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SYMPOSIUM

Opsin Expression Varies with Reproductive State in the Cichlid Fish *Astatotilapia burtoni*

Julie M. Butler[1,*,‡] and Karen P. Maruska*

*Department of Biological Sciences, Louisiana State University, 202 Life Sciences Bldg, Baton Rouge, LA 70803, USA;
‡Department of Biology, Stanford University, 304 Gilbert, 371 Jane Stanford Way, Stanford, CA 94305, USA

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[1]E-mail: jmbutler@stanford.edu

Synopsis Animals use visual communication to convey crucial information about their identity, reproductive status, and sex. Plasticity in the auditory and olfactory systems has been well-documented, however, fewer studies have tested for plasticity in the visual system, a surprising detail since courtship and mate choice are largely dependent on visual signals across taxa. We previously found reproductive state-dependent plasticity in the eye of the highly social cichlid fish *Astatotilapia burtoni*. Male *A. burtoni* increase their courtship, including multicomponent visual displays, when around ovulated females, and ovulated females are more responsive to male visual courtship displays than non-ovulated females. Based on this, we hypothesized that ovulation status impacts visual capabilities in *A. burtoni* females. Using electroretinograms, we found that ovulated females had greater visual sensitivity at wavelengths corresponding to male courtship coloration compared with non-reproductively-receptive females. In addition, ovulated females had higher neural activation in the retina and higher mRNA expression levels of neuromodulatory receptors (e.g., sex-steroids; gonadotropins) in the eye than non-ovulated females. Here, we add to this body of work by testing the hypothesis that cone opsin expression changes with female reproductive state. Ovulated females had higher expression of short wavelength sensitive opsins (*sws1*, *sws2a*, *sws2b*) compared with mouthbrooding females. Further, expression of *sws2a*, the most abundant opsin in the *A. burtoni* eye, positively correlated with levels of circulating 11-ketotestosterone and estradiol and estrogen, androgen, and gonadotropin system receptor expression in the eye in females. These data indicate that reproductive state-dependent plasticity also occurs at the level of photoreceptors, not just through modulation of visual signals at downstream retinal layers. Collectively, these data provide crucial evidence linking endocrine modulation of visual plasticity to mate choice behaviors in females.

Introduction

Across taxa animals use visual communication to convey information about their identity, motivation, reproductive state, sex, and species. Males often ramp up their coloration and courtship during reproductive seasons or when around reproductive females (Osorio and Vorobyev 2008). A male's body coloration or ornament size can be indicative of parasite load and overall health, which can provide females with crucial honest information during mate choice (Houde and Torio 1992; Thompson et al. 1997; Ness and Foster 1999; Molnár et al. 2013). Similarly, the intensity or vigorousness of courtship displays could also provide information on overall fitness that can be used to make mate choice decisions (Sargent et al. 1998). As such, animals that use visual courtship displays must be able to adequately detect these important signals to optimize communication.

Endocrine modulation of social communication has been demonstrated in several senses and across taxa. For example, female fishes, amphibians, and birds that are closer to reproduction are better able to detect their mate's call and/or are more responsive to the calls (Sisneros and Bass 2003; Lynch and Wilczynski 2008; Miranda and Wilczynski 2009; Caras et al. 2010; Maney and Pinaud 2011;

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Maruska et al. 2012; Maruska and Sisneros 2015). Similarly, metabolic and reproductive states are known to modulate chemosensory capabilities (Mousley et al. 2006; Palouzier-Paulignan et al. 2012; Nikonov et al. 2017). In fishes, visual capabilities can also be modulated by an animal's reproductive state. Androgens affect visual capabilities in male goldfish (Shao et al. 2014; Yue et al. 2018), and exogenous estrogens influence opsin expression in the eye of mosquito fish (Friesen et al. 2017). In humans, sex steroids are linked to healthy ocular function (Affinito et al. 2003), such that decreased estrogen signaling after menopause is linked to decreased tear production (Mathers et al. 1998) and lower protection against age-related eye diseases (e.g., glaucoma; Zhou et al. 2007; Vajaranant et al. 2010). In Túngara frogs, females treated with the reproductive hormone hCG exhibited higher visual sensitivity, but the same plasticity was not observed in hormonally-treated males (Leslie et al. 2020). Together, these studies suggest that reproductive hormones play a neuromodulatory role in vision and eye function across taxa.

