REPTILES & AMPHIBIANS

An Evaluation of Sexual Dimorphism in Head Size and Shape of Red Salamanders (*Pseudotriton ruber*)

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Abstract.—For many secretive species of amphibians and reptiles, observations of reproductive behavior are limited to sparse anecdotes from the field or to animals held in captivity. However, a careful examination of morphological differences between the sexes can help shape hypotheses about behaviors with which they may be correlated. For example, sexual dimorphism in head size and shape are correlated with courtship, territoriality, and mate-defense behaviors in some salamanders. One widespread species with a poorly described reproductive natural history is the Red Salamander (*Pseudotriton ruber*). Herein we measure head size and employ geometric morphometric methods to evaluate head shape in *P. ruber*, with the goals of (1) quantifying and visualizing sexual variation, and (2) forming hypotheses about reproductive behavior. We found preliminary evidence for differences in head size and shape that are consistent with mate-guarding behavior, and we remark upon directions for future research.

For many rare or secretive species of animals, direct observations of ecological interactions—including reproductive behavior—are scarce. However, the analysis of morphological traits from living or preserved specimens can help generate hypotheses about these behaviors. For example, sexual dimorphism—differences in the secondary sexual characteristics—is common and well-documented among many animals, including amphibians (Shine 1979; Delêtre and Measey 2004). The presence of male-biased sexual size dimorphism (SSD) is often interpreted as preliminary evidence suggestive of male-male combat, including in Dusky Salamanders (genus *Desmognathus*) (Camp 1996). The enlarged jaw musculature in Climbing Salamanders (genus *Aneides*) (Staub 1993) and some male Brook Salamanders (genus *Eurycea*) (Deitloff et al. 2014) has led the authors to form and test hypotheses about the function of these traits in agonistic behaviors. Because salamanders are generally secretive and exhibit cryptic behavior that often eludes direct human observation, investigating their phenotypic diversity may be especially useful for forming hypotheses about the behaviors and reproductive ecology of these amphibians.

Lungless salamanders (family Plethodontidae) have proven to be useful models for studying the evolution of courtship behavior. Although the reproductive natural history of some species (e.g., Red-Legged Salamander; *Plethodon shermani*) has been studied in great detail, the behaviors of others

are rather poorly described (Sever et al. 2016). This is true even for some widespread species, like the Red Salamander (*Pseudotriton ruber*). Bruce (1978) inferred the reproductive phenology of *P. ruber* from anatomical examination of field-collected specimens, and observations of courtship behaviors in the laboratory have been recorded by several authors (Thorn 1959; Organ and Organ 1968; Arnold 1972). Additionally, Arnold (1972) reported instances of male *P. ruber* "pursuing

Fig. 1. An adult Red Salamander (*Pseudotriton ruber*) from Greene County, Tennessee. This individual appeared to have enlarged jaw musculature, and the arrow indicates a scar suggestive of a bite mark. Photograph by T.W. Pierson.

and biting other males when they were housed in containers with females." This may be consistent with mate-guarding behavior, which has been documented in some members of the Two-Lined Salamander (*Eurycea bislineata)* species complex (Deitloff et al. 2014; Pierson et al. 2019).

Indirect evidence of agonistic behavior in many plethodontid salamanders can be found in distinct, "crescent-shaped" or "semicircular" wounds and scars created by bites from conspecifics (*Aneides*: Staub 1993; *Desmognathus:* Camp 1996; *Eurycea*: Deitloff et al. 2014; Pierson 2019; Pierson and Miele 2019; Pierson et al. 2019). In some of these genera (e.g., *Aneides* and *Desmognathus*), these scars are retained in preserved specimens, but in others (e.g., *Eurycea*), they seem to fade or disappear (Graham 2014; pers. obs.). Through the course of regular fieldwork in the southeastern United States, we noted similar scars on adult Red Salamanders (*P. ruber;* Figs. 1–3) and Mud Salamanders (*P. montanus*). We hypothesized that these scars result from mate-guarding behavior.

