Evolutionary developmental biology

UV wavelengths experienced during development affect larval newt visual sensitivity and predation efficiency

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We experimentally investigated the influence of developmental plasticity of ultraviolet (UV) visual sensitivity on predation efficiency of the larval smooth newt, Lissotriton vulgaris. We quantified expression of SWS1 opsin gene (UV-sensitive protein of photoreceptor cells) in the retinas of individuals who had developed in the presence (UV⁺) or absence (UV⁻) of UV light (developmental treatments), and tested their predation efficiency under UV⁺ and UV⁻ light (testing treatments). We found that both SWS1 opsin expression and predation efficiency were significantly reduced in the UV⁻ developmental group. Larvae in the UV⁻ testing environment displayed consistently lower predation efficiency regardless of their developmental treatment. These results prove for the first time, we believe, functional UV vision and developmental plasticity of UV sensitivity in an amphibian at the larval stage. They also demonstrate that UV wavelengths enhance predation efficiency and suggest that the magnitude of the behavioural response depends on retinal properties induced by the developmental lighting environment.

1. Introduction

Many animals possess ultraviolet (UV, 300–400 nm) vision and use UV cues (reflectance or absorbance) from food items or the environment while foraging for food (reviewed in [1]). When UV cues are not accessible, animals’ foraging efficiency is directly negatively affected [2,3]. A recent study demonstrated that, at least in zooplankton predators, UV vision allows location of UV-absorbing food items at greater distances and angles [4].

Variation in lighting environment directly affects development of visual systems [5]. Development in UV-poor environments results in a decreased expression of SWS1 opsin protein, the UV-sensitive molecule of retinal photoreceptor cells [6,7]. Differential expression of the opsin gene should result in differences in different cone type densities or in visual pigments within cones. This could impact colour discrimination [8], visual sensitivity and speed of phototransduction [9]. Interestingly, in the bluefin killifish, developmental plasticity in the retina induces variations in how individuals perceive colours in different lighting environments [10]. The critical question is whether such plasticity also has implications for fitness-related behaviours like foraging.

This is a major issue for freshwater-foraging UV-sensitive species because they frequently experience heterogeneous UV transmission conditions owing to strong spatial and temporal variation in UV-absorbing organic molecules [11]. Here, we investigated the importance of UV vision plasticity on foraging efficiency in the larval stage of the smooth newt, Lissotriton vulgaris, a species that breeds and develops in lentic habitats. Larvae are aquatic and feed on...
UV-absorbing zooplankton, whereas adults have a diversified carnivorous diet and alternate aquatic and terrestrial phases. Adults possess a retina with photoreceptor cells sensitive to UV, short and long wavelengths [12], and use UV signals in sexual selection [13].

We tested (i) whether the SWS1 opsin gene is expressed in the retina of newt larvae, (ii) whether UV light conditions experienced during development modulate SWS1 expression, and (iii) whether the effects of immediate lighting environment and developmental plasticity interact to affect foraging efficiency. To do so, we reared larvae from eggs in the presence (UV+) or absence (UV−) of UV light (developmental environments), measured the expression of the SWS1 gene, and assessed predation efficiency of each individual under both UV+ and UV− light (testing environments).

We predicted lower SWS1 expression level and predation efficiency in the UV− developmental group as well as lower predation efficiency in the UV− testing environment regardless of the developmental group.

2. Material and methods

(a) Sampling, rearing conditions and lighting treatments

Larvae from 11 gravid female L. vulgaris captured on April 2012 in a pond near Angers (western France) were reared from eggs laid in a UV-free lighting environment (see details in the electronic supplementary material, S1). From hatching, each larva was exposed daily to 15 min supplementary lighting. Half the larvae from each female were exposed to UV+ visible light (UV+ developmental group, DUV+), while the other half was exposed to visible light only (UV− developmental group, DUV−). To do so, rearing containers were moved into a darkroom equipped with a UV-B neon light (Repti Glo 5.0 UVB/T8, Exo Terra®), located at 20 cm above the water surface. This light was left uncovered for the DUV+ group, or covered with a UV-blocking filter (3 mm layer of clear DuraLar® polyester film, Grafix®) for the DUV− group (see electronic supplementary material, S2, for spectra of lighting conditions).

(b) Predation tests

At the final stage before metamorphosis (i.e. 58–64 days after hatching), visual foraging performance of 29 larvae from 11 females was tested. Tests took place in the darkroom with the lighting system described above after a 48 h fasting period. Before the test began, each larva was placed in a Petri dish (40 mm diameter × 17.4 mm height, 1 cm water column) under the neon light and, after 9 min of habituation, the light was turned on. One minute later, eight large Daphnia magna were gently introduced in the Petri dish and the test begun. To optimize detectability and attractiveness of prey, daphnia had been preliminarily fed with a lutein–yeast solution to enhance their reflectance in the visible and UV ranges (details in the electronic supplementary material, S3 and S4; [3]). The number of remaining live prey items was recorded every 5 min for 90 min. Each larva was tested twice, once under UV+ visible light (UV+ testing group, TUV+) and once under visible light (UV− testing group, TUV−) in a random presentation order, with a minimum 2 day interval between tests.

