Reproductive Biology and Population Structure of *Eurycea chamberlaini* in North Carolina

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*Eurycea chamberlaini* (Chamberlain’s Dwarf Salamander) is a small spelerpine salamander with scant reproductive and life-history data available. Therefore, the objectives of our study were to examine the reproductive life history and population structure of *E. chamberlaini* from a North Carolina population. From February 2008 to February 2009, monthly collections were made in Craven County, North Carolina. All specimens (n = 392) were histologically examined for reproductive life history and population characteristics by month. Overall, male and female *E. chamberlaini* follow a reproductive cycle similar to other spelerpine species with sperm in the Wolffian ducts of specimens from September to February; however, spermatidogenesis was delayed until August and September with the presence of mature sperm in testes from August through November. We captured 201 female salamanders of which 36 were considered immature. We caught 191 males of which 27 were considered immature. The mean snout–vent length (SVL ± 1 SD) of mature females (27.13 ± 2.33 mm) and males (25.84 ± 2.03 mm) was statistically different from one another. Females tended to be larger than males from our monthly samples, with a greater proportion of the largest specimens being female. Overall, population structure of *E. chamberlaini* appears similar to other coastal plain salamander species.

**E**urycea chamberlaini** (Chamberlain’s Dwarf Salamander) is a small spelerpine salamander from the coastal plain of North and South Carolina, USA (Harrison and Guttman, 2003). *Eurycea chamberlaini* was originally considered a color morph of *E. quadridigitata* (Bishop, 1943), but *E. chamberlaini* are morphologically and genetically distinct from *E. quadridigitata* (Harrison and Guttman, 2003; Lamb and Beamer, 2012; Wray et al., 2017). While life-history and reproductive data are available from members of the dwarf salamander complex from Alabama (*E. hillisi*; Trauth, 1983), Louisiana (*E. paludicola*; Sever, 1974, 1975a, 1975b; Wen et al., 2021), South Carolina (*E. quadridigitata*; Harrison, 1973; Semlitsch, 1980; Semlitsch and McMillan, 1980), and Florida (*E. quadridigitata*; Wray et al., 2017), very little data exist for *E. chamberlaini*. Only scant reproductive and life-history data have been published for *E. chamberlaini* in the original species description (Harrison and Guttman, 2003), a taxonomic revision of *E. quadridigitata* (Wray et al., 2017), and a few notes on reproductive biology published under the former *E. quadridigitata* nomenclature (Brimley, 1923; Bishop, 1943). Brimley (1923) hypothesized that the mating season was most likely October through April and noted that individuals were easier to capture during those months. Bishop (1943) observed that females may reach a larger maximum body size relative to males.

To date, no data on population structure of *E. chamberlaini* have been published since the species description in 2003 (Harrison and Guttman, 2003), although previous studies on populations of *E. quadridigitata* reported a 1:1 sex ratio (Harrison, 1973; Semlitsch, 1980; Semlitsch and McMillan, 1980), like other spelerpine salamanders (Bruce, 1978a, 1978b, 1988a). The lack of basic population-level data hampers management and conservation efforts and is cause for concern as other species of *Eurycea* with limited ranges are declining and are very susceptible to anthropogenic changes (Bendik et al., 2014; Diaz et al., 2020). While populations of *E. chamberlaini* do not appear to be in direct jeopardy of extinction, the paucity of population data makes it difficult for an accurate conservation assessment.

The objectives of our study were to examine the reproductive life history and population structure of *E. chamberlaini* from a North Carolina population. We expected that reproductive cycles would follow a similar pattern to those of other dwarf salamander populations. For example, in males, mature spermatozoa appear in the testes in August (Sever, 1974, 1975a) and peak secondary sexual characteristic (i.e., mental glands and cirri) hypertrophy occurs in October/November (Sever, 1974, 1975a, 1975b); in females, vitellogenesis takes place from September to February (Trauth, 1983) and sperm storage has been observed from November to February (Trauth, 1983). We expected to find more immature, recently metamorphosed individuals in our collections starting in July based on previous reports of the timing of metamorphosis of *E. quadridigitata* from South Carolina (Semlitsch, 1980). We also hypothesized that sex ratios would be 1:1 and that sexual-size dimorphism would not be recovered between the sexes, similar to findings from *E. quadridigitata* (Harrison, 1973; Semlitsch, 1980; Semlitsch and McMillan, 1980).

