The role of visual adaptation in cichlid fish speciation

Wright, Daniel Shane

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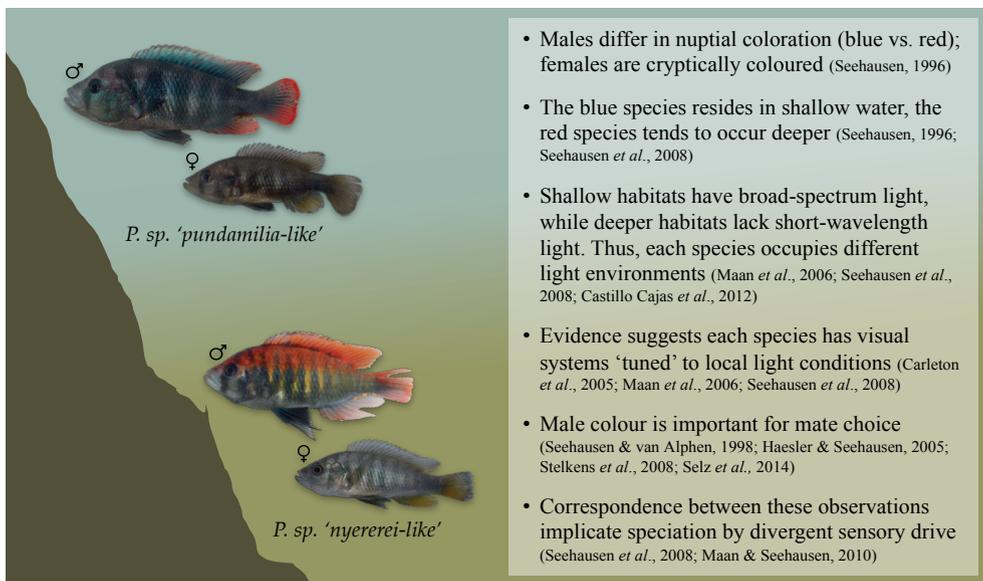
Chapter 7

Synthesis

Daniel Shane Wright

Sensory adaptation to divergent environmental conditions can be consequential for speciation, affecting both ecological performance and sexual communication. Aquatic environments have provided a natural arena to study these processes, as the attenuation of light through water results in distinct light environments, to which vision-dependent species must adapt. Many studies have explored visual adaptation in a number of fish species, from both marine and freshwater environments. In this thesis, I built upon such work, to test the role of visual adaptation in the speciation of *Pundamilia* cichlids from Lake Victoria.

Over the past two decades, great strides have been made to detail many aspects of visual perception and sexual selection in *Pundamilia*. Field and lab studies have combined to generate a wealth of knowledge, resulting in strong correlative evidence to suggest that visual adaptation to the local light environment is a source of divergent selection between sympatrically occurring blue and red forms (see Fig. 7.1). However, experimental evidence linking individual visual properties to behaviour and fitness is lacking. This was the goal of my thesis – to experimentally test how visual adaptation to the local light environment can drive species divergence. In the chapter that follows, I summarize the results of this work and discuss their implications. To end, I provide directions for future research.

- Males differ in nuptial coloration (blue vs. red); females are cryptically coloured (Seehausen, 1996)

- The blue species resides in shallow water, the red species tends to occur deeper (Seehausen, 1996; Seehausen *et al.*, 2008)

- Shallow habitats have broad-spectrum light, while deeper habitats lack short-wavelength light. Thus, each species occupies different light environments (Maan *et al.*, 2006; Seehausen *et al.*, 2008; Castillo Cajas *et al.*, 2012)

- Evidence suggests each species has visual systems 'tuned' to local light conditions (Carleton *et al.*, 2005; Maan *et al.*, 2006; Seehausen *et al.*, 2008)

- Male colour is important for mate choice (Seehausen & van Alphen, 1998; Haesler & Seehausen, 2005; Stelkens *et al.*, 2008; Selz *et al.*, 2014)

- Correspondence between these observations implicate speciation by divergent sensory drive (Seehausen *et al.*, 2008; Maan & Seehausen, 2010)

Figure 7.1. *Pundamilia sp.* in the Mwanza Gulf, Lake Victoria - Blue and red phenotypes of *Pundamilia* occur at many rocky island locations throughout the Mwanza Gulf. In this thesis, we focused on the species pair *P. sp. 'pundamilia-like'* and *P. sp. 'nyererei-like'* from Python Island.

