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No evidence for sexual differences in carapace color in a population of Allegheny crayfish

Sean M. Hartzell

Department of Biological and Allied Health Sciences, Bloomsburg University of Pennsylvania, 400 East 2nd Street, Bloomsburg, Pennsylvania 17815, USA (e-mail: seanhartzell77@gmail.com)

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Sexual dichromatism consists of inter-sexual differences in coloration within a species and is widespread among animal taxa. The presence of sexual dichromatism in crayfish has not been well-studied. However, spot patterns in a population of rusty crayfish (*Faxonius* [*Orconectes*] *rusticus*) have recently been demonstrated to be sexually dichromatic, which suggests sexual dichromatism might exist in other species of the genus *Faxonius*. Intraspecific carapace color variation varies considerably within some populations of Allegheny crayfish (*Faxonius* [*Orconectes*] *obscurus*) although the contributing factor to this variation has not been demonstrated conclusively. Here, I utilize digital image analysis to quantify carapace coloration of a sample of Allegheny crayfish from eastern Pennsylvania, USA to determine if carapace color variation in this population can be attributed to sexual dichromatism. No significant difference was found in carapace coloration between sampled male and female Allegheny crayfish, which suggests another factor may be responsible for the carapace color variation observed in this population.

Introduction

Sexual dichromatism is a form of sexual dimorphism in which there are consistent intra-specific sex differences in coloration (Bell & Zamudio 2012, Bartlett & Light 2017). Sexual dichromatism typically evolves through either sexual selection or natural selection due to different predation pressures between sexes in a species (Bell & Zamudio 2012, Lambert *et al*. 2017). This phenomenon is present among many vertebrate and invertebrate taxa (e.g., Davis & Grayson 2008, Kemp 2008, Bell & Zamudio 2012, Bartlett & Light 2017, Hartzell 2019). Sexual dimorphism is common among many crayfish species with the presence of larger and heavier chelae in males (Stein 1976). However, sexual dichromatism in crayfish has been understudied. Hartzell (2019) recently demonstrated that sexual dichromatism is present within the spots on the carapace of rusty crayfish (*Faxonius* [*Orconectes*] *rusticus*, Girard), which suggests this phenomenon might also be present in other crayfish species, particularly congeners.

Allegheny crayfish, *Faxonius (Orconectes) obscurus* (Hagen), vary in carapace coloration from olive green to tawny and chestnut to dark brown (Ortmann 1906, Loughman 2010, Loughman & Simon 2011, *see* also fig. 1C–D in Hartzell 2017). Ortmann (1906) attributed this variation in carapace color to ontogenetic shifts in coloration for populations of this species in Pennsylvania. Hartzell (2017) recently demonstrated that in eastern Pennsylvania, within-population ontogenetic color change of Allegheny crayfish is not related to carapace color variation.

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Sexual dichromatism is present in rusty crayfish (Hartzell 2019), and here, I examine whether sexual dichromatism in carapace color exists in Allegheny crayfish by quantifying carapace coloration using digital image analyses (Stevens *et al*. 2007, Davis & Grayson 2008).

Methods

The methodology here follows Hartzell (2017). Alleghany crayfish ($n = 50$, 33 males, 17 females) were collected on 16 October 2018 from a single ca. 100 m transect within Fishing Creek, a 5th order tributary of the North Branch Susquehanna River near Lightstreet, Columbia County, Pennsylvania USA, at the Jonson Flats section of Frank W. Kocher Memorial Park (41.046957°N, 76.429085°W). Crayfish were collected during this time (i.e., the fall season) because this corresponds to when many adult (> 20 mm carapace length) Allegheny crayfish are reproductively active (i.e., mature males are in mating form [Form I] and females have visible glair glands [Ortmann 1906, authro's unpubl. data]) and therefore sexual dichromatism, if present, is likely to be most prominent. Crayfish were collected by dip net and placed in a gray bucket filled with stream water for no longer than 45 min. Crayfish with worn or dirty carapaces or those that were juveniles (i.e., < 20 mm carapace length [Fielder 1972]) were released immediately and excluded from this study. Crayfish were photographed at the time of collection, on location using a Fujifilm FinePix XP75 16.5 megapixel digital camera (Fujifilm Holdings Corporation, Tokyo, Japan) with standardized settings and with reflectance standards as described in Hartzell (2017). Sex was determined for each crayfish via visual inspection for the presence or absence of gonopods (Wadena *et al*. 2018), although individuals were not assigned to male (or female) reproductive form (i.e., Form I; Form II [Ortmann 1906, Wetzel 2002]) in the present study. Carapace length from the tip of the rostrum to the posterior end of the cephalothorax (Fielder 1972) was measured in mm for each crayfish in the field with a metric ruler. After being photographed, crayfish were vouchered in 80% ethanol for use in a separate study.

