Thyroid hormone tinkering elicits integrated phenotypic changes potentially explaining rapid adaptation of color vision in cichlid fish

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Vision is critical for most vertebrates, including fish. One challenge that aquatic habitats pose is the high variability in spectral properties depending on depth and the inherent optical properties of the water. By altering opsin gene expression and chromophore usage, cichlid fish modulate visual sensitivities to maximize sensory input from the available light in their respective habitat. Thyroid hormone (TH) has been proposed to play a role in governing adaptive diversification in visual sensitivity in Nicaraguan Midas cichlids, which evolved in less than 4000 generations. As suggested by indirect measurements of TH levels (i.e., expression of deiodinases), populations adapted to short wavelength light in clear lakes have lower TH levels than ones inhabiting turbid lakes enriched in long-wavelength light. We experimentally manipulated TH levels by exposing 2-week-old Midas cichlids to exogenous TH or a TH inhibitor and measured opsin gene expression and chromophore usage (via cyp27c1 expression). Although exogenous TH induces long-wavelength sensitivity by changing opsin gene expression and chromophore usage in a concerted manner, TH-inhibited fish exhibit a visual phenotype with sensitivities shifted to shorter wavelengths. Tinkering with TH levels in eyes results in concerted phenotypic changes that can provide a rapid mechanism of adaptation to novel light environments.

KEY WORDS: Heterochrony, Midas cichlid, opsin, spectral sensitivity, thyroid hormone, visual sensitivity.

Changes in hormonal regulation are a potent force for adaptation (Young et al. 1989; Dufty et al. 2002; Ogawa et al. 2009; Bento et al. 2010; Sommer and Ogawa 2011). Such endocrine systems often have profound effects on whole organisms allowing for concerted phenotypic changes in morphology, life history, and behavior. For example, variation in steroid hormones in nematodes allowed for the evolution of dauer larvae and parasitism (Ogawa et al. 2009; Sommer and Ogawa 2011) as well as the development of novel trophic phenotypes (Bento et al. 2010). Thyroid hormone (TH) is one of the most important messenger molecules in vertebrates, mediating cell differentiation as well as developmental and physiological processes, which allows for integrated changes, that is, changes in multiple phenotypes that act synergistically (Huxley 1920; Evans 1988; Jannini et al. 1995; Forrest et al. 1996; Harpavat and Cepko 2003; Bernal 2007). Secreted by the thyroid gland or follicles, TH induces a cascade of regulatory mechanisms substantially altering gene expression patterns in target cells (reviewed in Evans 1988; Umesono and Evans 1989; Bernal 2007). Regulation of TH levels has been implicated in the evolution of several novel phenotypes. For instance, reduction in TH activity leads to heterochronic shifts and maintenance of a juvenile phenotype at maturity that allowed the evolution of mature neotenic newts, for example, Ambystoma mexicanum, adapted to permanent aquatic life (Huxley 1920; Page et al. 2008; Johnson...
and Voss 2013). Thus, due to its pleiotropic effects, TH could potentially produce rapid, integrated, and adaptive changes in response to natural selection.

