	0.6216771	0.23047347	0.124499559
Correlation length	0.6032925	−0.4901562	0.2028424	0.55386907	0.218789891
Low contrast					
Nearest neighbor distance	0.6911773	0.20390958	0.5670829	0.3112483	−0.249472
Nearest neighbor angle alignment	−0.2760528	−0.78401837	0.3880778	0.2888037	0.2740403
Average speed	0.8340566	−0.03428064	0.1824436	−0.4085915	0.3208453
Polarization	−0.2842564	0.85244538	0.136973	0.2375681	0.3425419
Correlation length	0.6637597	−0.13026273	−0.5997015	0.4111669	0.1172797

Each variable was scaled to unit variance, with no rotation. For each contrast level, scree plot analysis concluded that the first three principal components explained >80% of the variability in the data.

transfer ability across all levels of visual contrast highlights the potential for non-vision sensory modalities to contribute to the regulation of individual interaction rules to increase survival in dynamically changing conditions. As of now, we are unable to directly determine the exact contribution of the different sensory modalities to the interaction mechanisms that control the antipredator response, which provides an important avenue for further investigation.

Our study shows the underlying metrics that constitute the schooling tendency shift as a result of a changing contrast environment. In particular, we found that polarization, angle and distance to the nearest neighbor were strongly loaded on the first principal component in high contrast conditions, which ceased to be the case in low contrast conditions. In low contrast conditions, correlation length, distance to the nearest neighbor and swimming speed were loaded on the first principal component, with polarization and angle loaded on the second principal component; this shift in the correlation between these parameter groups as the contrast decreases implies a change in how sensory perception may be mapping to the behavioral response. The increased correlation between nearest neighbor distance, correlation length (information transfer) and speed as contrast level decreases further supports the idea that auxiliary sensory systems may be contributing more than vision does to the escape response. As contrast drops, schooling state metrics that are to a lesser degree controlled by vision (nearest neighbor distance, correlation length), but rather by other sensory systems, may become more correlated if the lateral line system is compensating for the loss of visual perception. Additionally, since the loss of visual perception directly influences speed, the grouping of speed with the non-visually mediated schooling metrics suggests that multiple sensory systems, not just vision, can control schooling behavior in conditions where information about the environment is unreliable. Therefore, our results provide evidence that low contrast environments may restrict the contribution of the different sensory pathways available to schooling prey, and their synergistic actions, to collectively react to threats. A predator in the same environment may experience a similar loss of visual perception; however, visually mediated piscivorous predators typically hunt in low light conditions (Cerri, 1983) and can actively select when to attack; a prey school can only respond when an attack is initiated and correctly detected.

The controlled nature of the study does present limitations on the extent to which our findings can be generalized towards freely behaving fish schools in the wild. The use of visual stimuli as a proxy for a predation scenario is widely used in behavioral laboratory studies, and our virtual arena is an advancement in presenting these stimuli in a manner more consistent with natural conditions. However, the combination of an expanding dot stimulus with dot densities controlling visual contrast in our virtual arena does not take into consideration other abiotic and biotic environmental factors that may play a role in regulating the anti-predator response of a fish school. Abiotic factors, such as light variability, scattering due to turbidity and flow conditions may influence the collective dynamics of a school, both prior to and during predator attack. Biotic factors, such as predator approach speed, species type, multi-species interactions, habituation and learning may also play a role in the spatiotemporal dynamics of the collective response; the effects of these environmental factors are critical efforts for future research. In response to our looming stimuli, the first individual fish response (mean±s.e.m.) occurred when the disk appeared to be 45.90±4.88 deg, expanding at 0.15±0.03 deg ms^{-1}, with an estimated time to collision of 351.11±50.93 ms. Over the course of the response, the average individual speed followed the same sinusoidal response pattern as the schools (Fig. S3), and the heading directions were largely uniform (Fig. S4). However, without more experiments we cannot currently estimate the time between a fish becoming alarmed and responding behaviorally, but by systematically varying approach speeds, this could be incorporated into future work.

Although an extended acclimation time was given to each school after each experiment, we cannot rule out the possible learning and habituation effect on an individual due to the experimental setup. Random sampling on the larger population may cause a single fish to be present in multiple experiments, which may ultimately bias the collective response (particularly the correlation length) due to habituation and learning. Our experimental results suggest that no bias is present; however future work would benefit from addressing the impacts of habituation and learning to ensure a rigorous experimental design.

In this study, we explored whether environmental conditions that influence visual perception of predators affects the antipredator schooling tendency of fish. We found that schooling behavior was modified in contrast-limited environments that influence the schools' ability to detect predators. In environments with lower visual contrast between a predator and its surroundings, escape responses of schooling fish were weaker, but only in behavioral metrics that are typically regulated by vision. When environmental conditions altered the ability to visually detect and respond to predators, the antipredator response may be driven more strongly by alternate sensory modalities (lateral line system) that act to increase probability of survival. Our results demonstrate that, in addition to the fact that environmental conditions modulate schooling behavior, antipredator responses of schools in visual information-constrained environments are not completely diminished but rather supplemented by other sensory systems. Understanding the contribution of the sensory systems of fish to schooling behavior under dynamic environmental conditions will improve our knowledge of the mechanisms that underlie collective reactions of free-ranging aggregated fish.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: I.I.R.-P., N.O.H., J.C.T.; Methodology: I.I.R.-P., N.O.H., J.C.T.; Software: I.I.R.-P., J.C.T.; Validation: I.I.R.-P., G.R., K.M.B., J.C.T.; Formal analysis: I.I.R.-P., G.R., N.H., K.M.B., J.C.T.; Investigation: I.I.R.-P., G.R., J.C.T.; Resources: J.C.T.; Data curation: I.I.R.-P., J.C.T.; Writing - original draft: I.I.R.-P.; Writing - review & editing: I.I.R.-P., G.R., K.M.B., J.C.T.; Visualization: I.I.R.-P., N.O.H., J.C.T.; Supervision: N.O.H., K.M.B., J.C.T.; Project administration: I.I.R.-P., K.M.B., J.C.T.; Funding acquisition: I.I.R.-P., J.C.T.

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Data availability
The data used in this paper are available from the Dryad Digital Repository (Rodriguez-Pinto et al., 2024): https://doi.org/10.5061/dryad.tx95x6b2p. Custom OpenGL software for the Holocube system is available upon request from Dr Jamie Theobald.

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