Developmental effects of environmental light on female preference

By rearing both species, *P. sp. 'pundamilia-like'* and *P. sp. 'nyererei-like'*, under light conditions mimicking the shallow vs. deep light environments of Python Island, Lake Victoria, we sought to provoke changes in visual system development, taking advantage of light-induced phenotypic plasticity in the expression of colour-sensitive opsin genes (common in fish). In **chapter 2**, we tested a behavioural consequence of this light manipulation: female mate preference. Prior work in *Pundamilia* had shown that male colour was important for female choice (Seehausen & van Alphen, 1998; Maan *et al.*, 2004; Haesler & Seehausen, 2005; Stelkens *et al.*, 2008; Selz *et al.*, 2014; Svensson *et al.*, 2017), which correlated with species differences in colour sensitivity (Maan *et al.*, 2006) and visual system properties (Carleton *et al.*, 2005; Seehausen *et al.*, 2008). Thus, by manipulating visual development, our goal was to examine the link between changes in visual perception and female colour preference.

We found that the light environment significantly influenced female mate preference: shallow-reared females (both species and hybrids) preferred blue males and deep-reared females tended to prefer red males. We also found that species-assortative preferences broke down when females were reared unnaturally (blue females in deep or red females in shallow). Female preferences were relatively weak (compared to prior studies) but our behavioural measures appear to be good predictors of female mate choice (see Box 1). Thus, our results were consistent with the predictions of divergent sensory drive, suggesting that female preference, and thereby reproductive isolation, is causally linked to heterogeneity in the local light environment. This means that divergence in visual properties might indeed have caused divergence in mate preference as a by-product, as hypothesized for *Pundamilia*. This also has implications for natural systems: changes in local conditions (by environmental change or migration) could influence sexual preferences and disrupt species barriers. However, as we found no effect of test light (females were tested in both light environments), we cannot say that these results were due solely to differences in visual perception - if they were, we would have expected an interaction between rearing light and test light (though the results of chapter 5 suggest visual perception is involved in preference determination; discussed in more detail below). It is also possible that other aspects of our experimental design – selective survival of differentially reared fish (Maan *et al.*, 2017) and/or prior female experience with male phenotypes (e.g. deep-reared females developed in an environment where the perception of blue coloration was not possible prior to experimental trials) – contributed to these results. Nonetheless, chapter 2 highlights how the local light environment experienced during development can affect reproductive isolation between species.

Box 1 – Does female preference predict mate choice?

Previous work in *Pundamilia* has shown that male coloration is important for female preference in both species (Seehausen & van Alphen, 1998; Maan *et al.*, 2004; Haesler & Seehausen, 2005; Stelkens *et al.*, 2008; Selz *et al.*, 2014; Svensson *et al.*, 2017). These studies have used behavioural preference (the observer scores female response to male courtship behaviour) and mate choice designs (preference is deduced from choice of spawning partner), with consistent results from both approaches. In chapter 2, we also used a mate preference design but found relatively weak preferences, with high inter-female variability, and no significant difference between species (prior studies found strong species-assortative preference). We attributed this to the fact that, in contrast to earlier studies, we reared females in manipulated light environments and removed eggs from brooding females ~6 days post fertilization (thus, little opportunity for imprinting; Verzijden & ten Cate, 2007). Nonetheless, this raises the question whether our preference measure was a reliable indicator of subsequent mate choice. To explore this, I designed a new experimental set-up to test if our assessment of female preference corresponds with mating decisions.