We recently found reproductive state-dependent plasticity in the visual system of female cichlid *Astatotilapia burtoni*, but not males (Butler et al. 2019). Male *A. burtoni* exist on a dominance continuum ranging from dominant to subordinate phenotypes, which they can rapidly and reversibly switch between. Dominant males are brightly colored, often with either blue or yellow body coloration and brightly colored orange/red spots on their fins (Fernald 1977; Fernald and Hirata 1977). They can turn on a dark eyebar and a red humeral patch depending on their social environment, with a dark eyebar often displayed in aggressive contexts and the red humeral patch more closely related to courtship (Leong 1969; Heiligenberg et al. 1972; Wapler-Leong 1974). To court females, males produce a series of visual displays, including a body quiver, tail waggle, and leading the female back to his territory. Subordinate males are drably colored, do not hold territories, and are often found with females. When around ready to reproduce females, dominant males increase their use of multicomponent courtship displays with twice as many displays performed toward ovulated females than non-ovulated gravid females that are still at least a day from reproducing (Butler et al. 2019). In turn, ovulated females are more responsive to male courtship displays. By orienting toward the behavior, following the male, and more time in the spawning territory, ovulated females perform more affiliative, mate choice-like behaviors than non-ovulated gravid and non-gravid females. Together, this indicates that both males and females modulate their reproductive behaviors based on the female's ovulation status, with males increasing their production of visual displays and females being more responsive to them.

Based on these ovulation-specific changes in intersexual behavior, we hypothesized that visual capabilities would vary with female reproductive state (Butler et al. 2019). Using electroretinograms we found that as a female approaches spawning, she has greater sensitivity to 500–550 nm wavelengths of light. After being induced to ovulate via injections of prostaglandin F2α, females had a two-fold increase in sensitivity across the visual spectrum (450–650 nm). In addition, ovulated females had higher neural activation in the inner nuclear and ganglion cell layers (INL, GCL) of the retina in response to a courting male than did non-ovulated gravid females. We also found that ovulated females had higher expression of gonadotropin system receptors and sex steroid receptors in the eye than did non-ovulated gravid females and non-reproductive mouthbrooding females. Together, these data suggest that ovulated females are better able to detect components of male visual courtship displays.

Here, we sought to further examine the potential mechanisms underlying visual plasticity in *A. burtoni*. Our previous measures (electroretinograms (ERGs) and neural activation) examined retinal plasticity from modulatory and information transfer cells in down-stream retinal layers. While an increase in ON-bipolar cell activity (ERGs) and higher activation in the INL and GCL could indicate increased sensitivity at the level of the photoreceptors, they could also reflect increased modulation of the signal as it is transferred through the retina. To examine if there is reproductive state dependent plasticity at the level of the photoreceptors, we used quantitative PCR to measure expression of cone opsin genes in the eyes of ovulated, non-ovulated gravid, and mouthbrooding females, and in dominant and subordinate males. We chose to measure only cone opsin genes because male body coloration is likely important for female mate choice and reproduction. *Astatotilapia burtoni* express seven cone opsins (Fernald and Liebman 1980; Fernald 1981; Carleton 2009; O'Quin et al. 2011): short wavelength sensitive *sws1*, *sws2a*, and *sws2b*, middle wavelength sensitive *rh2a-a*, *rh2a-b*, and *rh2b*, and long wavelength sensitive *lws*. Short wavelength sensitive opsins detect UV (*sws1*) and blue wavelengths of light, while *lws* is sensitive to more yellow/orange/red color ranges of light (Fernald and Liebman 1980; O'Quin et al. 2010). The middle wavelength sensitive opsins are more broadly tuned to blue/green/yellow color ranges of light (Fernald and Liebman 1980; O'Quin et al. 2010). We found that opsin expression varied with female reproductive state. Further, expression of *sws2a*, which comprises ~50% of all opsins in the female eye, positively correlated with levels of circulating sex steroids. When combined with past work on visual plasticity in *A. burtoni*, these data indicate plasticity is also occurring at the level of the photoreceptors and not just through downstream modulation or processing of visual signals. This provides further evidence to support that reproductive and ovulation state mediate visual capabilities in a species that is dependent on visuals signals for mate choice behaviors.