The degree of sexual dimorphism is variable among spelerpine plethodontid salamanders. For example, males of some species have specialized organs (e.g., cirri and mental glands) used in locating and courting mates (Sever 1979b). One species (Patch-Nosed Salamander; *Urspelerpes brucei*) is sexually dimorphic in color and pattern (Camp et al. 2009), and several species—including *P. ruber* (Bruce 1978)—exhibit female-biased SSD. In the Two-Lined Salamander (*Eurycea bislineata)* species complex, males that exhibit mate-guarding behaviors also have hypertrophied jaw musculature that presumably increases the force of their bites (Noble 1929; Sever 1979a; Alcorn et al. 2013; Siegel et al. 2020; Rainey et al. 2021). This musculature leads to gross morphological differences in head shape and size that are quantifiable without dissection. Plethodontine plethodontid salamanders in the genus *Aneides* have a superficially similar head morphology (Wake 1963), although the genus exhibits a variable degree of sexual

dimorphism across species (Staub 2021). Furthermore, their head dimorphism is apparently associated with more general agonistic behavior rather than strictly with mate-guarding (Staub 1993). In *Pseudotriton*, the only sexually dimorphic traits that have been reported other than snout-vent length (SVL) are related to cloacal morphology (Bruce 1978; Rucker et al. 2021). However, we have noted (Pierson et al. 2019) that some adult *Pseudotriton* found during our fieldwork appear to have enlarged jaw musculature superficially similar to what has been better documented in some *Eurycea* (Figs. 1–3). Nonetheless, this possible sexual dimorphism in head size and shape has not been clearly described in the scientific literature, and no work has been conducted to describe how these characters vary seasonally or geographically, or how they may reflect the behavioral and reproductive ecology of *P. ruber*.

Here, we examined and measured museum specimens and employed geometric morphometric methods to (1) quantify sexual dimorphism in the head size and shape of *P. ruber*, and (2) make cautious, indirect inferences about the plausibility of mate-guarding behavior in this species, for which few direct observations of any reproductive behavior exist.

Methods

We examined and measured ethanol-preserved specimens of *P. ruber* from the Georgia Museum of Natural History. All specimens originated from Georgia, North Carolina, or South Carolina, and collection localities included the Appalachian Mountains, Piedmont Valley and Ridge, and Coastal Plain physiographic provinces (Table 1). Additional systematic work on *P. ruber* is justified (Folt et al. 2016), but the specimens we examined represented the nominotypical subspecies—the Northern Red Salamander (*P. r. ruber*)—and the Southern Red Salamander (*P. ruber vioscai*), according to the current and most widely adopted taxonomy.

Fig. 2. An adult Red Salamander (*Pseudotriton ruber*) from Grundy County, Tennessee. This individual appeared to have enlarged jaw musculature, and the arrows indicate scars suggestive of bite marks. Photograph by T.W. Pierson.

Fig. 3. A pair of adult Red Salamanders (*Pseudotriton ruber*) found together under a single log in Greene County, Tennessee. The lower individual appeared to have enlarged jaw musculature, and the arrows indicate scars suggestive of bite marks. Photograph by T.W. Pierson.

Table 1. Metadata for all museum specimens used in analyses, including the accession number in the Georgia Museum of Natural History, snout-to-vent length (SVL), head width (HW), the presence or absence of cloacal papillae, and the collection locality and date.