As in the prior study, I used a two-way preference design to assay female preference for blue vs. red males. However, I altered the partitions separating males and females to allow interaction. Males were confined behind a two-part barrier; a fixed partition made of rubber coated 'chicken wire' and a removable sheet of transparent plastic. Jointly, the two parts of the barrier mimicked the setup of the prior mate preference study - fish could see each other and exchange chemical cues but not physically interact. When the plastic divider was removed, females could enter the male compartments but males could not exit (the holes in the 'chicken wire' were too small). Thus, the setup allowed sequential testing of female mate preference and female mate choice in the same experimental trial. As in chapter 2, I tested each female for preference behaviour (~20 minutes observation time). I then removed the solid divider to allow the fish to mate (for the remainder of the day). Subject females were chosen based on their gravidity status (on a scale 1-5 as in Seehausen & van Alphen (1998); only 4+ were used) and were tested in light conditions matching their rearing environment (female species identity was random). I tested 27 females, 5 of which spawned with one of the stimulus males (within ~8 hours).

In 4 of 5 trials, females spawned with one of the two males, while in the fifth, the camera failed before mating occurred. For this trial (open circle in Fig. 7.2), I inferred the female spawning decision from the observation that the brooding female was in the compartment with the blue male (had they not spawned, I would have expected the male to be aggressively courting the female – he was not). Female choice matched preference in all cases, except one female that showed an inconsistent preference (for blue in the lateral display-based measure and for red in the quiver-based measure). These results suggest that female preference, as assessed in our setup, is a good predictor of mate choice.
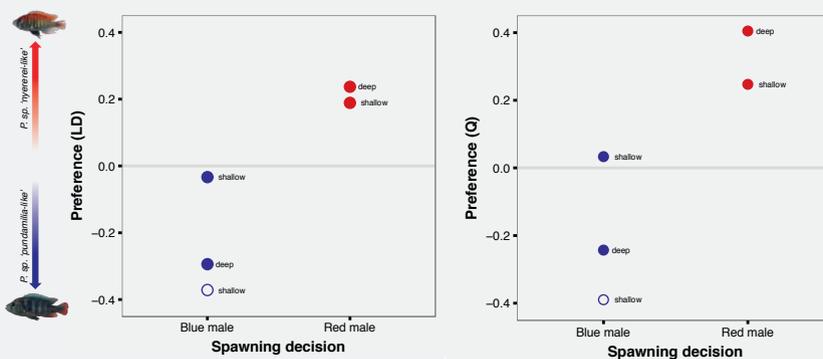
Figure 7.2. Preference is predictive of choice – Female preference correlated with spawning decisions. Solid circles indicate trials confirmed by video evidence (choice was inferred for one trial). The light environment females were reared (and tested) in is given next to each

Developmental effects of the environmental light on male coloration

In addition to light-induced changes in preference, there might be phenotypic plasticity in the expression of male colours, which could be adaptive. For example, males may optimize detectability in their present environment (as shown in other fish: Fuller & Travis, 2004; Lewandowski & Boughman, 2008; Hornsby *et al.*, 2013), thereby influencing mate choice dynamics (and reproductive isolation) in heterogeneous light environments. In **chapter 3**, we tested for plasticity in male nuptial coloration. From standardized photographs, we assessed the colour of differentially reared males (of both species and hybrids) and found little evidence for colour plasticity. We also moved adult males between the two light environments but again found no indications of colour change. Importantly, the species-specific colours, blue vs. red, did not change in response to the light treatments. These results are consistent with the predictions of sensory drive; male colour signals, which are subject to divergent selection by female choice, do not change.

