More than meets the eye: Predator-induced pupil size plasticity in a teleost fish

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Abstract

1. Most animals are visually oriented, and their eyes provide their ‘window to the world’. Eye size correlates positively with visual performance, because larger eyes can house larger pupils that increase photon catch and contrast discrimination, particularly under dim light, which have positive effects on behaviours that enhance fitness, including predator avoidance and foraging.

2. Recent studies have linked predation risk to selection for larger eyes and pupils, and such changes should be of importance for the majority of teleost fishes as they have a pupil that is fixed in size (eyes lack a pupillary sphincter muscle) and, hence, do not respond to changes in light conditions.

3. Here, we quantify eye and pupil size of individual crucian carp, a common freshwater fish, following controlled manipulations of perceived predation risk (presence/absence). We also tested if crucian carp responded to increased predation risk by shifts in diel activity patterns.

4. We found that crucian carp show phenotypic plasticity with regards to pupil size, but not eye size, as pupil size increased when exposed to predators (pike). Predator-exposed crucian carp also shifted from diurnal to nocturnal activity. Using a modelling exercise, we moreover show that the plastically enlarged pupils significantly increase visual range, especially for small objects under dim light conditions.

5. Overall, our results provide compelling evidence for predator-induced pupil enlargement resulting in enhanced visual capabilities in a teleost fish. Pupil size plasticity in combination with the observed shift towards nocturnal activity may allow for efficient foraging also under dark conditions when predation risk from diurnal and visually oriented predators is reduced. The data highlight the powerful role of predation risk for eye development and evolution.

KEYWORDS

crucian carp, eye evolution, inducible defence, phenotypic plasticity, predator–prey interactions, pupil size, vision, visual ecology
For prey living in a world full of natural enemies, it is essential to perceive and accurately respond to impending danger. Predation is a primary force driving adaptation in prey, involved in the evolution of a broad suite of prey traits that reduce the risk of predation, including morphological (Brönmark & Miner, 1992; Hodge et al., 2018; Young, Brodie Jr., & Brodie III, 2004), physiological (Clinchy, Sheriff, & Zanette, 2013; Furtbauer et al., 2015; Sapolsky, Romero, & Munck, 2000) and behavioural adaptations (Creel, 2018; Lima & Dill, 1990; Neill & Cullen, 1974; Sih, 1980; Ydenberg & Dill, 1986). Such predator-driven trait changes can become canalized and genetically fixed within populations and species, and thus always expressed, regardless of prevailing risk of predation. However, in nature, environmental conditions are inherently dynamic, and the risk of predation is often spatially and temporally variable, which may favour the evolution of phenotypic plasticity in traits that decrease predation risk (Pigliucci, 2001; Tollrian & Harvell, 1999). Via plasticity, prey can thus fine-tune anti-predator traits to increase survival chances when predators are present, but also save important expression and maintenance costs in their absence (Pigliucci, 2001).

Reliable assessment of the prevailing predation risk hinges on efficient sensory systems, including hearing, olfaction and vision (Banks, Sprague, Schmoll, Parnell, & Love, 2015; Jurcar & Moore, 2018; Kats & Dill, 1998; Møller & Erritzoe, 2010; Roberts et al., 2016; Suzuki, 2018). As constructing and maintaining well-functioning sensory systems is costly (Laughlin, de Ruyter van Steveninck, & Anderson, 1998; Moran, Softley, & Warrant, 2015; Niven & Laughlin, 2008; Sartori et al., 2014), one might expect that such costs can be reduced via plasticity also in the expression of sensory systems in response to variable and unpredictable risk of predation.