Materials and methods

Experimental animals

Laboratory-bred *A. burtoni* were maintained in community aquaria (114 L) with gravel substrate and at least two to three terra cotta pots to serve as spawning territories for males. Environmental conditions mimicked natural conditions (pH = 7.6–8.0; 28–30 C; 12 L:12 D diurnal cycle), and fish were fed cichlid flakes (AquaDine, Healdsburg, CA, USA) daily. All experiments were performed in accordance with the recommendations and guidelines stated in the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, 2011. All animal care and collection were approved by the Institutional Animal Care and Use Committee at Louisiana State University, Baton Rouge, LA, USA.

We used the same samples for the study reported here as those used for qPCR analyses in Butler et al. (2019). We collected eyes from five groups of fish: ovulated females, non-ovulated gravid females, mouthbrooding females, dominant males, and subordinate males. Gravid (ovulated and non-ovulated) females were selected based on the presence of a distended abdomen, slightly distended jaw, and presence of courting males. Ovulated females were visually distinguished from non-ovulated gravid females based on a slightly distended jaw and protruding urogenital papilla, and were confirmed to have ovulated (eggs released from follicular/ovarian membrane) during dissection. All gravid females (ovulated and non-ovulated) had high levels of reproductive investment (i.e., gonadosomatic index, GSI, >7.0). Mouthbrooding females were collected 5–10 days after the onset of brooding, with low levels of reproductive investment (GSI < 1.0). Males were collected from dyadic paradigms where they were in their respective social status for at least 30 days (GSI: dominant > 0.70; subordinate < 0.50). All fish were of approximately the same size (standard length: 41.54 ± 6.63 mm; body mass: 2.23 ± 1.02 g). Fish were collected over a two-year period but likely share similar genetic backgrounds because of being collected from a laboratory-bred stock.

Tissue collection and processing

All fish were collected at the same time of day to minimize any changes associated with diurnal opsin expression (Halstenberg et al. 2005). All fish were exposed to full-spectrum LED lights that did not differ between the groups, so any changes in gene expression are not due to differences in light environments (Nandamuri et al. 2017). Fish were quickly netted from their home aquaria, measured for standard length and body mass, blood collected via the caudal vein, and sacrificed via rapid cervical transection. Both eyes were removed from the head by clipping the optic nerve as close to the eye as possible, the lens and any excess tissue surrounding the eye removed, and immediately frozen and stored at 80 C until processing. Serum was isolated from blood samples and stored at 80 C until processing. RNA extraction from eye samples was done following the manufacturer's protocol (RNeasy Plus Mini Kit, Qiagen) and consistent RNA amounts were used in cDNA synthesis reactions (iScript, BioRad).

Quantitative PCR

We measured expression of six cone opsin genes (*sws1*, *sws2a*, *sws2b*, *rh2a*, *rh2b*, and *lws*) using previously published primers (Carleton and Kocher 2001; O'Quin et al. 2011; Supplementary Table S1). The primers for *rh2a* amplify both *rh2a-a* and *rh2a-b*, so our data are presented as just *rh2a*. qRT-PCR was performed on a CFX connect Real-Time system (BioRad) using the following reaction parameters: 95 C for 30 s, 45 cycles of 95 C for 1 s, and 60 C for 15 s; and followed by a melt curve analysis. Although these primers were designed for a taqman protocol, each primer pair produced a single melt peak at the expected temperatures. PCR Miner (Zhao and Fernald 2005) was used to calculate reaction efficiencies and cycle thresholds. The relative amount of mRNA was normalized to the expression of *gnat2* (cone-specific alpha subunit of transducin), which does not vary with reproductive/social state (females: $F_{2,26} = 1.278$, $P = 0.296$; males: $F_{1,28} = 3.176$, $P = 0.086$), using the following formula: Relative target gene mRNA levels $= \leftarrow [1/(1 \quad E_{target})\hat{\ }CT_{target}]/[1/(1 \quad E_{geomean})\hat{\ }CT_{geomean}]$ 100, where E is the reaction efficiency and CT is the average cycle threshold of the duplicate wells. Cycle threshold values and primer efficiencies were checked for all samples for all genes (average CT values = *gnat2*: 24–26; *sws1*: 32–36; *sws2a*: 23–28; *sws2b*: 26–32; *rh2a*: 24–28; *rh2b*: 32–34; *lws*: 22–26). All primer efficiencies were in the same range (88–94%), allowing data to be combined across multiple plates. We did not compare expression between males and females because *gnat2* expression, as well as other commonly used reference genes (e.g., *eef1a*, *18 s*, *rpl32*, *gapdh*), is significantly different with sex ($F_{1,56} = 35.517$, $P < 0.001$).