The fish eye has emerged as a compelling system to study adaptive evolution (Fuller and Claricoates 2011; Novales Flamarique 2013; Härer et al. 2017; Torres-Dowdall et al. 2017; Carleton et al. 2020; Zheng et al. 2021) and it is a major target of TH (Eldred et al. 2018; Volkov et al. 2020). The spectral sensitivity of the visual pigments in the photoreceptors of the retina is dependent on the interaction of their two constituent components: an opsin protein and a light-absorbing retinal chromophore (Govardovskii et al. 2000, Cronin et al. 2014). Both these constituents have been shown to be sensitive to alterations in circulating TH levels so that an integrated response, that is, changes in opsin gene expression and changes in chromophore usage, can be evoked (Volkov et al. 2020). For example, artificially increasing TH levels in trout juveniles leads to changes in photoreceptor identity by switching opsin gene expression from the UV-sensitive sws1 to the more blue-sensitive sws2 (Cheng et al. 2009; Novales Flamarique 2013). On the other hand, when TH-metabolizing genes are knocked out in zebrafish, that is, fish could not transform TH into its active form, photoreceptors express short-wavelength sensitive opsins instead of more long-wavelength sensitive ones (Houbrechts et al. 2016). Additionally, changes in TH level are known to affect chromophore usage in fish (i.e., vitamin A1-derived or the more long wavelength sensitive vitamin A2-derived; Temple et al. 2008; Suliman and Novales Flamarique 2014; Volkov et al. 2020). Changes in chromophore usage are indirectly modulated by circulating TH levels through an enzyme (Cyp27c1) that catabolizes the production of vitamin A1 from vitamin A2. Elevated TH levels induce the upregulation of the expression of cyp27c1, which increases usage of the vitamin A2-derived chromophore resulting in a more long wavelength shifted spectral sensitivity of photoreceptors (Enright et al. 2015; Volkov et al. 2020; Corbo 2021). To summarize previous studies on the effect of TH on the visual system: tinkering with TH levels has a large impact on the spectral sensitivity of vision in fish, and hence, it might play an important role during adaptation of the visual system to different light environments.

TH has also been suggested to play an important role in the adaptive divergence of visual sensitivity in Nicaraguan Midas cichlid fishes (Amphilophus citrinellus) (Günther 1864) (Härer et al. 2017; Karagic et al. 2018). Midas cichlids are a young and small species flock composed of at least 13 species (Torres-Dowdall and Meyer 2021). These species are part of an adaptive radiation associated with the recent colonization (less than 4000 generations ago) of numerous deep and clear-water crater lakes from a source population that inhabited two shallow and extremely turbid old great lakes Nicaragua and Managua (Kautt et al. 2020). These colonization events resulted in a drastic change in the photic environment experienced by Midas cichlids, as some of the newly colonized crater lakes have light spectra significantly enriched in short-wavelength light resulting in a broader photic environment that also expands into deeper waters compared to that seen in the turbid great lakes (Torres-Dowdall et al. 2017; Härer et al. 2018). Accordingly, the spectral sensitivity of crater lake Midas cichlids has adaptively diverged from the ancestral form in the great lakes by shifting toward shorter wavelengths through changes in both components of their visual pigment, chromophore type usage and identity of opsin proteins (Torres-Dowdall et al. 2017; Härer et al. 2018). There is evidence suggesting that this change might be driven by changes in the circulating TH level during development (Härer et al. 2017; Karagic et al. 2018).

Most cichlid fish, including Midas cichlids, undergo ontogenetic changes where the opsin gene expression in their single and double cone photoreceptors changes (Carleton et al. 2008; O’Quin et al. 2011; Härer et al. 2017). As larvae and juveniles, the visual system of Midas cichlids from the turbid great lakes is most sensitive to short wavelengths due to expression of UV- or violet-sensitive opsin genes in single cones (sws1 or sws2b, respectively) and blue-green and green-sensitive opsin genes in double cones (rh2b and rh2a, respectively). As development progresses, sensitivity shifts toward longer wavelengths and individuals express the blue-sensitive sws2a in single cones and the green-sensitive rh2a and red-sensitive lws in double cones (Härer et al. 2017). Derived populations of Midas cichlids from the clear-water crater lakes follow a similar ontogenetic progression; however, developmental rates of crater lake Midas cichlids are slower and the ontogenetic progression is terminated at an earlier developmental stage compared to that seen in the turbid great lake cichlids (Härer et al. 2017). Thus, divergence among great lake and crater lake Midas cichlids might have been aided by heterochronic changes because adults of the derived species resemble juveniles of the source one (i.e., pedomorphosis). Importantly, the differentiation in ontogenetic progression between species has been suggested to be correlated with differences in circulating TH levels. Crater lake species have lower TH levels than fish from the great lakes (indirectly measured via differences in deiodinase expression) (Härer et al. 2017). Moreover, changes in the expression of different genes involved in TH metabolism are observed when the ontogenetic progression of spectral sensitivity is altered by manipulating light conditions (Karagic et al. 2018). Previous studies used the expression of TH-metabolizing genes as proxies for circulating TH levels, so it is unclear whether direct manipulations of TH levels can elicit a response in visual sensitivities. However, this prompts the hypothesis that modulation of TH levels might cause integrated changes of two major
components that determine visual sensitivity (opsin expression and chromophore usage). Such integrated changes, in turn, might have contributed to the adaptive evolution of a short wavelength shifted visual sensitivity in Midas cichlids after their colonization of clear-water crater lakes from turbid great lakes.