Accession number	SVL (mm)	HW (mm)	Papillae	State	County	Date
UGAMNH 13135	64.6	11	absent	Georgia	Fulton	15-Aug-1953
UGAMNH 13138	90	15.5	present	Georgia	Fulton	4-Aug-1966
UGAMNH 13139	89.1	13.5	absent	Georgia	Fulton	4-Aug-1966
UGAMNH 13140	70.5	11.5	absent	Georgia	Fulton	7-Apr-1963
UGAMNH 13141	91.8	13	absent	Georgia	Fulton	7-Apr-1963
UGAMNH 13155	69.5	10.5	present	Georgia	Union	16-May-1970
UGAMNH 13156	73.1	11	present	Georgia	Towns	27-Nov-1966
UGAMNH 13157	72.9	12.5	absent	Georgia	Towns	26-Mar-1966
UGAMNH 13169	61.87	9.69	present	Georgia	Rabun	6-Jul-1960
UGAMNH 13174	55.1	8.6	absent	Georgia	Floyd	28-Jun-1970
UGAMNH 13175	51.5	8.3	absent	Georgia	Floyd	28-Jun-1970
UGAMNH 13176	54.3	8.5	absent	Georgia	Floyd	28-Jun-1970
UGAMNH 13179	65.4	9.7	absent	Georgia	Taylor	29-Apr-1967
UGAMNH 13180	56.1	8.4	absent	Georgia	Taylor	29-Apr-1967
UGAMNH 13181	68.4	11.4	absent	Georgia	Fulton	3-May-1963
UGAMNH 13184	69	11.5	absent	Georgia	Towns	3-Jun-1960
UGAMNH 13185	71.53	11.03	absent	Georgia	Rabun	29-Aug-1953
UGAMNH 13189	78.8	12.1	absent	Georgia	Fannin	1-Sep-1951
UGAMNH 13191	107.3	17.5	absent	Georgia	Floyd	8-Jun-1968
UGAMNH 13192	76.51	10.58	absent	Georgia	Richmond	1-Jul-1954
UGAMNH 13193	72.28	10.61	present	Georgia	Richmond	1-Jul-1954
UGAMNH 13194	61.73	9.39	absent	Georgia	Richmond	1-Jul-1954
UGAMNH 13195	61.87	8.81	absent	Georgia	Richmond	1-Jul-1954
UGAMNH 13196	72.93	9.98	absent	Georgia	Richmond	1-Jul-1954
UGAMNH 13197	78.77	11.08	absent	Georgia	Richmond	1-Jul-1954
UGAMNH 13198	56.82	8.96	absent	Georgia	Richmond	26-Dec-1959
UGAMNH 13199	64.88	9.98	absent	Georgia	Rabun	29-Apr-1972
UGAMNH 13200	70.24	11.18	absent	Georgia	Rabun	29-Apr-1972
UGAMNH 13204	66.47	10.9	absent	Georgia	Rabun	9-May-1959
UGAMNH 13205	64.62	9.74	absent	Georgia	Rabun	9-May-1959
UGAMNH 13207	68.84	11.14	present	Georgia	Rabun	9-May-1959
UGAMNH 13210	73.08	11.37	absent	Georgia	Rabun	9-May-1959
UGAMNH 13211	68.77	10.26	absent	Georgia	Rabun	9-May-1959
UGAMNH 13212	70.2	11.44	present	Georgia	Rabun	9-May-1959
UGAMNH 13213	71.6	11.48	present	Georgia	Rabun	9-May-1959
UGAMNH 13215	64.62	10.79	absent	Georgia	Rabun	9-May-1959
UGAMNH 13228	70.5	10.4	absent	Georgia	Union	1-Jul-1950
UGAMNH 13230	68.6	10.1	present	Georgia	Union	3-Jun-1953
UGAMNH 13231	71.15	10.35	absent	Georgia	Rabun	27-Mar-1954
UGAMNH 13232	73.76	11.01	present	Georgia	Rabun	27-Mar-1954
UGAMNH 13233	65	9.79	absent	Georgia	Rabun	27-Mar-1954
UGAMNH 13234	68.56	10.2	absent	Georgia	Rabun	27-Mar-1954
UGAMNH 13238	85.28	12.39	absent	North Carolina	Transylvania	10-Apr-1955
UGAMNH 13239	68.08	10.22	absent	North Carolina	Transylvania	10-Apr-1955