Hormone assays

We measured circulating levels of 11-ketotestosterone (11-KT), estradiol (E2), and progestins (P4) using enzyme-linked immunosorbent assays on serum collected from ovulated, non-ovulated gravid, and mouthbrooding females (Cayman Chemical; estradiol: 582251; 11-KT: 582751; progestins: 582601) as part of the previous study (Butler et al. 2019). We did not perform hormone assays on males, but readers are referred to the extensive published data on circulating steroids in dominant and subordinate males (e.g., Maruska et al. 2012; Maruska 2014, 2015). Kits have been previously validated for this species (Maruska and Fernald 2010). Intra-assay CVs were 9.94%, 9.27%, and 10.10% for 11-KT, E2, and P4, respectively.

Statistical analyses

All statistics were done in R. Briefly, we first tested for normality and outliers (Iglewicz and Hoaglin 1993) in all data. qPCR data were analyzed using ANCOVAs with reproductive state and sex as fixed effects, standard length as a covariate, and Tukey's tests for pairwise comparisons. Discriminant function analysis was used to group animals based on opsin composition using within-groups covariances and all groups considered equal (package: MASS; Venables and Ripley 2020). Missing values were replaced with the group mean. Correlations were assessed using Pearson product moment tests. All data and code for analyses will be provided upon reasonable request.

Results

Opsin expression varies with reproductive state

All six of the measured opsins are detectable in the eye, most in a reproductive state-dependent manner (**Fig. 1**). Expression of short wavelength sensitive opsins (*sws1:* 360 nm, *sws2a*: 456 nm, and *sws2b:* 423 nm) varies with female reproductive status (*sws1*: $F_{2,28} = 4.256$; $P < 0.023$; *sws2a*: $F_{2,28} = 6.138$, $P = 0.007$; *sws2b*: $F_{2,28} = 5.659$, $P = 0.009$), but not male social status (*sws1*: $F_{1,28} = 0.022$; $P = 0.884$; *sws2a*: $F_{1,28} = 0.130$, $P = 0.863$; *sws2b*: $F_{1,28} = 1.852$, $P = 0.185$). For all three short wavelength sensitive opsins, ovulated females have higher expression than mouthbrooding females (*sws1*: $P = 0.010$; *sws2a*: $P = 0.002$; *sws2b*: $P = 0.005$), and non-ovulated gravid females are intermediate between the two groups. *Rh2a* (523 nm) expression does not vary with female reproductive state ($F_{2,26} = 0.129$, $P = 0.880$) or male social status ($F_{1,28} = 1.122$, $P = 0.299$). In contrast, *rh2b* (472 nm) expression varies with female reproductive state ($F_{2,26} = 3.566$, $P < 0.043$), such that brooding females have higher expression than ovulated and gravid females. In males, subordinate males have higher *rh2b* expression than dominant males ($F_{1,28} = 6.905$, $P = 0.014$). Finally, *lws* (561 nm) expression does not vary with either female reproductive state ($F_{2,26} = 0.414$, $P = 0.615$) or male social status ($F_{1,28} = 0.046$, $P = 0.831$).