To test whether TH changes cause an integrated response of the visual system and, thus, could have contributed to adaptation, we directly altered TH levels to investigate its potential role during adaptive evolution of the visual system of Midas cichlids. Here, we present the results of a split-brood design experiment where TH levels were manipulated either by adding exogenous TH to increase circulating levels or by reducing those levels with a TH inhibitor, thiourea (Prazdnikov and Shkil 2019). We conducted this experiment in fish from the turbid great lake Nicaragua, which serves as a proxy for the ancestral state of fish that colonized clear-water crater lakes (Torres-Dowdall et al. 2017). Fish were exposed to the treatments during early stages of development (14 days post hatching) as this is the age when the largest changes in ontogenetic progression of spectral sensitivity were detected (Härer et al. 2017). If TH were to play a significant role in the adaptive evolution of visual sensitivity, we would predict an integrated change in the visual system in response to TH manipulations, with expected changes in the expression of different opsin genes and the enzyme catalyzing changes from vitamin A1- to A2-derived chromophore.

**Methods**

**THYROID HORMONE AND THIOUREA TREATMENTS**

TH levels were artificially manipulated in 149 individuals from eight separate broods (Table S1; Karagic et al. 2022) of laboratory-reared _Amphilophus citrinellus_ from great Lake Nicaragua (one tank per brood per treatment; 24 tanks in total), which is the source population to the colonization of clear-water crater lakes (Kautt et al. 2016; Kautt et al. 2020). To increase TH concentrations, we dissolved L-thyroxine in the tank water to a final concentration of 300 ng/ml (Prazdnikov and Shkil 2019; Volkov et al. 2020). To decrease endogenous TH concentrations, we dissolved thiourea to 0.01% (Prazdnikov and Shkil 2019). Concentrations were chosen to induce phenotypic differences without increased mortalities in either treatment group. Midas cichlid juveniles were raised together until 14 days posthatching. Afterward, fish were equally distributed among three treatments: one untreated control, thiourea, and TH treatments. Fish were kept in the treatments for 14 days. To circumvent degradation of exogenous L-thyroxine or thiourea, water with respective additives was replaced every 3 days. Fish were maintained under a 12:12 h light:dark cycle under full spectrum white light and fed with _Artemia_ spp. nauplii ad libitum.

**GENE EXPRESSION ANALYSIS**

After 14 days in the treatments (i.e., 28 dph), we euthanized all fish simultaneously and at the same time of day for all broods, after 6 h of light exposure (Yourick et al. 2019), with an overdose of MS-222 and stored samples in RINalater (Sigma-Aldrich, St. Louis, Missouri) at −20°C until RNA extraction. RNA was extracted from whole fish with the Tissue MiniPrep kit (Qiagen, Hilden, Germany), because extracellular opsin gene expression is negligible (Fig. S1; Karagic et al. 2018) and cDNA was synthesized with the GoScript Reverse Transcription system (Promega, Madison, Wisconsin). Expression levels were measured with quantitative real-time PCR for six cone opsin genes commonly expressed by Midas cichlids ((_sws1, sws2b, sws2a, rh2b, rh2a, lws), _cyp27c1_, as well as two housekeeping genes (_gapdh2, _imp2_; efficiencies as well as primer sequences are given in Table S2). One of the two green-sensitive paralogs, _rh2aa_, is not expressed in Midas cichlids (Härer et al. 2017; Torres-Dowdall et al. 2017); hence, it was not investigated in this study. Expression of cone opsin genes is given as proportional expression of each gene compared to the expression of all opsin genes and was calculated with the following equation:

\[
\text{Proportional expression} = \frac{1}{\sum \left(1 + E_i C_i \right)^{-1}}
\]

with \(E_i\) and \(C_i\) as the efficiency and critical cycle number of the gene of interest, respectively. \(E_i\) and \(C_m\) represent efficiencies and critical cycle numbers for all other opsin genes. Expression of _cyp27c1_ was normalized to housekeeping gene expression using their geometric mean according to the following equation:

\[
\text{Relative expression} = \text{Efficiency}^{\left(C_{gm} - C_i \right)}
\]

with \(C_{gm}\) being the geometric mean of the critical cycle numbers of the housekeeping genes and \(C_i\) the critical cycle number of the gene of interest. We computed the mean of each brood per gene and treatment to perform statistical analysis using a linear model with gene expression as the response and treatment as the explanatory variable. Using an ANOVA, we tested for the effect of the treatment on gene expression and then performed pairwise comparisons to determine significant differences between treatments for each gene using the estimated marginal means and a post hoc Tukey test (\(a = 0.05\)).

**ESTIMATIONS OF PREDICTED VISUAL SENSITIVITIES**

Predicted visual sensitivities of control, TH-treated, and thiourea-treated fish were estimated according to Rennison et al. (2016) using the mean expression levels of each opsin gene per treatment and absorbance spectra templates from Govardovskii et al. (2000). Additionally, we took chromophore usage into account based on _cyp27c1_ expression levels using the threshold of 1 that we assumed to be mostly vitamin A2-derived chromophore usage.
Eldred et al. 2018). It was previously hypothesized that changes in opsin expression, which should increase spectral sensitivities in the blue-green portion of the spectrum (i.e., between 400 and 500 nm wavelengths), compared to control fish. For TH-treated fish, differences in opsin gene expression should increase spectral sensitivity in both the short blue (i.e., 370–420 nm) and red (i.e., 560–700) portions of the spectrum. Comparing the derived and ancestral states (Fig. 2B), we found that derived fish show higher predicted spectral sensitivities in the UV and blue part of the spectrum (300–450 nm), similar to thioaura treated fish.

**Discussion**

TH is known to be an important signaling factor during the development of the retina, determining photoreceptor fate and, therefore, affecting overall spectral sensitivity (Roberts et al. 2006; Temple et al. 2008; Novales Flamarique 2013; Viets et al. 2016; Eldred et al. 2018). It was previously hypothesized that changes in circulating levels of TH in the retina might be the underlying mechanism responsible for the evolutionary changes seen in

Figure 1. Relative expression of cone opsins and cyp27c1. Expression of all six cone opsin genes (sws1 = 360 nm, sws2b = 425 nm, sws2a = 456 nm, rh2b = 472 nm, rh2a = 517 nm, lws = 560 nm; wavelengths of maximum sensitivities are derived from microspectrophotometry data from Torres-Dowdall et al. (2017); sensitivities are based on vitamin A1-derived chromophore usage) is shown in the left panel (means ± standard deviation). Cyp27c1 expression across treatments is shown in the right panel. In total, we used eight broods, each with six to 10 individual fish per brood and treatment. Letters indicate differences between treatments according to a post hoc Tukey test on estimated marginal means (see Fig. S3 for distribution of residuals). Expression levels for individual broods are shown in Figures S4–S11.

Based on Torres-Dowdall et al. (2017). We acknowledge that A1/A2 chromophore usage varies continuously (Corbo 2021), but it is likely that below certain threshold of cyp27c1 expression, mainly vitamin A1-derived chromophores are used. Wavelengths of maximum absorbance for Midas cichlids were used from Torres-Dowdall et al. (2017), except for sws1 that was taken from Spady et al. (2006) for tilapia. For the comparison between ancestral (A. citrinellus) and derived (A. astorquii) visual sensitivities, expression data were taken from Härer et al. (2017).