To assess the population structure and reproductive life history of *E. chamberlaini*, we modeled our study after natural history studies of other plethodontid salamanders (e.g., Bruce 1971, 1978a, 1978b). We assessed sex, body size, and reproductive condition of every individual from a large collection of *E. chamberlaini* made monthly throughout an entire year.

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MATERIALS AND METHODS

We made monthly visits to the Croatan National Forest near Otter Creek in Craven County, North Carolina (34.98°N, 76.95°W) from February 2008 to February 2009. Most E. chamberlaini were found beneath leaf litter along the banks of a swamp. Each month approximately 30 individuals were collected, after which time we ceased searching. Supplementary collections were made in March, April, and August 2019.

Three hundred ninety-two metamorphosed E. chamberlaini, representing every month of the year were collected during the study. Specimens were euthanized upon capture by submergence in chlorethone and subsequently fixed in neutral buffered formalin. After at least one week in fixative, snout to the posterior angle of the vent (SVL) and cirr length (males only) were recorded for each specimen and reproductive maturity was assessed, i.e., mature or immature. Males were considered adults based on the presence of cirri, or definitive testes and opaque Wolffian ducts. Females were considered adults based on the presence of ovarian follicles and opaque oviducts.

 Entire urogenital tracts and lower jaws were removed from five adult males from each collection month and cloacae were removed from five adult females from each collection month. Male urogenital tracts, male lower jaws, and female cloacae were rinsed in distilled water, dehydrated with ascending concentration of ethanol, cleared in toluene, and embedded in paraffin wax for histological examination. Histological sections were obtained at seven microns with a rotary microtome and affixed to albuminized slides. Male urogenital tracts were sectioned on the frontal plane, whereas male lower jaws and female cloacae were sectioned on the transverse plane. All slides were stained with hematoxylin and eosin following the protocol of Kiernan (1990) for general histological examination.

Serial transverse sections of lower jaws of males were obtained to assess seasonal secretory activity of mental glands, a known pheromone-producing gland in plethodontid salamanders (for review see Sever et al., 2016). The mean diameter of mental gland tubules for each specimen was determined through measurement of ten mental gland tubules per specimen. Mean mental gland diameter for each month was then calculated using the means of each specimen within a month.

Frontal sections of entire male urogenital tracts were obtained to assess the spermaticogenic cycle and sperm transport in E. chamberlaini. Germ cell types (i.e., spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and mature spermatozoa) were recorded for each testis following Uribe (2003). The presence or absence of spermatozoa in the Wolffian ducts was also recorded for each specimen. Serial transverse sections of female cloacae were obtained to assess sperm storage in effort to pinpoint the timing of sperm transfer in E. chamberlaini. All female plethodontid salamanders store spermatozoa in a complex spermatheca that opens through the roof of the cloaca (Sever, 1994). Sperm were recorded as present or absent in the spermathecae.

The total number of immature and mature individuals were enumerated by sex and month for statistical comparisons. A χ² test was conducted to test for deviations from a 1:1 sex ratio. The mean SVL of mature and immature males and females were compared with a t-test.

RESULTS

Male reproductive cycle.—Tests of all mature male E. chamberlaini possessed spermatogonia throughout the year (Figs. 1, 2A, B). Most specimens during November and December possessed primary spermatogonia that were sporadically found in connective tissue of the testes. Distinct lobules filled with secondary spermatogonia were present in specimens collected in January and remained a common feature of the testes through the April collection. Three of the five adult males from the May collection and all adult males sectioned from June to September possessed enlarged lobules filled with primary spermatocytes (Fig. 2C). Lobules with primary spermatocytes were most numerous in the months of June and August, immediately before and during the commencement of meiosis I, respectively.

The meiotic stages of spermaticogenesis were restricted to August and September in male E. chamberlaini (Fig. 2D). The first secondary spermatocytes arising from meiosis I were observed in a few caudally positioned testicular lobules from specimens captured in August. Similar to August specimens, secondary spermatocytes were only present in a few lobules from September specimens, and these spermatocytes were found in more cranially positioned testicular lobules, indicative of the stereotypical caudal to cephalic meiotic wave of spermatidogenesis that was previously described in plethodontid salamanders (Trauth, 1983). In specimens collected during September, lobules containing cysts at different stages of meiosis II were observed adjacent to and caudal to lobules containing secondary spermatocytes. Immediately caudal to lobules containing cysts undergoing meiosis II were lobules with elongating spermatids undergoing spermiogenesis (Fig. 2E), and even more caudally were cysts containing spermatozoa that could be considered mature (Fig. 2F). Mature spermatozoa were also found in the testes of four out of the five specimens examined from November, after which lobule number decreased considerably and the only lobules remaining were filled with enlarged Sertoli cells indicative of recent spermiation (Fig. 2G).