The pattern of opsin expression generally follows that previously published for adult *A. burtoni*, with high levels of *sws2a*, *rh2a*, and *lws*, low expression of *sws2b*, and little-to-no-expression of *sws1* or *rh2b* (**Fig. 2A**). In all fish, *sws2a* is the most abundant opsin expressed, comprising 48% and 40% of total opsin expression in females and males, respectively. *Rh2a* comprised 16% of female opsin expression, but 25% of male opsin expression. Expression of *lws* makes up ∼35% of total opsin expression in all fish. *Sws2b* comprises ∼2% of total opsin expression, and *rh2b* and *sws1* expressions are each less than 1% of total opsin expression. A discriminant function analysis of opsin expression produced three significant functions, with function 1 explaining 53.94% of data variance, and functions 2 and 3 explaining 31.63% and 11.72% of the variance, respectively (**Fig. 2B**). Function 1 is positively loaded by *sws1* and *lws*, negatively loaded by *rh2a* and *rh2b*, and separates females from males. Function 2 is loaded most negatively by *rh2b* and positively by *sws1* expression. This roughly separates ovulated from brooding females and dominant from subordinate males. The DFA correctly classifies 37 total fish, with only 5 fish being predicted as the incorrect sex. Dominant and subordinate males are commonly misidentified as each other based on opsin expression. While ovulated females are distinguishable from brooding females (0% misidentified as brooding), two fish are predicted as non-ovulated gravid. Gravid females are incorrectly predicted as ovulated (two of eight) or brooding (three of eight), further indicating

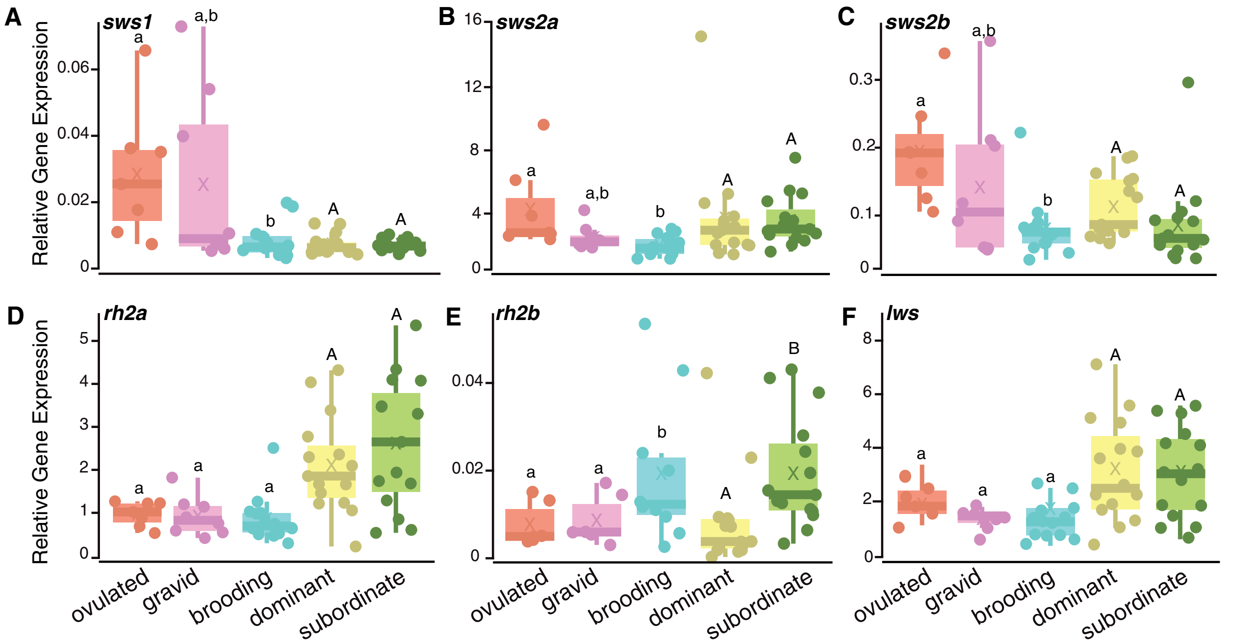

Fig. 1. Opsin expression is reproductive state-dependent. Ovulated females have higher expression of *sws1* (**A**), *sws2a* (**B**), and *sws2b* (**C**), compared with brooding females, with gravid females as an intermediate. There are no differences in *rh2a* (**D**) expression, but *rh2b* (**E**) expression is higher in subordinate males than dominant males, and in brooding females than ovulated and gravid females. Expression of *lws* (**F**) is not different with female reproductive state or male social status. Different lower- and upper-case letters represent significant differences ($P < 0.05$) within females and males, respectively. All data points are represented as closed circles, data mean as an "X," and data median as a solid line.