Visual pigment expression covaries with light environment in wild fish

Before examining the underlying visual system properties of our light-manipulated population, it was first necessary to characterize natural variation in opsin expression of wild caught fish and to establish how this variation contributes to divergent visual adaptation. Prior work in *Pundamilia* had detailed allelic variation in the long-wavelength sensitive (LWS) opsin gene (Carleton *et al.*, 2005; Terai *et al.*, 2006; Seehausen *et al.*, 2008) but a systematic analysis of opsin expression patterns was lacking. Only two studies had previously examined *Pundamilia* opsin expression, both of which used few, lab-bred fish, from different populations (Carleton *et al.*, 2005; Hofmann *et al.*, 2009). In **chapter 4**, I sampled blue and red *Pundamilia* males from multiple island locations across southeastern Lake Victoria and reported patterns of opsin expression, in relation to the local light environment and variation in LWS allele type.

We found that species-specific patterns of opsin expression were variable and could not be explained by variation in the light environment. At clearer water locations (i.e. Makobe Island), the deeper occurring, red phenotypes expressed relatively more LWS but at more turbid locations (i.e. Python Island), the shallow-residing, blue phenotypes expressed more LWS. This is despite the fact that LWS genotype differentiation is consistent between the blue and red species at the different locations (Seehausen *et al.*, 2008). Estimates of visual performance by visual modeling showed that the reversal in opsin expression in turbid waters may be beneficial - the Python Island phenotypes (both species) captured more light than the Makobe phenotypes, at both locations - suggesting that the expression profiles in the Makobe phenotypes are suboptimal. However, recent work has given us a better understanding of the (short) evolutionary history of the populations surveyed in chapter 4 (Meier *et al.*, 2017, 2018); our results may reflect this variable history. The blue and red forms at Python Island represent a secondary speciation event, following the hybridization of *P. pundamilia* and *P.*

nyererei from locations outside the Mwanza Gulf (i.e. Makobe Island). Subsequent divergence into a *'pundamilia-like'* blue phenotype and *'nyererei-like'* red phenotype likely corresponded with visual adaptation to turbid photic conditions of the Mwanza Gulf (plus potential hybridization with other Gulf-adapted species). Thus, the results of chapter 4 highlight the need to explore other mechanisms of visual tuning, as well as more sophisticated ways to assess visual performance, to better understand how visual properties adapt and co-evolve in particular environments.

Linking opsin expression, opsin genotype, and mate preference

Having characterized the natural opsin expression profiles of wild caught fish from Lake Victoria, the focus of **chapter 5** returned to our light-manipulated population, to explore the causal link between visual perception and female preference behaviour. As stated above, heterogeneity in the local light environment corresponds with differences in male colour, female mate preference, and visual system properties in *Pundamilia*, thereby implicating sensory drive (Seehausen *et al.*, 2008; Maan & Seehausen, 2010). In chapter 5, we tested for experimental evidence of this hypothesis.

In our lab population, the blue phenotypes expressed relatively more LWS and the red phenotypes expressed relatively more RH2 (middle wavelength sensitive opsin; hybrid expression was intermediate). These patterns were consistent with the species-specific expression profiles we reported for wild fish from Python Island in chapter 4. Independent of species, the light manipulations influenced opsin expression; deep-reared fish expressed more LWS and shallow-reared fish expressed more SWS (short wavelength opsin; the effect for both opsins was strongest in the red types). This result suggests that plasticity in opsin expression may be adaptive, as the expression patterns were reflective of the locally available light (e.g. increased LWS expression in the long wavelength-shifted light environment). However, opsin expression plasticity may not be enough to compensate for underlying genetic differences, as each species (non-hybrids) survived less when reared in the 'wrong' light environment (blue fish in deep or red fish in shallow; Maan *et al.*, 2017).