UGAMNH 13240	50.81	8.89	absent	North Carolina	Clay	30-Mar-1963
UGAMNH 13242	64.8	9.7	present	Georgia	Fannin	15-Aug-1953
UGAMNH 13249	62.81	9.1	absent	Georgia	Clarke	27-Feb-1954
UGAMNH 13250	62.14	8.46	absent	Georgia	Clarke	6-May-1963
UGAMNH 13257	69.04	10.04	absent	Georgia	Rabun	14-Jun-1955
UGAMNH 13258	62.68	9.12	present	Georgia	Rabun	27-Mar-1954
UGAMNH 13259	64.53	10.13	absent	Georgia	Rabun	27-Mar-1954
UGAMNH 13265	65.4	10.4	absent	Georgia	Union	31-May-1953
UGAMNH 13273	63.24	10.29	present	South Carolina	Pickens	18-Aug-1954
UGAMNH 13277	56.06	9.59	absent	South Carolina	Aiken	27-Mar-1952
UGAMNH 20824	68.27	10.13	absent	North Carolina	Macon	5-Mar-1989
UGAMNH 20825	78.55	11.56	absent	North Carolina	Macon	5-Mar-1989
UGAMNH 21469	62.32	10.48	absent	Georgia	Richmond	2-Feb-1958
UGAMNH 21471	84	12.9	absent	Georgia	Towns	1-May-1954
UGAMNH 21481	69.2	10.4	absent	Georgia	Fannin	3-Jun-1953
UGAMNH 21483	61.9	8.7	absent	Georgia	Union	3-Jun-1953
UGAMNH 21485	57.8	9.9	absent	Georgia	Union	27-Oct-1951
UGAMNH 21486	53.6	8.4	absent	Georgia	Union	27-Oct-1951
UGAMNH 21487	48.1	8.1	absent	Georgia	Union	27-Oct-1951
UGAMNH 21488	54.1	9.3	absent	Georgia	Union	27-Oct-1951
UGAMNH 32738	65.39	10.83	absent	North Carolina	Macon	16-May-1995
UGAMNH 45575	88.12	15.05	present	South Carolina	Aiken	27-Aug-1979
UGAMNH 45576	60.5	9.49	absent	South Carolina	Aiken	25-Apr-1979
UGAMNH 45577	57.84	6.98	absent	South Carolina	Aiken	25-Apr-1979
UGAMNH 45578	77.84	10.93	absent	South Carolina	Aiken	25-Apr-1979
UGAMNH 45579	55.7	7.92	absent	South Carolina	Aiken	25-Apr-1979
UGAMNH 45580	77.84	11	absent	South Carolina	Aiken	4-Oct-1979
UGAMNH 45581	75.79	10.07	absent	South Carolina	Aiken	23-Feb-1979
UGAMNH 45582	72.64	9.52	absent	South Carolina	Aiken	23-Feb-1979
UGAMNH 45586	62.6	9.89	absent	South Carolina	Aiken	16-May-1979
UGAMNH 45588	80.28	11.83	absent	South Carolina	Aiken	31-Oct-1980
UGAMNH 45589	77.18	11.84	absent	South Carolina	Aiken	28-May-1982
UGAMNH 45591	70.39	10.46	absent	South Carolina	Aiken	24-May-1980
UGAMNH 45594	58.62	8.95	absent	South Carolina	Barnwell	3-Jan-1979
UGAMNH 45595	67.53	10.59	absent	South Carolina	Barnwell	9-Apr-1979
UGAMNH 45596	65.45	9.85	absent	South Carolina	Barnwell	27-Feb-1979
UGAMNH 45597	52.52	7.54	absent	South Carolina	Barnwell	2-Feb-1980
UGAMNH 45598	79.58	12.64	absent	South Carolina	Barnwell	5-Jul-1980
UGAMNH 45600	71.46	11.07	absent	South Carolina	Barnwell	17-Jun-1982
UGAMNH 45601	74.96	12.11	absent	South Carolina	Barnwell	17-Jun-1982
UGAMNH 45602	50.43	6.83	absent	South Carolina	Barnwell	31-Aug-1981
UGAMNH 45603	77.1	11.96	absent	South Carolina	Barnwell	17-Jun-1982
UGAMNH 45605	59.74	8.4	absent	South Carolina	Barnwell	7-Jun-1981
UGAMNH 45610	54.61	8.22	absent	South Carolina	NA	17-Apr-1979
UGAMNH 49983	70.7	11.3	present	Georgia	Taylor	11-Mar-2006

Because our museum loan conditions prohibited the dissection of these specimens, we non-destructively determined the sex of each specimen by visually examining the cloaca under a Leica EZ4 dissection stereo microscope. We categorized salamanders into two groups—those with papillae lining the inside of the cloaca and those without (Fig. 4). In *Pseudotriton,* these cloacal papillae are reportedly always present in mature males during the breeding season, are present—but perhaps not prominent—in mature males outside of the breeding season, and are absent from all females and immature males (Bruce 1975, 1978). Because this character may depend upon age and season, the presence of papillae is sufficient to identify a male, but the absence of papillae does not definitively identify a salamander as a female. Consequently, the categorizations we use here are not completely analogous to sex.

To test for differences in head size, we measured snout-to-vent length (SVL) and head width (HW) with Neiko digital calipers. We measured SVL as the distance between the tip of the snout and the posterior end of the cloaca, and we measured HW as the width at the widest part of the head. In R v4.1.2 (R Core Team 2021), we fit a linear model with HW as a function of SVL, presence of papillae, and the interaction between the two variables ($\alpha = 0.05$). We also calculated HW/SVL ratios and compared means using a one-tailed Welch's t-test ($\alpha = 0.05$).