Visually guided behaviours, such as finding food and detecting potential mates and/or predators, are principal drivers of the evolution of the eye, and have produced a plethora of eye morphologies and visual strategies across taxa and environmental gradients (Banks et al., 2015; Brischoux, Pizzatto, & Shine, 2010; Hall & Ross, 2007; Land & Fernald, 1992; Land & Nilsson, 2012; Schwassmann & Kruger, 1966). The size of the pupil is of fundamental importance for visual performance as the pupil area determines the light-gathering capacity, and, hence, contrast sensitivity and acuity (Caves, Sutton, & Johnsen, 2017; Land & Nilsson, 2012; Veilleux & Kirk, 2014). The size of the pupil is especially important under dim light conditions, and the vast majority of vertebrate taxa are capable of enhancing visual performance via dynamic control of the pupil size in response to changes in light levels using the muscle of the iris (Douglas, 2018; McDougall & Gamlin, 2015). Recent studies have linked selection for large eye/pupil size to e.g. dim light habitats and nocturnal behaviour (Martinez-Ortega, Santos, & Gil, 2014; Schmitz & Wainwright, 2011; Warrant, 2004) or to competition (Beston & Walsh, 2019) and predation risk per se (Freund & Olmstead, 2000; Glazier & Deptola, 2011; Møller & Erritzoe, 2010; Nilsson et al., 2012). Such changes may be of particular importance in the many teleost fish that lack a pupillary sphincter muscle and, hence, have a pupil that is fixed in size and not responsive to ambient light intensity (Douglas, 2018; Helfman et al., 2009). Yet, while numerous studies have unravelled how predation risk can affect prey eye and pupil size, both at micro- and macro-evolutionary scales (Banks et al., 2015; Beston, Dudycha, Post, & Walsh, 2019; Land & Nilsson, 2012; Møller & Erritzoe, 2010; Nilsson et al., 2012), few have examined whether individuals can implement changes to eye morphology traits in response to environmental variation within their lifetime (but see Ab Ghani, Herczeg, & Merilä, 2016; Svanbäck & Johansson, 2019 for examples of predator-induced plasticity in overall eye size). The high energetic costs of neural tissue make the eye one of the most energetically expensive organs in the vertebrate body (Laughlin, de Ruyter van Steveninck, & Anderson, 1998; Moran, Softley, & Warrant, 2015; Niven & Laughlin, 2008). By adopting an inducible strategy in the visual system, prey should enjoy a closer phenotype-environment match where investments into eye and pupil size are tuned to the prevailing level of predation risk and where unnecessary costs of expression thus can be saved in the absence of predators (Tollrian & Harvell, 1999).

Here, we tested for predator-induced plasticity in eye and pupil size in a teleost fish, the crucian carp Carassius carassius, a common freshwater fish that occupies small and shallow lakes and ponds in temperate regions across the northern hemisphere. Crucian carp constitute a well-established model system to study phenotypic plasticity and, particularly, inducible morphological defences (Brönmark & Miner, 1992; Brönmark & Pettersson, 1994; Holopainen et al., 1997; Hultén et al., 2014). Previous field and laboratory studies have shown that crucian carp respond to chemical cues released by predators, such as pike Esox lucius, by an increase in body depth (Brönmark & Miner, 1992; Brönmark & Pettersson, 1994). The morphologically defended phenotype constitutes less desirable prey for gape-limited predators (Nilsson, Brönmark, & Pettersson, 1995), and the deep body improves escape performance via enhanced locomotor capacity (Domenici et al., 2008). However, many prey species also respond behaviourally to increased predation risk by, for example, changes in diel activity patterns (Bakker, Reiflers, Off, & Gleichman, 2005; Lima & Dill, 1990; Monterroso, Alves, & Ferreras, 2013; Reebs, 2002; Weiss, 2018). When prey are confronted by visually oriented and diurnal enemies, a shift towards nocturnal activity would directly reduce encounter rates. However, for previously diurnal species, predator-induced increases in nocturnality can cause mismatches between eye morphology and the new and novel visual environment and thus interfere with, for example, foraging efficiency. An increase in nocturnality may thus select for facultative and rapid adaptations of the visual system, enabling night-time activity. Such changes in diel rhythms have recently been suggested as the selective agent driving evolutionary shifts in eye size among populations of the Trinidadian killfish Rivulus hartii (Beston & Walsh, 2019). Furthermore, a recent study demonstrated that selection pressures on activity patterns have been the main drivers for evolutionary divergence in the mammalian eye (Baker & Venditti, 2019).
In this study, we quantified predator-induced changes in eye and pupil size at the level of individuals among mature, wild-caught and previously predator-naïve crucian carp. We also examined diel activity patterns in crucian carp held in the presence or absence of predatory pike. We predicted that individuals exposed to perceived predation risk would develop larger eyes capable of holding a larger pupil. The increased light-gathering capabilities should result in enhanced contrast detection and visual range, and thus aid in predator detection. We also predicted that crucian carp should shift activity patterns towards a higher degree of nocturnality in the presence of pike, a strategy assumed to be adaptive as it would directly reduce the risk of capture from diurnal and visually oriented pike predators (Skov & Nilsson, 2018). Finally, to shed light on how plastic changes to pupil size may affect visual performance, we modelled how the observed trait changes affect maximum detection distances of objects of varying size under diverse light regimes.

