J Exp Biol Advance Online Articles. First posted online on 21 April 2017 as doi:10.1242/jeb.149575
Access the most recent version at http://jeb.biologists.org/lookup/doi/10.1242/jeb.149575

Comparison of functional and anatomical estimations of visual acuity in two species of coral reef fish

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Keywords: visual acuity, retinal topography, visual behaviour, spatial frequency, marine signaling, reef fish

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ABSTRACT

The high contrast, complex patterns typical of many reef fish serve several purposes, including providing disruptive camouflage and a basis for vision-based communication. In trying to understand the role of a specific pattern it is important to first assess the extent to which an observer can resolve the pattern, itself determined, at least in part, by the observer's visual acuity. In this study, we study the visual acuity of two species of reef fish using both anatomical and behavioural estimates. The two species in question share a common habitat but are members of different trophic levels (predator vs. herbivore/omnivore) and perform different visual tasks. On the basis of the anatomical study we estimated visual acuity to lie between 4.1 – 4.6 cycles per degree (cpd) for *Pomacentrus amboinensis* and 3.2 – 3.6 cpd for *Pseudochromis fuscus*. Behavioural acuity estimates were considerably lower, ranging between 1.29 and 1.36 cpd for *Pomacentrus amboinensis* and 1.61 and 1.71 cpd for *Pseudochromis fuscus*. Our results show that two species from the same habitat have only moderately divergent visual capabilities, despite differences in their general life histories. The difference between anatomical and behavioural estimates is an important finding as the majority of our current knowledge on the resolution capabilities of reef fish comes from anatomical measurements. Our findings suggest that anatomical estimates may represent the highest potential acuity of fish but are not indicative of actual performance, and that there is unlikely to be a simple scaling factor to link the two measures across all fish species.

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INTRODUCTION

Many coral reef fish use visual signaling for a range of behaviours, including maintaining and defending territories, recognizing individuals and/or species and attracting potential mates (Cott, 1940; Endler, 1991; Marshall and Vorobyev, 2003; Siebeck, 2004; Siebeck et al., 2010). Furthermore, prey species often become aware of the presence of a predator through visual signals. In order to survive, they need to identify the nature of the potential attack and respond appropriately (Kelley and Magurran, 2003). Errors in the interpretation of these signals by the receiver can be costly and lead to a loss of fitness. Because the visual system of fish has evolved in response to a range of social and environmental pressures (Dobberfuhl et al., 2005), the visual capabilities of different species are highly variable (Lythgoe, 1979; Endler, 1990, 1993), and can be described using a range of measures including (but not limited to) acuity, temporal resolution and absolute visual sensitivity. Visual acuity, or spatial resolution, is a measure of the minimum separable angle that can be resolved by the eye (Neave, 1984) and is one of the most common measures to assess the visual capability of an animal (Reymond, 1985; Harman et al., 1986; Collin and Pettigrew, 1989; Aho, 1997; Haug et al., 2010). Knowledge of visual acuity of an animal allows us to evaluate the level of detail an animal can see in a visual scene, which is important if we wish to understand various aspects of their visual behaviour independently of the human visually guided behaviour perception.

Differences in lifestyle and habitat complexity lead to differences in visual tasks and with that high variability in acuity among species. For example, the wedge tailed eagle (*Aquila audax*), has a high behavioural acuity of 143 cycles per degree (cpd), as it relies on vision to spot prey from a great distance (Reymond, 1985). In contrast, the domestic chicken (*Gallus g. domesticus*), which feeds at a much closer distance, has a much lower behavioural acuity of 7 cpd (Jarvis et al., 2009). Acuity is also through to be influenced by an organism's light environment (Lee and O'Brian, 2011). A study conducted on a temperate (*Hippocampus abdominalis*) and tropical (*Hippocampus taeniopterus*) species of seahorse found that tropical species had a higher acuity compared to their temperate counterparts, despite living in similar habitats. The difference in acuity may be attributable to the two species' light environment as temperate waters tend to be more turbid with a narrow spectrum of light, whilst tropical coral reefs have a broad spectrum of light (Mosk et al., 2007). The increased visual resolution of *Hippocampus taeniopterus* may enhance its ability to capture prey in the bluer water of the tropical environment (Lee and O'Brian, 2011). Amongst fishes, reports indicate a wide range

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in spatial resolution capabilities, commensurate with their wide range of habitats and lifestyles (for review see Douglas and Hawryshyn, 1990). One study which used anatomical measurements to estimate acuity for 15 species of reef fish from varying habitats found that acuity varied between 4 and 27 cpd (Collin and Pettigrew, 1989). Although in some instances fish living in similar habitats may have similar acuities (for example, *Halophryne diemensis* and *Pomacanthus semicirculatus*; 4 and 7 cpd respectively), this is not always the case. Species found inhabiting open water can have much larger differences in acuity (for example *Lethrinus chrysostomus* and G*ymnocranuis bitorquatus;* 19 and 27 cpd respectively) (Collin and Pettigrew, 1989).

The visual acuity of fish has been measured using a range of behavioural (Brunner, 1934; Yamanouchi, 1956; Nakamura, 1968; Hodos and Yolen, 1976; Hairston et al., 1982; Neave, 1984; Pankhurst et al., 1993; Neumeyer, 2003b; Haug et al., 2010; Lee and O'Brian, 2011; Champ, 2012) and anatomical methods (Hodos and Yolen, 1976; Collin and Pettigrew, 1989; Fritsches et al., 2003; Matsuda et al., 2005; Theiss et al., 2007; Litherland and Collin, 2008; Matsuda et al., 2008; Kino et al., 2009; Carton and Vaughan, 2010; Temple et al., 2010; Lee and O'Brian, 2011; Champ, 2012). However, due to the many differences in methodology, comparisons between studies are difficult. In cases where both anatomical and behavioural methods have been used to measure acuity, there are some examples where the acuity estimates are similar (Brunner, 1934; Marc and Sperling, 1976; Neumeyer, 2003b; Temple et al., 2013). However, it is more common that behavioural acuity estimates are lower than acuity estimated from anatomical measures (see table 1 for review). There are several possible explanations for this discrepancy. Anatomical measures do not account for higher order processing beyond the retina, which would lead to an underestimate of behavioural abilities. At the same time this approach also fails to take into account any optical properties of the eye that may limit acuity (Browman et al., 1990; Haug et al., 2010; Lee and O'Brian, 2011), possibly leading to an overestimate of the capabilities of the fish. Behavioural estimates of visual acuity are likely to yield a more accurate estimate of an animal's actual visual ability as behavioural is the output of both the retinal and neural systems (Browman et al., 1990; Douglas and Djamgoz, 1990). However, behavioural experiments can be impractical species which cannot be tested in captivity. In addition, the results of behavioural experiments can be variable and time consuming due to differences in motivation amongst individuals (Dickinson et al., 1995; Niv et al., 2006). As a result, less than 15 studies have compared both behavioural and anatomical estimates of acuity (but see table 1 for a review).

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The purpose of this study is to investigate the visual acuity of two species of reef fish using both anatomical and behavioural techniques. Photoreceptor and ganglion cell densities within the retina were measured to provide an estimate of the maximum optical acuity of the visual system. Behavioural experiments were then used to determine how the acuity of each species is expressed functionally. Fish were trained to discriminate between horizontal and vertical broad-spectrum gratings. The spatial frequency of the gratings was then systematically increased until the fish could no longer discriminate the gratings. The Ambon damselfish (*Pomacentrus amboinensis*; Bleeker 1868) and one of its predators, the Yellow dottyback (*Pseudochromis fuscus*; Müller & Troschel, 1849) were selected as test species as they often share the same habitat, but come from different trophic levels and undertake different visual tasks. Also, both species of fish adapt well to captivity and readily perform visual discrimination tasks (Parker, unpublished results; Siebeck, 2004; Siebeck et al., 2008; Siebeck et al., 2010; Cripps et al., 2011).

Pomacentrus amboinensis is known to use unique facial patterns for species and individual recognition (Siebeck et al., 2010). These facial patterns are complex and discrimination requires the ability to discern fine detail. On the other hand, *Pseudochromis fuscus* is a solitary, opportunistic predator known to prey on slow moving benthic crustaceans as well as newly settled fish, including juvenile *Pomacentrus amboinensis* (McCormick and Meekan, 2007; Cortesi et al., 2015a). Whilst *Pseudochromis fuscus* must learn to avoid unpalatable or poisonous prey, they have no obvious patterns themselves that differ between individuals nor are they able to see the facial patterns on *Pomacentrus amboinensis* as they are UV blind (Siebeck and Marshall, 2001; Cortesi et al., 2015b). The difference in visual tasks performed by each species suggests that, despite living in the same visual environment, *Pomacentrus amboinensis* are expected to have a slightly higher acuity, at least for the static patterns forming the focus of this study.