We then used geometric morphometric methods to better quantify variation in head shape between the two groups. First, we took dorsal photographs with an Olympus Tough TG-6 Camera positioned directly above the head of the salamander. We enabled in-camera lens corrections to remove lens distortions that may affect accurate morphometry. We excluded some specimens that lacked the rigidity characteristic of formalin fixation because differences in preservation method can bias morphological measurements of salamanders (Pierson et al. 2020). We excluded specimens that were contorted or preserved in a position that might bias measurements (e.g., with the tongue protruding) and two specimens that had outlier HW measurements, which likely resulted from transcription error during data collection. We did not measure larvae, but we did include small, metamorphosed specimens that may have represented juveniles. In total, our dataset included 89 specimens (15 with papillae; 74 without papillae).

We placed seven fixed landmarks and extracted coordinates using ImageJ v1.53a (Schneider et al. 2012). We used the following seven landmarks: 1) the posterior edge of the forelimb, where it attaches to the body; 2) the widest point of the head; 3) the part of the snout with the sharpest angle; 4) the very tip of the rostrum, at the midline of the body; and 5–7 as the mirror image of 3–1, respectively. Although landmarks 1 and 7 are not on the head, we included them because
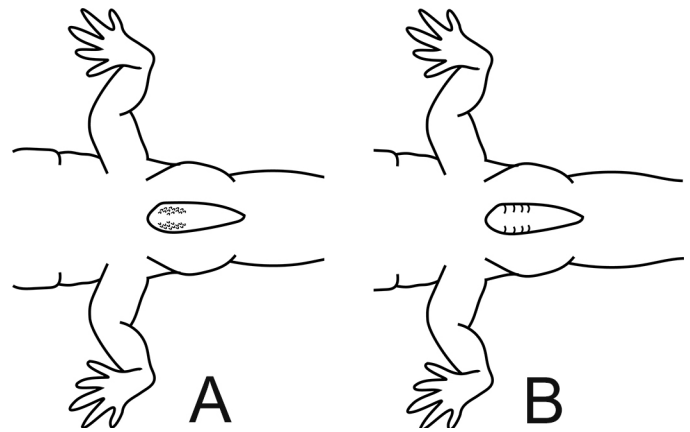
Fig. 4. Cartoon representation of differences in cloacal anatomy of Red Salamanders (*Pseudotriton ruber*), showing a swollen cloaca and internal papillae—which are often most visible in the anterior of the cloaca—indicative of an adult male in breeding condition (A); and a smooth cloaca with folds, usually indicative of a juvenile or female (B). Anatomical features are not drawn perfectly to scale.

they represent more unambiguously identifiable positions than alternatives (e.g., the gular fold) in the dorsal view. To minimize potential bias in landmark placement, the author (KW) who placed the landmarks was blind as to the presence of papillae in the specimens.

Following landmark placement and coordinate extraction, we used Generalized Procrustes Analysis (GPA) in R v4.1.2 (R Core Team 2021) to remove the effects of position, rotation, and scale (Rohlf and Slice 1990). Using the packages "geomorph" and "RRPP" (Collyer and Adams 2018, 2020; Adams et al. 2020), we used a Procrustes ANOVA fitted by Residual Randomization in Permutation Procedures (RPPP) to test whether variation in head shape was explained by SVL, the presence of papillae, and their interaction ($\alpha = 0.05$).

To visualize differences in mean head shape between specimens with and without papillae, we generated deformation grids, which interlay mean coordinates along distorted grid markings (Bookstein 1989). We used a magnification factor of 3 and plotted these deformation grids using the package "geomorph" (Adams et al. 2020). All data and code are available for download from Zenodo (10.5281/zenodo.6977639).