2 | MATERIALS AND METHODS

2.1 | Fish and maintenance

We used wild-caught and previously predator-naïve crucian carp [Experiment 1 (eye plasticity): \( n = 96, \) size range 83–93 mm; Experiment 2 (diel activity): \( n = 70, \) size range 83–110 mm] collected with fyke and sink nets in two small lakes in the vicinity of Lund, southern Sweden. After capture, fish were immediately transported to experimental facilities at Lund University and acclimatized to laboratory conditions in a 700 L tank (aerated and filtered water) and fed a varied diet consisting of chironomids, Daphnia and commercial carp pellets ad libitum. In order to enable identification of individuals throughout the experiments, a passive integrated transponder tag (HDX, Oregon RFID, size: 12.0 mm long and 2.12 mm diameter, weight 0.1 g) was surgically implanted into the abdominal cavity of each crucian carp prior to experiments, following Skov et al. (2005). Predatory pike were caught by electrofishing in the lake Kranksjön, southern Sweden. The laboratory experiments were conducted in a constant temperature of 18°C and a 12:12 hr light:dark regime. We set up 24 aquaria (152 L; 95 × 40 × 40 cm), each divided into two compartments of equal size by a perforated transparent acrylic glass partition. This set-up allowed crucian carp in the predator presence treatment to perceive both visual and chemical cues from pike. We provided artificial vegetation, one in each of the two sections, and in order to prevent visual interactions between replicates, two sides of each aquarium were externally covered with a black/blue plastic film. We examined the effect of predation risk on diel activity patterns in crucian carp in a behavioural experiment. After 166–176 days of treatment exposure, the diel activity patterns of individual fish were recorded within each aquarium. During the diel activity trials we employed different light regimes simulating natural conditions: (a) day (7.00–19.00; ~540 lx), (b) evening (19.00–20.58; ~18 lx), (c) night (21.00–5.00; 0 lx) and (d) dawn (5.00–7.00; ~18 lx). To allow of approximately 5% of the total body mass in each replicate tank. A single pike (Experiment 1: \( n = 12, \) standard length: 31–41 cm, Experiment 2: \( n = 7, \) standard length 23–31 cm) were introduced to the other compartment in aquaria used for the predator treatment (pike treatment aquaria assigned by random permutation). Predatory pike received a strict crucian carp diet and were fed on a weekly basis during the acclimatization and experimental period.