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Methods

Animal collection and housing

Male fish of each species (7-8 cm SL) were caught in shallow reefs near Lizard Island, on the Great Barrier Reef (14°40'S 145°28'E) using custom made hand nets during SCUBA, with permission from the Great Barrier Reef Marine Park Authority (G08/27055.1) and the Queensland Fisheries Service (PRM377271). Fish were sent to Brisbane (in individual plastic bags half-filled will with fresh seawater and topped up with oxygen) using a commercial aquarium trader (Cairns Marine Aquarium). All experiments were conducted in accordance with the University of Queensland Animal Ethics guidelines (AEC approval numbers: VTHRC/194/08/ARC/UQ, VTHRC/212/09/AUSTRALIA PACIFIC FOUNDATION/U). Due to their territorial nature, all fish were housed in separate aquaria (35x26x20 cm), each containing a PVC pipe (10cm length, 5cm diameter) that served as a shelter. High water quality was maintained as each aquarium contained an internal water filter (Aqua one 101F), which was cleaned every second day. In addition, regular water changes were carried out every second day. Fish were fed a mixture of water and fish flakes (HBH: flake frenzy marine flakes, Springville, Utah, USA) daily as part of the experiments. Also, due to their territorial nature, the fish were not moved to a different aquarium for testing but tested in the aquarium that contained their new territory. The food reward was administered by hand with a syringe and plastic tubing. The aquarium room was illuminated using standard fluorescent 60W strip lighting (ambient light: 349 Lx) on a 12 h: 12 h light:dark cycle. Following the behavioural experiments fish were kept in captivity at the University of Queensland and used for further visual behavioural experiments.

Retinal anatomy

Photoreceptor and ganglion cell density counts

Six light-adapted fish from each species were euthanized by severance of the spinal cord. The standard length (SL) of each fish was recorded to the nearest millimeter. Once enucleated, the diameter of the eye was measured to the nearest 0.1 mm along the dorsal-ventral, axial (corneal-scleral), and equatorial (anterior-posterior) axis (Ullmann et al., 2012). The technique for dissecting the retinal layer from the eye and subsequent mounting was largely derived from Stone (1981) and only variations to the technique are described below. A ventral orientation slit was initially made to ensure correct orientation of the retina. Once dissected, the vitreous humour and retinal pigment epithelium were gradually removed before the retina was fixed in 4% paraformaldehyde in 0.1M phosphate buffer solution (pH 7.4) and

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stored at 4°C for 1-2 hours. A wall surrounding the flattened retina was made with duct tape, and 100% glycerol was then added. A cover slip was placed over the retina and sealed with nail polish.

The same individual retina used for photoreceptor cell topography was also used for ganglion cell topographic studies. Mounting and drying methods followed procedures described by Curcio et al. (1987). After retinas were mounted and dried, they were rehydrated through a descending alcohol series followed by a 2 min rinse in distilled water. Each retina was then nissl stained using a 0.01% cresyl violet solution (pH 4.3) for 15 seconds. After staining, slides were dipped in 0.025% acetic acid solution to remove any excess cresyl violet. Slides were then dehydrated in an ascending alcohol series before being cleared in xylene. Each retina was cover slipped in a DEPEX mounting medium (Aldrich Chemical Company Inc., USA).

Topography maps

Initially an outline of each retina for each cell layer examined was mapped onto 1.0 mm^2 lined graph paper, viewing the retina with a Zeiss Axioskop compound microscope (40x magnification). For photoreceptor counts, photographs were taken at 100x magnification with a SPOT digital camera (Diagnostic Instruments Inc., Sterling Heights, MI, USA). Photographs were taken at every 1 mm and 0.5 mm (in low and high density areas respectively) and analysed by counting the double and single cone photoreceptors using a standardised grid. For ganglion cells, counts were made at the same distances at 100x magnification, resulting in approximately 150-200 points being sampled across the retina. All clearly identifiable neural elements lying within the ganglion cell layer were counted irrespective of size (Collin and Pettigrew, 1988, 1988b; Litherland and Collin, 2008; Temple et al., 2010; Lee and O'Brian, 2011; Champ et al., 2013). Due to the distinct elongated shape and dense staining (Collin and Pettigrew, 1988c), glial cells were easily identifiable and were excluded from cell counts. Sampling areas at each point were defined by a 10x10 square eyepiece graticule (magnification calibrated for objective used). Cells that touched the top of the left-hand side of the sampling grid were omitted from counts to prevent oversampling, as they would be counted in the previous grid above and to the side.

Several adjustments were made to ganglion cell counts, including accounting for shrinkage and for the inclusion of non-neuronal elements (displaced amacrine cells) (Stone, 1981;

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Curcio et al., 1987; Collin and Collin, 1988; Mednick and Springer, 1988; Hart, 2002; Fritsches et al., 2003). Final counts were then converted to cells per square millimetre. From the data points for both cell densities, topography maps were constructed linking areas of similar cell density (Stone, 1981). In both photoreceptor and ganglion cells estimates, a density map was then created by labelling the number of cells counted at each point to the relevant location on the graphed retinal outline.

Anatomical estimates of visual acuity

Summation ratios were calculated between the density of photoreceptors and that of ganglion cells at overlapping points of each cell layer. With the aid of Matthiessen's ratio (Matthiessen, 1880), the anatomical visual acuity, sometimes referred to as spatial resolving power (SRP) can be calculated. Assuming a square mosaic visual acuity was calculated in the regions of highest cell density for both photoreceptor and ganglion cell layers (for equations see Collin and Pettigrew, 1989). For the anatomical visual acuity estimate it was assumed that all of the photoreceptors (double and single cones) in the mosaic were being used for the animal's visual acuity (Matsuda et al., 2008; Haug et al., 2010; Temple et al., 2010; Champ et al., 2013; Temple et al., 2013).

Behavioural acuity

Apparatus and stimuli

Experiments were run in the home tank of each fish. A transparent Perspex barrier was placed eight centimeters (cm) from the stimuli to control the minimum distance at which the fish could examine the stimuli during experimentation. An opaque, white Perspex separator was attached to the middle of the barrier to ensure that the fish could only see one stimulus at a time once it approached the barrier (Fig. 1A). The barrier was placed in the tank before each session and removed upon completion of the session.

The stimuli consisted of a series of square wave gratings. Gratings were chosen because they have the same brightness and are commonly used to estimate acuity (Yamanouchi, 1956; Nakamura, 1968; Srinivasan and Lehrer, 1988; Macuda et al., 2001; Champ et al., 2013). Widths of gratings (consisting of one black and white bar) ranged from 10 mm (corresponding to 0.17 cpd) during training, and 3.33 mm to 0.87 mm (corresponding to 0.50 and 1.90 cpd, respectively) during testing. Stimuli comprised of equal black and white bars printed on paper and laminated (2x2 cm). When displayed, gratings were always rotated to

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ensure the black bar was not always at the bottom or to the left. This was to ensure fish did not always select the pattern that had a lower dark center of gravity.

Procedure

Training

A number of learning steps, following the protocol described in Siebeck et al (2009), were used to train fish to associate a particular orientation with a food reward. Briefly, fish were trained to initially feed of a feeding tube attached to a plastic syringe (containing a mixture of fish flakes and water). The next step was to associate the food reward with a particular stimulus (henceforth referred to as reward stimulus). Fish were only fed when they interacted by tapping or rubbing their mouth against the reward stimulus. Half the fish (n=3) were trained to select the horizontal grating and the other half (n=3) were trained to the vertical grating. Once fish could successfully associate a food reward with the reward stimulus (by tapping at least two times), and they showed their typical anticipatory behaviour such that the food reward could be delivered at the far end of the tank, a distance barrier was added along with the distracter stimulus. Fish were then required to continue to select the reward stimulus by tapping or rubbing their mouth on the Perspex distance barrier (set at 8 cm away from stimuli) directly in front of the selected stimulus (Fig. 1B). A trial ended when the fish selected the correct stimulus twice in succession. The number of correct and incorrect taps were tallied and used to calculate the frequency of correct choices for each session. Fish were considered to have learned the task when they reached 75% correct choices or greater for at least 5 consecutive sessions.

