

**EFFECTS OF BACKGROUND COLOR AND PREDATION RISK ON COLOR  
CHANGE IN FIRE SALAMANDER LARVAE**

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ABSTRACT

The threat-sensitivity hypothesis assumes individuals should demonstrate flexibility in response to perceived predation risk and vary the intensity of anti-predator responses in concert with perceived risk of predation. Substrate color matching is adaptive as it enables organisms to become less conspicuous to both their prey and predators. I hypothesized that newborn fire salamander (*Salamandra atra*) larvae will respond fast through physiological color change to contrasting backgrounds, becoming lighter against a white background and darker against a black background. Additionally, in accordance with the threat-sensitivity hypothesis, I expected a background color  $\times$  predator interaction—i.e., that predator presence will further enhance the focal larvae color-matching response.

To explicitly test these hypotheses I conducted a replicated outdoor mesocosm experiment. I used a two-by-two factorial design: pools of black or white background color crossed with the presence or absence of a larger cannibalistic conspecific. Digital photos of the focal larvae's dorsal view revealed that larval brightness and chroma changed accordingly against the contrasting black and white backgrounds to increase background matching. Although not statistically significant, larvae tended to show a stronger color-change response towards enhanced background matching in the presence of the free predator. Larval survival was strongly reduced in the presence of the larger conspecific, with no apparent effect of background color. This study demonstrates that *Salamandra* larvae are capable of environmentally induced physiological color change and highlights the need for further investigation into the interplay between threat intensity, mechanisms of risk assessment, and physiological antipredator responses.

*Keywords:* amphibian, crypsis, physiological color change, pigmentation, predator, prey, background, threat sensitivity

## INTRODUCTION

The threat-sensitivity predator avoidance hypothesis assumes individuals should demonstrate flexibility in response to perceived predation risk (Helfman, 1989). Escalation in antipredator responses is expected with increased perceived predation risk, to maximize potential survival benefits. In situations where perceived predation risk is low, individuals are expected to exhibit less intense response to reduce energy costs and/or allow the engagement in alternative activities (Brown, 2005). Predator-induced physiological responses are poorly explored compared to other behavioral, morphological, and life history traits (Noonburg and Nisbet, 2005; Steiner and Van Buskirk, 2009).

Adaptive cryptic coloration, also known as background matching, crypsis, or camouflage, has been empirically shown to reduce predation risk and detection by visual predators (Feltmate and Williams, 1989; Donnelly and Whoriskey, 1991; Cooper and Allen, 1994; Merilaita et al., 2001; Johannesson and Ekenbhal, 2002). The ability to rapidly (on the order of minutes) and reversibly change color has evolved independently in a variety of taxonomic groups including cephalopods, crustaceans, spiders, amphibians, reptiles, and fish (Stuart-Fox and Moussalli, 2008). Color change in amphibians not only reduces conspicuousness to predators (Heinen, 1994; Garcia and Sih, 2003; Garcia et al., 2004), but is also involved in reduction of UV damage (Endler, 1988; Garcia et al., 2004), and thermoregulation (Kats and Van Dragt 1986; Hutchinson and Dupre, 1992; Garcia et al., 2003). The process entails pigment movement within special cells in the animal integument. Physiological color change occurs primarily through dispersion, contraction, and migration of melanin within the dermal melanophores, the cells containing melanin (Herman, 1992; Viertel and Richter, 1999). The resulting pigmentation changes enable several amphibian species to quickly adjust to different combinations of light and substrate color.

Larvae of the fire salamander (*Salamandra infraimmaculata*) experience high density-dependent mortality rates throughout their aquatic larval phase (Eitam et al., 2005). Larval skin, unlike that of the terrestrial post-metamorphic stages, does not contain toxins (Daly, 1995), making larvae highly palatable and susceptible to predation, which is primarily inflicted by larger cannibalistic conspecifics (Warburg, 1992; Degani, 1993). Larvae of *Salamandra* and other urodeles experience both inter and intra-cohort cannibalism which intensifies with larval development and size variation (Maret and Collins, 1994; Cohen-Koren et al., 2005; Eitam et al., 2005). Moreover, cannibalism in *S. infraimmaculata* is common as breeding sites are often ephemeral ponds of restricted size (Segev et al., in press) where food resources are limited.

In this study, I quantified the effect of contrasting black and white background color and a predator (large conspecific) on *S. infraimmaculata* larval pigmentation. I hypothesized that larvae will respond fast through physiological color change to contrasting backgrounds, becoming lighter against the white background and darker against the black. Additionally, in accordance with threat-sensitivity hypothesis, I expected a background color  $\times$  predator interaction—i.e., that the predator presence will further enhance the focal larvae color-matching response.