2.2 | Experiment 1 – Eye plasticity

Trait changes in overall eye and pupil size at the level of individuals were quantified from digital photographs. We photographed all crucian carp prior to treatment exposure and once again when the experimental period was over (after 193 days). The camera [digital single lens reflex (DSLR), Canon EOS 80D; Canon Inc.] equipped with a 18–35 mm lens (f/1.8 DC HSM, Sigma Inc.) was vertically mounted on a copy stand, connected to a desktop computer and images were captured using the live-view function of the DSLR and the EOS Utility 3 software (Canon Inc.). Fish were netted from their experimental aquaria, anaesthetized (benzocaine; Sigma Aldrich, Ethyl p-Aminobenzoate) and laterally placed on a white foam rubber board, always with the anterior part facing left. A ruler was included in each image for scale calibration. The effect of muscle relaxation from anaesthesia allowed for standardized images for eye size measurements, i.e. the eye always pointed vertically towards the camera lens. In events of mortality, we added new fish of similar size to maintain density. However, only fish allocated to experimental tanks at the initiation of the experiment were included in the analysis (\( n = 73 \)).

2.2.1 | Image analyses

From the digital images we extracted morphological variables using the image analysis software ImageJ v. 1.49 (https://imagej.nih.gov/ij/). We measured the widest part of the eye and pupil at the horizontal plane (Beston, Wostl, & Walsh, 2017; Svanbäck & Johansson, 2019). In addition, we measured standard length (the distance between the tip of the snout to the end of the last scale anterior to the caudal fin) of all fish to account for potential body size differences and enable calculation of relative eye and pupil size.

2.3 | Experiment 2 – Diel activity

We examined the effect of predation risk on diel activity patterns in crucian carp in a behavioural experiment. After 166–176 days of treatment exposure, the diel activity patterns of individual fish were recorded within each aquarium. During the diel activity trials we employed different light regimes simulating natural conditions: (a) day (7.00–19.00; ~540 lx), (b) evening (19.00–20.58; ~18 lx), (c) night (21.00–5.00; 0 lx) and (d) dawn (5.00–7.00; ~18 lx). To allow...
monitoring of fish activity also under low light conditions, we used digital infrared scouting cameras (UOVISION UV572 12 MP HD, infrared wavelength: 960 mm) horizontally facing the centre of the crucian carp compartment. Some fish species have evolved a visual sensitivity for near-infrared light (Enright et al., 2015). However, pilot experiments in our model system showed that crucian carp did not respond behaviourally to the IR light during dark hours, i.e. we could not detect any behavioural response upon the onset of the IR light. The camera was set to record for 1 min each hour for 24 hr, starting at 9.00. All fish were fed 24 hr before each trial started to standardize hunger levels prior to activity measurements. Pike in the predator treatment were fed one crucian carp the day before we recorded activity. A grid (4 × 4 squares) was taped on the front of each aquarium to enable quantification of fish activity. From the recordings we quantified the total number of squares entered by each individual during 1 min at 12 different time points during 24 hr (day: 08.00, 11.00, 14.00, 18.00; twilight: 05.00, 06.00, 19.00, 20.00; night: 01.00, 03.00, 21.00, 23.00), resulting in four time points per light condition). In case of mortality in experimental tanks, fish were immediately removed and replaced with a similar-sized individual so that the initial density level was held constant throughout the experiment.

2.4 | Modelling of visual range

The maximum distance for visual detection of dark objects was calculated according to theory developed for aquatic vision (Nilsson, Warrant, & Johnsen, 2014). The pupils were assumed to be circular, and we used the estimated means of the pupil diameters from each treatment group (predator-exposed or control), although we modelled visual performance for the complete pupil range of 0–4 mm. Estimated means were predicted by an ANCOVA model at the centre of the covariate (standard length: 88.7 mm), using the post-treatment size of the pupil diameters (to the nearest 0.01 mm) as the dependent variable and the post-treatment standard length of fish (to the nearest 0.01 mm) as a covariate. Tank identity was used as a random factor, nested within treatment. We modelled the maximum distance (visual range) for detection of circular black targets with diameters of 1 mm (planktonic prey), 10 cm (predators) and an intermediate value of 1 cm. Maximum detection distances were calculated for targets seen against measured intensities of downwelling light, horizontal light and upwelling light (Nilsson, Warrant, & Johnsen, 2014). Optical properties of lake water were assumed to agree with values for estuarine (Maclver et al., 2017) water with a secchi depth of 2.7 m: beam attenuation coefficient 2.19, background attenuation coefficient 0.566 and an intensity drop of 0.0324 log units per m water. Average background radiances, just below a single aquarium divided by the total number of fish per aquaria (n = 5), was used as response variable. Treatment (predator-exposed or control) was used as a between-subject factor to examine the factor of primary interest (time × treatment). Our data did not meet the assumptions of sphericity, and were therefore corrected by the conservative Greenhouse–Geisser procedure (Greenhouse & Geisser, 1959).

