
SEASONAL CHANGES OF PRIMARY AND SECONDARY SEXUAL CHARACTERISTICS IN THE SALAMANDERS *EURYCEA AQUATICA* AND *E. CIRRIGERA*

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Abstract.—*Eurycea aquatica* and *E. cirrigera* are closely-related plethodontid salamanders with overlapping geographic ranges in the southeastern United States. These species generally occupy separate habitats: *E. aquatica* occurs primarily in springs, while *E. cirrigera* occurs in and around streams and swamps. To test whether these environments influence reproduction in these salamanders, we analyzed seasonal variation of putative secondary sexual characteristics, such as cirri length and head width. To explore whether relationships exist between these characteristics and reproductive anatomy, we also analyzed seasonal variation in testis size and vas deferens diameter (i.e., primary sexual characteristics), and determined the relationship between these primary and secondary sexual characteristics within each species. For *E. cirrigera*, testis size was largest in summer and fall, followed by enlargement of the vas deferens and simultaneous lengthening of the cirri in winter and early spring. *Eurycea aquatica* becomes difficult to locate during the non-breeding season, precluding a thorough seasonal analysis and comparison of the reproductive cycle between the two species. However, the secondary sexual characteristics of *E. cirrigera* and *E. aquatica* exhibit different temporal relationships to testis size, and testis size and vas deferens size (standardized by body size) differ between the two species, suggesting that these features may represent adaptations for different male reproductive strategies.

Key Words.—Caudata; cirri; *Eurycea bislineata* complex; Plethodontidae; reproductive cycles

INTRODUCTION

Plethodontid salamanders exhibit a range of different mating systems, which has made them excellent subjects for comparative studies involving courtship evolution (Arnold 1977), behavioral ecology (Jaeger 1993), pheromone function (Houck et al. 2007), and life-history evolution (Tilley and Bernardo 1993; Chippindale et al. 2004). However, previous research has focused on a small number of model species (for example, the genus *Plethodon*), which have been the subject for numerous studies involving courtship (Gergits and Jaeger 1990; Gillette et al. 2000) and inter- and intraspecific competition (e.g., Jaeger 1971, 1984; Anthony et al. 1997; Adams and Rohlf 2000; Deitloff et al. 2009), while other species within the plethodontidae have received relatively less attention. Similarly, while the reproductive cycles for many plethodontids are known, other species remain uncharacterized. Testis size in salamanders fluctuates seasonally in many species, becoming largest and coinciding with spermatogenesis prior to the breeding season (Woodley 1994; Jensen et al. 2002; Rastogi et al. 2005). The testis then decreases in size during the breeding season as spermiation begins (movement of sperm from the testes to the vas deferens), at which time the vas deferens becomes convoluted and

increases in size (Woodley 1994; Jensen et al. 2002; Rastogi et al. 2005). Although histological preparations are necessary to confirm stages of spermatogenesis, these indirect measures of testicular activity have been used to determine general patterns (Meshaka and Trauth 1995; Jensen et al. 2002; Aranzábal 2003) and require less time and effort.

Certain secondary sexual characteristics of plethodontid salamanders have courtship function and are closely associated with gametogenic cycles and hormone secretion (Rastogi et al. 2005). For example, the mental glands and enlarged premaxillary teeth of *Eurycea* are described as male pheromone delivery systems that influence female receptivity. These traits typically are influenced by reproductive hormones (Houck and Sever 1994; Kozak 2003; Woodley 1994). Cirri (fleshy protuberances distal to the nasolabial grooves) have chemosensory function (Dawley and Bass 1988), and have been linked to male chemoinvestigatory (Schubert et al. 2006) and courtship behaviors (Jaeger 1986). In addition, cirri and nasolabial grooves are activated by testosterone (Sever 1975; Schubert et al. 2006), suggesting that these structures also are closely associated with endogenous reproductive cycles.

Species in the *Eurycea bislineata* complex exhibit a

spectrum of secondary sexual characteristics and morphologies: some species have elongate cirri and a gracile morphology (*E. wilderae*, *E. cirrigera*), and other species have enlarged heads, small cirri, and a robust morphology (*E. aquatica*). Some populations may be polymorphic and include males of both gracile and robust types (e.g., *E. wilderae* populations in North Carolina, USA; Sever 1979, 1989, 1999; and *E. cirrigera* populations in Georgia, USA; Jensen et al. 2008). Head size of male salamanders of the *E. bislineata* complex can be markedly different from females (e.g., *E. bislineata*; Noble 1929; *E. aquatica*; Sever 1999; Graham et al. 2010; and *E. cirrigera*; Brophy and Pauley 2002). Also, head size may vary seasonally in *E. bislineata* (Petraska 1998).

The array of secondary sexual characteristics exhibited by the *Eurycea bislineata* complex is expected to reflect phylogenetic or ecological differences among species (Kozak 2003; Kozak et al. 2006); however, the adaptive significance of the entire suite of characteristics is unknown. If they are related to courtship and reproduction, these features may exhibit seasonal variation associated with seasonal hormone secretion and/or the testicular cycle. Seasonal variation of head size has not been previously analyzed quantitatively in any member of this species complex, and cirri length has not been quantitatively compared across seasons in a member of the *E. bislineata* complex (but see Sever 1975 for a study of seasonal variation of cirri in *E. quadridigitata*). The relationship between these features and the male reproductive cycle has not been explored until now.