Results

Our linear model revealed a significant interaction between the presence of cloacal papillae and SVL upon HW ($\beta = 0.07$; $p = 0.005$); larger specimens that had cloacal papillae exhibited larger relative HW (Fig. 5). However, the t-test showed that overall, there was no significant difference ($T = -1.51$; $p = 0.07$; Fig. 5) in the mean HW/SVL ratio in specimens with papillae (mean = 0.153) and those without papillae (mean = 0.156). Warp grids appeared to show a relative narrowing of the snout and widening of the jaws in specimens with papillae
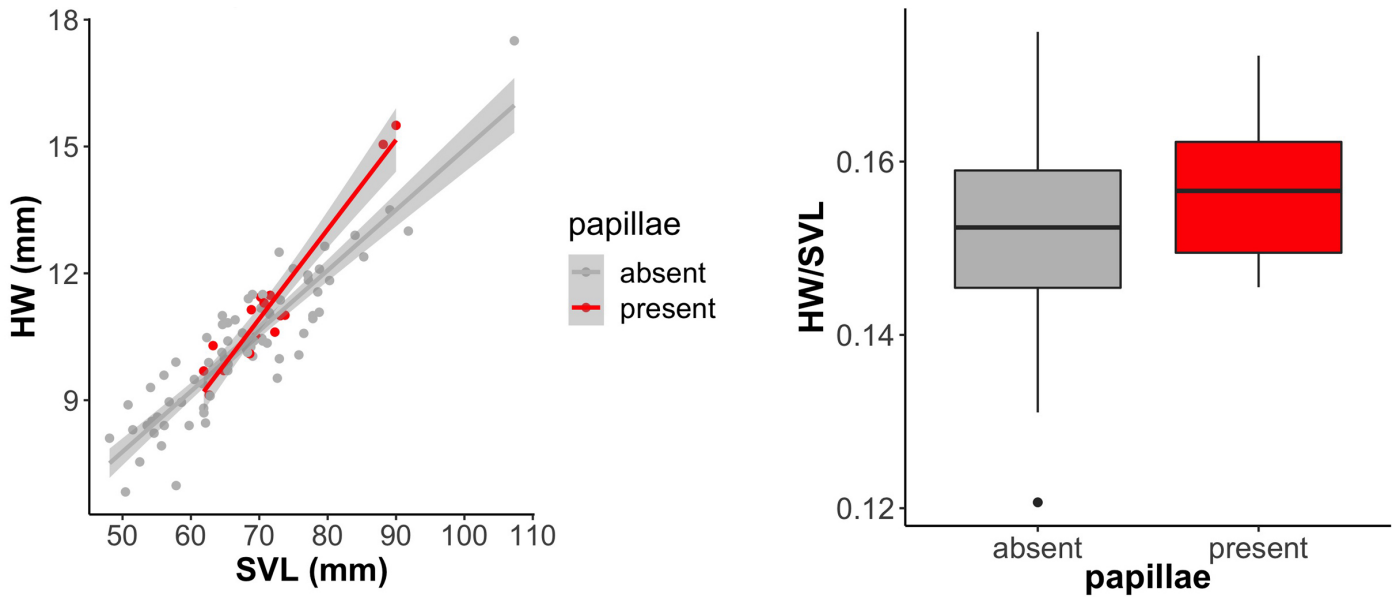
Fig. 5. An interaction plot showing head width (HW) as a function of snout-vent length (SVL) and the presence of cloacal papillae (left) in Red Salamanders (*Pseudotriton ruber*); and standard box plots (center line = median; box = interquartile range [IQR; first quartile (Q1) to third quartile (Q3)]; whiskers = minimum [Q1 -1.5 x IQR] and maximum (Q3 + 1.5 * IQR]; points = outliers) showing differences in HW/SVL ratio between specimens without and with papillae (right).

(Fig. 6), but the ANOVA did not demonstrate any significant effects of papillae (F = 0.65; p = 0.44), SVL (F = 2.01; p = 0.118), or the interaction between the two (F = 0.61; p = 0.34) on head shape.
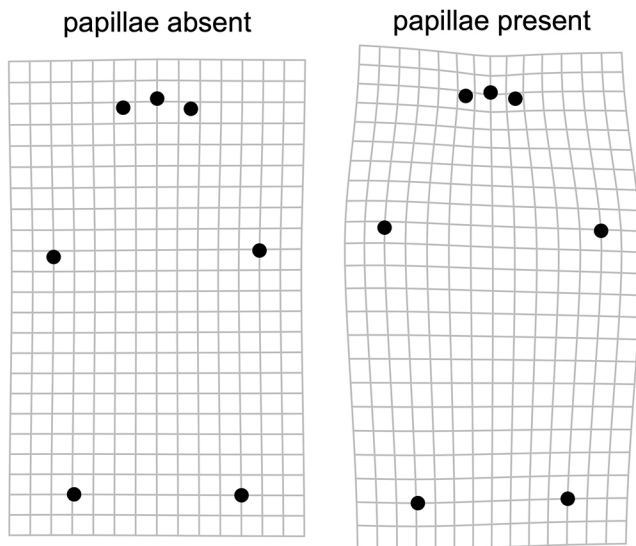
Fig. 6. Deformation (warp) grids demonstrating differences in mean head shape between specimens of Red Salamanders (*Pseudotriton ruber*) without and with papillae. Note that the salamanders with papillae have a markedly pinched rostrum and relatively broader head, although overall differences in head shape were not significant. Deformation grids are magnified by a factor of 3.