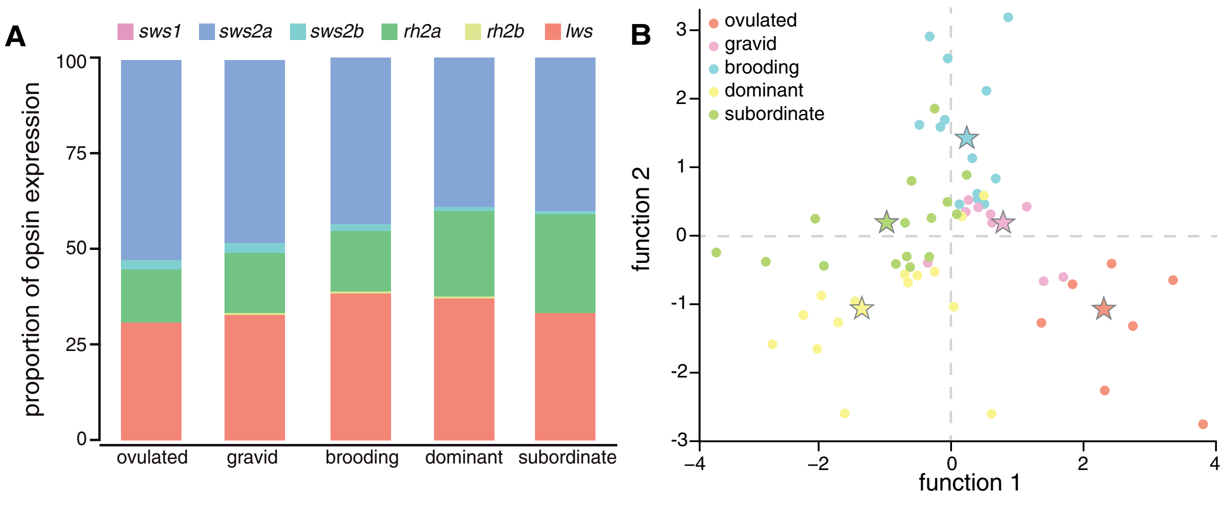

Fig. 2 Cone opsin composition differs between males and females. (**A**) Expression of each opsin as a fraction of the total opsin expression (except *rh1*). *sws2a*, *rh2a*, and *lws* are the dominant cone opsins expressed (98%) in all fish. (**B**) Discriminant function analysis roughly separates males and females along function 1 and function 2 roughly separates dominant from subordinate males and ovulated from brooding females. Stars represent group centroids.

that the transition in opsin composition happens as a female approaches reproductive readiness.

In females, *sws2a* expression positively correlates with circulating levels of both 11-KT ($R = 0.469$, $P = 0.012$) and estradiol ($R = 0.486$, $P = 0.009$) (Fig. 3), but there are no other correlations between circulating sex steroids and expression of any other opsin. However, these correlations may be driven by differences in opsin and/or circulating steroid differences among female groups, as there are no significant correlations within each

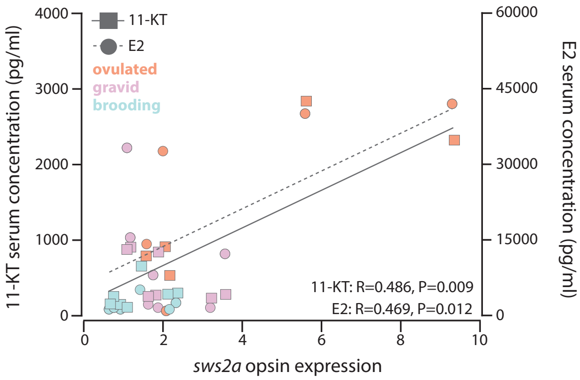

Fig. 3 *sws2a* opsin expression positively correlates with 11-KT [estradiol (E2) in females]. Expression of *sws1* in ovulated (orange), non-ovulated gravid (purple), and brooding (blue) females positively significantly correlates with circulating levels of 11-KT (squares, solid line) and estradiol (circles, dashed line).