Mature spermatozoa were found in the Wolffian ducts of three of the five specimens from September and were present in all specimens until February, whereas only two of the five specimens possessed spermatozoa in the Wolffian ducts (Fig. 2H). Based on these data, we attributed possible timing of mating to the months of September through February. This coincided perfectly with hypertrophy of the mental glands, which showed obvious enlargement because of the synthesis of secretory granules from September to February, with the largest mean glandular tubules found in specimens from October (Fig. 3A–D). A similar pattern was found for seasonal length of cirri, with longer cirri found during the fall and winter months.

Size at maturity was difficult to determine in the male sample. During non-reproductive months (March, April, May, June, and July) all male salamanders, regardless of size, possessed small testes that were easily distinguished as testes, even in the 14.00 mm SVL range. All those salamanders also lacked measurable cirri. The possible mating season months (September, October, November, December, January, and February) provided more insight as smaller individuals lacked cirri, enlarged testes, enlarged mental gland tubules, and enlarged pigmented Wolffian ducts (n = 6) in comparison to mating males that possessed all those features (n = 58). The
smallest specimen found with measurable cirri, enlarged mental gland tubules, enlarged testes, and enlarged pigmented Wolffian ducts was 22.20 mm SVL and was captured in the October collection. The smallest specimen found with enlarged testes was 19.41 mm SVL and was captured in the September collection. That specimen lacked measurable cirri, a mental gland, and enlarged pigmented Wolffian ducts, and it is questionable if this specimen would actually mate without hypertrophy of secondary sexual structures. Interestingly, the testes were in the same spermatogenic stage as mature males from the month of September and no sperm were present in the Wolffian ducts. Similar to the other non-mating months, secondary sexual structures were not hypertrophied. All other males less than 22.20 mm that were captured during the reproductive months lacked cirri, enlarged mental gland tubules, enlarged testes, and pigmented Wolffian ducts and, thus, we determined that 22.20 mm was a good approximation for size at maturity.

**Female reproductive cycle.**—The female reproductive cycle commenced in September with the appearance of enlarged, yellowish vitellogenic follicles in the ovaries of *E. chamberlaini* (Fig. 1). Sperm were found in the spermathecae of females with vitellogenic follicles from October to March. Only a subset of the histologically examined specimens possessed sperm in the October and March samples, and sperm in the March sample were far fewer in density in the August collection. Histologically, the testes were in the same spermatogenic stage as other mature specimens from August and no sperm were present in the Wolffian ducts. Similar to the other non-mating months, secondary sexual structures were not hypertrophied.
comparison to the spermathecae from individuals from October to February, consistent with ovulation occurring between the time of our February and March collections (Fig. 4A–C, a–c). These data are consistent with sperm presence in the Wolffian ducts of males, and further confirmed mating as a fall and winter event in *E. chamberlaini*.

The smallest female (SVL) that was found to be vitellogenic during months when vitellogenic follicles were common in Fig. 2.

Histological micrographs depicting germ cell development and sperm transport within male testes throughout the year (hematoxylin and eosin). (A) April; (B) March; (C–F) August; (G) November; (H) January; M1c, cyst containing primary spermatocytes undergoing meiosis I; M2c, cyst containing secondary spermatocytes undergoing meiosis II; Mpt, mid-principal piece of sperm tails; Msc, cyst containing primarily mature sperm; Ppt, principal piece of sperm tails; Sc, Sertoli cell; Sc1c, cyst containing primary spermatocytes; Scn, Sertoli cell nuclei; Sg1l, lobule containing primary spermatogonia; Sg2l, lobule containing secondary spermatogonia; Sn, sperm nuclei; Soc, cyst containing spermatids in early spermiogenesis; Sp, sperm; Stl, post-spermiating lobule; Wen, nuclei of Wolffian duct epithelium. Scale bar = 50 μm.
the ovaries (October, November, January, and February) was 22.67 mm (November female). Mean SVL of vitellogenic \((n = 51)\) and non-vitellogenic \((n = 28)\) females during these months was 28.05 mm \((SD = 1.75)\) and 25.71 mm \((SD = 4.05)\), respectively. Removing non-vitellogenic females from the February sample that were over 22.67 mm \((females that had most likely just oviposited based on distended oviducts; \(n = 17)\) from the mean reduced the mean to 21.37 mm \((SD = 2.15)\). A 27.15 mm non-vitellogenic female collected in January was removed from our analyses. Removing this specimen from the non-vitellogenic mean further reduced the mean to 20.79 mm \((SD = 1.03)\), which we interpreted as the mean size of immature metamorphs in the sampled population, at least during the reproductive months. Based on the finding of a high percentage (~70%) of larger females that were non-vitellogenic and with distended oviducts in February, oviposition occurred between the months of February and the date of our March collection. No females from our March collection were vitellogenic, including those over 22.67 mm \((n = 27)\). During the other months (October, November, December, and January), all females over 22.67 mm except one aforementioned January specimen (27.15 mm) and another smaller January specimen (22.73 mm) possessed vitellogenic follicles and, thus, it is assumed that adult female \(E.\ chamberlaini\) yolk follicles annually.