## METHODS

I used a two-factorial design to identify how background color, predation risk in the form of a larger cannibalistic conspecific, and their interaction might affect rapid (physiological) changes in color in recently born *Salamandra* larvae. I used 100 two-week-old larvae (mean total length: 32.33 mm, SE = 0.12; mean weight: 0.21 g, SE = 0.003) deposited in captivity by 5 different females. The gravid females were collected at a breeding site, Ein-al-Balad (32°43'13"N; 35°04'17"E) in Mt. Carmel National Park. Prior to the experiment, larvae were kept outdoors in 5 plastic tubs (length, width, depth: 60 × 40 × 14 cm). To test the effect of background color and conspecific predator, for each replicate, I placed 5 larvae in a 33 L plastic tub (length, width, depth: 60 × 40 × 14 cm) and attached either a black or white plastic sheet to the bottom of each tub, with 10 replicates for each color. Treatments were randomized in a 2 × 10 array under a 90% shade net suspended 1.5 m above the ground. I added a single predator—a larger conspecific larva 3–4 times the mass of the focal “prey” larvae—into five light-background tubs and five dark-background tubs. These larger larvae were collected at the same breeding site as the gravid mothers.

Larvae were assigned to the experimental tubs such that each treatment replicate included larvae from all 5 mothers, to reduce the possibility for a maternal effect. Forty-eight hours after introducing the larvae into the tubs, I collected all the surviving larvae and photographed a dorsal view of each larva at the lab. I used a digital Leica DFC 300 color camera mounted on a Leica MZ 125 binocular. Illumination conditions (distance from, and angle to, the subject) were kept constant throughout the photographic procedure using a cold-light source (Leica CLS 150X). I used three color components to define larval color: brightness (i.e., amount of black versus white), chroma (i.e., amount of grey and white mixed with the “pure” focal color), and hue (the actual color, e.g. yellow, red, blue, etc.). An increase in brightness and hue and a decrease in chroma values mean higher resemblance to solid white, while a decrease in brightness and hue and an increase in chroma values mean higher resemblances to a solid black background. Photos were saved in TIFF format (Stevens et al., 2007) and analyzed using an image analyzing software (Photoshop 10.0). Using Photoshop, I randomly selected 3 equal size patches (10,000 pixels each) from the dorsal view (head and body) of each larva and quantified the mean number of pixels on every color intensity level, i.e., measured the brightness of the selected area in a grayscale mode as well as the average intensity of red, green, and blue that I transformed into values of chroma and hue (Garcia and Sih, 2003; Garcia et al., 2004). To test for predation and background-color effects on larval survival, I used a non-parametric test (Mann-Whitney U) due to the lack of variance in larval survival in the absence of a predator (100% survival in the predator-free tubs). For the color-response variables, I used each tub average and performed a two-way ANOVA on the values brightness, chroma, and hue.

I chose to expose the focal larvae to a free predator and not to a caged predator or predator-conditioned water to maximize the focal larvae exposure to predator cues: visual, mechanical, and chemical. Moreover, in a cannibalistic system prey may respond

only to chemical components released from injured and/or consumed individuals (“alarm cues”), and not to chemical components specific to the predator (“kairomones”). As the large conspecific larvae were not caged and could consume the smaller focal larvae, it was possible that predation may selectively remove individuals that are less “background matched” and consequently reduce within-tub variance, I therefore tested for a treatment effect using a two-way ANOVA on within-tub variance in color values and found no statistically significant differences in either of the three color variables.

### RESULTS

Prior to treatment assignment of the larvae, there was no significant difference in any of the color variables— brightness, chroma, or hue (ANOVA:  $p > 0.98$  for all three color variables). However, 48 hours post-treatment exposure, larval color changed to resem-

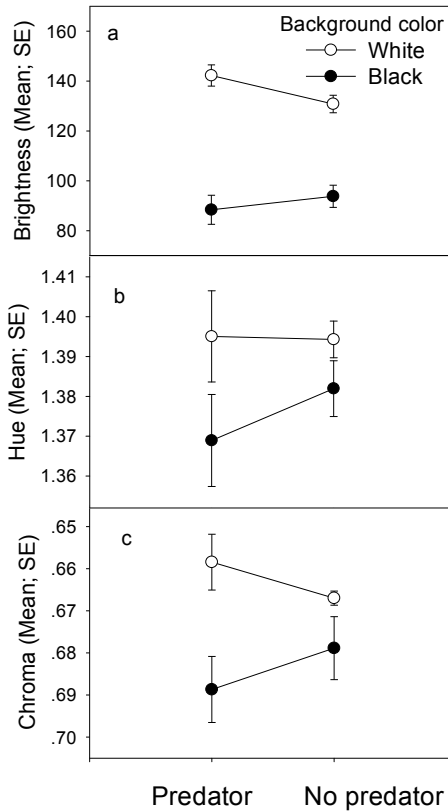


Fig. 1. Effects of background color and potential predator (large cannibalistic conspecific) on larval *Salamandra* (tub mean  $\pm$  SE): (a) brightness; (b) hue; (c) chroma.

ble the background more closely, resulting in a significant change in brightness (increase against the white background and decrease against the black) and chroma (decrease against the white background and increase against the black) and a marginal effect on larval hue (Figs. 1, 2; Table 1). There was no overall effect of the predator on prey larval color. Although the predator  $\times$  color interactions were not statistically significant, prey larvae tended to show a directional response towards enhanced background matching under predation risk. Statistical power, using the measured effect size, was very low for the interactive terms (Table 1).