2.5 | Data treatment and statistical analyses

Statistical analyses were performed using SPSS v. 23.0 for Mac OS X (SPSS Inc.).

2.5.1 | Eye plasticity

We used a GLMM to analyse the effect of predator presence/absence on eye morphology. The change in horizontal (0°) size (nearest 0.01 mm) of eyes and pupils of individual fish were used to analyse the degree of predator-induced eye plasticity, where trait changes of individual fish were used as response variables, calculated as post-treatment values minus pre-treatment values. Moreover, we tested for differences in relative eye and pupil size, using the post-treatment measurements as dependent variables, without and with standard length as a covariate respectively. Tank identity was used as a random factor, nested within treatment to compensate the degrees of freedom according to the experimental design. All data met the assumptions of normality and equality of variances.

2.5.2 | Diel activity

We used a general linear mixed repeated measures model (GLMMR) to test if pike exposure induces a shift towards nocturnality in crucian carp. The mean activity for each time point (n = 12 time points) and aquaria, calculated as the total sum of entered squares within a single aquarium divided by the total number of fish per aquaria (n = 5), was used as response variable. Treatment (predator-exposed or control) was used as a between-subject factor to examine the factor of primary interest (time × treatment). Our data did not meet the assumption of sphericity, and were therefore corrected by the conservative Greenhouse–Geisser procedure (Greenhouse & Geisser, 1959).
3 | RESULTS

3.1 | Eye plasticity

Fish allocated to treatments (predator presence/absence) did not differ in body size before (88.7 ± 2.8 mm standard length, M ± SD, 88.6 ± 2.5 mm, respectively, t_{71} = −0.143, p = 0.886) or after (88.8 ± 2.8, 88.7 ± 2.7, respectively, t_{71} = −0.160, p = 0.874) the experimental period.

Analysing the individual change in pupil size over the course of the experiment revealed that predator exposure induced larger pupils in crucian carps (F_{1,23.93} = 15.03, p < 0.001, Figure 1). Similarly, at the end of the experiment, the relative pupil size (pupil size in relation to body-length) were larger in predator-exposed fish (estimated mean: 2.77 mm ± 0.014 SE) as compared to fish held in the absence of predators (estimated mean: 2.70 mm ± 0.014 SE); GLMM with SL as covariate (F_{1,23.81} = 7.689, p < 0.011). However, we found no effect of predator exposure on overall eye size, neither on relative (estimated mean predator exposed: 6.76 mm ± 0.029 SE; estimated mean control: 6.72 mm ± 0.029 SE (F_{1,23.15} = 0.49, p = 0.490)) or absolute size (F_{1,24.12} = 0.25, p = 0.621), nor for changes in horizontal eye size (F_{1,23.14} = 0.33, p = 0.572).

3.2 | Diel activity

During the 24-hr period of activity recordings, predator-exposed fish were less active than control fish, and activity patterns differed significantly between treatments over the diel cycle (time × treatment interaction term: F_{3.204,38.450} = 2.909, p = 0.044), with predator-exposed fish being more active during darkness whereas control fish demonstrated activity peaks during daylight conditions and were relatively less active during low light conditions (Figure 2).