We have observed oviposition sites of \(E.\ chamberlaini\) where the eggs were scattered singly or in small groups between damp decaying leaves either in water or immediately adjacent to water. Eggs were observed in Craven County, North Carolina among leaves in a seepage on 18 February 2005 and 12 February 2007. This seepage was dry on 16 February 2008 and no eggs were observed. At a site near the Tar River in Nash County, several gravid females were observed on 6 February 2011, but no nests were observed. This site was revisited on 12 February 2011, and two nests were observed, at least one of which was almost certainly deposited between the two visits because the area where this nest was located was searched thoroughly during the previous visit. While females were sometimes found adjacent to eggs, it did not appear that they were actively guarding eggs. Instead, it is likely they were still in the process of ovipositing. The scattered nature of the eggs combined with the small body size of \(E.\ chamberlaini\) make it unlikely that females could effectively guard their scattered egg complement.

**Metamorph population structure.**—Based on the above reproductive data, we used 22.20 mm and 22.67 mm SVL as the minimum size for maturity in males and females, respectively. Individuals from non-reproductive months (March through July for males and March through September for females) were reported as immature if they were under these thresholds and mature if they were over these thresholds. We define immature as a high probability that the individual has never reproduced before the time of capture; however, considering \(E.\ chamberlaini\) is a seasonally reproducing species, it is possible that individuals in the 22 mm SVL range that were captured early in the year would have reached minimum reproductive size in time for gametogenesis in the late summer to early fall of that same year.

We caught 201 female salamanders of which 36 were immature and 191 males of which 27 were immature. Mature female mean SVL \(\pm SD\) \((27.13 \pm 2.33)\ mm) were larger than mature males \((25.84 \pm 2.03)\ mm; t_{327} = 5.31, P < 0.001)\). Females tended to be larger than males from our monthly samples with a greater proportion of the largest specimens being female (Figs. 5, 6). All individuals collected during the reproductive season (September–February) were significantly larger \((27.01 \pm 2.08)\ mm versus \((26.19 \pm 2.35)\ mm)\) than individuals collected during the non-reproductive season (March–August; \(t_{312} = 3.23, P = 0.005)\; (Fig. 6). Females, across both mature and immature stages, were significantly larger \((25.93 \pm 3.43)\ mm) than males \((25.04 \pm 2.80)\ mm; t_{1356} = 10.21, P = 0.002)\). The sex ratio of the total mature 165 males and 164 females did not deviate from a 1:1 sex ratio \((\chi^2 = 0.003, P = 0.956)\).
DISCUSSION

A wealth of information exists on plethodontid reproductive life history and/or population structure for a variety of genera including *Desmognathus* (Bruce, 1988b), *Eurycea* (Bruce, 1982), *Gyrinophilus* (Bruce, 1978a), *Plethodon* (Organ, 1960; Bruce, 1967), *Pseudotriton* (Bruce, 1978b), and *Stereochilus* (Bruce, 1971, 2008). These (and other) natural history studies have provided the basic data that shape our understanding of complex life cycles (Bruce, 2005), ecology (Bruce, 2011), and evolution (Ryan and Bruce, 2000) of plethodontid salamanders. For brevity, we limit most of our discussion to comparisons between *E. chamberlaini* and other dwarf salamander species, all of which were referred to as *E. quadridigitata* as recently as 2017.

