

Spots of adult male red-spotted newts are redder and brighter than in females: evidence for a role in mate selection?

Andrew K. Davis¹ & Kristine L. Grayson²

¹D.B. Warnell School of Forestry and Natural Resources, The University of Georgia, Athens, Georgia, USA

²Department of Biology, University of Virginia, Charlottesville, Virginia, USA

As aquatic adults, eastern red-spotted newts (*Notophthalmus v. viridescens*) are generally green with two rows of dorsal spots, which vary in number between individuals and range in colour from orange to red. The function of these spots is unknown, but it is possible that they serve as sexual ornamentations and we examined this hypothesis by testing for sexual dimorphism in spot characteristics. We used an image analysis approach that has been used previously with this and other amphibian species to compare the number, size and colour – redness (hue score) and brightness – of spots in 100 male and 100 female aquatic adult newts captured during the breeding season in Virginia. While we found no apparent sex-related differences in the numbers of spots, and only a minor difference in spot sizes, we discovered that adult male red-spotted newts had significantly redder and more brightly coloured dorsal spots than did females. We also found that spot brightness is positively associated with body size. As male red-spotted newts entice females to mate in part with a highly visual display (the “hula dance”), the sexual dimorphism in spot colour we found could be associated with this display. Our results, taken together with current knowledge on the carotenoid pigments behind the red spot colour, suggest a role for spot coloration in the mating system of *N. v. viridescens* that should be examined further and considered in other studies of mate choice. In a more general sense, this discovery also leads us to question if this phenomenon is present in other amphibian species previously thought to be “monomorphic”.

Key words: coloration, image analysis, *Notophthalmus v. viridescens*, sexual dimorphism, spot pattern

INTRODUCTION

Amphibians display a wide diversity of skin pigmentation patterns and colours, but for many species their function is unknown. Some species have cryptic patterns that no doubt aid in camouflage (Fernandez & Collins, 1988; Storfer et al., 1999). Others have brightly coloured patterns, which advertise their toxicity or mimic the toxicity of another species (Daly & Myers, 1967; Howard & Brodie, 1971; Brodie & Brodie, 1980). Sexual dimorphism in pigmentation is also known to exist in certain amphibian species (Kramek & Stewart, 1980; Buchanan, 1994; Hoffman & Blouin, 2000; Todd & Davis, 2007), and in most cases where this is found the hypothesis is that it serves a role in courtship or mate attraction (Vásquez & Pfennig, 2007). This biological phenomenon is present in many other animal taxa; within sexually dimorphic bird species, more brightly coloured males are known to be selected by females (e.g. Hill et al., 1999), and the same is true in some lizards (Kwiatkowski & Sullivan, 2002) and fish (Godin & Dugatkin, 1996). Even among insects, variation in male colour has been shown to be related to mating success in butterflies (Davis et al., 2007). Thus, it is certainly reasonable to expect a similar situation among amphibians that show sex-related differences in colour traits, especially in pigments that are energetically expensive to synthesize, such as carotenoids, which form the red and orange colours of animal integument (Hill, 1992; 2000).

Eastern red-spotted newts (*Notophthalmus v. viridescens*), as their name implies, have distinct red dorsal spots, and little is known about their function. Histological work has demonstrated that the spots of red-spotted newts are made up of carotenoid pigments, which are deposited by specialized chromatophore cells (erythrophores) in the spot regions (Forbes et al., 1973). In *N. v. viridescens* these dorsal spots are generally small, in the order of 1% of the dorsal body surface area combined (Davis & Grayson, 2007), and therefore may be poorly suited for advertising toxicity or for camouflage. Most *Notophthalmus* species develop these dorsal spots in the larval stage and retain them throughout their lifecycle, while their background coloration changes dramatically between life stages (Conant & Collins, 1998; Petranka, 1998). After metamorphosis, in the terrestrial eft stage, individuals are highly toxic to predators (Brodie, 1968) and have bright red skin that serves an aposematic function (Howard & Brodie, 1971). Upon returning to the aquatic habitat to breed, the background coloration of adults transforms to green, although there is wide variation in the degree of green (Davis & Grayson, 2007). In this stage, individuals are approximately ten times less toxic than in the eft stage (Brodie, 1968). Furthermore, background coloration changes with habitat, as adults migrating to the forest for the non-breeding season (winter) become browner, suggesting background colour serves a camouflage function in the adult stage (Davis & Grayson, 2007). Thus, most research concerning colour in