In this study, we used museum specimens to compare seasonal changes in male reproductive anatomy and secondary sexual characteristics of two members of the *E. bislineata* complex; *E. aquatica* and *E. cirrigera*, which are species with overlapping ranges in Alabama and Georgia, USA (Timpe et al. 2009). Typically, *E. aquatica* occurs in permanent springs, while *E. cirrigera* is found in other wetland habitats, such as streams and swamps (Rose and Bush 1963; Mount 1975; Timpe et al. 2009). Springs typically have different physical properties than other wetland habitats (e.g., minimal variation in water temperature and flow rate, low turbidity, and high pH; Hubbs 1995). Therefore, the behavioral and reproductive strategies of these species may reflect these differences (e.g., Graham et al. 2010). We tested for seasonal variation in the primary sexual characteristics of testis size and vas deferens size, as well as secondary sexual characteristics in these two species. These traits have been studied in numerous vertebrates and results typically demonstrate a link between testis size and male sexual strategies and/or sperm competition (e.g., Harcourt et al. 1981; Møller and Briskie 1995; Emerson 1997). We tested whether secondary sexual characteristics were correlated with

measurements of male reproductive tract anatomy and whether differences occur between testis size (adjusted for body size) or vas deferens size in our two species. By comparing male *E. cirrigera* (gracile, cirrigerous morphotype) and *E. aquatica* (robust, big-head morphotype), we analyzed two extremely different male morphologies exhibited by the *Eurycea bislineata* complex, hypothesizing that such closely related, yet morphologically and ecologically divergent species, would be characterized by distinct reproductive adaptations. Specifically, we predicted that because *E. aquatica* occupies springs, which presumably have higher local population densities than swamps and streams, they would show adaptations for higher male-male competition (e.g., larger testis size and stronger associations between testis size and secondary sexual characteristics).

MATERIALS AND METHODS

Museum specimens.—We measured morphological characters on museum specimens of *E. aquatica* (males: n = 50; females: n = 31) and *E. cirrigera* (males: n = 62; females: n = 65) from the Auburn University Herpetological Collections (AUM). We analyzed females to confirm that putative secondary sexual characteristics were sexually dimorphic and to associate events in the male's reproductive cycle to those in the female cycle. We examined specimens from each month with specimens available in an attempt to evenly represent each species, sex, and month. We gathered data from male *E. aquatica* during January, February, March, April, October, and December, but we omitted data from October from the analyses because only one sample was available from that month. We gathered data for female *E. aquatica* collected during January, February, March, April, July, and December. However, we omitted data from July from the analyses because only one sample was available from that month. We collected data from male and female specimens of *E. cirrigera* during all months; however we omitted data from July and December, again because only one sample was available from each of these months. We retained those single sample months in our graphical representations of results.

Measurement protocol.—We generated measurements from multiple individuals of known species assignment based upon Timpe et al. (2009). We used the same *E. aquatica* specimens as those used by Timpe et al. (2009), or used specimens collected from the same sites analyzed by Timpe et al. (2009) after they were confirmed to contain *E. aquatica*. We selected *E. cirrigera* specimens from sites and physiographic regions not known to harbor *E. aquatica*. These specimens were morphologically consistent with *E.*

cirrigera, corresponded to “clade L” *E. cirrigera* localities sampled by Kozak et al. (2006), and included the same individuals used in the analysis of Timpe et al. (2009).

We measured snout-vent length (SVL; distance from the snout to the anterior margin of the cloaca) and head width of all specimens using vernier calipers. Because the cirri were too small to accurately measure using calipers, we photographed the lateral view of the head of the salamanders using a 8.0 megapixel digital camera (Canon PowerShot S5 IS, Canon Inc., Tokyo, Japan) while including a ruler in the image for scale. We used a scaling function provided by the software tpsDig2 (Rohlf 2008) to record cirri length. We used a similar methodology to record morphological measurements of the testis and vas deferens. We dissected male specimens by opening the abdomen via a sagittal incision and two transverse incisions. We then pinned the flaps back and manipulated the viscera to allow the right testis and vas deferens to be photographed for digital measurement. We examined each female specimen for the presence of eggs, which are visible through the abdomen. We counted eggs and stored them in a vial with the corresponding specimen’s museum number. The same individual (MAA) recorded all morphological measurements.

Data analysis.—To test whether the various reproductive parameters (response variables) varied among months, we performed several analyses of covariance (ANCOVAs) with month as the independent variable and SVL as a covariate (sample sizes are shown in Appendix). For males of *E. aquatica*, we tested head width, length and width of testis, and width of vas deferens as response variables. For males of *E. cirrigera*, we tested head width, length of cirri, length and width of testis, and width of vas deferens as response variables.