Two previous studies reported the spermatogenic cycle of dwarf salamanders (Sever, 1975a; Trauth, 1983). Similar to other plethodontids (for review see Siegel et al., 2014), Sever (1975a) reported spermatidogenesis in July and August, with mature sperm appearing by August, in populations of *E. paludicola* from Louisiana. By the end of October, spermiation was complete and testes were devoid of mature spermatozoa. Spermatozoa could be found in the Wolffian ducts from September to February. Trauth (1983) reported results similar to Sever (1975a) for a population of *E. hillisi* from Alabama with the exception that mature sperm were present in testes until December. Similar to both previous studies, we found sperm in the Wolffian ducts of specimens of *E. chamberlaini* from September to February; however, spermatidogenesis was delayed until August and September with the presence of mature sperm in testes from August

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**Fig. 4.** Histological micrographs depicting complex spermaticae of females throughout the year. (A,a) September; (B,b) January; (C,c) March; Clo, cloacaal orifice; Nep, nuclei of spermatic tubule epithelium; Sp, sperm; Spt, spermatic tubules; Vt, vent. Scale bars = 200 μm (A–C) and 20 μm (a–c).

**Fig. 5.** Number of male and female *Eurycea chamberlaini* collected from different size classes. Note that a higher proportion of females attain greater lengths in comparison to males.
through November (Fig. 1). Harrison (1973) reported that the Wolffian ducts were packed with sperm in the fall from a South Carolina population of *E. quadridigitata*, but further details were not provided. Whether differences between these four populations represent “real” species/geographic variation or simply annual variation is debatable until multiyear studies from each of these species are undertaken. Only a single previous study (published as a dissertation and two manuscripts; Sever, 1974, 1975a, 1975b) detailed aspects of seasonal variation of male secondary sexual structures in dwarf salamanders. Sever (1974, 1975b) measured cirri from 335 dwarf salamanders of which a minimum of 286 represent samples of *E. paludicola* from Louisiana. A maximum of 49 specimens could represent either *E. quadridigitata* or *E. hillisi* from Georgia based on Sever’s collection records. Similar to the results in Sever (1974, 1975b), we found that cirri were most enlarged in male *E. chamberlaini* from September to February with a peak in length between October and December. We concluded that cirri were not measurable from March to August; however, Sever (1974, 1975b) provided measurements of cirri of specimens of *E. paludicola* from March to August in the range of 0.05 mm to 0.15 mm, which we consider not visible. During their hypertrophy, Sever (1974, 1975b) provided similar cirrus length measurements to what we found for *E. chamberlaini*. Mental gland hypertrophy was also similar between Louisiana *E. paludicola* and *E. chamberlaini*; i.e., noticeable enlargement of the mental gland tubules occurring from September to February, with maximum hypertrophy from October to December, coincident with the time period characterized by greatest cirri length (Sever, 1974, 1975a). Harrison (1973) noted that secondary sexual characteristics were hypertrophied during the fall in South Carolina *E. quadridigitata*, but no mensural data were provided for a more in-depth comparison.

Our results on the female reproductive cycle of *E. chamberlaini* coincide relatively well with the results obtained from a population of Alabama *E. hillisi* (Trauth, 1983). Vitellogenic follicles were found in both populations from October to February with sperm presence in spermathecae from October to March in *E. chamberlaini* and November to February in *E. hillisi* (Trauth, 1983); however, we considered the sperm reported in the spermathecae from March specimens from our collection as residual, based on the fact that they were few in number and that March specimens were devoid of eggs with distended oviducts. In both populations, no vitellogenic females were found after February, indicating the approximate time of ovulation and oviposition is between February and March.

Trauth (1983) provided more details than in our study on sperm presence in cloacae and determined that fresh sperm were entering cloacae in specimens from November to February, indicative of a mating season that extends from the fall through the entire winter. Wen et al. (2021) reported the presence of sperm caps in the cloaca of four female *E. paludicola* from Louisiana. One observation was made on 19 November 2017, and three observations were made on 29 November 2017. These data coincide perfectly with the hypertrophy of secondary sexual characteristics and presence of sperm in the Wolffian ducts in male *E. chamberlaini* from our study and *E. paludicola* from Louisiana (Sever, 1974, 1975a).