Correspondence: Andrew K. Davis, D.B. Warnell School of Forestry and Natural Resources, The University of Georgia, Athens, GA 30602, USA. *E-mail:* akdavis@uga.edu

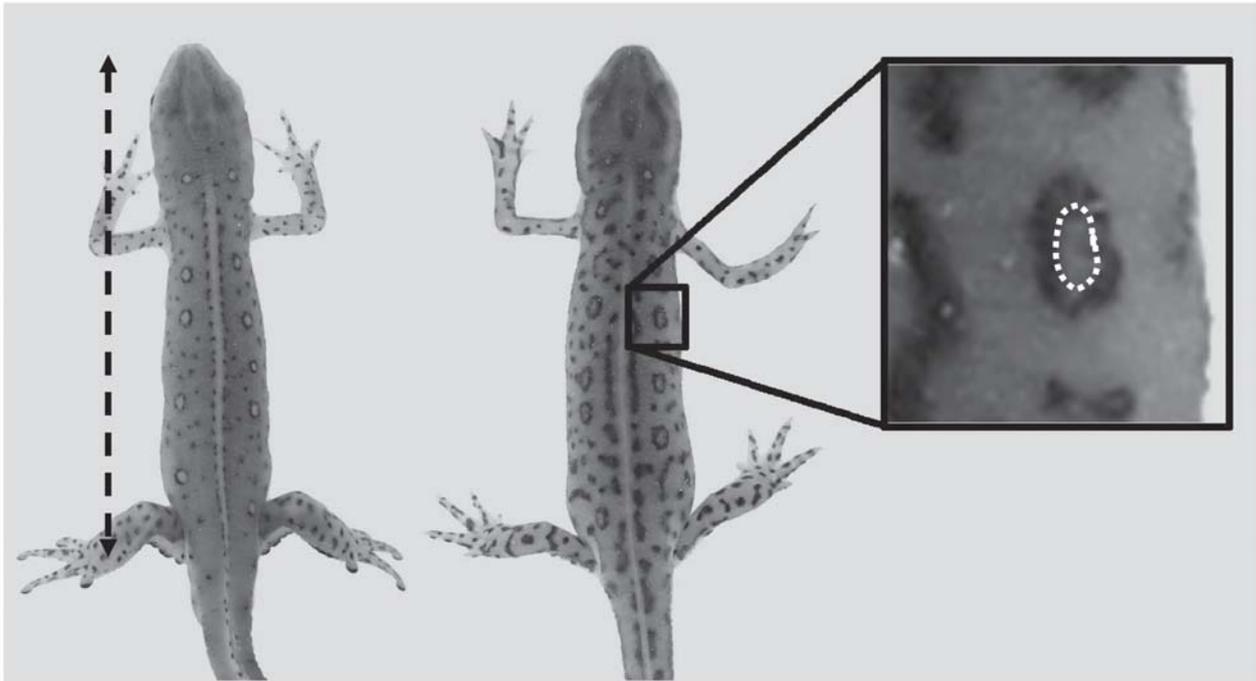


Fig. 1. Male (left) and female (right) red-spotted newt shown in photographing position. All newts were photographed under the same conditions with the same camera setup following Davis and Grayson (2007). Spots are coloured in shades of red to orange, and have a ring of black around them in some individuals (inset). Black dashed line indicates torso length measurement (from nose to posterior point of hindlimb attachment). White dashed circle indicates area of spot measurement.

this species has been directed toward the variation in background skin colour or how it relates to toxicity in the eft stage, with surprisingly no efforts to examine the significance of the conspicuous red spots in adults.