3.3 | Modelled visual range

Modelling the effect of the observed increase in pupil diameter on visual performance revealed a corresponding increase in the visual detection range. The pupil diameter increases by 2.52% (area increases by 5.1%) and depending on light conditions and target size this extends the visual range by 0.28%–2.0% (see Figure 3; Table S1). The gain in visual range depends only marginally on viewing direction but has a strong dependence on light intensity and on the size of the objects that are visually detected. The strongest performance gain (response) is found for detection of very small targets (prey) in dim light. The results are robust for major variation of input assumptions to the modelling (Table S2). Even though the visual range depends to some degree on water quality (beam and background attenuation coefficients), this has very little effect on the calculated response values.

4 | DISCUSSION

Our results show that perceived predation risk can induce plastic changes in the vertebrate eye, and also suggest behavioural changes in response to ecological shifts in predator communities. Crucian carp exposed to a live predator (elevated perceived risk) increased pupil but not overall eye size, and also showed a higher degree of nocturnal activity as compared to fish held in the absence of predators. Next, we applied a theoretical model to shed light on the adaptive value of such trait changes and showed that inducing a larger pupil is a specialization that enhances visual performance, specifically under dim light conditions. Our results thus imply that predator-induced plasticity in a key eye trait may act in concert with behavioural shifts to enable crucian carp to increase survival chances and maintain...
efficient foraging under dim light while reducing the odds of visual detection by their predators.

As a principal sensory organ, the eye provides immediate information on the surrounding environment. The selective advantages of using light as a source of information relate to habitat-specific properties, and, hence, numerous solutions and adaptions to obtain adequate visual information are found in nature (Land & Nilsson, 2012). Eye size is positively correlated with visual acuity and limits visual sensitivity by setting the maximum possible size of the pupil; pupil size ultimately constrains the light-gathering capacity of the eye (Land & Nilsson, 2012). The visual conditions of freshwater habitats are characterized by high absorption and scattering of downwelling light (Holopainen, Tonn, & Paszkowski, 1997), where a larger pupil size significantly improves contrast detection and visual range (Caves, Sutton, & Johnsen, 2017; Nilsson et al., 2012; Veilleux & Kirk, 2014). However, with a few exceptions, teleost fish lack a sphincter pupillae muscle (Douglas, 2018; Helfman et al., 2009) and therefore cannot autonomically regulate the amount of light reaching the retina by changing pupil size in accordance to changes in ambient light. The capability of increasing pupil size in response to changes in perceived predation pressure should hence be adaptive. Relatively large pupils is of particular importance for animals living in environments with poor light conditions, where contrast detection is of more significance than resolution (Land & Nilsson, 2012; Warrant, 2004). Perhaps the most striking example is provided by the giant squid Architeuthis sp. which has evolved spectacularly large eyes and pupils, up to three times larger than any other animal (Land & Nilsson, 2012), as an adaptation for early detection and successful avoidance of predatory sperm whales in the dim light of the deep-sea environment (Nilsson et al., 2012). Moreover, light deterioration in turbid and brown water results in shorter detection and escape distances in common roach Rutilus rutilus attacked by pike, and, hence, pike demonstrate higher attack success and prey fish a lower survival probability in poor light conditions (Ranåker et al., 2012). However, both prey and predators suffer much shorter visual detection distances in dim light.

To evaluate how changes in pupil size affect visual performance in crucian carp we used a model developed for calculating visual performance in water from data on pupil diameter and ambient light intensity (Nilsson, Warrant, & Johnsen, 2014). One important component of the model is the law of diminishing returns, i.e. the marginal value of a relatively larger pupil size decreases with increased pupil size. This general phenomenon is an important limiting factor for eye size in aquatic animals (Nilsson, Warrant, & Johnsen, 2014), but also a reason why small fish larvae have disproportionally large eyes. The observed change in
crucian carp pupil area is 5.1% and corresponds to a symmetric change in pupil diameter by 2.5%. This improves the visual range with 0.28%–2.0%, depending on light conditions and visual target size (Figure 3; Table S1). These seemingly small changes should be viewed in light of the cubic relationship with volume visibility. A 2.5% increase in visual range is associated with a 6.1% increase of the water volume that can be scanned to detect predators and prey, a significant increase that should be of importance for survival and growth opportunities. For example, Brandon, James, and Dudycha (2015) showed that eye size is under selection in a natural population of Daphnia, and also that changes in eye size have clear fitness consequences; small increases (1%) in the eye diameter of *Daphnia obtusa* increase reproductive outputs by 20%.