Discussion

We found preliminary, but inconclusive, evidence for differences in head size and shape that were consistent with our hypothesis that adult males in breeding condition would exhibit enlarged jaw musculature. In particular, our linear model suggests that large *P. ruber* with cloacal papillae have disproportionately wide heads, similar to patterns described in some *Aneides* (Staub 2021). Although the results of our analysis of head shape were not statistically significant, they were directionally consistent with our hypothesis, suggesting that specimens with cloacal papillae may have more pointed snouts and wider jaws.

Our study was limited by two factors: (1) a relatively small sample size; and (2) our reliance upon secondary sexual characteristics that do not require dissection. We examined most of the suitable *P. ruber* specimens available at the Georgia Museum of Natural History, but our final sample included only 15 specimens with papillae and 74 without papillae. Because any existing head shape differences may be subtle, larger sample sizes may be necessary to define significant head shape variation. Fortunately, future studies can use the abundant material available from other museums. For example, the North Carolina Museum of Natural Sciences includes > 2,000 *P. ruber* specimens in its online database. A larger sample size would not only increase power to detect differences between sexes, but it would also allow researchers to investigate seasonal and geographic variation in these traits. In *Eurycea*, male reproductive morphology and behavior vary geographically, perhaps

reflecting differences in opportunities for courtship (Pierson et al. in review). We encourage further study to examine other populations and identify any spatial variation in head shape.

Because we did not dissect specimens to look for reproductive organs, we relied solely upon the presence or absence of a single secondary sexual character—cloacal papillae. This is a potential problem for several reasons. First, cloacal papillae are reported to be obvious in adult male *P. ruber* during the breeding season and less obvious during the non-breeding season, but they are absent from both adult females and juveniles (Bruce 1975, 1978). Thus, it is likely that our category of specimens without cloacal papillae included all juveniles and some adult males collected from outside of the breeding season. In *Eurycea*, hypertrophied jaw musculature is most pronounced in "guarding" males during the breeding season (Siegel et al. 2020). Although a reasonable hypothesis might suggest that the hypertrophy of jaw musculature and the enlargement of cloacal papillae are simultaneous, this should be evaluated. In future studies, the dissection of specimens (or use of already-dissected specimens) to more confidently determine sex would be helpful. Although "candling"—or shining a light through the venter of a salamander—is sometimes effective for visualizing testes or eggs, it has not been evaluated for *P. ruber* (Rucker et al. 2021) and in our experience is not effective with formalin-fixed specimens of other species.

We found partial support for a larger relative head width in reproductive adult male *P. ruber*. Additionally, we found (statistically insignificant) differences in head shape suggestive of enlarged jaw musculature (Fig. 6). Enlarged jaw musculature and larger relative head width are consistent with differences between mate-guarding male *Eurycea* and female *Eurycea* (Pierson 2019; Siegel et al. 2020; Rainey et al. 2021). Because our linear model suggests that larger males exhibit greater relative head widths, male sexually dimorphic characters may become more pronounced with age and/or reproductive fitness. We hypothesize that these differences in head shape and head size—together with observations of crescent-shaped scars on adult *Pseudotriton* and preliminary observations of male–male aggression (Arnold 1972)—reflect mate-guarding behavior. However, we acknowledge that we cannot exclude alternative explanations. For example, enlarged jaw musculature could be used in more general agonistic behavior, like what is found in *Aneides*. In other organisms, like some freshwater turtles, sexual dimorphism in head size and shape instead reflect dietary niche partitioning (Lindeman 2000). Figure 6 also suggests that males may possess pinched rostrums. The presence and possible function of pinched rostrums in males warrant future investigation.

Finally, although existing studies of head dimorphism in spelerpine plethodontid salamanders have focused on soft tissues, differences in musculature may be mirrored by osteological differences, like they are in *Aneides* (Wake 1963) and

in Musk Turtles (genus *Sternotherus*) (Pfaller et al. 2009). Increased accessibility of micro computed tomography (microCT) data may provide one avenue to test this hypothesis. We encourage future researchers to employ a variety of methods—including field observations, laboratory trials, and careful examination of museum specimens—to better explore reproductive behavior and morphology in *P. ruber* and other spelerpine plethodontid salamanders.

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