**Results**

**OPSEN EXPRESSION DEPENDS ON THYROID HORMONE LEVELS**

Cone opsin gene expression responded to manipulations in TH level as predicted (Fig. 1; Tables S3–S8). Overall, according to our models the opsin genes sws2a, rh2b, rh2a, and lws were

**CHROMOPHORE USAGE SHIFTS WITH INCREASING TH LEVELS**

Chromophore usage, measured indirectly by expression of cytochrome cyp27c1 (Enright et al. 2015; Volkov et al. 2020), was significantly altered by our experimental treatments (Fig. 1; Table S9). We observed a significant 3.0-fold increase in cyp27c1 expression when fish were treated with exogenous TH. In contrast, TH inhibition with thioaura led to a 0.7-fold, but nonsignificant, change compared to the untreated control fish.

**INTEGRATED CHANGES OF VISUAL PIGMENTS PRODUCE ADAPTIVE VISUAL SENSITIVITIES**

As a response to changes in opsin gene expression and chromophore usage, predicted visual sensitivities differed among experimental treatments (Fig. 2A). Thioaura-treated fish showed changes in opsin expression, which should increase spectral sensitivities in the blue-green portion of the spectrum (i.e., between 400 and 500 nm wavelengths), compared to control fish. For TH-treated fish, differences in opsin gene expression should increase spectral sensitivity in both the short blue (i.e., 370–420 nm) and the red (i.e., 560–700) portions of the spectrum. Comparing the derived and ancestral states (Fig. 2B), we found that derived fish show higher predicted spectral sensitivities in the UV and blue part of the spectrum (300–450 nm), similar to thioaura treated fish.
the visual system of Midas cichlid fishes after the colonization of clear-water crater lakes from turbid great lakes (Härer et al. 2017; Karagic et al. 2018). However, this evidence was indirect, and the direct effect of TH remained unclear. Here, we found strong support for this hypothesis, as both components of visual pigments, opsin proteins’ spectral class and chromophore identity, varied in a predictable way associated with TH manipulations (Fig. 1). Most importantly, by manipulating TH, we were able to induce the expression of the visual phenotype usually observed in derived fish from clear-water crater lakes in specimens from the source population (Fig. 2).

Previous experiments have shown that artificially increasing TH levels results in shifts in spectral sensitivity toward longer wavelengths by affecting the expression of opsin genes (Cheng et al. 2009; Novales Flamarique 2013; Viets et al. 2016), by changing chromophore usage from A1- to A2-derived (Temple et al. 2008; Suliman and Novales Flamarique 2014; Enright et al. 2015) or both (Volkov et al. 2020). Notably, we found that the opposite pattern, an increase in expression of short-wavelength-sensitive opsins, could also be induced by reducing circulating TH levels during development (Fig. 1). Across our treatments, we found that manipulations of TH levels affected chromophore usage and the expression of most opsin genes, resulting in visual sensitivities shifted in the same direction (Fig. 1). This means that TH induces an integrated change in spectral sensitivity in Midas cichlids, as both constituents of visual pigments, opsin protein and chromophore, were affected by our treatments in a concerted manner. Together, these integrated changes produced drastic differences in the spectral sensitivity of great lakes Midas cichlid fish across treatments (Fig. 2). More importantly, these changes mimicked not only the intraspecific differences during ontogeny of the ancestral species from the turbid great lakes (Härer et al. 2017), but also interspecific adaptive divergence in spectral sensitivity of Midas cichlids from Nicaraguan clear-water crater lakes (Torres-Dowdall et al. 2017).