In this study we quantified spot characteristics in a sample of wild-caught red-spotted newts to determine if sexual dimorphism in spot traits exists in this species. If so, it would suggest they serve as sexual ornaments during courtship. We used an image analysis approach (Davis & Grayson, 2007) to examine spots from 100 male and 100 female aquatic adult individuals captured during the breeding season. Specifically, we measured the number, size and colour (redness and brightness) of all dorsal spots and statistically tested whether any of these traits differ between sexes. If spots function as sexual ornaments, we expected males to have more spots, larger spots or more brightly coloured spots. We simultaneously asked if any of these characteristics are associated with body size.

METHODS

Animal capture and processing

Aquatic, adult newts were captured using a seine from Station Pond (pond area = 0.65 hectares, estimated newt population = 7,600–12,700; Grayson, unpubl. data) at Mountain Lake Biological Station (Giles County, Virginia, USA 37°22'32"N, 80°31'20"W, elevation 1160 m), as part of an unrelated study. A random subset of 100 males and 100 females was selected for this study from the 720 newts captured during the sampling period from 22 to 24 April 2007. At this site, this period fell within the breeding sea-

son for *N. v. viridescens*, which extends from mid-March to June (Massey, 1988).

Upon capture, newts were transported to an indoor laboratory next to the pond for processing. Sex was determined for each newt based on the yellow glandular spot on the posterior cloaca present in males. Newts were individually placed in a white plastic container filled with clean water (such that the newt was just submerged) and photographed from above using a Canon PowerShot A95 digital camera (Canon Inc., Lake Success, New York, USA) at a fixed distance from the stage, following Davis & Grayson (2007). All newts were photographed using this setup, which was in the same room with the same lighting conditions. In our experience, the submersion of the newt in water not only reduced the discomfort of the animal (i.e. it minimized escape behaviour), but eliminated glare from the overhead lights in the photos. Newts were then released into artificial enclosures in the same pond as part of the unrelated study.

Processing images

All newt images were imported into Adobe Photoshop with Fovea Pro (Reindeer Graphics, Inc.) plugins installed, which combined is an image analysis software package that allows for evaluation of morphological as well as colour parameters in animals, provided that all animals are photographed under similar conditions (Davis et al., 2004; Davis et al., 2007; Davis & Grayson, 2007; Davis & Maerz, 2007; Todd & Davis, 2007; Davis & Grosse, 2008). In our case, the green-coloured newts were readily visible against the white background in the images (Fig.

Table 1. Summary of spot characteristics of 100 male and 100 female red-spotted newts. Means are shown with 1 standard deviation in parentheses. Total and single spot areas are expressed as a percentage of the dorsal surface area of the torso. Significant differences from statistical comparisons (t-tests, $P < 0.05$) are indicated with an asterisk.

	Females	Males
Number of spots/side	5.19 (1.56)	5.51 (1.53)
Total number of spots	10.38 (3.13)	11.02 (3.06)
Total spot area (%)	1.01 (0.35)	0.96 (0.33)
Single spot area (%)	0.10 (0.03)	0.09* (0.02)

1). For each newt, we digitally traced the outline of the head and body minus the legs and tail (i.e. the “torso”) and the surface area of this selection was obtained in mm² based on prior calibration of actual dimensions using a photograph of a ruler taken by us with the same camera setup. The length of this selection (hereafter called “torso length”) was also obtained. Next, we digitally traced the contours of all dorsal spots on each newt and ran a Fovea Pro measurement routine that returns the dimensions and the colour parameters of the selected areas. In this case, the parameters of interest to us were the number of spots, single spot sizes (i.e. spot surface areas, in mm²) and the colours of all spots. We note here that we considered the newt “spots” to be the reddish-orange coloured portion of the dorsal spots, and did not include the dark ring of pigment that surrounds the spots of some individuals (Fig. 1). This is because not all spots within individuals have this ring, and the ring is not present in all individuals (pers. obs.).