Testing

Once each fish within both groups fulfilled the criterion for learning, they advanced to testing. A two-alternative forced choice procedure was used to test the fish, following methods described by Siebeck et al (2010). Both the reward and distracter stimuli were shown an equal number of times in each of the two positions on the plank with a constraint that the same stimulus was never presented in the same position more than twice in succession. This was done to prevent development of a side bias. Each session began with placement of the stimulus holder and barrier at the far end of the aquarium, with an opaque board placed in front of the holder to hide the stimuli. Once the fish had moved back to its home the opaque board was removed and the trial started. This was done to ensure the fish had the same viewing distance at the beginning of each trial. The number of taps the fish

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made for each stimulus was recorded. A trial ended when the fish selected the correct stimulus twice in succession. After a correct choice the fish was rewarded using the same method that was used during the training phase. If fish took longer than 2 min to make a decision, the opaque board was placed into the tank and once fish moved to their home, the trial was repeated. If there was still no response from the fish after 2 min, the session was terminated for that individual. The experimenter would return to this individual to complete the session only after all other fish had completed a full session.

The gratings consisted of vertical and horizontal black and white striped patterns with spatial frequencies ranging between 0.50 and 1.90 cpd. The patterns were presented in frequency-matched pairs during five sessions. Each session consisted of eight trials resulting in 40 choices per spatial frequency. Testing began with the lowest spatial frequency (0.50 cpd) and then proceeded to successively higher spatial frequencies until the fish could no longer discriminate between the two orientations. The threshold criterion was set at 72.5% correct choices. Upon completion of the experiment, fish were given a further five sessions with the lowest spatial frequency to ensure the decrease in performance was not due to a lack of motivation.

Calculation of behavioural visual acuity

The spatial frequency (SF) of teach pattern was calculated based on the grating size and viewing distance using the following formula adapted from Nakamura (1968):

$$SF = \frac{1}{\left(2tan^{-1}\left(\frac{0.5CW}{D}\right)\right)\left(\frac{180}{\pi}\right)} \quad (1)$$

Where CW is the width of one cycle (in mm) and D is the minimum viewing distance (96 mm). One cycle is defined as the combined width of one black and one white band. The distance of discrimination in these experiments included the distance from the decision point to the placement of the stimuli set at 80 mm (Fig. 1B) and the anterior nodal point (the distance from the end of the snout to the anterior surface of the cornea – 16 mm).

Statistical analysis

The number of correct and incorrect taps were tallied and used to calculate the percentage of correct choices for each spatial frequency tested. Data for each fish were fit with a logistic

function using the Palamedes toolbox (version 0.8.1. Prins and Kingdom, 2009) for Matlab (Mathworks version 2014b). Slope and threshold parameters were allowed to freely vary. The guess rate was fixed at 50% and the lapse rate set such that the function asymptote matched the maximum performance level of each individual fish across all frequencies. From these functions, the threshold of discrimination was then calculated for each fish based on achieving a level of accuracy of 72.5% (chosen as the threshold for significant deviation from chance in a 2AFC, based on a binomial test, n=40, $p<0.01$).

Results

Anatomical estimates of visual acuity

The spatial distributions of photoreceptor and ganglion cells were measured for both *Pomacentrus amboinensis* and *Pseudochromis fuscus*. Standard body lengths were on average larger in *Pomacentrus amboinensis* (9 cm ± 0.36) than *P. fuscus* (7.23 cm ± 0.33). The mean size of the eye and lens was larger in *Pomacentrus amboinensis* (length: 6.65 mm ± 1.9 mm; lens: 2.32 ± 0.1 mm) than in *Pseudochromis fuscus* (length: 4.62 mm ± 0.22; lens: 1.6 mm ± 0.15). Within the photoreceptor layer, cone arrangements in both species generally followed a square mosaic pattern (Fig. 2C), with a single central cone, surrounded by four double cones and at times accessory single cones in the corners of the configuration. Row mosaics were also found in areas of lower cell densities (Fig. 2A). Two distinct size groups of ganglion cells were found within the retina of both species (Fig. 2B,D), but were combined into a single density count.

The representative isodensity topography maps of both total photoreceptor cell populations match well with ganglion cell populations within both species, with arrangement of the recorded centro-peripheral cell density gradients varying topographically and quantitatively between *Pomacentrus amboinensis* (Fig. 3A,B) and *P. fuscus* (Fig. 3C,D). *Pomacentrus amboinensis* had a peak density of cones (28,850 ± 2511 cells/mm^2) and ganglion cells (23,100 ± 2617 cells/mm^2) in the dorso-temporal region of the retina that extended into the centro-temporal region of the retina. A smaller, secondary peak of cell density was also found in the nasal retinal quadrant. In contrast to *Pomacentrus amboinensis*, *Pseudochromis fuscus* featured a slight horizontal streak across the dorsal meridian, containing two *area centralis* with the highest density of cones (33,750 ± 3572 cells/mm^2) and ganglion cells (26,550 ± 4791 cells/mm^2) found in the dorso-temporal quadrant.

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Based on our measurements, the visual acuity calculated from cone photoreceptor counts were generally higher than those estimated from ganglion cell densities. The visual acuity estimates based on cone photoreceptor counts ranged from 4.6 ± 0.35 cpd and 3.6 ± 0.27 cpd for *Pomacentrus amboinensis* and *Pseudochromis. fuscus* respectively. The visual acuity estimates based on ganglion cell density was 4.1 ± 0.36 cpd for *Pomacentrus amboinensis* and 3.2 ± 0.32 cpd in for *Pseudochromis fuscus*. Summation ratios between photoreceptors and ganglion cells varied from 1.4:1 in the *areae* to more than 2.9:1 in the peripheral retina in *Pomacentrus amboinensis*, and 1.6:1 in the *areae* to more than 3.5:1 in *Pseudochromis fuscus*.

Behavioural limit of visual resolution

For each species, the percentage of correct choices (based on the tapping distribution) for all fish was grouped and shown for each spatial frequency (Fig. 4). For *Pomacentrus amboinensis*, all individuals (*n*=6) preformed best at the lowest spatial frequency at 0.50 cpd, reaching on average 81% (correct choice frequencies for the group ranged between 80 – 93% correct). The group maintained this level of accuracy until 1.25 cpd at which point discrimination rate decreased to 78% ± 2.23. Acuity thresholds for individual *Pomacentrus amboinensis* ranged between 1.29 – 1.36 cycles per degree (72.5% correct choices, binomial test, n=40, $p<0.01$). A similar pattern was found for *Pseudochromis fuscus* individuals (*n*=6). The highest accuracy was observed at the lowest spatial frequency tested (0.50 cpd) reaching between 75 – 93% correct. Performance was maintained for the first five spatial frequencies tested, then dropped rapidly. Acuity thresholds of individual *Pseudochromis fuscus* ranged between 1.61 – 1.71 cycles per degree (72.5% correct choices, binomial test, n=40, $p<0.01$).

There was no significant difference in the acuity threshold for the two groups (horizontal or vertical trained) for either species of fish (Wilcoxon Rank Sum = 11, n1 = n2 = 3, n.s. two-tailed, in both cases). Overall the median threshold for *Pseudochromis fuscus* was 1.69 cpd and for *Pomacentrus amboinensis* was 1.29 cpd with the distributions for the two species being significantly different (Wilcoxen Rank Sum = 21, n1 = n2 = 6, $p = 0.0022$ two-tailed).

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Discussion

The aim of this study was to assess the validity of anatomical approaches to estimating visual acuity and to compare measures across species. Our approach involved a combination of classical anatomical and behavioural techniques to estimate the visual acuity of two coral reef species that live in the same visual environment but have different visual behaviours. To facilitate comparisons with other studies we employed both photoreceptor and ganglion cell counts to calculate the anatomical limit of acuity. In practice the two anatomical measures produced similar results, with the ganglion cell measure providing slightly more conservative estimates. As the difference between these two retinal layers reflects the convergence of visual information between the two layers, we focus on the acuity calculated from the ganglion cells in all subsequent comparisons with the behavioural measures.