Photographs were processed utilizing the Image Calibration and Analysis Toolbox (Troscianko & Stevens 2015) in the program ImageJ (National Institute of Health, Bethesda, MD). Each photograph was linearized and equalized using the Image Calibration and Analysis Toolbox as described in Hartzell (2017) to limit the effects of varying light conditions when photographing. Subsequently, the Image Calibration and Analysis Toolbox was used to select a "region of interest" (ROI) with the freehand selection tool in ImageJ in each image. ROIs consisted of the cephalothorax and abdomen of each Allegheny crayfish photographed. The Batch Multispectral Image Analysis function in the Image Calibration Analysis Toolbox was then used to calculate the mean red, green, and blue (R, G, B) luminance of each ROI. Subsequently, percentage of "green dominance" (Hartzell 2017) was calculated (= mean green luminance/[mean red luminance + mean blue luminance]) as a metric of the quality of green coloration of the carapace of each crayfish.

Statistical analysis was conducted in the program R version 3.3.2 (R Core Team 2016) using the "stats" package. Preliminary analyses revealed green dominance data fit the assumptions of parametric tests. Therefore, a two-sample *t*-test was used to examine if a significant difference was present in mean green dominance between male and female Allegheny crayfish, with $\alpha = 0.05$ for the test.

Results

Carapace length of Allegheny crayfish sampled ranged from 20 to 42 mm, encompassing the approximate size range of adult male and female specimens collected during early fall (Fielder 1972). Mean (± SD) green dominance score was not significantly different between female (0.51 ± 0.14) and male (0.47 ± 0.11) Allegheny crayfish in the population sampled ($t_{48} = 1.090, p = 0.281$).

Discussion

This study appears to be the first that tests for sexual dichromatism of the carapace color in adult (> 20 mm carapace length) Allegheny crayfish. These results, although limited, suggest that carapace coloration in Allegheny crayfish is not sexually dichromatic (or is sexually monochromatic), at least during the early fall season during which the crayfish were collected. This differs from a recent study that demonstrated sexual dichromatism of carapace spots in congeneric rusty crayfish (Hartzell 2019). For this study, the crayfish were sampled when most adults (> 20 mm carapace length) are reproductively active. Therefore, further sampling is needed in other seasons to determine if sexual dichromatism may occur between adult Allegheny crayfish when they are not reproductively active. Additionally, further sampling is needed in order to determine whether sexual dichromatism occurs at other life stages, such as in juveniles.

The results of this study, while limited, suggest that carapace color variation observed in this population during fall may not be attributable to sexual differences. Likewise, a previous study (Hartzell 2017) demonstrated that ontogenetic color change is not likely a source of color variation observed within this population of Allegany crayfish. Other factors may be responsible for the carapace color variation observed within this population. The color of crayfish may change over time to match background substrata and may also be influenced by diet (Bowman 1942, Kaldre *et al*. 2015). That the sampling of this population occurred at the same location and on the same day indicates these crayfish would have a similar diet and would be unlikely to vary in any diet-related variation in color. In addition, in-stream substrate at this site consist of a relatively heterogenous background coloration, which thus likely cannot be attributed to carapace color variation within this population (Hartzell 2017). Therefore, variation in the carapace color of this Allegheny crayfish population might be attributable to some other factor, such as genetic variation within the population (Black & Huner 1980) or possible introgression of genes from other *Faxonius* species (e.g., *Faxonius rusticus*) introduced into the region which may hybridize with Allegheny crayfish (Herman 2012).

Little work appears to have been done to quantitatively test for sexual dichromatism in crayfish (Hartzell 2019), and therefore, even though this study is limited in scope and presents negative results, it contributes to a field of study that appears to have received little attention in Astacology. Because this study only examined one population of Allegany crayfish, future studies could examine populations in other portions of the native and introduced range of this species (reviewed by Adams *et al*. 2010) to elucidate if other populations of this species are sexually monochromatic. In addition, because sexual dichromatism is present in at least one member of the crayfish genus *Faxonius* (Hartzell 2019) future works could examine if sexual dichromatism is present or absent in other species of this genus as well as in other crayfish genera.

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