Midas cichlids from the clear-water crater lakes adapted to the novel environments by shifting their spectral sensitivities toward shorter wavelengths compared to their source population in the great lakes (Torres-Dowdall et al. 2017). Vision in Midas cichlids from the great lakes mainly relies on a vitamin A2-derived chromophore together with a long-wavelength-sensitive set of opsins, resulting in visual pigments with peak sensitivities at long wavelengths (e.g., sws2a: $\lambda_{\text{MAX}} \sim 455$ nm, rh2aβ: $\lambda_{\text{MAX}} \sim 528$ nm, and lws: $\lambda_{\text{MAX}} \sim 602$ nm; Torres-Dowdall et al. 2017). In comparison, in crater lake fish a vitamin A1-derived chromophore is preferentially used, shifting sensitivity of the visual pigments toward shorter wavelengths (e.g., sws2a: $\lambda_{\text{MAX}} \sim 450$ nm, rh2aβ: $\lambda_{\text{MAX}} \sim 509$ nm, and lws: $\lambda_{\text{MAX}} \sim 559$ nm) and the expression of alternative opsin genes with short-wavelength sensitivity is upregulated (e.g., s2s2b: $\lambda_{\text{MAX}} \sim 431$ nm and rh2b: $\lambda_{\text{MAX}} \sim 476$ nm; based on opsins with vitamin A1-derived chromophores; Torres-Dowdall et al. 2017). All these changes occurred relatively rapidly, as the crater lakes were colonized only less than 4000 generations ago (Kau et al. 2018; Kau et al. 2020). It has been proposed that this rapid evolution occurred through ontogenetic changes, where the crater lake Midas cichlids show a paedomorphic phenotype, as their visual system develops slower and its progression is halted at an earlier developmental stage than that of their ancestors from the turbid great lakes (Härer et al. 2017; Karagic et al. 2018). Here, we provide empirical support for this hypothesis by showing that the
phenotype of the derived species can be artificially induced in the ancestral species merely by decreasing circulating TH levels with thiourea (Fig. 2).

We envision multiple potential mechanisms by which TH could have affected the rapid evolution of visual sensitivities in the Midas cichlid fish after the colonization of clear-water crater lakes. First, overall circulating TH levels could have been decreased after colonization of crater lakes from the great lakes. Stickleback fish (*Gasterosteus aculeatus*) populations that have adapted to streams repeatedly evolved to have lower circulating TH levels by downregulating the expression of a TH stimulating gene (Kitano et al. 2010). In these stickleback populations, a reduction of overall metabolic rates due to lower TH levels is adaptive, but this might not be generalized to other species where an overall change in circulating TH levels could be maladaptive due to pleiotropic effects during development (Inui and Miwa 1985; Brown 1997; Gamborino et al. 2001; Johnson and Voss 2013). Second, local control of TH metabolism could be driving adaptive divergence in the retina of fish while avoiding negative pleiotropic effects. TH concentrations can be controlled in a tissue-specific manner by differential regulation of TH-metabolizing genes, for example, deiodinases (Bianco and Kim 2006). An increase in retinal expression of the TH-inactivating deiodinase 2 (dio2) together with a decrease in expression of the TH-activating deiodinase 3 (dio3) could lower the levels of TH present in the eye without having extraocular pleiotropic effects (Bianco and Kim 2006). A previous study has identified such differences in local TH control within retinas of clear-water crater lake compared to the turbid-water great lake Midas cichlids, where crater lake fish express more dio2 and less dio3 than fish from the ancestral great lake environment (Härer et al. 2017). Our TH treatments evoked the same response with thiourea-treated fish expressing more dio2 and less dio3 compared to TH-treated fish (Fig. S2). Finally, another potential mechanism to change the retinal response to circulating TH levels can be achieved by locally lowering the expression of TH receptors (Mader and Cameron 2006; Roberts et al. 2006; Raine and Hawryshyn 2009; Volkov et al. 2020). A reduction in TH receptor sensitivity due to structural changes could have a similar effect (Adams et al. 1994; Hayashi et al. 1995). However, structural changes will affect TH sensitivity in the organism as a whole. Therefore, it is likely that regulatory evolution is driving retina-specific changes in the expression of TH-related genes (e.g., deiodinases and/or TH receptors).