Pomacentrus amboinensis was found to have a behavioural acuity of 1.29 cpd, which was significantly lower than the anatomical limit of 4.1 cpd. *Pseudochromis fuscus* had a slightly higher behavioural acuity of 1.69 cpd but a lower anatomical acuity of 3.6 cpd. The acuity and retinal topography assessed in both species fits within the typical range of shallow water reef fish (Collin and Pettigrew, 1989; Champ et al., 2013). Our results indicate that, despite the fact that the social system of *Pomacentrus amboinensis* requires that they discern the fine detail of facial patterns of conspecific individuals, their visual abilities are similar to that of *Pseudochromis fuscus*. Based on their behavioural acuity, *Pomacentrus amboinensis* can likely resolve the larger components of their facial patterns (approx. 3 mm in diameter) in distances up to 44 cm away (for calculation methods see Marshall, 2000). The smaller facial pattern components around the eye (≤ 1 mm diameter) can be resolved at distances of less than 15 cm. The predator, *Pseudochromis fuscus* on the other hand, has the potential to resolve pattern components of this size up to 62 cm and 21 cm, respectively.

It is important to note that our observed acuity measurements are based on data collected from broad-spectrum patterns viewed in well-lit, clear waters of our aquarium system. To understand the full visual capability of a species, including their ability to discern natural patterns and objects, other visual elements must be taken into account. For example, how a species' visual system processes the contrast between the pattern component/object and its background can affect how a pattern/object is perceived. In addition, contrast depends on a range of variables, including pattern wavelengths and water quality, factors which need to be carefully evaluated when considering specific abilities performed in the context of their

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habitat (Lythgoe, 1979; Douglas and Djamgoz, 1990).

Looking beyond this study, one should be a little wary when attempting to compare the behavioural results of different studies because visual estimates can depend on the specific method used. Of the 13 fish species for which both anatomical and behavioural acuity has been measured, anatomical estimates of acuity were higher than behavioural acuity in 7 cases and similar in 4 cases (see table 1). The discrepancy could be due to the dependency of the behavioural results on the particular paradigm and stimuli used to determine functional acuity. Srinivasan and Lehrer (1988) found different outcomes in the acuity of honeybees depending on whether radial (sectored) or linear (square-wave) gratings were used. Srinivasan (1988) suggested that the higher acuity found with square-wave gratings is likely due to motion cues as freely-flying bees may experience greater stimulation of horizontal motion sensitive cells when approaching vertical gratings, and vice versa. Triggerfish have also been noted to perform poorly in acuity tests when trained to circular stimuli as opposed to grating stimuli (Champ et al., 2013). The authors suggested that this might be due to the fact that circular stimuli used in the experiment subtended a smaller angle on the retina than the much larger grating stimuli. Overall, it appears that there is no perfect way to measure functional acuity as a number of factors, such as the experimental paradigm as well as the stimuli used can influence acuity measures. For example, the ability to detect the misalignment of two lines yields a measure of hyperacuity rather than acuity. In humans this task can be solved by single ganglion cells that are potentially much bigger than the stimulus (Westheimer, 1976; Fahle, 2002). Also, studies in humans suggest that the acuity measured using the Landolt-C test produce values, which are around twice as high as those established using more conventional gratings stimuli. This can be explained by the fact that the C-shaped test patterns also possessing energy in frequencies lower than the gap in the test shape (Bondarko and Danilova, 1997). This, in turn, might explain why one study reported similar results for anatomical and behavioural measures (3.23 – 3.52 cpd) (Temple et al., 2013). The Landolt-C may not be a valid measure of acuity as a different mechanism may be at work (i.e. hyperacuity) as this task could be solved by single ganglion cells that are potentially much bigger than the stimulus. Overall, it may be possible that the behavioural acuity measured in our study could be improved to match anatomical acuity if a different type of behavioural test or different stimulus set were used. For example, by repeating the experiment using a grating that is tested against a uniform grey stimulus, this may reduce the discrepancy between behavioural and anatomical results (Reymond, 1985, 1987; Neumeyer,

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2003b). Using this method, Neumeyer (2003b) found similar results when measuring acuity in the goldfish. However, it may be possible that there were slight differences in brightness between the stimuli that the goldfish in fact were basing their decision on brightness discrimination at spatial frequencies beyond their acuity.

On the other hand, it is possible that the anatomical values in our case in fact overestimate acuity if the assumption of the validity of Matthiessen's ratio is incorrect for the study species (Matthiessen, 1880). Matthiessen's ratio of 2.55 is commonly used to calculate anatomical visual acuity and was also used in this study. Matthiessen found that although focal lengths varied between 2.2 and 2.8 lens radii, nearly all fish have focal lengths of about 2.5 lens radii (Walls, 1943). However, even if ratios were reduced to 2.2 in both species studied here, acuity is only reduced by ~0.4 cpd. Furthermore, for anatomical acuity to match the acuity estimated from behavioural experiments, focal length would need to be reduced to <0.5, which is unlikely. On thing that is certainly true is that the anatomical methods fail to take into account any processing that occurs beyond the retina. In the case of hyperacuity this later processing stage is crucial to determining behavioural ability.

The analysis of retinal topography in both species shows similar areas of visual importance which is not surprising as it fits with 'terrain theory' described by (Hughes, 1977). According to this theory the topography of cells across the retina is a representation of the symmetry of the habitat (Hughes, 1977). Species living in complex environments, such as coral reefs, often possess peak densities of retinal ganglion cells, known as *area centralis*, in the temporal or dorso-nasal visual field (Collin and Pettigrew, 1988). In contrast, pelagic species or ones that live over sandy bottoms near coral reefs have an uninterrupted view of the sand-water horizon and tend to possess a *horizontal streak* of high cell density (Collin and Pettigrew, 1988b). Both species investigated here fit the general pattern found for individuals coexisting in the same highly complex visual environment such as the reef (Collin and Pettigrew, 1988; Lee and O'Brian, 2011; Champ, 2012).

The highest area of cell density recorded for *Pomacentrus amboinensis* was found in the dorso-temporal region (extending centrally), with a slightly smaller area found in the dorso-nasal region. The location of high cell density in *Pomacentrus amboinensis* is consistent with Ali and Anctil's (1976) findings on other species from the same family (Pomacentridae). For *Pomacentrus amboinensis*, the location of these areas may facilitate the detection of small

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objects, such as algae on the substrate and/or zooplankton within the water column. Furthermore, the extension of this *area centralis* to the central field may enhance individual and species recognition of fish which can be best identified using their patterns (Siebeck, 2004; Siebeck et al., 2010). In addition, the dorso-nasal region with slightly lower cell density projects to the caudal visual space. Collin and Pettigrew (1988) suggest that this *area centralis*, found also in the Staghorn damselfish, *Amblyglyphidodon curacao* may enable fish to negotiate the finger-like projections of the staghorn coral in escape situations. This zone may also be important for predator detection and/or territorial defense. As *Pomacentrus amboinensis* live in small groups with a single dominant male surrounded by a number of females, this *area centralis* may help males to keep an eye on their females and eggs from potential predators (McCormick, 1999).

Pseudochromis fuscus was found to possess a high density of cells across the dorsal meridian and two small areas of high cell density in the dorsal-temporal region of the visual field. A band of high cell density across the meridian indicated the presence of a weak *horizontal streak* with a prominent area of high cell density in the same location as *Pomacentrus amboinensis*. This increase of cell density across the retinal meridian may allow *Pseudochromis fuscus*, a sit and wait predator, to maintain their position and spend their time scanning their environment for benthic prey and potential threats. The area of high cell density found in the dorsal-temporal region, may enhance prey capture once prey is detected, which has also been described in *Balistoides conspicillum* and *Aulostoma chinensis* (Collin and Pettigrew, 1988b).

It is assumed that predatory fish have higher acuities than prey fish (Collin and Pettigrew, 1989). Our results show comparable values for both species although note that the behavioural measures, at least, were statistically significantly different and not in the direction predicted by Collin and Pettigrew (1.29 cpd and 4.1 cpd recorded for *Pomacentrus amboinensis*; 1.69 cpd and 3.6 cpd recorded for *Pseudochromis fuscus* using anatomical and behavioural measures, respectively). In practice, the differences are minor when the size of fish (in particular lens size) is taken into account. A number of studies record visual acuities that vary in proportion to eye size and lens diameter (Walls, 1943; Collin and Pettigrew, 1989; Kiltie, 2000; Bozzano et al., 2001). Having a larger lens provides fish with the ability to detect smaller prey or the same size prey at a greater distance (Bozzano et al., 2001). In this study, the lens size in *Pomacentrus amboinensis* was on average slightly larger than

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Pseudochromis fuscus, resulting in the higher anatomical acuity. If *Pseudochromis fuscus* possessed the same lens size as *Pomacentrus amboinensis* acuity would only vary between both species by ~0.2 cpd which is a negligible difference.