Our calculations of the functional response, i.e., how much performance is gained by an incremental increase in pupil area, also provides important insight into the most likely reasons for increasing the pupil area (right panels of Figure 3). The response is almost the same for all viewing directions but increases strongly with lower light intensities and smaller target sizes. This suggests that the predator-induced change towards a nocturnal activity pattern and the resulting need for efficient foraging in dim light can be related to the plastic increase in pupil area. The improvement in detecting larger objects, such as potential predators, in bright light is only marginally increased, indicating that the larger pupil may be a secondary effect of the more crepuscular/nocturnal lifestyle adopted by crucian carp in the presence of predators.

Previous studies have linked the evolution of larger overall eye size to predation risk. For example, Ab Ghani et al. (2016) found that three-spined sticklebacks *Gasterosteus aculeatus* show plasticity in eye size and induce relatively larger eyes when reared in presence of chemical cues from predatory Eurasian perch *Perca fluviatilis*. Furthermore, juvenile perch have recently also been shown to express plasticity in overall eye size as a response to predation risk (Svanbäck & Johansson, 2019). However, in our study, we found no evidence of predator-induced plasticity in overall eye size. This lack of response in overall eye size among predator-exposed fish can be due to various reasons. Phenotypic plasticity and capability of trait modulation can vary substantially over ontogeny (Fawcett & Frankenhuys, 2015; Hochberg et al., 2011; Hoverman & Relyea, 2007; Meuthen et al., 2018), and while the sticklebacks and perch in the studies mentioned above were exposed to chemical cues from predators from the onset of the larval/juvenile stage (Ab Ghani et al., 2016; Svanbäck & Johansson, 2019), we performed our experiments on larger and sexually mature fish (the majority of experimental subjects showed fully developed gonads). Moreover, as pigmented eyes with high contrast against the background can increase the risk of detection by predators, small eyes may have been suggested to be favoured under predator-driven selection (Beston et al., 2017; Svanbäck & Johansson, 2019). Furthermore, as eyes are energetically costly to develop and maintain (Moran, Soffley, & Warrant, 2015), the costs associated with changes to pupil size may be significantly lower than investment into construction and maintenance of larger eyes, especially, as an increased eye size ultimately requires a reconstruction and enlargement of the orbit part of the cranium (Goatley, Bellwood, & Bellwood, 2010; Rohner et al., 2013). A predator-induced change only in pupil size while maintaining overall eye size could be an efficient and fast-acting strategy to enhance vision in dim light. However, for teleost fish this strategy is limited as pupil diameter typically is only marginally smaller than lens diameter, and light entering the eye outside the lens will degrade the image (Douglas, 2018; Land & Nilsson, 2012). Prey adopting inducible defence strategies are often exceptionally vulnerable to predation prior to the expression of the defended phenotype (Holopainen, Tonn, et al., 1997), where rapid increases of pupil size followed by altered diel activity patterns may serve to increase survival before additional anti-predator traits have been expressed.