reproductive state. Further, *sws1* and *sws2b* expression positively correlate with expression of estrogen, androgen, and gonadotropin system receptor (i.e., luteinizing hormone receptor, gonadotropin releasing hormone receptors) expression in the eye ($P < 0.05$ for all); however, both *sws1* and *sws2b* are expressed at relatively low levels (0.31% and 2.26%, respectively) compared with other opsins. Expression of the other four opsins does not correlate with any of these reproductively-important neuromodulatory receptors in the eye.

Discussion

Here, we show that opsin expression varies with female reproductive state, further demonstrating endocrine-mediated visual plasticity in *A. burtoni*. We previously found that visual sensitivity varies with female ovulation status (Butler et al. 2019). Using integrative techniques, we showed that ovulation status was linked to increased visual sensitivity, higher neural activity in the retina, higher levels of neuromodulatory receptors in the eye, and an increase in affiliative mate-choice like behaviors. Here, we expand on that work to show that opsin expression also varies with female reproductive state, but in a different wavelength-dependent manner than that determined by electroretinograms. Ovulated females had higher expression of short wavelength sensitive opsins (*sws1*, *sws2a*, and *sws2b*) than mouthbrooding females, with gravid females as an intermediate between the two, suggesting that as a female approaches reproductive readiness and ovulates, expression of the opsin responsible for detecting the UV/violet/blue color range of light also increases. Male *A. burtoni* have both a blue and yellow morph, with some males having both blue and yellow pigmentation. Their fins also have iridescent-like pigments. As such, short wavelength sensitive opsins likely detect components of male body coloration, but not the red humeral patch often associated with reproduction. The positive significant correlation between circulating sex steroids and *sws2a* expression, the predominant opsin expressed in females, further suggests endocrine-mediated plasticity in the visual system; however, this correlation was only significant when all females were combined and not within female reproductive states. Manipulating estrogen signaling impacted expression of cone opsins in western mosquitofish (*Gambusia affinis*) and sailfin mollies (*Poecilia latipinna*; Friesen et al. 2017). Female mosquitofish supplemented with estradiol had higher expression of *sws2a* and *rh2* compared with vehicle-injected females, and *lws* expression was decreased in tamoxifen (estrogen receptor antagonist) treated females. The results presented here, and those from Friesen et al. (2017), suggest that circulating estradiol levels likely mediate opsin expression, but the effects themselves, as well as the opsins influenced, appear to be species specific.

Using electroretinograms to measure the b-wave (primarily an ON-bipolar cell response) in dark-adapted fish, we previously found that gravid females had increased sensitivity to 500 and 550 nm light stimuli compared with non-gravid recovering females (Butler et al. 2019). After ovulation was hormonally induced, we found an increase in sensitivity across the visual spectrum, with the largest gain in sensitivity in the yellow–green color range. Despite changes in female visual sensitivity to yellow–green wavelengths of light measured via ERGs, we did not find any changes in the predominant middle wavelength sensitive opsins (*rh2a*) with female reproductive state. The difference between ERG data and opsin expression is not surprising. Because of using dark-adapted animals, it is likely that our ERGs measured a visual response that was dominated by rods, not cones. It is also important to note that the wavelengths measured by ERGs were not at the peak sensitivity of each opsin. In contrast to *rh2a* expression, brooding females and subordinate males have higher expression of *rh2b* than ovulated/gravid females and dominant males, respectively. However, *rh2b* expression only comprises 0.25% of the total opsin expression, with *rh2a* expression over 10 times higher than *rh2b* expression. Further, past studies have suggested that *rh2b* may be a pseudogene in *A. burtoni* because of its low to nonexistent expression across developmental stages and in adults (O'Quin et al. 2011). So the functional implications of this difference in *rh2b* expression are questionable. No other differences were found with male social status, further supporting our previous findings that visual plasticity is found in female but not male *A. burtoni*.