For females, we performed an ANCOVA with month as the independent variable, SVL as a covariate, and number of eggs as the response variable (sample sizes are shown in Appendix). Number of eggs in females of *E. aquatica* and *E. cirrigera* was not normally distributed; therefore, we assessed significance of these ANCOVAs by comparing the observed *F* values to randomized values, following the procedure outlined in Adams and Collyer (2007) and Collyer and Adams (2007). In our analyses, we performed the residual randomization procedure 999 times, and we treated the proportion of random values (plus the observed) greater or equal to the observed value as the significance of the effect.

For each of the above ANCOVAs, we included an interaction term between month and SVL to test whether the slopes of the relationship between the reproductive parameter and SVL were homogenous among months

(i.e., interaction term was not significant). If slopes were homogeneous (interaction term was not significant), SVL covaries with the reproduction parameter in the same way every month; therefore, we removed the interaction term and reported results from the ANCOVA without this term. If slopes were not homogenous (interaction term was significant), SVL covaries with the reproduction parameter differently among months, and an overall result for SVL is not reliable. Therefore, for these analyses we report the results for months and the correlation between the reproductive parameter and SVL separately for each month. We used graphs to show variation among months for each characteristic, standardizing for SVL (dividing by SVL) when SVL significantly covaried with the response variable.

We predicted that secondary sexual characteristics correlated with reproductive parameters. To test this prediction, we compared head width in males of *E. aquatica* to length and width of testis and width of vas deferens ($n = 26$), and we compared head width and length of cirri in males of *E. cirrigera* to those same parameters ($n = 40$) with correlation analysis. We used an ANOVA to determine whether species and sexes differ in head width of *E. aquatica* and *E. cirrigera*. We used species and sex as the independent variables and included a term for the interaction between these factors. For the response variable, we divided head width by SVL to standardize for SVL. We performed pairwise comparisons of head width/SVL for males and females of *E. aquatica*, males and females of *E. cirrigera*, and between males of both species. Bonferroni correction was used so that $\alpha = 0.0167$ for each comparison, to maintain an overall α of 0.05.

We also tested the prediction that *E. aquatica* and *E. cirrigera* differed in average length and width of testis and average width of vas deferens during January, February, March, and April to control for seasonal effects. These were months for which we had large sample sizes for both species (see Discussion). We used ANCOVAs with species as the independent variable, reproductive characters as response variables, and SVL as a covariate (slopes were tested for homogeneity).

RESULTS

Seasonal patterns.—We found several differences among months for the reproductive parameters measured. Mean testis length was not significantly different among months and did not covary with SVL, and slopes were homogeneous (Table 1). Mean testis width significantly differed among months (Table 1); however, slopes were not homogenous (interaction between month and SVL was significant; Table 1), and correlation analysis showed that none of the relationships between testis width and SVL were significantly different than zero. Width of the vas deferens did not differ among months

TABLE 1. Results for ANCOVAs examining differences in reproduction parameters using month as a factor (e.g., differences among months) and SVL as the covariate. Sample sizes are provided in Appendix.

		df	F	P
Male <i>E. aquatica</i>				
Head width	Month	4	3.65	0.01*
	SVL	1	88.79 < 0.001*	
Testis length	Month	4	0.58	0.68
	SVL	1	3.53	0.08
Testis width	Month	4	30.87 < 0.001*	
	Month x SVL	4	3.23	0.04*
Vas deferens width	Month	4	1.69	0.20
	Month x SVL	4	3.21	0.04*
Male <i>E. cirrigera</i>				
Head width	Month	9	8.59 < 0.001*	
	SVL	1	35.42 < 0.001*	
Cirri length	Month	9	2.94	0.007*
	SVL	1	4.29	0.04*
Testis length	Month	9	29.80 < 0.001*	
	SVL	1	0.16	0.69
Testis width	Month	9	15.64 < 0.001*	
	SVL	1	11.25	0.002*
Vas deferens width	Month	9	2.84	0.02*
	SVL	1	1.75	0.20
Female <i>E. aquatica</i>				
Eggs (number)	Month	4	5.06	0.02*
	SVL	1	1.51	0.25
Female <i>E. cirrigera</i>				
Eggs (number)	Month	9	16.82	0.001*
	SVL	1	1.43	0.25

*Overall P < 0.05

(Table 1); however, slopes were not homogenous (Table 1), and correlation analysis showed that none of the relationships between testis width and SVL were significantly different than zero.