There was a weak, positive relationship between mean female preference for red males and LWS expression, suggesting a role for opsin expression in preference determination. This pattern, however, was inconsistent with the species-specific expression profiles; the red phenotypes expressed less LWS. At Python Island, male coloration is both necessary and sufficient for assortative mating (Selz *et al.*, 2014), thus our results indicate that species differences in opsin expression and mate preference do not align. We also found that deep- and shallow-reared females displayed similar relationships between LWS expression and preference and that light-induced differences in the preference slopes were absent in the females tested for preference behaviour. This suggests that our light manipulations influenced female mate preference (shallow-reared females preferred blue males, deep-reared preferred red males) and opsin expression (deep-reared fish expressed more LWS and less SWS) but that we lack evidence for a causal link between light-induced

changes in expression and preference. We, therefore, did not find support for the hypothesis that variation in opsin expression serves as a 'magic' trait in *Pundamilia* speciation, pleiotropically affecting both visual adaptation and assortative mating (i.e. Smith, 1966). Other visual system components, however, have shown a more consistent relationship with female preference behaviour. LWS genotype, nearly fixed in each species, has been consistently associated with assortative female preference at multiple locations throughout the Mwanza Gulf (Seehausen *et al.*, 2008). Thus, in chapter 5, we also explored the link between allelic variation in LWS and female preference.

From prior work (Seehausen *et al.*, 2008), we expected two forms of the LWS gene in our experimental population, which is indeed what we found: blue types were predominantly homozygous for the 'P' allele and red types for the 'H' allele. Female preference was significantly associated with LWS genotype, but only in the shallow light treatment (there was no preference variation in deep test light). This result suggests that LWS genotype (or genetic factors that are linked to it) influences female preference but only when light conditions permit full-spectrum colour signaling (prior studies observed similar results: Seehausen & van Alphen, 1998; Haesler & Seehausen, 2005; Selz *et al.*, 2014). When considering the different combinations of rearing light and test light for all genotypes, we found more light-dependent preference variation in the 'HH' genotypes, all of which were red females. As mentioned above, we also found stronger evidence of opsin expression plasticity in the red fish (all 'HH' genotypes were red types). Thus, our results may indicate that, if only in the red phenotypes, several aspects of visual perception interact to influence preference. We tested the combined influence of opsin expression and LWS genotype on female preference but found no significant indications of this interaction. However, we had opsin expression data for only 5 'HH' females, thus exploring this interaction in more fish could provide greater statistical power to detect an interaction.

Taken together, the results of chapter 5 confirm a role of visual perception in determining female preference for differently coloured males. However, they also highlight the complexity of the relationship between visual adaptation and visually mediated mate choice. We did not find experimental evidence for opsin expression serving as a 'magic trait' but it does not mean that one does not exist. LWS genotype, on the other hand, may be more influential, as it was significantly associated with female preference. It is also possible that visual properties do not directly influence mate preference - the correlation between visual properties and mate preference (as demonstrated at a population level: Haesler & Seehausen, 2005; Seehausen *et al.*, 2008; van der Sluijs *et al.*, 2008; Selz *et al.*, 2014) may have evolved indirectly, by selection favouring assortative mating among individuals with similar visual adaptations (Maan & Seehausen, 2012). To test these alternative scenarios, further manipulative studies, potentially incorporating other aspects of visual perception (e.g. differential chromophore usage, higher-level neural processes), are required. The availability of techniques to target specific visual loci (as recently demonstrated in medaka: Homma *et al.*, 2017) also raises the possibly of manipulating the opsin genotype (discussed in more detail at the end).

Environmental light influences foraging performance

The sensory drive hypothesis not only predicts correspondence between signals and preferences in a given environment, but also natural selection for sensory systems that are properly tuned for the local conditions - a mismatch between visual system properties and the visual environment should result in lower fitness. In **chapter 6**, I tested this aspect of sensory drive by measuring foraging performance.