When considering the different values of acuity one should bear in mind that the acuities were calculated based on the controlled minimum distance. Although this was fixed throughout experiments it is not known at what distance the fish made the decision about which stimulus to choose. Whilst it could be argued that fish may have made their decision from their home this is unlikely in this case. Generally speaking, during each trial fish would often move towards the perspex barrier looking at both stimuli before making a choice. Furthermore, recalculating the acuity from the added distance the home may give (i.e 5 cm) acuity would only be increase from 1.35 cpd to 2.05 cpd in *Pomacentrus amboinensis* and from 1.71 cpd to 2.6 cpd in *Pseudochromis fuscus* (values based on highest acuity recorded in each species). Whilst this does increase the acuity value a discrepancy is still found within both anatomical and behavioural results.

The behavioural visual acuity estimates for *Pomacentrus amboinensis* and *Pseudochromis fuscus* are similar to those found in the goldfish (Hester, 1968; Wilkinson, 1972; Neumeyer, 2003b) and triggerfish (Champ et al., 2013). These studies reported acuities between 1.5 and 2.0 cpd. Interestingly, these values are based on different arbitrary thresholds for calculating behavioural acuity. In fact, in addition to the previously described sources for variability in the reported behavioural acuity measures, some variability in the literature is also likely to stem from the definition of the threshold. Detection thresholds are most commonly set at 70% and 75% discrimination accuracy (Hodos and Yolen, 1976; Neumeyer, 2003b; Temple et al., 2013). However, detection thresholds as high as 80% (Weiler, 1966) and as low as 65% accuracy (Champ et al., 2013) have also been reported. It is possible that pre-determined thresholds may contribute to the mismatch between values for anatomical and behavioural acuity. If we applied the threshold criterion of 65% used by Champ (2013) to our study, behavioural acuity would be increased to 1.44 and 1.76 cpd from 1.29 cpd and 1.69 cpd for *Pomacentrus amboinensis* and *Pseudochromis fuscus* respectively. We followed recently published studies in which a threshold of 72.5% was used (Lind and Kelber, 2011; Potier et al., 2016). It is important to remember that by increasing the number of sessions, any accuracy level different from chance can become statistically significant, and that therefore the threshold also depends on the number of trials and sessions carried out. We used 40 trials

per spatial frequency, which was identical to Lind and Kelber (2011) but higher than Potier (2016).

In summary, this study is one of three studies that we know of that explores visual acuity for more than one species of reef fish using both behavioural and anatomical methods. We have highlighted some of the problems associated with the different methods used in the field and believe that there is a need to standardize methods to facilitate comparison and knowledge transfer across species. It is likely that the large differences observed between behavioural and anatomical visual acuity are not only a reflection of the optical properties (i.e. lens size, focal length) of individuals or species but are in fact indicative of mechanisms that occur post retina, however, this requires further experimentation. Our data also suggest that environmental complexity may be a more important driver for visual acuity than life history, as the predator and prey species studied here were found to have similar visual capabilities.

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Acknowledgements

We would like to thank staff at Lizard Island Research station for their support and use of equipment. We also thank the Marshall and Collin labs for the use of equipment throughout the anatomical studies. In particular, we also thank Darryl Whitehead from SBMS histology for his help with the preparation of ganglion cells for the anatomical component. We thank Connor Champ for valuable feedback.

Competing interests

No competing interests declared

Author contributions

A.N.P. and U.E.S. designed the behavioural study. A.N.P. and K.A.F. designed the anatomical study. A.N.P carried out the experiments. G.W analysed the behavioural results. A.P drafted the original manuscript. A.N.P., K.A.F., C.N., G.W and U.E.S. helped revise it.

Funding

This research was supported by the Australian Research Council (grant # DP140100431).

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References

Aho, A. C. (1997). The visual acuity of the frog (*Rana pipiens*). *Journal of Comparative Physiology A* **180**, 19-24.

Ali, M. A. and Anctil, m. (1976). Retinas of fishes. An atlas. Berlin: Springer-Verlag.

Bondarko, V. M. and Danilova, M. V. (1997). What spatial frequency do we use to detect the orientation of a Landolt C? *Vision Res.* **37**, 2153-2156.

Bozzano, A., Murgia, R., Vallerga, S., Hirano, J. and Archer, S. (2001). The photoreceptor system of the retinae of two dogfishes, *Scyliorhinus canicula* and *Galeus melastromus*: possible relationship with depth and predatory lifestyle. *Journal of Fish Biology* **59**, 1258-1278.

Browman, H. I., Gorden, W. C., Evans, B. I. and O'brian, W. J. (1990). Correlation between histological and behavioural measures of visual acuity in a zooplanktivorous fish, the white crappie (*Pomoxis annularis*). *Brain, Behaviour and Evolution* **35**, 85-97.

Brunner, G. (1934). Uber die Seh-scharfe der Elritze (*Phoxinus laevis*) bei verschiedenen Helligkeiten. *Zeitschrift fuer Vergleichende Physiologie* **21**, 296-316.

Carton, A. G. and Vaughan, M. R. (2010). Behavioural and anatomical measures of visual acuity in first-feeding yellowtail kingfish (*Seriola lalandi*) larvae. *Environmental Biology of Fishes* **89**, 3-10.

Carvalho, L. S., Noltie, D. B. and Tillitt, D. E. (2004). Biochemical, histological and behavioural aspects of visual function during early development of rainbow trout. *Journal of Fish Biology* **64**, 833-850.

Champ, C., Wallis, G., Vorobyev, M., Siebeck, U. E. and Marshall, J. (2013). Visual acuity in a species of coral reef fish: *Rhinocanthus aculeatus*. *Brain Behav. Evol.* **83**, 31-42.

Champ, C. M. (2012). Colour and spatial vision in the reef fish, *Rhinecanthus aculeatus*, vol. Ph.D Thesis. Brisbane: University of Queensland.

Collin, S. P. and Collin, H. B. (1988). The morphology of the retina and lens of the sandlance, *Limnichthyes fasciatus* (Creeiidae). *Experimental Biology* **47**, 209-218.

Collin, S. P. and Pettigrew, J. D. (1988). Retinal topography in reef teleosts. I. Some species with well-developed areae but poorly developed streaks. *Brain, Behaviour and Evolution* **31**, 269-282.

Collin, S. P. and Pettigrew, J. D. (1988b). Retinal topography in reef teleosts. II. Some species with prominent horizontal streaks and high-density areae. *Brain, Behaviour and Evolution* **31**, 283-295.

Collin, S. P. and Pettigrew, J. D. (1988c). Retinal ganglion cell topography in teleosts: a comparison between nissl-stained material and retrograde labelling from the optic nerve. *The Journal of Comparative Neurology* **276**, 412-422.

Collin, S. P. and Pettigrew, J. D. (1989). Quantitative comparison of the limits on visual spatial resolution set by the ganglion cell layer in twelve species of reef teleosts. *Brain, Behaviour and Evolution* **34**, 184-192.

Cortesi, F., Feeney, W. E., Ferrari, M. C., Waldie, P. A., Phillips, G. A., McClure, E. C., Skold, H. N., Salzburger, W., Marshall, N. J. and Cheney, K. L. (2015a). Phenotypic plasticity confers multiple fitness benefits to a mimic. *Curr. Biol.* **25**, 949-954.

Cortesi, F., Musilová, Z., Stieb, S., Hart, N. S., Siebeck, U. E., Malmstrøm, M., Tørresen, O. K., Jentoft, S., Cheney, K. L., Marshall, J. N. et al. (2015b). Ancestral duplications and highly dynamic opsin gene evolution in percomorph fishes. *Proceedings of the national academy of sciences* **112**, 1493-1498.

Cott, H. B. (1940). Adaptive coloration in animals. London, UK: Methuen.