Male and female reproductive parameters and secondary sexual characteristics.—For all of the analyses examining males of *E. cirrigera*, slopes were homogeneous. Mean head width was significantly different among months and did covary with SVL (Table 1; Fig. 1B). Mean cirri length was significantly different among months and significantly covaried with SVL (Table 1, Fig. 3). Mean testis length was significantly different among months, but did not covary with SVL (Table 1; Fig. 2B). Mean testis width was significantly different among months and significantly covaried with SVL (Table 1; Fig. 2B). Mean width of vas deferens significantly different among months, but did not covary with SVL (Table 1; Fig. 2B). The mean number of eggs of females of both *E. aquatica* and *E. cirrigera* significantly differed among months, but did not covary with SVL (Table 1), and slopes were homogeneous. For

TABLE 2. Results from correlation analysis between secondary and primary reproduction parameters. * Overall P < 0.05

	Secondary	Primary	df	r	P
<i>E. aquatica</i>					
Head width	Testis length	24	-0.05	0.79	
Head width	Testis width	24	-0.49	0.01*	
Head width	Vas deferens width	24	0.45	0.02*	
<i>E. cirrigera</i>					
Head width	Testis length	38	-0.20	0.22	
Head width	Testis width	38	0.11	0.48	
Head width	Vas deferens width	38	0.14	0.39	
Cirri length	Testis length	38	0.03	0.87	
Cirri length	Testis width	38	0.06	0.70	
Cirri length	Vas deferens width	38	0.27	0.90	

E. cirrigera, eggs were present from September through March and completely absent from April through August. For *E. aquatica*, eggs were present in January and March but not in February, April, July, or December.

Relationship between reproductive parameters and secondary sexual characteristics.—We tested whether size of secondary sexual characteristics in males correlated with measurements of reproductive anatomy. Sample sizes were small, making detection of correlations difficult. However, we detected that in *E. aquatica*, head width was not significantly correlated with testis length but did correlate with testis width and width of vas deferens (Table 2; Fig. 4). Head width of *E. cirrigera* was not significantly correlated with testis length, testis width, or width of vas deferens (Table 2). Also, in *E. cirrigera*, cirri length was not significantly correlated with testis length, testis width, or width of vas deferens (Table 2).

We found that species ($F_{1,204} = 125.88$; $P < 0.001$) and sexes differed ($F_{1,204} = 42.16$; $P < 0.001$) in head width/SVL, and the interaction between species and sex was significant ($F_{1,204} = 34.77$; $P < 0.001$; Fig. 5). After correction for SVL, males of *E. aquatica* have significantly wider heads than females (male $\bar{x} = 0.15 \pm 0.01$; female $\bar{x} = 0.13 \pm 0.009$; $P < 0.001$) and males of *E. cirrigera* (male $\bar{x} = 0.13 \pm 0.008$; $P < 0.001$; Fig. 5). Males and females of *E. cirrigera* do not differ in head width (female $\bar{x} = 0.13 \pm 0.009$; $P = 0.110$; Fig. 5). Testis length in *E. aquatica* is significantly longer than *E. cirrigera* (*E. aquatica* $\bar{x} = 2.62 \pm 1.00$ [SD]; *E. cirrigera* $\bar{x} = 1.92 \pm 0.58$; $F_{1,30} = 5.62$; $P = 0.024$), SVL did not significantly covary ($F_{1,30} = 0.062$; $P = 0.810$), and slopes were homogeneous. Testis width did not differ between the two species (*E. aquatica* $\bar{x} = 0.53 \pm 0.19$; *E. cirrigera* $\bar{x} = 0.51 \pm 0.28$; $F_{1,30} = 0.05$; $P = 0.820$), SVL did not covary ($F_{1,30} = 0.09$; $P = 0.760$), and slopes were homogeneous. Vas deferens is significantly wider in *E. aquatica* than in *E. cirrigera* (*E. aquatica* \bar{x}