Image analysis programs measure colour based on the hue, saturation and luminance values associated with on-screen pixels in the image. Fovea Pro measures the average hue, saturation and luminance values for all pixels in the areas selected, which in this case were the individual spots. Hue generally refers to what most people think of as colour (i.e. the difference between green, red or blue). Saturation is the intensity of a hue from grey tone (no saturation) to pure, vivid colour (high saturation). The difference between pink or red is an example of low saturation versus high saturation. Luminance is the lightness or darkness of that colour if the image were in black and white. For our purposes, we were interested in the first two measures, the hue and saturation of spot colours. Hue was measured on a scale of 0–360, though the range in our data was between 16 and 37, with lower values representing redder colours (perfect red is 0). Saturation was measured on a scale of 0–255 (our data ranged from 92 to 204) with higher values representing more saturated, brighter colours. We point out that these colour variables do not necessarily represent the true colour of the spots, but more so the colour as measured under our camera setup and lighting conditions. As such, they would not be comparable to other newt measurements taken with a different setup. However, since all

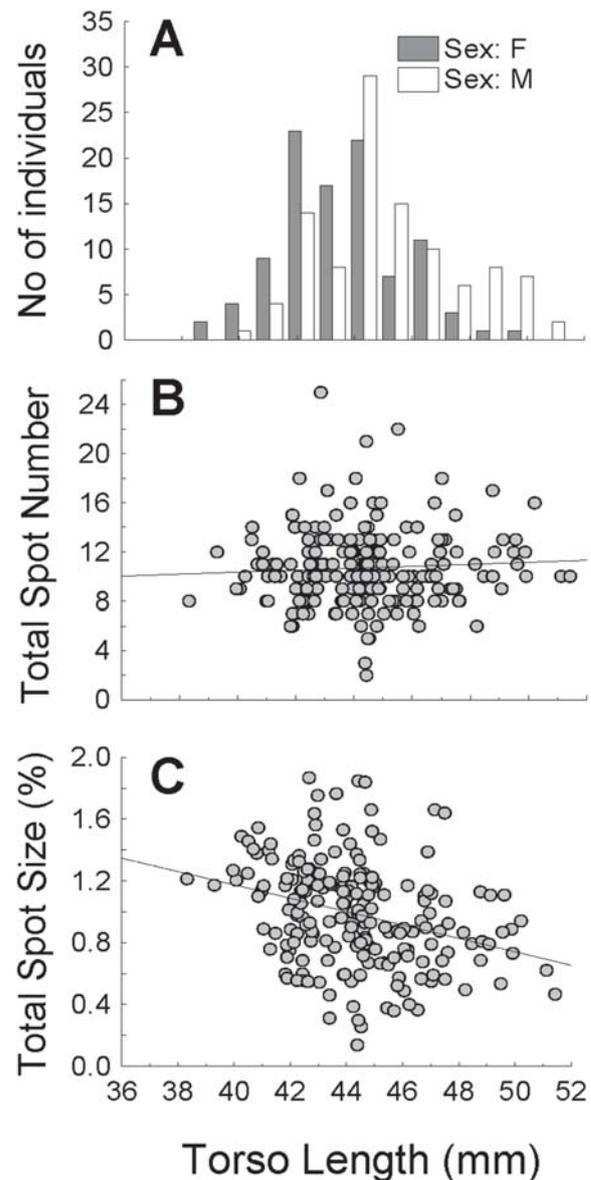


Fig. 2. Relationships between newt body size (torso length) and sex (A), spot number (B) and spot size (C). Spot size is expressed as the total surface area of all spots as a percentage of the dorsal surface area of the torso. There was no significant linear relationship between body size and spot number, but a significant negative relationship between body size and spot size (see results).

newts in *this* study were photographed similarly by us, these colour measures are useful for detecting *relative* differences among individuals, sexes, etc.