      Prior work in *Pundamilia* had documented consistent correlations between visual system characteristics and the local light conditions (Seehausen *et al.*, 2008), with each species surviving best in its natural light environment (Maan *et al.*, 2017). Together, these studies imply species-specific, visual adaptation. The results of chapter 6 were consistent with this – non-hybrids tested in 'natural' light (blue fish in shallow or red fish in deep) caught more live prey than their 'unnaturally' tested counterparts (blue fish in deep or red fish in shallow). Thus, we have evidence to support the hypothesis that species differences in visual properties are adaptive (or are the result of divergent adaptation). However, we also found that hybrids suffered no ill consequences. This is consistent with other laboratory studies: hybrid survival did not differ from the parental species (Maan *et al.*, 2017) and hybrids suffered no intrinsic fitness reduction (Van der Sluijs *et al.*, 2008). So, while our results are consistent with divergent selection on visual system properties, they also suggest that other factors may be involved. To further explore the role of divergent visual adaptation, future studies may benefit from inducing stronger fitness-related consequences. This could be accomplished by creating a more challenging foraging environment (mean success rate in chapter 6 was ~85%) by using different prey items or by altering the light conditions (discussed more at the end).

      We also found a weak effect of the developmental light environment; deep-reared fish tended to capture more prey. These results are consistent with the developmental light effects reported in chapters 2 (female mate preference) and 5 (relative opsin expression). This effect could be due to higher LWS expression in deep-reared fish, which could have aided prey capture. However, as we did not measure opsin expression in fish tested for foraging performance, this has yet to be confirmed. Interestingly, we also found that deep-reared fish had smaller eyes and that foraging performance was negatively related to eye size. We could not infer causality in this relationship but light-induced changes in eye size are intriguing. Plasticity in eye size may represent another form of visual adaptation, perhaps related to light-induced changes in retinal development (as documented in other cichlids: Van der Meer, 1993). In box 2, I explore light-induced differences in eye size further.

Box 2: Light influences eye size

In a recent meta-analyses of ray-finned fishes, Caves *et al.* (2017) found that eye size was positively correlated with visual acuity. This is not surprising; in many species, larger eyes are associated with higher visual ability. Caves and coauthors also reported an influence of the local light environment –visual acuity was highest in clear, bright water – but an underlying covariance with size complicated their interpretations – body and eye size were typically smaller in turbid and dark environments (Caves *et al.*, 2017). In chapter 6, we also found that eye size was light-dependent; deep-reared fish had smaller eyes. Here, I explored this relationship further, also incorporating data from chapter 3 (Wright *et al.*, 2018).