In some fishes, long wavelength sensitive opsins have been tied to reproductive state and sexual maturity. In guppies, expression of long wavelength sensitive opsins (*A180*, *S180*) increased with sexual maturity, and in adult fish, females had higher expression of both *A180* and *S180* than males (Laver and Taylor 2011). The authors attributed this increase in red-sensitive opsins in sexually-mature females to their need to discriminate male red body coloration during mate choice. Further, androgens increase *lws* expression in male three-spined sticklebacks (Shao et al. 2014). Despite these differences in *lws* expression in other teleosts, we found no reproductive state differences in *lws* expression in *A. burtoni*. There were no correlations between androgens and *lws* expression in females. Despite dominant male *A. burtoni* often having higher levels of circulating androgens compared with subordinate males (Maruska 2014), *lws* expression did not differ with male social status.

When combined with our previous work on visual system plasticity in *A. burtoni*, we show that female reproductive state mediates visual sensitivity, likely through multiple different mechanisms. The changes in opsin expression with female reproductive state demonstrate that visual capabilities are modulated at the level of the photoreceptors, not just through downstream modulation or processing. While studies have reliably shown that hormonal systems can mediate sensory plasticity, the underlying mechanisms remain poorly understood. However, research into the role of estrogens in human ocular health has demonstrated that estrogens have protective effects against macular degeneration and can help prevent age-related decreases in photoreceptor density (Chui et al. 2012; Wang et al. 2017). Photoreceptors themselves have not been found to express sex steroid receptors, but estrogen receptors have been localized to the retinal pigmented epithelium cells (Kobayashi et al. 1998; Gupta et al. 2005), which signal directly to photoreceptor cells and play a vital role in phototransduction. While we and others have found an increase in opsin expression related to endocrine state, it remains unknown if this is due to an overall increase in the number of photoreceptors, increased opsin expression within cones, or a shift in opsin expression within a cone. Photoreceptors take several weeks to differentiate in goldfish (Wu et al. 2001), so it seems unlikely that photoreceptor density can change on the same rapid timescale as opsin expression. It has also been proposed that the retina has maximized morphology for proper lamellar packing and phototransduction, such that increasing lamellar volume (i.e., from increased opsins within a cone) could interfere with proper phototransduction (Wen et al. 2009). As such, the changes observed in opsin expression likely suggest that cones shift their expression from one opsin to another, which would change overall opsin composition of the retina leading to changes in spectral sensitivity. Changes in cone identity from one opsin to another have been demonstrated in several fishes (Cheng et al. 2006, 2009; Flamarique et al. 2013), but remains untested in *A. burtoni*. It was previously found that middle and long wavelength sensitive opsins have higher diurnal variation than short-wavelength sensitive opsins (Halstenberg et al. 2005). The authors proposed that this could be because blue opsins are smaller, and therefore, potentially more stable than green and red opsins. Although we collected fish at the same time of day to avoid changes associated with time of day and light exposure, it is possible that diurnal changes in green and red opsins are greater than the influence of reproductive state on opsin expression. Another possibility is that the smaller size of short wavelength sensitive opsins allows for more plasticity before cones reach detrimental levels. Our discriminant function analyses separated males and females, and largely distinguished dominant from subordinate males and ovulated from brooding females, demonstrating that overall opsin composition differs based on reproductive and social state, but more research is still needed to identify the mechanism underlying these changes. As neuroscience techniques continue to improve and become more accessible and applicable to non-model systems, future studies using these approaches will better reveal *how* hormones mediate plasticity.

In summary, high levels of parental investment associated with maternal mouthbrooding make reproduction extremely costly for female *A. burtoni*. After a female ovulates, she has approximately 24 h to find, choose, and reproduce with a male. If she is unsuccessful, she will pick up unfertilized eggs, and negate the energetic demands that went into egg production. As such, the ability to adequately detect visual components of male courtship displays and to make appropriate mate choice decisions is extremely important in this species, and likely many others that cycle in and out of breeding condition. Future work is needed to elucidate the mechanisms of how endocrine systems modulate sensory capabilities at the periphery and the central processing of social signals.

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Conflict of interest

The authors declare no competing or conflicting interests.

Supplementary data

Supplementary data are available at *ICB* online.

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