When spot measurements were complete we were left with three parameters per spot (area, hue and saturation), as well as counts of spots per individual, which we also divided into left-side spots or right-side spots. We suspected that spot size would be related to the size of individuals; therefore we transformed each raw spot area value into a percentage of the torso area, which was calculated previously. Next we computed the total surface area of all spots per newt, by summing all spot size values,

Table 2. Results of ANCOVAs examining factors influencing newt spot colours (either spot hue or saturation), as determined from image analysis of 100 males and 100 females. The interaction effects of sex*torso length and sex*total spot number were initially included in both analyses but removed when found to be not significant.

Colour variable	Independent variable	df	MS	F	P
Hue	Sex	1	236.08	20.01	<0.001
	Torso length	1	22.22	1.88	0.171
	Total number of spots	1	0.22	0.02	0.891
	Error	196	11.80		
Saturation	Sex	1	17319.84	48.59	<0.001
	Torso length	1	1433.02	4.02	0.046
	Total number of spots	1	394.96	1.11	0.294
	Error	196	356.43		

and the average spot size per newt using the same values. For the spot colour variables, we computed the within-individual average spot hue and saturation, so that each newt had a single value for the hue and saturation of their spots which we used for analyses.

Data analysis

Basic sex-related differences in spot characteristics such as number and size were examined with *t*-tests. We further explored relationships between body size (i.e. torso length) and spot characteristics using Pearson correla-

tions. For the spot colour variables (hue and saturation), we sought to examine the possible differences between sexes but also potential influences of body size and spot number. Further, since the hue and saturation of spots may be related, we initially examined each of these response variables simultaneously using a MANCOVA design, with hue and saturation as the dependent variables, sex as a categorical factor, and the continuous effects of torso length and total spot number. If significant effects of independent variables were found in this analysis, we followed up with independent ANCOVA analyses of each response variable, using the same independent variables. The interaction terms of sex*torso length and sex*total spot number were initially included in all analyses. All analyses were conducted using Statistica 6.1 software (Statistica, 2003).

RESULTS

General spot characteristics

Of the 100 male and 100 female red-spotted newts examined in this study, males and females had approximately five spots per side on average (Table 1), and there was no significant difference between sexes in this number ($t=-1.4$, $df=198$, $P=0.161$). Nor was there a difference in average total number of spots ($t=1.11$, $df=198$, $P=0.269$), which was between 10 and 11 and ranged from two to 25 in all 200 individuals. The approximate amount of dorsal surface area covered by spots (on the torso) in both sexes was 1% and this average was not different between sexes ($t=-1.41$, $df=198$, $P=0.161$). However, there was a slight but significant sex-related difference in the average single spot area (relative to torso area), with males spots averaging 0.09% and female spots 0.1% each ($t=2.95$, $df=198$, $P=0.004$).

Body size effects

Male newts generally had larger torso lengths than females (Fig. 2A), being 45.1 mm on average compared to 43.6 mm for females. This difference was significant ($t=-4.92$, $df=198$, $P<0.001$). However, there was no significant relationship between torso length and total number of spots ($r=0.06$, $P=0.391$; Fig. 2B). There was a significant negative relationship between torso length and total relative spot size ($r=-0.30$, $P<0.001$; Fig. 2C), suggesting that larger individuals had smaller relative spot coverage. This