Cripps, I., Munday, P. L. and McCormick, M. I. (2011). Ocean acidification affects prey detection by a predatory reef fish. *Plos One* **6**, 1-7.
Curcio, C. A., Packer, O. and Kalina, R. E. (1987). A whole mount method for sequential analysis of photoreceptor and ganglion cell topography in a single retina. *Vision Res.* **27**, 9-15.
Dickinson, A., Balleine, B., Watt, A., Gonzalez, F. and Boakes, R. A. (1995). Motivational Control after Extended Instrumental Training. *Anim Learn Behav* **23**, 197-206.
Dobberfuhl, A. P., Ullmann, J. F. P. and Shumway, C. A. (2005). Visual acuity, environmental complexity, and social organisation in african cichlid fishes. *Behavioural Neuroscience* **119**, 1648-1655.
Douglas, R. and Djamgoz, M. (1990). The visual system of fish. London: Chapman and Hill.
Douglas, R. H. and Hawryshyn, C. W. (1990). Behavioural studies of fish vision: an analysis of visual capabilities. In *The Visual System of Fish*, eds. R. H. Douglas and M. B. A. Djamogoz), pp. 373-418. Cambridge, Great Britain: Chapman and Hall.
Endler, J. A. (1990). On the measurement and classification of colour in studies of animal colour patterns. *Biological Journal of the Linnean Society* **41**, 315-352.
Endler, J. A. (1991). Variation in the appearance of guppy color patterns to guppies and their predators under difference visual conditions. *Vision Res.* **31**, 587-608.
Endler, J. A. (1993). The colour of light in forests and its implications. *Ecological Monographs* **63**, 1-27.
Fahle, M. (2002). Learning to perceive features below the foveal photoreceptor spacing. In *Perceptual Learning* eds. M. Fahle and T. Poggio). Cambridge: The MIT Press.
Fritsches, K. A., Marshall, J. N. and Warrent, E. J. (2003). Retinal specializations in the blue marlin: eyes designed for sensitivity to low light levels. *Marine and Freshwater Research* **54**, 333-341.
Hairston, N. G., Li, K. T. and Easter, S. S. (1982). Fish vision and detection of planktonic prey. *Science* **218**, 1240-1242.
Harman, A. M., Nelson, J. E., Crewther, S. G. and Crewther, D. P. (1986). Visual acuity in the nothern native cat (*Dasyurus hallucatus*) - behavioural and anatomical estimates. *Behav. Brain Res.* **22**, 211-216.
Hart, N. S. (2002). Vision in the peafown (Aves: *Pavo cristatus*). *The Journal of Experimental Biology* **205**, 3925-3935.
Haug, M. F., Biehlmaier, O., Mueller, K. P. and Neuhauss, S. C. F. (2010). Visual acuity in larval zebrafish: behaivor and histology. *Frontiers in Zoology* **7**.
Hester, F. J. (1968). Visual contrast threshold of the goldfish (*Carassius auratus*). *Vision Res.* **8**, 1315-1336.
Hodos, W. and Yolen, N. M. (1976). Behavioral correlates of 'tectal compression' in goldfish. II. Visual acuity. *Brain, Behaviour and Evolution* **13**.
Hughes, A. (1977). The topography of vision in mammals of contrasting life style: comparative optics and retinal organisation. In *The visual system of vertebrates*, vol. VII (ed. F. Crescitelli), pp. 613-756. Berlin: Springer.
Jarvis, J. R., Abeyesinghe, S. M., McMahon, C. E. and Wathes, C. M. (2009). Measuring and modelling the spatial contrast sensitivity of the chicken (Gallus g. domesticus). *Vision Res.* **49**, 1448-1454.
Kelley, J. L. and Magurran, A. E. (2003). Learned predator recognition and antipredator responses in fishes. *Fish and Fisheries* **4**, 216-226.

Kiltie, R. A. (2000). Scaling of visual acuity with body size in mammals and birds. *Functional Ecology* **14**, 226-234.
Kino, M., Miayzaki, T., Iwami, T. and Kohbara, J. (2009). Retinal topography of ganglion cells in immature ocean sunfish, *Mola Mola*. *Environmental Biology of Fishes* **85**, 33-38.
Lee, H. R. and O'Brian, K. M. B. (2011). Morphological and behavioral limit of visual resolution in temperate (*Hippocampus abdominalis*) and tropical (*Hippocampus taeniopterus*) seahorses. *Vis. Neurosci.* **28**, 351-360.
Lind, O. and Kelber, A. (2011). The spatial tuning of achromatic and chromatic vision in budgerigars. *Journal of Vision* **11**, 1-9.
Litherland, L. L. and Collin, S. P. (2008). Comparative visual function in elasmobranchs: spatial arrangement and ecological correlates of photoreceptor and ganglion cell distributions. *Vis. Neurosci.* **25**, 549-561.
Lythgoe, J. N. (1979). The Ecology of Vision. New York: Oxford University Press.
Macuda, T., Gegear, R. J., Laverty, T. M. and Timney, B. (2001). Behavioural assesment of visual acuity in bumblebees. *The Journal of Experimental Biology* **204**, 559-564.
Marc, R. E. and Sperling, H. G. (1976). The chromatic organisation of the goldfish cone mosaic. *Vision Res.* **16**, 1211-1224.
Marshall, J. (2000). Communication and camouflage with the same 'bright' colours in reef fishes. *Philosophical transactions of the Royal Society of London. B* **355**, 1243-1248.
Marshall, J. N. and Vorobyev, M. (2003). The design of color signals and color vision in fishes. In *Processing in the Aquatic Environment*, eds. S. P. Collin and J. N. Marshall), pp. 194-222. New York: Springer.
Matsuda, K., Torisawa, S., Hiraishi, T. and Yamamoto, K. (2008). Comparison of visual acuity and visual axis of three flatfish species with different ecotypes. *Fisheries science* **74**, 562-572.
Matsuda, K., Torisawa, S., Hiraishi, T., Nashimoto, K. and Yamamoto, K. (2005). Visual acuity and spectral sensitivity of the elkhorn sculpin *Alcichtys alcicornis*. *Fisheries Science* **71**, 1136-1142.
Matthiessen, L. (1880). Untersuchungen iiber dem aplanatismus und die periscopie der kristallinsen in den augen der fische. *Pflugers Arch* **21**, 287-307.
McCormick, M. I. (1999). Experimental test of the effect of maternal hormones on larval quality of a coral reef fish. *Oecologia* **118**, 412-422.
McCormick, M. I. and Meekan, M. G. (2007). Social facilitation of selective mortality. *Ecology* **88**, 1562-1570.
Mednick, A. S. and Springer, A. D. (1988). Asymmetric distribution of retinal ganglion cells in goldfish. *The Journal of Comparative Neurology* **268**, 49-59.
Mosk, V., Thomas, N., Hart, N. S., Partridge, J. C., Beazley, L. D. and Shand, J. (2007). Spectral sensitivities of the seahorses *Hippocampus subelongatus* and *Hippocampus barbouri* and the pipefish *Stigmatopora argus*. *Vis. Neurosci.* **24**, 345-354.
Nakamura, E. L. (1968). Visual acuity of two tunas, *Katsuwonus pelamis* and *Euthynnus affinis*. *Copeia* **1968**, 41-49.
Neave, D. A. (1984). The development of visual acuity in larval plaice (*Pleuronectes platessa* L.) and turbot (*Scophthalmus maximus* L.). *Journal of Experimental Marine Biology and Ecology* **78**, 167-175.
Neumeyer, C. (2003a). Color vision in fishes and its neural basis. In *Processing in the Aquatic Enivironment*, eds. S. P. Collin and J. N. Marshall). New York: Springer.