(c) Quantification of opsin gene expression

After behavioural tests, we selected two larvae at the final larval stage, one from each developmental group, from nine females (for two females, all larvae of one group had metamorphosed before analysis). After euthanasia and enucleation, relative opsin mRNA expression of SWS1 opsin gene in the eye was quantified using methods described by Dupuis et al. [14] and Piafli et al. [15] (see electronic supplementary material, S5 and S6 for method details). In order to ensure that experimental conditions affected SWS1 opsin expression only, we also quantified the long-wavelength-sensitive (LWS) opsin expression.

(d) Statistical analyses

All analyses were carried out using R v. 3.1.1 [16]. The differences in relative mRNA expression levels of SWS1 and LWS genes between the two developmental treatments were tested using paired Wilcoxon tests. To analyse predation tests, we tested the effects of developmental and testing treatments and their interaction on daphnia survival using a mixed-effects Cox model (coxme package [17]), with larva identity nested within the random effects. The model included binary covariates representing the developmental treatment (UV+ or UV−) and the testing treatment (UV+ or UV−), with their interaction. The model was run both with (i) a null model, where the effects of immediate lighting environment and developmental plasticity interact to affect foraging efficiency and (ii) whether UV light conditions experienced during development modulate SWS1 expression, and (iii) whether the effects of immediate lighting environment and developmental plasticity interact to affect foraging efficiency. To do so, we reared larvae from eggs in the presence (UV+) or absence (UV−) of UV light (developmental environments), measured the expression of the SWS1 gene, and assessed predation efficiency of each individual under both UV+ and UV− light (testing environments). We predicted lower SWS1 expression level and predation efficiency in the UV− developmental group as well as lower predation efficiency in the UV− testing environment regardless of the developmental group.

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female identity as a random factor. Test order was introduced as covariate. Surviving daphnia were treated as right-censored (i.e. still alive) cases. Analysis began with a full model including all experimental effects as fixed effects. The minimum adequate model was obtained using likelihood ratio tests (LRTs).

3. Results

Developmental treatment strongly affected SWS1 gene expression (paired Wilcoxon test, \( V = 45, N = 9, p = 0.004 \)) but not LWS opsin gene expression (\( V = 35, N = 9, p = 0.16 \), figure 1). UV light deprivation during development resulted in a 53.1% average decrease in SWS1 opsin expression at the final larval stage.

Predation tests reveal that daphnia prey survival was significantly affected by both developmental (\( \chi^2 = 6.59, p = 0.010 \)) and testing treatment (\( \chi^2 = 10.83, p = 0.001 \)). Survival probability of daphnia was 71.1 \( \pm \) 2.7% higher in tests with a DUV– newt predator than in tests with a DUV+ newt (figure 2). Regardless of developmental treatment, daphnia survival was 46.6 \( \pm \) 1.4% higher during TUV– tests than during TUV+ tests (figure 2; see also the electronic supplementary material, S7, for a representation of the inter-individual variation of the total number of eaten prey at the end of the test). Prey survival did not depend on test order (TUV+ or TUV– in first test, LRT: \( \chi^2 = 0.14, p = 0.91 \)).

4. Discussion

We found low, but substantial levels of SWS1 gene expression in the eyes of larvae, of the same order as those found in several fish species (e.g. [11,18]). This is the first demonstration to our knowledge that UV cones are present and functional in the retina of an amphibian at the larval stage. In addition, we demonstrated developmental plasticity in the expression of the SWS1 gene. Expression of SWS1 was halved in larvae deprived of UV light during development, whereas LWS expression remained unchanged. SWS1 opsin was still expressed in UV-deprived larvae, which suggests that its expression is partly genetically determined. Our results are consistent with other findings in vertebrates [6,7,18], and support the idea that the visual system is plastic and changes with the quantity and quality of wavelengths available in the environment [19].

UV light conditions experienced during development can affect behaviour [10,20]. For example, bluefin killifish reared in a UV-rich environment shifted colour-guided foraging preferences when tested in a UV-poor environment, whereas those reared in a UV-poor environment did not shift when tested in a UV-rich environment [10]. By contrast, we found additive, not interactive, effects of the immediate and developmental lighting environment on predation efficiency in newts. Regardless of developmental group, prey survival was higher in the UV– testing environment, meaning that, as in fishes [2–4], the use of UV cues enhances predation efficiency in newt larvae. We also found that UV-deprived larvae foraged less efficiently than larvae reared with UV light. Thus, developing in UV-poor environments such as coloured water strongly reduces the performance of visually guided behaviours like foraging, which potentially negatively affects fitness components at the larval or later stage.

Overall, this study suggests that the magnitude of visually guided behavioural response depends on the retinal properties induced by the developmental lighting environment. We can hypothesize that early UV light exposure is required for the proper development of UV-sensitive visual systems; otherwise the performance of visually guided tasks can be jeopardized. Thus, UV transmission properties of the environmental medium might be an unrecognized but important factor for the selection of breeding sites in UV-sensitive animals.

Ethics. All experiments were conducted under the approval of the Préfecture de Maine-et-Loire (permit 04/2012) and in accordance with the current laws in France.

Data accessibility. Data are available at Dryad: http://dx.doi.org/10.5061/dryad.444s1.

Authors’ contribution. J.S., G.R., S.S., M.T. conceived and designed the study. G.R., S.S., J.S. performed the behavioural experiment. M.M., G.R., J.S. analysed data. D.G. carried out the genetic labwork. P.M. developed primers. M.M., J.S., G.R., M.T., D.G., S.S., P.M. drafted and revised the manuscript. All authors gave final approval for publication and agree to be held accountable for the work performed.

Competing interests. Authors have no conflict of interests.

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