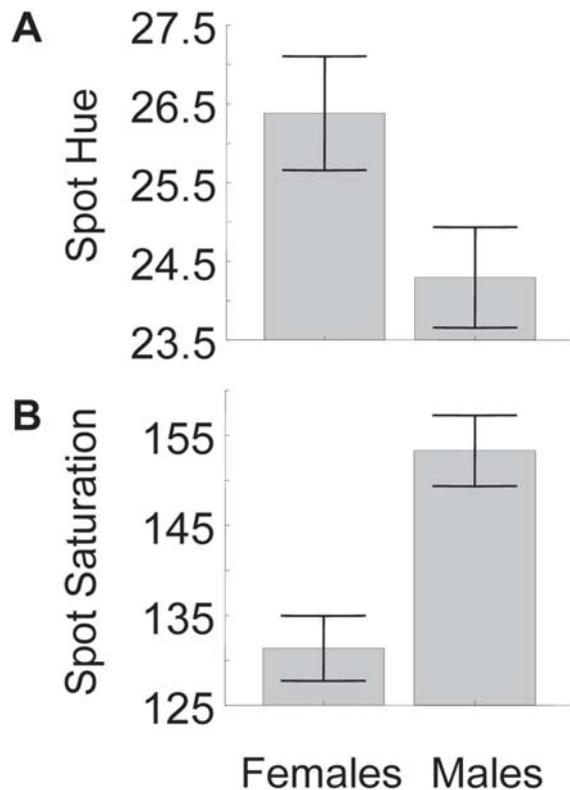


Fig. 3. Differences between male and female spot colours, assessed using image analysis. Male spots have significantly lower hue scores (i.e. are more red; A), and significantly higher saturation scores (i.e. are brighter, more intense colours; B) than female spots. Error bars represent 95% confidence intervals.

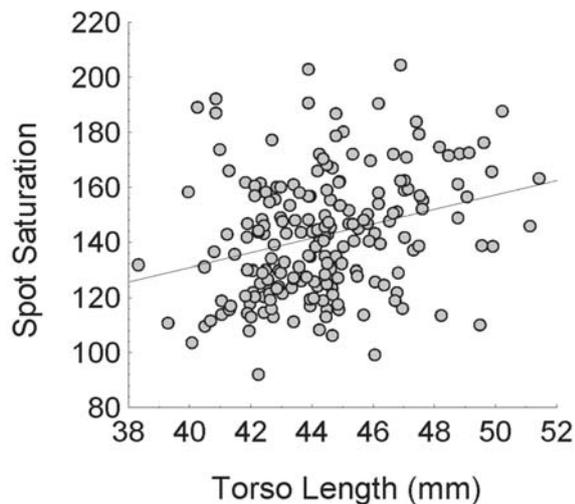


Fig. 4. Relationship between body size (torso length) and average spot saturation score in all red-spotted newts in this study.

is not because they have fewer numbers of spots (Fig. 2B). This could be due to allometry, but a further comparison of torso length and average single spot area (raw values) showed a shallow but significant negative relationship ($r = -0.17$, $P = 0.014$). Combined, our results therefore indicate that smaller individuals have larger spots.

Spot colour

In the initial MANCOVA examining factors influencing spot hue and saturation, there was no support for the interaction terms of sex*torso length and sex*total spot number ($P > 0.05$ for both). Results of a simplified model with main effects only revealed a significant effect of sex ($F_{2,195} = 31.07$, $P < 0.001$) and of torso length ($F_{2,195} = 3.28$, $P = 0.040$), but not of total spot number ($F_{2,195} = 0.55$, $P = 0.577$). When spot hue was examined individually with these variables there was again a significant effect of sex (Table 2), but no effects of either torso length or total number of spots. The sex-related difference was such that male newts had lower spot hue scores than females (Fig. 3A), indicating that their spots were more red than those of females. We also found an effect of sex in our separate analysis of spot saturation (Table 2), with males having higher saturation scores (i.e. their spots were brighter) than females (Fig. 3B). As with hue, there was no effect of total spot number on spot saturation scores, but interestingly, there was a significant effect of torso length (Table 2). In this case, larger individuals tended to have higher saturation scores (i.e. their spots were brighter; Fig. 4).