Survival of *Salamandra* larvae was strongly reduced in the presence of a larger conspecific; 100% survived in the absence of the larger conspecific, while only 60% (mean per pool: 3; SE = 0.39) survived in the presence of the larger conspecifics (Mann-Whitney:  $U = 95$ ;  $p < 0.0001$ ). Background color had no significant effect on larval survival in the predator tubs:  $2.8 \pm 2.2$  (Mean  $\pm$  SE) larvae survived against the black background and  $3 \pm 1.5$  (Mean  $\pm$  SE) survived against the white background (Mann-Whitney:  $U = 48.5$ ;  $p = 0.900$ ).

#### DISCUSSION

This study was designed to assess two levels of prey cryptic response: a “general” or “baseline” background matching response, and an increased color-change response



Fig. 2. Larval pigmentation following 48 h exposure to a black background (left) and a white background (right).

Table 1

Two-way ANOVA assessing the effect of background color, predator (large cannibalistic conspecific), and their interaction on larval *Salamandra* brightness, hue, and chroma (df = 1.16)

Variable	Treatment	F-ratio	<i>p</i>	Power
Brightness	Background Color	42.88	<0.0001	0.999
	Predator	1.39	0.255	0.073
	Color × Predator	2.29	0.149	0.157
Hue	Background Color	4.43	0.052	0.357
	Predator	0.45	0.512	0.05
	Color × Predator	0.57	0.463	0.05
Chroma	Background Color	10.81	0.005	0.78
	Predator	0.01	0.923	0.05
	Color × Predator	2.06	0.171	0.134

induced by the presence of a free predator. Results confirm for the first time, that *Salamandra inframaculata* larvae are capable of physiologically changing dorsal pigmentation to achieve a higher degree of background matching. Substrate color matching in the absence of perceived risk of predation may reduce conspicuousness to undetected predators and can also have an adaptive value in the absence of immediate predation threat by rendering larvae less detectable to their own prey. Substrate color matching has been demonstrated in other species (Kats and Vandragt, 1986; Thurman, 1990; Stegen et al., 2004; Théry, 2007; Hanlon et al., 2009; Whiteley et al., 2009), and urodeles (*Ambystoma opacum*, *A. punctatum*, and *A. tigrinum*—Pietsch and Schneider, 1985, 1990; *Ambystoma texanum* and *A. barbouri* - Garcia and Sih, 2003).

Predator-induced responses are expected to be adaptive when predation risk varies spatially and temporally and when prey can reliably detect and estimate immediate predation risk (Lima and Steury, 2005). The predator effect was non-significant because, without reference to the background color, there was no directional change in the focal larvae pigmentation that increases crypsis. Moreover, since *Salamandra* larvae use a sit-and-wait tactic to ambush prey (Duellman and Trueb, 1994), background color matching may only partly increase the prey's safety. This may also reduce the effect of the predator × color interaction term.

The results did not demonstrate conclusively color matching enhancement under perceived risk of predation. However, given the trend observed in these data, a predator-induced augmentation in color matching cannot be ruled out; increased sample size (not possible in this study due to limits set by the Israel Nature and Parks Authority) might demonstrate a predator × background interaction effect on larval brightness and chroma (Table 1). Furthermore, the high variability in chroma and hue values in the predator treatments suggest that larvae varied in their predator-induced color change response. This plasticity may result from high variation in predation intensity between

tubs, although there was no correlation between predation intensity and the focal larvae color change. The variation in color and brightness values may also reflect individual genetic variation in the ability to change color and/or to perceive predation risk. Fine tuning of predation risk, for example with the use of different concentrations of alarm chemicals (Brown et al., 2004; Mizra et al., 2006), may reveal directional color change response in this system.

The stimulus that triggers antipredator responses is often species-specific (Garcia and Sih, 2003; Lehtiniemi, 2005) and context-dependent, as different sensory modalities may be involved in both predator detection and assessment (Barbosa and Castellanos, 2005). The use of free predator in the experimental design was intended to allow local predation risk assessment based on damage-released chemicals, a widely common signal in freshwater aquatic systems (Chivers and Smith, 1998). A plausible mechanism for predator-induced color change response may involve detection of chemical alarm cues coupled with an antipredator behavioral response (Houtman and Dill, 1994). Focal larvae may behaviorally change their spatial arrangement within the experimental tub, i.e., aggregate or stay closer and longer near the tub's circumference, consequently changing the interaction between direct and background reflected light. This interaction has been experimentally shown to elicit background-matching response in other salamanders, *Ambystoma tigrinum* (Pietsch and Schneider, 1985, 1990). To further our understanding of color-change plasticity within the framework of threat-sensitivity and risk allocation hypothesis, more empirical work is needed to address explicit hypotheses concerning adaptive value, mechanisms, and costs.

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