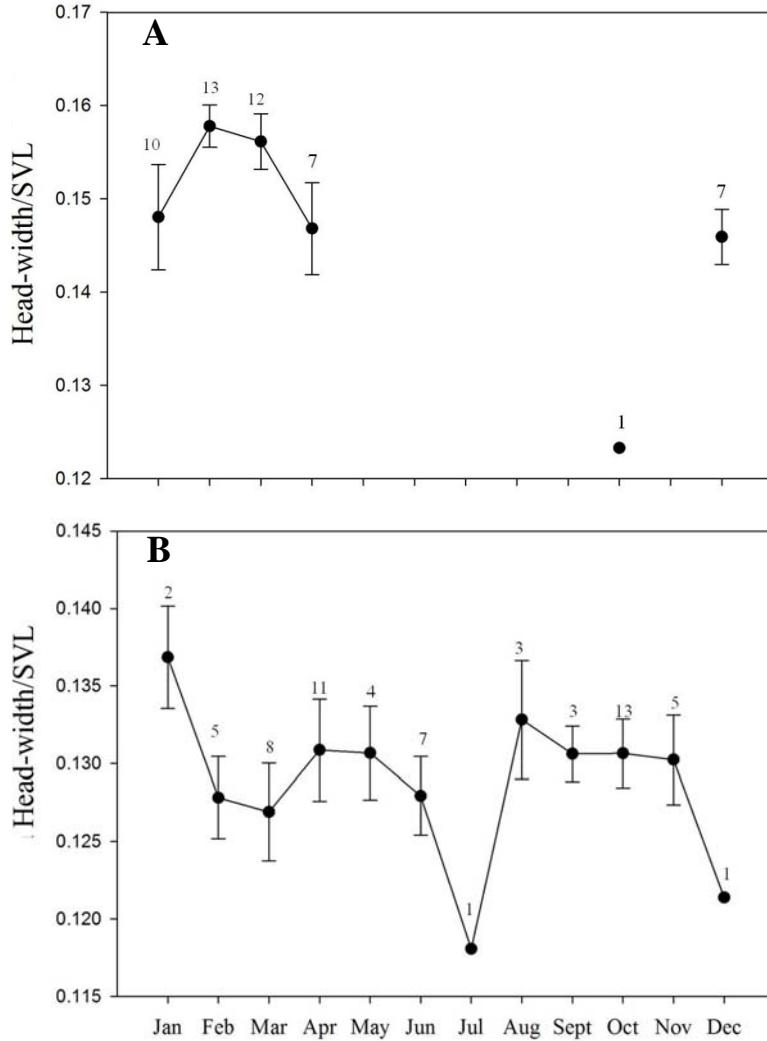


FIGURE 1. A) Monthly variation in head width, standardized for SVL, in males of *Eurycea aquatica*. Dots represent means and vertical lines represent standard errors for each month. Gaps are months for which no specimens were available (see text). B) Monthly variation in head width, standardized for SVL, in males of *Eurycea cirrigera*. Symbols are the same as in A. Small numerals within graphs indicate monthly sample sizes.

$= 0.30 \pm 0.09$; $E. cirrigera \bar{x} = 0.17 \pm 0.06$; $F_{1,30} = 21.59$; $P < 0.001$), SVL did not covary ($F_{1,30} = 1.40$; $P = 0.250$), and slopes were homogeneous.

DISCUSSION

We tested whether primary and secondary sexual characteristics vary monthly in both *E. aquatica* and *E. cirrigera*. We found that for *E. aquatica*, head width and width of testis varied monthly, but length of testis and width of vas deferens did not. For male *E. cirrigera*, all measured reproductive parameters (testis, vas deferens, and cirri length) varied monthly. *Eurycea aquatica* did not exhibit seasonal variation for all

variables as did *E. cirrigera*, but the temporal range of specimens for *E. aquatica* available to us made detection of this variation difficult. We confirmed that head width was sexually dimorphic and correlated with the width of testis and width of vas deferens in males of *E. aquatica*. Cirri length is also sexually dimorphic (Sever 1979; Sever 1989); however, cirri length and head width were not correlated with testis or vas deferens size in *E. cirrigera*. Both testis width and vas deferens width were significantly correlated with head width in *E. aquatica*. Therefore, the available evidence supports our hypothesis that the two species, despite being sympatric and closely related, exhibit several reproductive distinctions that may be associated with different male