DISCUSSION

While we found no apparent sex-related differences in the numbers of spots in this study, and only a minor difference in spot sizes, our results indicate that adult male red-spotted newts have significantly redder and more intensely coloured dorsal spots than females. To our knowledge, this discovery represents the first evidence

of a sexually dimorphic spot colour pattern in this species, although this difference is not necessarily obvious to the naked eye. Indeed, our results in reality demonstrate a *statistical* difference in spot colour, rather than the more commonly thought of concept of sexual dimorphism, where one sex is strikingly different in colour than the other. This is similar to prior findings where the white “saddle” patterns of marbled salamanders (*Ambystoma opacum*) were examined and a subtle but statistically significant difference in whiteness was found between males and females (Todd & Davis, 2007). Combined, these discoveries beg an important question: if colour differences exist in these species, which have historically not been thought of as monomorphic, how many other “monomorphic” amphibian species are there where this is also true? Clearly, there are a suite of research questions waiting to be answered and we point out that such questions concerning animal coloration are now quick and inexpensive to address using digital cameras, scanners and image analysis software, provided animals are photographed in a standardized fashion (Davis et al., 2004; Davis et al., 2005; Davis et al., 2007; Davis & Grayson, 2007).

The most likely explanation for the difference between males and females in spot colour in this species is that the trait is involved in mate selection, although we caution that further study would be needed to test for female preference for this trait. However, it is reasonable to suspect that spots have some function related to mate signalling, since a component of male courtship is a highly visual dance, in which a male prominently displays himself in front of a female in order to initiate courtship or to entice the female to pick up a deposited spermatophore (Verrell, 1982). Moreover, the spots seem poorly suited for camouflage or to serve an aposematic function, since they make up only 1% (combined) of the dorsal surface area (this study; Davis & Grayson, 2007). Additionally, the variation in the degree of redness and/or brightness we found in male newts is similar to that seen in carotenoid-based pigmentation of male birds; this variation in birds is clearly associated with mating success (Hill, 1990, 1993) and competitive ability (Pryke & Andersson, 2003). Lastly, carotenoid-based pigments are known to be energetically expensive to synthesize (Hill, 2000), so forming and maintaining red spots must serve some biological purpose. All evidence together therefore supports our hypothesis that spots on red-spotted newts serve as sexual ornaments.

Despite the sexual dimorphism in body size of this species, large body size has not been found to be a sexually selected trait in male red-spotted newts (Gabor et al., 2000). In fact, studies in two species of European newt, *Triturus helveticus* and *Euproctus asper*, found a female preference for smaller males (Haerty et al., 2007; Poschadel et al., 2007). While more research is needed on female choice in red-spotted newts, our data show that smaller individuals (both males and females) have larger spots than large individuals (both relative to their torso size and in raw surface area). In addition to suggesting that spot pattern is linked to sexual selection, this result also emphasizes that spots would be poorly suited to serve an aposematic function in the aquatic stage of this

species, as large individuals should have the largest spots if they served a warning purpose.

There is one other possible explanation for our results that is worth exploring in the future. The differences in spot colour we observed may simply be related to sex-based differences in physiological processes that regulate pigment formation. Indeed, evidence from ornithological research has shown that carotenoid pigments are energetically costly to synthesize (Hill, 1996). Moreover, recent evidence from other salamanders indicates that reproduction in females is more stressful than in males (Davis & Maerz, 2008). Thus, it is possible that reproductive females may not be able to divert energy and/or physiological resources to pigment production as much as males. This idea certainly warrants further study, perhaps by examining spot redness or brightness in reproductive and non-reproductive females.

We conclude from this study that the carotenoid-based spots of red-spotted newts differ in the degree of redness and brightness between males and females, which strongly suggests a role in mate selection or courtship and clearly warrants further research, particularly concerning female choice. Furthermore, the discovery of this sex-based coloration difference in this species, though it is subtle and not necessarily detectable by the human eye, also makes us wonder if similar results would be found in other amphibian species with carotenoid-based pigmentation that are not obviously sexually dimorphic. Indeed, any species with yellow, orange or red patterning may be a candidate for future study.

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