The combined dataset included 91 fish (31 blue, 29 red, 31 hybrid), split evenly between the rearing environments ($\geq$14 for each species, from each light condition). As in chapter 6, I used eye measurements corrected for body size (SL); body size did not differ between differentially reared fish (P = 0.28) or species (P = 0.21). Rearing light significantly influenced relative eye length (χ^2 (1) = 9.25, P = 0.002) and depth (χ^2 (1) = 10.55, P = 0.001); across species, deep-reared fish had smaller eyes (Fig. 7.2). Consistent with recent data from wild caught fish (van Rijssel *et al.*, 2018), relative eye size did not differ between the species groups (P > 0.27). Light-induced differences in absolute eye size (not corrected for SL) was greatest in larger fish (Fig. 7.3).
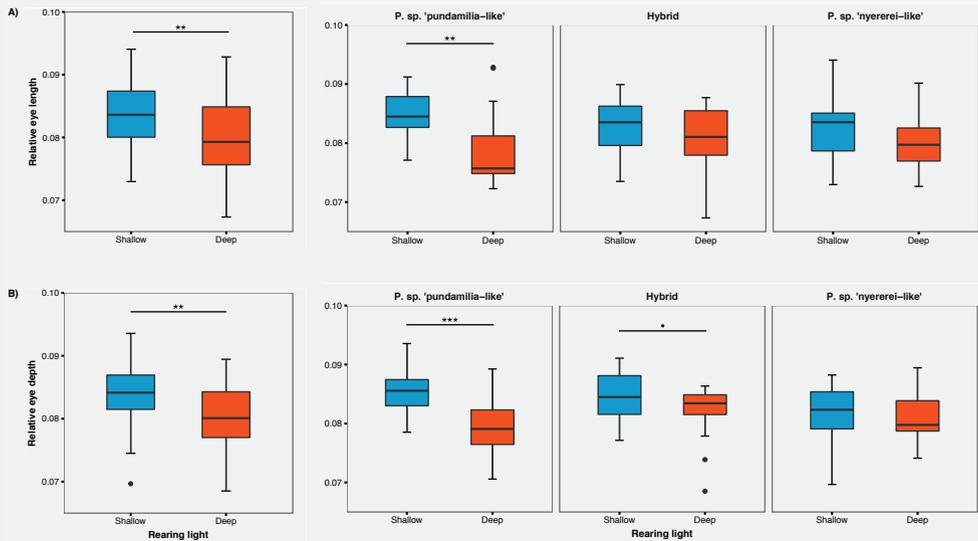
Figure 7.2. Light influences eye size – (A) Relative eye length and (B) relative eye depth was smaller in deep-reared fish. This effect was consistent across species, though strongest in the blue phenotypes.
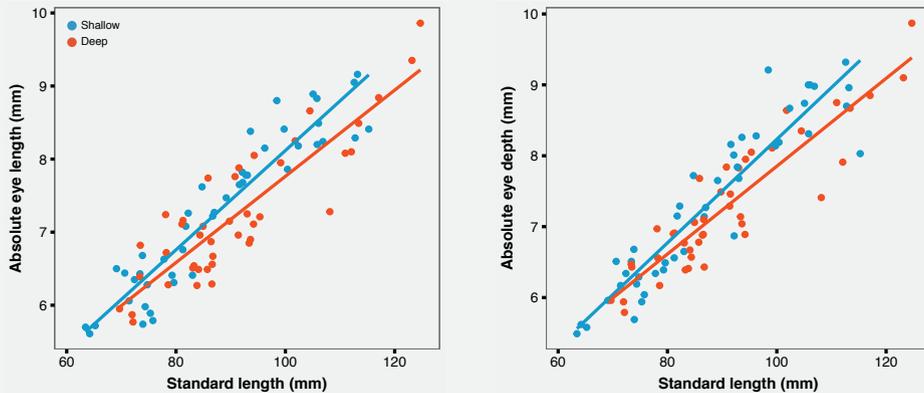
Figure 7.3. Relationship between eye and body size – Light-induced differences in absolute eye size were greatest in larger fish.

Though light-induced changes in eye size have not been studied in *Pundamilia*, Van der Meer (1993) did report light-induced effects on retinal development in a different species of Lake Victoria cichlid. Fish reared in light environments lacking short-wavelength light had reduced photoreceptor density, with fewer single (short wavelength sensitive) cones and enlarged double (including long wavelength sensitive) cones (Van der Meer, 1993). I did not measure cone density/composition of fish used in this thesis, but it is possible that our light manipulations induced similar changes. Deep-reared fish expressed more LWS and less SWS - consistent with the patterns reported by Van der Meer (1993). Possibly, the changes in opsin expression we observed also coincided with changes in photoreceptor density. These patterns are intriguing but, at this point, speculative. Nonetheless, my results here are consistent with the patterns reported Caves *et al.* (2017) and potentially highlight another form of visual adaptation. Further work is required to confirm these predictions and to assess their role in visual functioning.

What is the role of visual adaptation in speciation?

This thesis has used an integrative approach to answer the question: *what is the role of visual adaptation in speciation*? Using *Pundamilia* cichlids from Lake Victoria, we tested predictions of the sensory drive hypothesis with behavioural assays (chapters 2 & 6), colour analysis (chapter 3), visual system characterization (chapters 4 & 5), and field surveys (chapter 4). Each chapter provided evidence to support the role of visual adaptation in the divergence of the blue and red phenotypes. Both foraging and mate preference were influenced by the light environment experienced during development and species-specific visual properties, thereby providing experimental evidence that visual adaptation to the local light environment can act as a source of divergent selection. However, the mechanistic link between visual perception and reproductive isolation remains unresolved. As this thesis has shown, it is likely that the relationship is complex, involving multiple aspects of visual tuning. Future work may benefit from integrating multiple visual system elements and by further manipulating particular aspects of visual perception. I end by highlighting a few of these research opportunities:

1) **Can plasticity in visual development be 'pushed' further?** – The light treatments used in this thesis were designed to mimic the light environments experienced by each species at Python Island. These 'natural' treatments allowed us to test realistic scenarios of environment-dependent performance and the consequences of fish migrating between environments. As discussed above, our treatments were successful in manipulating opsin expression and influencing visually mediated behaviours but did not allow us to identify underlying causal links. This could be due to the fact that the changes in expression we observed were relatively subtle (though still significant). More dramatic changes in opsin expression, achieved by rearing fish in more extreme light manipulations (i.e. larger differences between treatments), may help future studies to better explore the link between expression and visual performance.

2) **How do opsin expression and LWS genotype combine to influence visual perception?** – Prior work in *Pundamilia* has documented a consistent relationship between LWS genotype and female mate preference (Seehausen *et al.*, 2008); a pattern we also observed in chapter 5. In chapters 4/5, we also explored the relationships between opsin expression and 1) LWS genotype and 2) mate preference but found that species differences in expression did not consistently align with either. This creates a situation where similar blue and red phenotypes from different locations (and visual environments; turbid vs. clear water) are nearly fixed for the same LWS genotype ('P' in blue, 'H' in red) and exhibit strong female preferences for male colour but differ dramatically in their opsin expression profiles. Thus, a new question arises: *how do opsin genotype and opsin expression together shape visual performance in a particular environment*? To address this question, future studies would benefit from isolation and manipulation of the LWS genotype. LWS knockdown has been successfully employed in medaka (Homma *et al.*, 2017), which has direct effects on mate preference (Kamijo *et al.*, 2018). In *Pundamilia*, these techniques could be used to manipulate LWS genotype, so as to disrupt the consistent relationship between genotype and preference. Researchers could then isolate the role of LWS in preference determination and explore the consequences of manipulating the relationship between opsin expression and LWS genotype.

3) **How does differential chromophore usage contribute to visual perception?** – Visual tuning in cichlids is accomplished by at least three mechanisms: changes in relative opsin expression, sequence variation within the opsin genes, and differential usage of vitamin-A based chromophores. While the first two elements have been explored in detail (previous work, plus the results presented within this thesis), the last – chromophore usage – has not been measured in *Pundamilia*. Cichlids can use either Vitamin A1- or Vitamin A2-based chromophores (Torres-Dowdall *et al.*, 2017) and shifts between the two can greatly influence visual sensitivity (Dartnall & Lythgoe, 1965; Hárosi, 1994; Toyama *et al.*, 2008). Future studies should measure differential

chromophore usage and how visual perception is influenced by its interaction with the other mechanisms of visual tuning. It may be that the blue and red phenotypes (or shallow- vs. deep-reared fish) preferentially use different chromophores, which may help to resolve the mismatch between opsin expression and preference we discussed above.

4) **What makes a mate attractive?** – Throughout this thesis, we have assumed that a given mate characteristic (e.g. colour) is attractive because it maximally stimulates the sensory (visual) system. While this is most certainly true, it also tends to diminish the complexities of a mating interaction. Mates must first be detected at a distance and then, after initial attraction, inspected at closer ranges. It is likely that these different contexts, as well as the different background environments over which this can take place, greatly influence the attractiveness of a mate. To assume that any single aspect of visual perception completely explains mate preference, without accounting for other environmental (and visual) factors, is naïve. This thesis has provided tangible directions for future research: LWS genotype is a key factor in preference determination, while opsin expression appears less influential. More sophisticated visual modeling, incorporating LWS genotype and other visual/environmental factors, may aid future researchers in their quest to better understand the dynamics of visual perception and mate choice.