The integrated response in the retina to changes in TH levels also provides a logical scenario explaining how all the changes seen in the visual system of Midas cichlids (Torres-Dowdall et al. 2017) could have occurred in the short time since the colonization of the crater lakes (Kautt et al. 2020). This integrated response also goes in line with the highlighted role of heterochrony in the evolutionary diversification of the visual system of African cichlids (Carleton et al. 2008; Carleton et al. 2016; O’Quin et al. 2011) and other vertebrates (Wikler and Finlay 1989). Such heterochronic shifts evoking integrated adaptive changes in the eye might be more common among vertebrates than appreciated and more effort needs to be focused on elucidating such questions in other systems. Adaptations of the visual system of Midas cichlids, thus, illustrate how changes in the endocrine system can produce a more integrated adaptive response of multiple phenotypes in a relatively short evolutionary timeframe. Generally, such conclusions are scarce in the current literature, because most studies focus on physiological or developmental processes of hormonal signal transduction (NG et al. 2001, Harpavat & Cepko 2003, Roberts et al. 2006, Temple et al. 2008, Cheng et al. 2009, Suliman & Novales Flamarique 2014, Viets et al. 2016). Here, we present an evolutionary perspective on changes in TH metabolism eliciting integrated adaptive phenotypic responses.

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**AUTHOR CONTRIBUTIONS**

JTD and NK conceived the idea. All authors designed the experiment. NK conducted treatments, experimental procedures, and data analysis. NK and JTD wrote the manuscript with comments from all authors.

**DATA ARCHIVING**

Data from this manuscript have been deposited to the Dryad repository and can be accessed via https://doi.org/10.5061/dryad.fzfr34tw9.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**LITERATURE CITED**


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Supplementary Information
Additional supporting information may be found online in the Supporting Information section at the end of the article.

Supplementary material

Supplementary Table 1: Proportional expression for all opsin genes and relative expression for cyp27c1. Data is given as mean per treatment per brood ± sd. Sample sizes for the expression of opsin genes are given under n and numbers in brackets denote the sample size used for investigating the expression of cyp27c1.

Supplementary Table 2: Efficiencies and primer sequences used for all opsin genes, cyp27c1 and two housekeeping genes, imp2 and gapdh2.

Supplementary Table 3: Results of a linear model using sws2a expression as a response variable and treatment as a fixed effect. The models are based on the gene expression of sws2a using brood means as data. Shown are the results of the ANOVA and the estimated marginal means to compare differences among groups. TH = thyroid hormone treatment, Tu = thiourea treatment, df = degrees of freedom, sum sq = sum of squares, mean sq = mean of squares, emmean = estimated marginal mean, se = standard error, CL = confidence level.

Supplementary Table 4: Results of a linear model using sws2b expression as a response variable and treatment as a fixed effect. The models are based on the gene expression of sws2b using brood means as data. Shown are the results of the ANOVA and the estimated marginal means to compare differences among groups. TH = thyroid hormone treatment, Tu = thiourea treatment, df = degrees of freedom, sum sq = sum of squares, mean sq = mean of squares, emmean = estimated marginal mean, se = standard error, CL = confidence level.

Supplementary Table 5: Results of a linear model using sws1 expression as a response variable and treatment as a fixed effect. The models are based on the gene expression of sws1 using brood means as data. Shown are the results of the ANOVA and the estimated marginal means to compare differences among groups. TH = thyroid hormone treatment, Tu = thiourea treatment, df = degrees of freedom, sum sq = sum of squares, mean sq = mean of squares, emmean = estimated marginal mean, se = standard error, CL = confidence level.

Supplementary Table 6: Results of a linear model using rh2b expression as a response variable and treatment as a fixed effect. The models are based on the gene expression of rh2b using brood means as data. Shown are the results of the ANOVA and the estimated marginal means to compare differences among groups. TH = thyroid hormone treatment, Tu = thiourea treatment, df = degrees of freedom, sum sq = sum of squares, mean sq = mean of squares, emmean = estimated marginal mean, se = standard error, CL = confidence level.