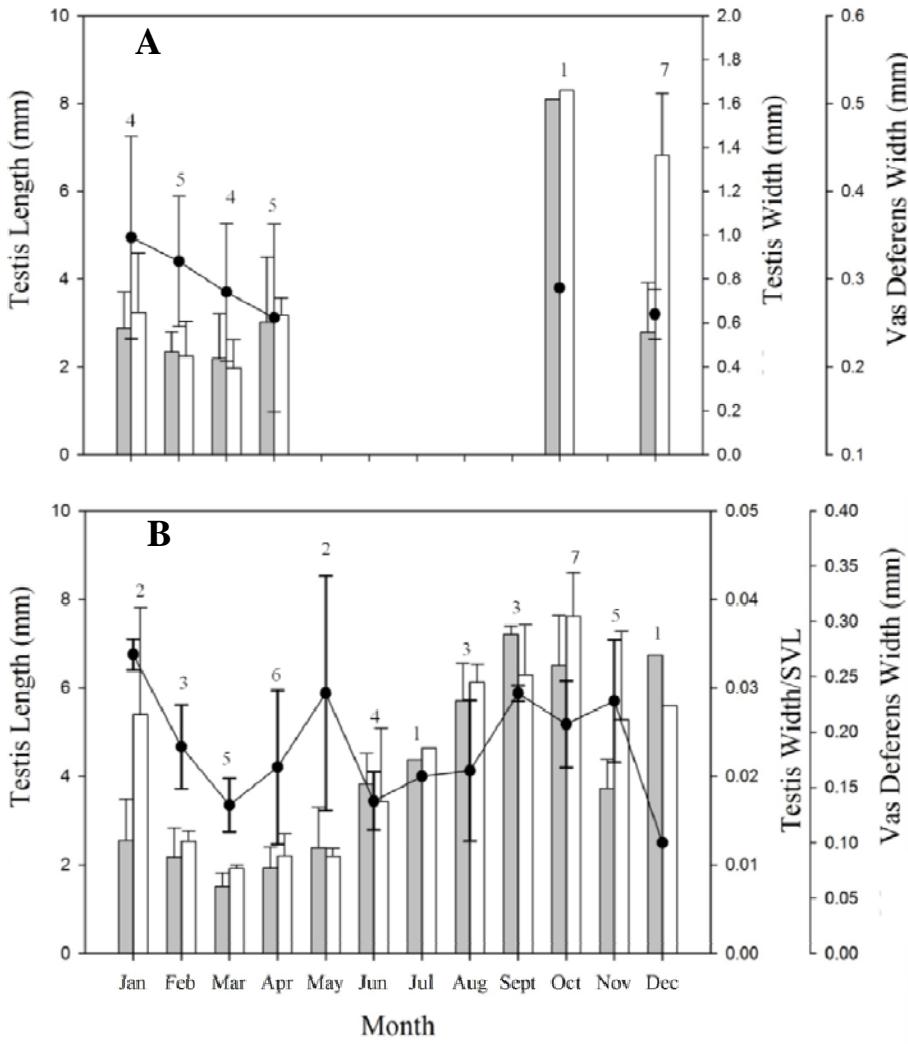


FIGURE 2. A) Monthly variation in reproductive anatomy of male *Eurycea aquatica*. Grey bars represent mean testis length, open bars represent mean testis width, and line plot indicates mean width of vas deferens. Sample sizes and standard error bars for each month are shown. Gaps are months for which no specimens were available (see text). B) Monthly variation in reproductive anatomy in males of *Eurycea cirrigera*. Symbols are the same as in A, except the sample size for vas deferens width in August and September is two rather than three due to inability to view and measure vas deferens in two specimens. Small numerals within graphs indicate monthly sample sizes.

mating tactics.

The seasonal pattern of testis and vas deferens size in *E. cirrigera* is consistent with numerous studies describing the male reproductive pattern of eastern U.S. plethodontids (Petránka 1998). Our results show that maximum testis length and width in *E. cirrigera* occurs during October–September, presumably coincident with peak spermatogenesis (Rastogi et al. 2005), and steadily declines through winter and spring. Width of vas deferens generally tracked testis size, presumably as spermatozoa were evacuated from the testis and into the vas deferens (Jensen et al. 2002; Rastogi et al. 2005).

Mean cirri length varied significantly in *E. cirrigera*, with maximum cirri length (November) occurring immediately following maximum testis size, and a secondary peak of cirri development occurring in February. However, our small sample size from December–January ($n = 3$) does not eliminate the possibility that peak cirri length may continue through these months as well. Testosterone is required for cirri development (Sever 1975; Schubert et al. 2006), and in *E. cirrigera*, this is probably activated by the peak of presumed testis activity (September–October) that occurs immediately prior to cirri elongation. The timing of

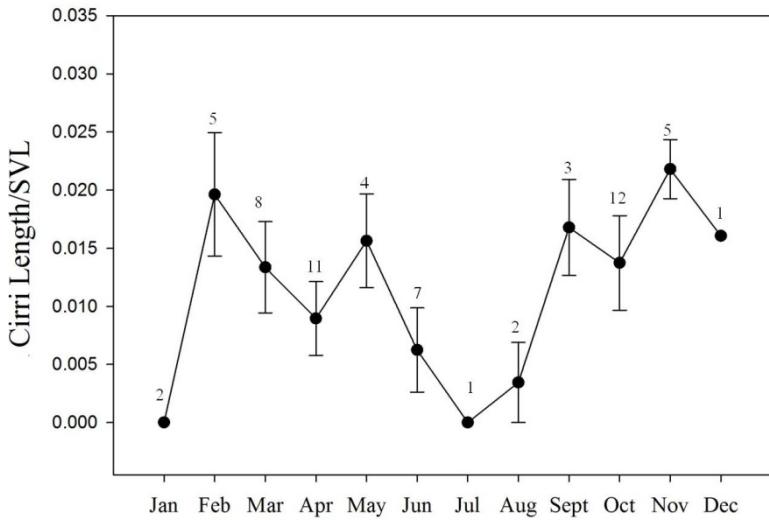


FIGURE 3. Monthly variation in length of cirri, standardized for SVL, in males of *Eurycea cirrigera*. Dots represent means and vertical lines represent standard errors for each month. Small numerals within the graph indicate monthly sample sizes.

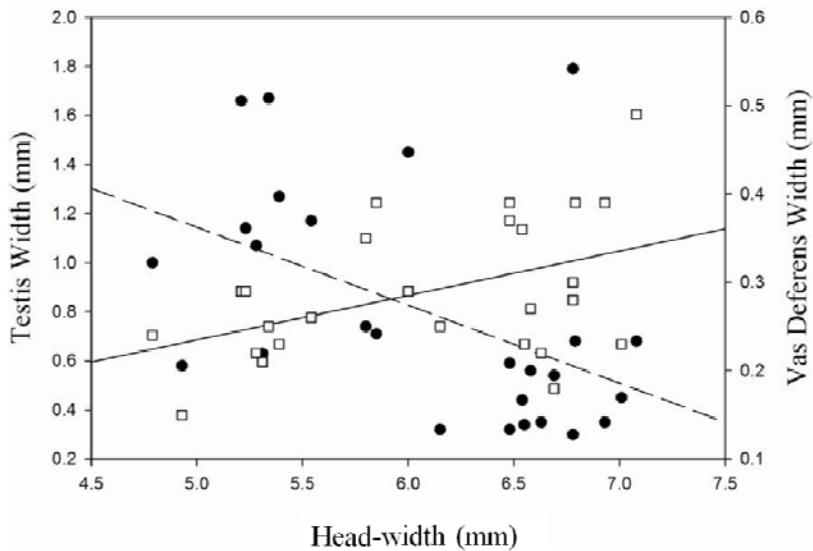


FIGURE 4. Correlation between head width and width of testis (open squares and solid regression line) and correlation between head width and width of vas deferens in males of *E. aquatica* (solid dots and dashed regression line).