The behavioural experiment showed that the presence of a natural predator alters the diel activity patterns in crucian carp. While non-exposed fish maintained a typical diurnal diel rhythm with a peak of activity around noon, pike-exposed crucian carp both reduced general activity and distinctly shifted to a nocturnal activity. This is in line with earlier field studies, where introduction of predatory perch and pike to a natural pond resulted in crucian carp residing in inshore habitats and shifting to nocturnal behaviour (Tonn, Paszkowski, & Holopainen, 1989). Such changes in activity patterns and habitat use are shared across different taxa and a common response in prey, particularly if reliable cues regarding the prevailing risk are present (Lima & Dill, 1990). For crucian carp, a nocturnal lifestyle, enabled by phenotypically plastic pupil enlargement, would lead to reduced encounter rates with typically diurnal pike predators (Skov & Nilsson, 2018). The change to crepuscular/nocturnal activity is expected to have a dramatic effect on predator vision (see Figure 3). For pupil diameters of 5–7 mm (the pike in our experiments), the visual range shrinks to about half from sunlight to twilight, and is further reduced to 12% at starlight. Compared to sunlight this implies that the water volume covered by vision is reduced with a magnitude of eight times in twilight and with a magnitude of 500 times in starlight. Consequently, the risk of swimming into the detection range of an ambush predator is massively reduced by changing to a more crepuscular or nocturnal activity.

Phenotypic plasticity must theoretically involve costs; if there are no associated costs, trait fixation would be the evolutionary stable strategy (Auld, Agrawal, & Relyea, 2010; Dewitt, Sih, & Wilson, 1998; Tollrian & Harvell, 1999). Empirically, however, costs of plasticity have been shown to be elusive and weak (Van Buskirk & Steiner, 2009), and even though vision is a metabolically expensive sensory system to develop and maintain (Laughlin, de Ruyter van Steveninck, & Anderson, 1998; Moran, Soffley, & Warrant, 2015), specific energetic costs associated with investment into a larger pupil are unclear. However, a pupil that is (too) large would increase the risk of direct sunlight entering through a gap between the pupil margin and the lens. Such straylight would seriously compromise contrast sensitivity in exposed parts of the retina. The predator-induced pupil size in crucian carp might therefore not be advantageous when predators are absent and...
day-time foraging is preferred. In addition, the typical diurnal activity patterns of crucian carp in predator-free environments (Tonn, Paszkowski, & Holopainen, 1989), together with our results, indicate that crucian carp are trading-off their optimal diel activity pattern against safety from predation. Earlier experiments have confirmed that predation risk can reduce the growth rate of crucian carp in the field (Tonn, Paszkowski, & Holopainen, 1992), and numerous studies have shown the importance of light and visual performance to successful detection and attack of prey in planktivorous fish species (Confer et al., 1978; De Robertis, Jaffe, & Ohman, 2000; Hairston, Li, & Easter, 1982; Varpe & Fiksen, 2010).

In our experiment, we found no differences in overall body size between predator-exposed and unexposed individuals, i.e. no evidence of foraging costs associated with the shift in diel activity patterns under these experimental conditions.

In summary, our results demonstrate predator-induced changes in pupil size and diel activity patterns in crucian carp. Coupled with plastic changes in body morphology (Brönmark & Miner, 1992), physiology (Holopainen, Aho, et al., 1997) and behaviour (Höglund et al., 2005; Hulthén et al., 2014; Pettersson, Nilsson, & Brönmark, 2000), our findings highlight that crucian carp have evolved phenotypic plasticity in a broad suite of traits that, when combined, produce an adaptive, integrated anti-predator phenotype.

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**AUTHORS’ CONTRIBUTIONS**

All authors contributed to the design of the study. J.V. performed the experiments; D.E.N. did the modelling of visual range; J.V. and D.E.N. performed the statistical analyses and prepared figures and tables; J.V. led the writing and revisions and K.H., D.E.N., P.A.N. and C.B. contributed substantially. All authors have approved the submitted manuscript and we declare no conflict of interest.

**DATA AVAILABILITY STATEMENT**

Data are available from the Dryad Digital Repository https://doi.org/10.5061/dryad.op2ngf1xr (Vinterstare, Hulthén, Nilsson, Nilsson, & Brönmark, 2020).

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