Supplementary Table 7: Results of a linear model using rh2a expression as a response variable and treatment as a fixed effect. The models are based on the gene expression of rh2a using brood means as data. Shown are the results of the ANOVA and the estimated marginal means to compare differences among groups. TH = thyroid hormone treatment, Tu = thiourea treatment, df = degrees of freedom, sum sq = sum of squares, mean sq = mean of squares, emmean = estimated marginal mean, se = standard error, CL = confidence level.
**Supplementary Table 8**: Results of a linear model using lws expression as a response variable and treatment as a fixed effect. The models are based on the gene expression of lws using brood means as data. Shown are the results of the ANOVA and the estimated marginal means to compare differences among groups. TH = thyroid hormone treatment, Tu = thiourea treatment, df = degrees of freedom, sum sq = sum of squares, mean sq = mean of squares, emmean = estimated marginal mean, se = standard error, CL = confidence level.

**Supplementary Table 9**: Results of a linear model using cyp27c1 expression as a response variable and treatment as a fixed effect. The models are based on the gene expression of cyp27c1 using brood means as data. Shown are the results of the ANOVA and the estimated marginal means to compare differences among groups. TH = thyroid hormone treatment, Tu = thiourea treatment, df = degrees of freedom, sum sq = sum of squares, mean sq = mean of squares, emmean = estimated marginal mean, se = standard error, CL = confidence level.

**Supplementary Figure 1**: For measurements of opsin gene expression, we used whole embryos for RNA extractions. To exclude an effect from extraocular opsin gene expression on our measurements on visual sensitivities, we compared intraocular to extraocular opsin gene expression. For this, we extracted RNA from six Amphilophus citrinellus embryos using only the eyes and the rest of the body for each individual. Subsequently, we measured opsin expression in eyes and whole bodies using quantitative real-time PCR. Depending on the opsin gene of interest, expression in eyes is between ~30-10,000 times higher compared to the rest of the body.

**Supplementary Figure 2**: Expression of genes involved in TH-metabolism. We measured gene expression using quantitative real-time PCR. Gene expression was normalized using two housekeeping genes (imp2 and gapdh2). For TH-receptors, no significant effect of TH-treatments on expression was observed. The TH-metabolizing genes dio2 and dio3 are used as proxies for circulating TH-levels. dio2 catabolizes the reaction from inactive T4 to active T3 with a negative feedback, i.e. high T3 levels inhibit dio2 expression. In contrast, dio3 catabolizes the reaction from active T3 to inactive T4, with a positive feedback, i.e. high levels of T3 activate expression of dio3. Hence, high dio2 expression indicates low circulating T3 levels, whereas, high dio3 expression indicates high levels of circulating TH.

**Supplementary Figure 3**: We plotted the residuals of our linear models with gene expression of each opsin gene against treatment to look for heteroscedasticity in the data.

**Supplementary Figure 4**: Proportional opsin gene expression for brood I. Individual data points show the expression of a single individual of A. citrinellus.

**Supplementary Figure 5**: Proportional opsin gene expression for brood II. Individual data points show the expression of a single individual of A. citrinellus.

**Supplementary Figure 6**: Proportional opsin gene expression for brood III. Individual data points show the expression of a single individual of A. citrinellus.

**Supplementary Figure 7**: Proportional opsin gene expression for brood IV. Individual data points show the expression of a single individual of A. citrinellus.

**Supplementary Figure 8**: Proportional opsin gene expression for brood V. Individual data points show the expression of a single individual of A. citrinellus.

**Supplementary Figure 9**: Proportional opsin gene expression for brood VI. Individual data points show the expression of a single individual of A. citrinellus.

**Supplementary Figure 10**: Proportional opsin gene expression for brood VII. Individual data points show the expression of a single individual of A. citrinellus.

**Supplementary Figure 11**: Proportional opsin gene expression for brood VIII. Individual data points show the expression of a single individual of A. citrinellus.

**Supplementary Figure 12**: Predicted visual sensitivities comparing fish from the experimental TH treatment by assuming pure vitamin A1-derived chromophore usage (black) or pure vitamin A2-derived chromophore usage (red). Predicted sensitivities are based on a combination of gene expression levels for each opsin gene (Govardovskii et al. 2000; Rennison et al. 2016) and their respective peaks of maximum absorbance (Torres-Dowdall et al. 2017a). Solid lines illustrate the mean ± standard deviation (shaded areas).