maximum cirri length and vas deferens size likely corresponds with peak courtship and mating in this species, although the precise timing of these events in the region is unknown (Mount 1975; Petranka 1998; Sever 1999). These results are similar to those describing the reproductive cycle of *E. bislineata* (Weichert 1945; Stewart 1958), which reportedly has two mating seasons associated with spermiogenesis, vas deferens enlargement, presence of spermatozoa in female spermathecae, mental gland development

(Weichert 1945), and premaxillary tooth development (Stewart 1958). The ovarian cycle of female *E. cirrigera* provides a complement to the male cycle: yolked eggs are found in the fall up to the single early spring nesting period (Petranka 1998; Sever 1999), and all females from late spring through summer lack yolked eggs.

Unlike *E. cirrigera*, which appears to be more common than *E. aquatica* and can be collected at any time of the year, *E. aquatica* becomes difficult to collect during summer and fall (Graham et al. 2010). For this

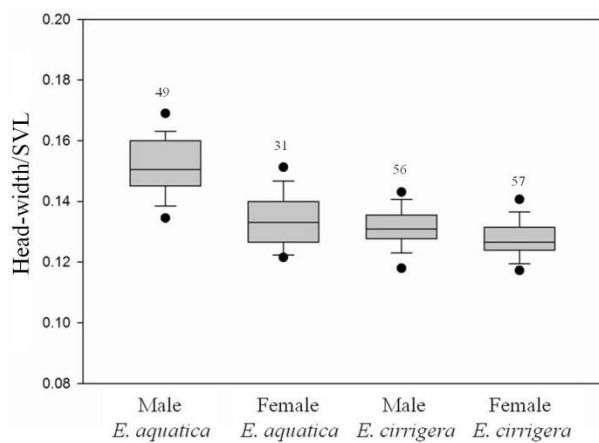


FIGURE 5. Head width, corrected for SVL, in both sexes of *E. aquatica* and *E. cirrigera*. The horizontal line within the innerbox indicates the median, outer box represents the 25th and 75th percentiles, error bars represent the 10th and 90th percentiles, and dots indicate the 5th and 95th percentile. Small numerals within the graph indicate sample sizes.

reason, we could not thoroughly analyze seasonal reproduction of this species.

The difficulty in locating *E. aquatica* adults was previously mentioned by Rose (1971), and is also a characteristic of *E. aquatica*'s sister taxon *E. junaluska*, adults of which are typically difficult to find and are usually encountered only on rainy nights crossing roads (Sever et al. 1976; Dodd 2004). This may indicate that behavioral characteristics are shared by *E. junaluska* and *E. aquatica* (e.g., migrating to and from breeding sites from unknown terrestrial retreats), but not by other members of the *E. bislineata* complex.

Climatic factors can influence salamander life histories (Camp and Marshall 2000), and springs, such as those inhabited by *E. aquatica*, exhibit far less seasonal variation in temperature and other physical factors than other aquatic habitats (Jones 1980; Hubbs 1995), such as those occupied by *E. cirrigera*. Despite this, within the sample of *E. aquatica* we analyzed, there appeared to be seasonal variation in testis width and ovarian development. In general, the pattern observed in *E. aquatica* was similar to that observed in *E. cirrigera*; mean testis width reached a maximum in December, and head size varied seasonally. However, nest dates suggest a much earlier oviposition and brooding period for *E. aquatica* (February–mid March) relative to *E. cirrigera* (April) in Alabama and nearby areas (Mount 1975; Niemiller and Miller 2007; Graham et al. 2010). This pattern is similar to that documented for cave-nesting *E. cirrigera* populations in Tennessee (Niemiller and Miller 2007). The relatively constant water temperature of large limestone springs (Hubbs 1995) appears to

enable *E. aquatica* to breed much earlier than *E. cirrigera*. Unfortunately, evidence for the exact timing and location (e.g., terrestrial or aquatic) of mating is scarce for both *E. aquatica* and *E. cirrigera* (Petránka 1998; Sever 1999; Brophy and Pauley 2002), so a thorough picture of the mating system of these species awaits further study. However, besides subtle differences in timing of certain events (e.g., nesting), the gametogenic cycles appear similar between *E. aquatica* and *E. cirrigera*.