Reproductive data from females that currently represent *E. quadridigitata* do not coincide as closely with the results provided by Sever (1974), Trauth (1983), and our study. Semlitsch (1980) reported that vitellogenesis occurred between September and November in a South Carolina population of *E. quadridigitata*, and that no gravid females were reported after November (Semlitsch and McMillan, 1980). Goin (1951) reported oviposition in November in a Florida population of *E. quadridigitata* and both Goin (1951) and Carr (1940) reported discovering eggs during January in Florida populations. Harrison (1973) witnessed oviposition in the lab as early as October in wild-caught *E. quadridigitata* from South Carolina. Brimley (1923) reported that oviposition occurred during February in a North Carolina population of *E. chamberlaini*, but also noted that eggs were found once in late December. Out of all reproductive characteristics, timing of egg deposition appears to be the most variable both within and between dwarf salamander species with recorded dates of oviposition extending from October to potentially March.

All previous studies with data on age at reproductive maturity refer to *E. quadridigitata sensu stricto* and concluded that both males and females reach reproductive maturity in the same year as metamorphosis (Harrison, 1973; Semlitsch, 1980). After deposition, eggs hatch within 30–40 days (Goin, 1951; Harrison, 1973) and the larval period lasts approximately 3 to 6 months (Harrison, 1973; Semlitsch, 1980). For example, Semlitsch (1980) found larvae as early as February with metamorphosis commencing in July in South Carolina *E. quadridigitata*. Wen et al. (2021) reported first detecting larvae of *E. paludicola* in a Louisiana population on 12 March 2018. They reported observing a metamorphed individual on 29 April 2018. They failed to detect any larvae on 11 May 2018, which suggests that
metamorphosis had been completed by that date. In our study, if sexual maturity was achieved by the fall mating season in *E. chamberlaini*, we would not expect to find non-reproductive post-metamorphic individuals in autumn; however, not only did we find small, recently metamorphosed individuals from May through August (Fig. 4), we also found small, non-reproductive individuals in September through November and January. Our data indicate that reproductive maturity is not reached in the first autumn after metamorphosis, at least not in every individual from our sampled population. Small male and female post-metamorphic individuals during the reproductive season (fall and winter) possessed undeveloped gonads and lacked secondary sexual characteristics that were nearly always present in larger, reproductively active specimens from the fall through the spring. We believe that further research with other dwarf salamander populations will be required to make sound comparisons of metamorphosis phenology between populations.

Previous studies indicate that no sexual-size dimorphism exists between male and female of *E. quadridigitata* from multiple populations, at different life-history stages (Harrison, 1973; Semlitsch, 1980; Semlitsch and McMillan, 1980). In our sample of a metamorphosed North Carolina population of *E. chamberlaini*, we found that mean female SVL was significantly greater than mean male SVL (Fig. 4). Bishop (1943) also reported that males are smaller than females from North Carolina *E. chamberlaini*, but the sample size assessed was small, and no statistical analyses were used. Whether females reach larger size in *E. chamberlaini* because of selective pressures (e.g., increased fecundity; Salthe, 1969) or some other mechanism is unknown. Regardless, female-biased sexual-size dimorphism is not uncommon in salamanders (Shine, 1979; Bruce, 2000). For example, Duellman and Wood (1954) found that larger individuals of *E. cirtiraga* (referred to as *E. bislineata* in that study) in their study populations were mostly females. It is also possible that smaller female specimens spend less time on the surface and are not as easy to detect or collect; however, similar to studies on *E. quadridigitata* at different life-history stages (Harrison, 1973; Semlitsch, 1980; Semlitsch and McMillan, 1980; Bailey et al., 2004), the sex ratio we recorded for *E. chamberlaini* did not significantly deviate from 1:1.

In conclusion, our study is the first to report detailed, monthly reproductive and population-level data on *E. chamberlaini*. In a North Carolina population, this species can be found year-round in appropriate habitats (e.g., beneath leaf litter in forested areas above floodplain swamps) but is more easily observed when larger individuals are more active during the reproductive season (October through April), which also reflects observations by Brimley (1923). Overall, the population structure of *E. chamberlaini* appears similar to other coastal plain *Eurycea* (Harrison, 1973; Semlitsch, 1980; Semlitsch and McMillan, 1980). We documented some variability in reproductive timing and efforts between *E. chamberlaini* and other dwarf salamander species. This variability reinforces the importance of basic natural history data and will further the knowledge of this species that has continued to be largely unstudied across its limited range. Indeed, future research can build on our data to inform future conservation efforts.

**DATA ACCESSIBILITY**

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**LITERATURE CITED**