Neumeyer, C. (2003b). Wavelength dependence of visual acuity in goldfish. *Journal of Comparative Physiology A* **189**, 811-821.
Niv, Y., Joel, D. and Dayan, P. (2006). A normative perspective on motivation. *Trends Cogn Sci* **10**, 375-381.
Pankhurst, P. M., Pankhurst, N. W. and Montgomery, J. C. (1993). Comparison of behavioural and morphological measures of visual acuity during ontogeny in a teleost fish, *Forsterygion varium*, Tripterygiidae (Forster, 1801). *Brain, Behaviour and Evolution* **42**, 178-188.
Potier, S., Bonadonna, F., Kelber, A., Martin, G. R., Isard, P., Dulaurent, T. and Duriez, O. (2016). Visual abilities in two raptors with different ecology. *J. Exp. Biol.* **219**, 2639-2649.
Prins, N. and Kingdom, F. A. A. (2009). Palamedes: Matlab routines for analyzing psychophysical data.
Reymond, L. (1985). Spatial visual acuity of the eagle *Aquila audax*: A behavioural, optical and anatomical investigation. *Vision Res.* **25**, 1477-1491.
Reymond, L. (1987). Spatial visual acuity of the falcon, *Falco berigora*: A behavioural, optical and anatomical investigation. *Vision Res.* **27**, 1859-1874.
Siebeck, U. E. (2004). Communication in coral reef fish: the role of ultraviolet colour patterns in damselfish territorial behaviour. *Animal Behaviour* **68**, 273-282.
Siebeck, U. E. and Marshall, J. N. (2001). Ocular media transmission of coral reef fish - can coral reef fish see ultraviolet light? *Vision Res.* **41**, 133-149.
Siebeck, U. E., Wallis, G. M. and Litherland, L. (2008). Colour vision in a coral reef fish. *J. Exp. Biol.* **211**, 354-360.
Siebeck, U. E., Litherland, L. L. and Wallis, G. (2009). Shape learning and discrimination in reef fish. *J. Exp. Biol.* **212**, 2112-2118.
Siebeck, U. E., Parker, A. N., Sprenger, D., Mathger, L. M. and Wallis, G. (2010). A species of reef fish that uses ultraviolet patterns for covert face recognition. *Curr. Biol.* **20**, 407-410.
Srinivasan, M. V. and Lehrer, M. (1988). Spatial acuity of honeybee vision and its spectral properties. *Journal of Comparative Physiology A* **162**, 159-172.
Stone, J. (1981). The wholemount handbook. Sydney: Maitland Publications.
Tamura, T. and Wisby, W. J. (1963). The visual sense of pelagic fishes especially the visual axis and accommodation. *Bulletin of the Japanese Society of Scientific Fisheries* **13**, 433-448.
Temple, S., Hart, N. S., Marshall, J. and Collin, S. P. (2010). A spitting image: specializations in archerfish eyes for vision at the interface between air and water. *Proceedings of the Royal Society. Series B.* **277**, 2607-2615.
Temple, S. E., Manietta, D. and Collin, S. P. (2013). A comparison of behavioural (Landolt C and anatomical estimates of visual acuity in archerfish (*Toxotes chatareus*). *Vision Res.* **83**, 1-8.
Theiss, S. M., Lisney, T. J., Collin, S. P. and Hart, N. S. (2007). Colour vision and visual ecology of the blue-spotted maskray, *Dasyatis kuhlii* Muller & Henle, 1814. *J Comp Physiol A* **193**, 67-79.
Ullmann, J. F. P., Moore, B. A., Temple, S. E. and Fernández-Juricic E. (2012). The retinal wholemount technique: A window to understanding the brain and behaviour. *Brain Behav. Evol.* **79**, 26-44.
Walls, G. L. (1943). The Vertebrate Eye and its Adaptive Radiation. Bloomfield Hills: Cranbook Press.

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Weiler, I. J. (1966). Restoration of visual acuity after optic nerve section and regeneration, in *Astronotus ocellatus*. *Exp. Neurol.* **16**, 377-386.
Westheimer, G. (1976). Spatial-frequency and light spread descriptions of visual-acuity and hyperacuity. *Journal of The Optical Society of America* **66**, 1078-1078.
Wilkinson, F. (1972). A behavioral measure of grating acuity in the goldfish. Halifax: Dalhousie University.
Yamanouchi, T. (1956). The visual acuity of the coral fish. *Publications of the Seto Marine Biological Laboratory* **2**, 133-156.

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Figures

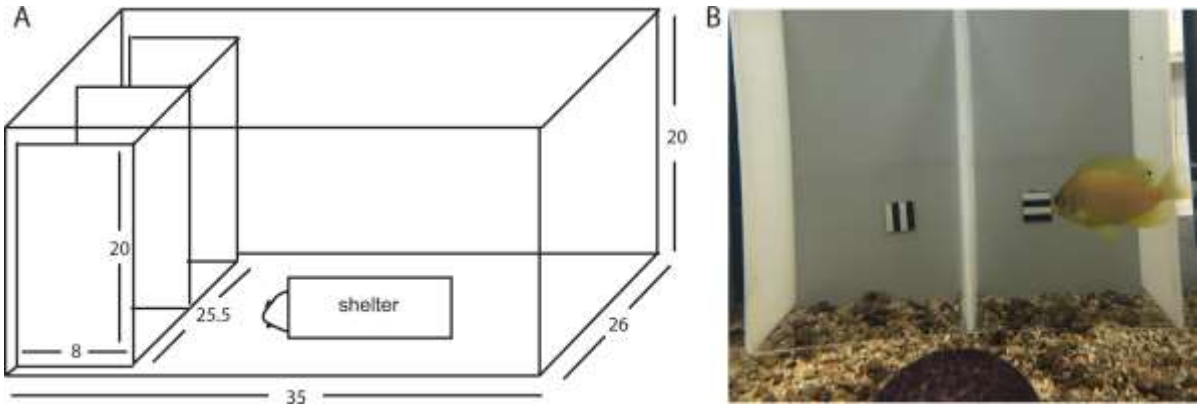

Fig. 1. Experimental apparatus. (A) Schematic of the aquarium showing the Perspex barrier Dimensions are given in centimeters. (B) View from the perspective of the experimenter showing the Perspex barrier, stimuli and a fish performing a trial.

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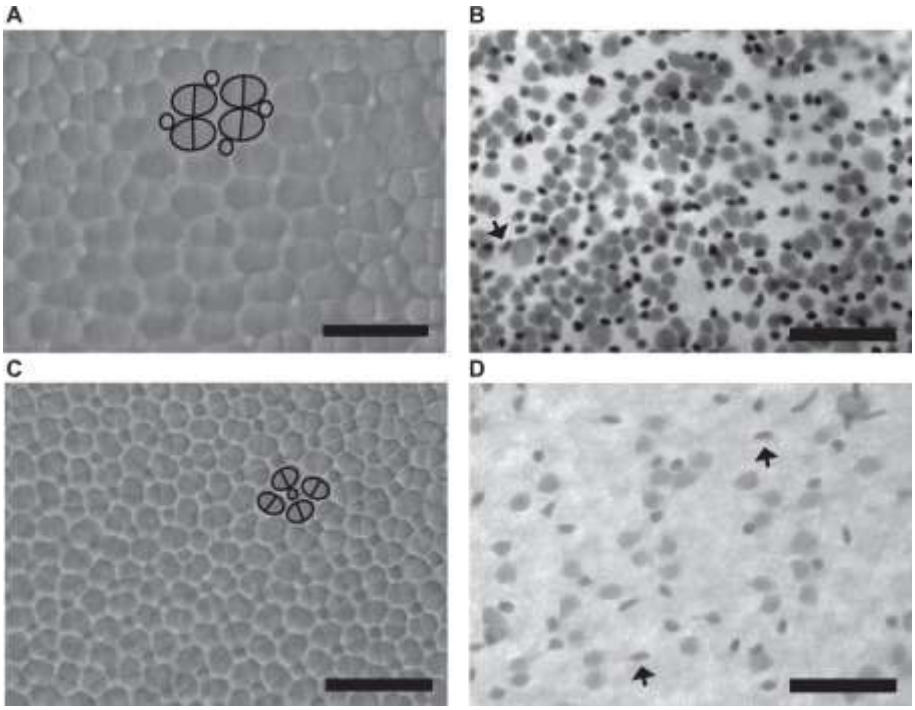

Fig. 2. Photographs showing the photoreceptor (A and C) and ganglion cell layer (B an D) of each species. (A) Row photoreceptor mosaic and (B) high density ganglion cells of *Pomacentrus amboinensis*; and (C) square photoreceptor mosaic and (D) low density ganglion cells of *Pseudochromis fuscus*. Outlines have been included to highlight the position of the double cones (ellipses with central bisectors) and single cones (circles). Arrows point to elongated, cigar-shaped cells presumed to be glial cells, which were not counted in the study. Scale bars = 25μm.