Despite these similarities, we found differences between the species in the way male primary reproductive parameters are associated with secondary sexual characteristics. We predicted that seasonal cycles of either testicular or vas deferens recrudescence would covary with cirri elongation (in *E. cirrigera*) and head width (in *E. aquatica*), consistent with studies that have demonstrated a link between testicular activity (e.g., testosterone secretion) and development of these structures (Sever 1975; Schubert et al. 2006). Interestingly, in *E. cirrigera*, no obvious association exists between measurements of testis or vas deferens anatomy and mean cirri length. This possibly reflects a time delay between maximum androgen secretion and cirri elongation, or a delay in the androgen sensitivity of cirri tissue. On the other hand, we confirm that head width in *E. aquatica* is sexually dimorphic and appears to be correlated with testis and vas deferens width. Head width also varies seasonally in males of *E. cirrigera*, but does not differ significantly between males and females, and does not correlate with testis parameters. Average testis length and vas deferens width was significantly larger in males of *E. aquatica* relative to *E. cirrigera*. In many vertebrate species, testis size and/or size of seminal support structures (e.g., vas deferens, epididymis) relative to body size can reflect the degree of competition for access to mates and sperm competition (Birkhead and Møller 1998).

These results provide support for the hypothesis that the secondary sexual characteristics exhibited by the *E. bislineata* complex reflect adaptations associated with different male sexual strategies (Kozak 2003). Mate competition between males typically results in an increase in sperm competition (Parker 1970; Harcourt et al. 1981; Birkhead and Hunter 1990) and intrasexual aggression (Emlen and Oring 1977), and these changes are usually reflected in increased development of testis size and secondary sexual characteristics (e.g., enlarged head size for aggressive competition for females: Bakkegard and Guyer 2004; larger testis size for increased sperm competition: Birkhead and Møller 1998). Therefore, larger relative testis size, larger relative head size, and the association between testis size and head size exhibited by *E. aquatica* may be associated with increased competition for access to mates, in contrast to the pattern displayed by *E.*

cirrigera. However, a thorough characterization of behavioral patterns in these members of the *E. bislineata* complex awaits further study.

Future studies should combine information on the reproductive anatomy, secondary sexual characteristics, and field observations of courtship and agonistic behaviors to elucidate the adaptive significance of the morphological and ecological variation displayed by these salamanders. These studies will benefit from the ability to place this information in a robust phylogenetic framework (Jacobs 1987; Kozak et al. 2006; Timpe et al. 2009), which will ultimately determine the evolutionary significance of these patterns.

Acknowledgments.—We would like to thank Roger Birkhead, Shannon Hoss, and Megan Loraas for assistance in collecting salamanders, and Dean Adams for statistical advice. We also thank the Guyer lab (Auburn University) and Bronikowski lab (Iowa State University) for helpful suggestions and criticisms on earlier versions of this manuscript. We have complied with all applicable institutional animal care guidelines (IACUC # 2007-1207), and all state and federal permits were obtained. Salamanders were collected under Alabama Department of Conservation and Natural Resources scientific collecting permit # 4268. Daniel Drennen (US Fish and Wildlife Service) assisted in locating collecting sites, and we appreciate the permission of landowners, especially the Sanders family, for access to their properties. Sean Graham and Michael Alcorn were supported by NIH grant # R01-A149724 to Tom Unnasch. We dedicate this paper and our sincerest sympathies to the Sanders family, whose lives and property (Sander's Spring) were devastated by a catastrophic tornado in May 2011.

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ELIZABETH K. TIMPE is a Ph.D. student at the University of Connecticut, working on phylogenetic aspects of salamanders. Her phylogenetic assessment of *Eurycea aquatica* versus *E. cirrigera* and other contributions to this project made this paper possible. (Photographed by Sean Graham)



MICHAEL A. ALCORN produced a great deal of the data analyzed in the manuscript, as part of his undergraduate Honor's Thesis research at Auburn University under the cruel tutelage of Sean Graham, Jennifer Deitloff, and Craig Guyer. He is now a Ph.D. student at the University of Chicago, and his current interests include biomechanics, functional morphology, and locomotion, especially in amphibians. (Photographed by Sean Graham)



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Graham et al.—Seasonal Changes in Sexual Characteristics in *Eurycea*.

Appendix. Summary statistics for characteristics that were measured from males and females of *Eurycea aquatica* and *E. cirrigera*. Sample size is represented by “n” and standard deviation is represented by “SD.”

	n	Mean	SD	Range
<i>Male E. aquatica</i>				
Snout-vent-length (mm)	50	40.55	3.31	33.78–47.82
Head width (mm)	50	6.16	0.72	4.79–7.70
Testis length (mm)	26	2.87	1.46	1.53–8.09
Testis width (mm)	26	0.80	0.46	0.30–1.79
Vas deferens width (mm)	26	0.29	0.08	0.15–0.49
<i>Male E. cirrigera</i>				
Snout-vent-length (mm)	62	38.75	3.62	28.19–45.33
Cirri length (mm)	62	0.44	0.37	0.00–1.21
Head width (mm)	62	5.07	0.43	3.69–5.88
Testis length (mm)	42	3.94	2.18	1.09–8.91
Testis width (mm)	42	0.86	0.46	0.30–1.70
Vas deferens width (mm)	41	0.19	0.059	0.05–0.31
<i>Female E. aquatica</i>				
Snout-vent-length (mm)	31	40.94	3.71	32.68–49.79
Eggs (number)	26	16.88	29.63	0–91
<i>Female E. cirrigera</i>				
Snout-vent-length (mm)	65	38.42	4.73	22.14–46.72
Eggs (number)	61	17.59	30.74	0–102