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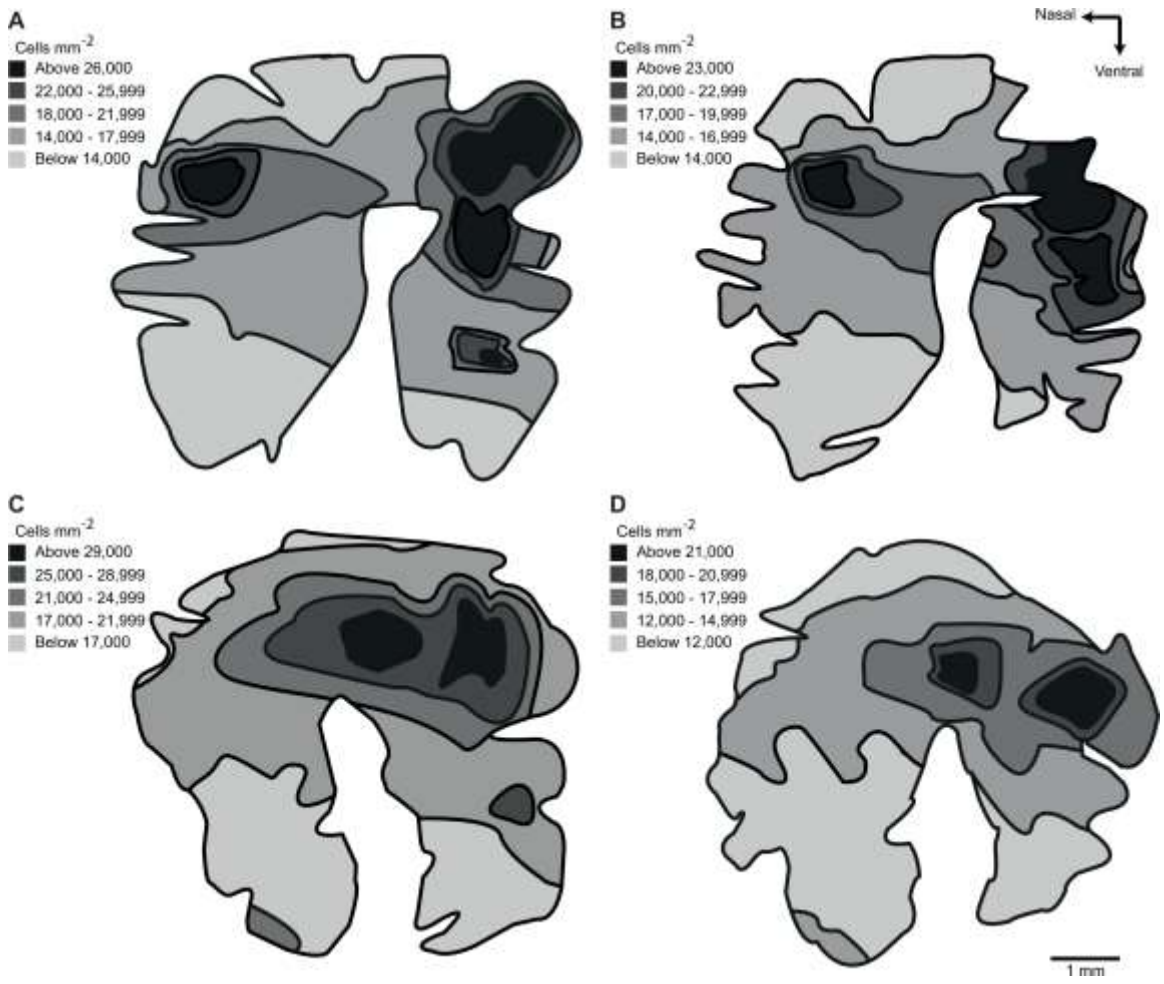

Fig. 3. Representative topographic retinal maps of each species. *Pomacentrus amboinensis* (A) photoreceptors and (B) ganglion cells; and *Pseudochromis fuscus* (C) photoreceptors and (D) ganglion cells.

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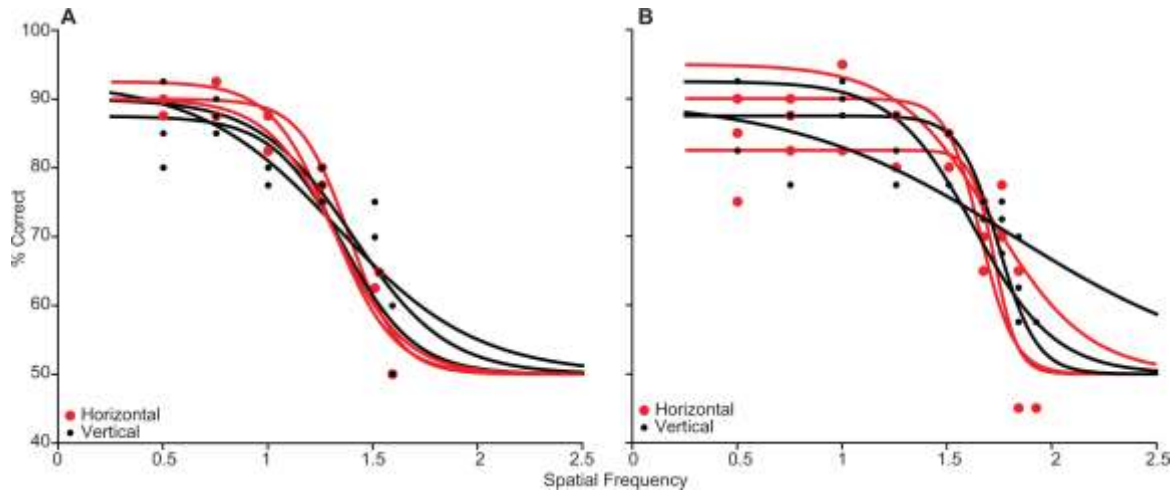

Fig. 4. Results for behavioural acuity experiment. (A) *Pomacentrus amboinensis* and (B) *Pseudochromis fuscus*. Individual dots represent the percentage correct choices of each fish at each of the tested spatial frequencies (n=40). Lines show fitted psychometric functions. Grey represents results obtained from fish trained to horizontal gratings and black from fish trained to vertical gratings.

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Table 1. Visual acuity estimates of fish species for which both anatomical and behavioural estimates were determined.

Species	Common Name	Behaviour	Anatomy	Ecology	Reference
Anatomical acuity based on photoreceptor cells					
Danio rerio	Larval zebrafish	0.16 (OR)	0.24 (PRS)	Freshwater Omnivorous Prey sp.	(Haug et al., 2010)
Toxotes chatareus	Archerfish	3.33 (MDA)	3.57 (PRS)	Freshwater Omnivorous Predatory sp.	(Temple et al., 2013)
Microcanthus strigatus	Convict fish	13.04 (MDS)	16.67 (PRS)	Marine/non-pelagic Omnivorous Prey sp.	(Yamanouchi, 1956)
Lepomis macrochirus	Bluegill sunfish	4.3 (RD)	22.2 (PRS)	Freshwater Omnivorous Prey sp.	(Hairston et al., 1982)
Oncorhynchus mykiss	Rainbow trout	0.625 (OR)	4.35 (PRC)	Marine/Freshwater Carnivorous Predatory sp.	(Carvalho et al., 2004)
Forsterygion varium	Striped triplefin	1.11 (RD)	6.67 (PRC)	Marine/non-pelagic Omnivorous Prey sp.	(Pankhurst et al., 1993)
Phoxinus Laevis	Minnow	5.45 (MDA)	5.45 (PRC)	Freshwater Omnivorous Prey sp.	(Brunner, 1934)
Carassius auratus	Goldfish	2 (MDA)	2.2 (PRC)	Freshwater Herbivorous Prey sp.	(Marc and Sperling, 1976; Neumeyer, 2003a)
Katasuwonus pelamis	Skipjack tuna	10.7 (MDA)	26 (PRC)	Marine/Pelagic Carnivorous Predatory sp.	(Tamura and Wisby, 1963; Nakamura, 1968)

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Anatomical acuity based on ganglion cells					
Hippocampus abdominalis	Big-belly seahorse	0.06 (RD)	6.12 (GCC)	Marine/non-Pelagic Carnivorous Predatory sp.	(Lee and O'Brian, 2011)
Hippocampus taeniopterus	Common seahorse	0.09 (RD)	6.64 (GCC)	Marine/non-Pelagic Carnivorous Predatory sp.	(Lee and O'Brian, 2011)
Rhinocanthus aculeatus	Picasso triggerfish	1.75 (MDA)	3.4 (GCC)	Marine/non-Pelagic Carnivorous Predatory sp.	(Champ et al., 2013)
Astronotus ocellatus	Oscar	11.32 (MDA)	12 (unknown)	Freshwater Omnivorous Predatory sp.	(Weiler, 1966)

All estimates are given in cpd. The method used in each particular study is noted: OR – optomotor/optokinetic response; RD – reactive distance; MDA – minimum distinguishable acuity; PRS – photoreceptor spacing; PRC – photoreceptor counts; GCC – ganglion cell counts.