# Sexual Dimorphism in the Three-toed *Amphiuma*, *Amphiuma tridactylum*: Sexual Selection or Ecological Causes?

## Clifford L. Fontenot, Jr.[1] and Richard A. Seigel[2]

**Sexual dimorphism is widespread among vertebrates, and may be attributable to sexual selection, differences in ecology between the sexes, or both. The large aquatic salamander, *Amphiuma tridactylum*, has been suggested to have male biased sexual dimorphism that is attributable to male–male combat, although detailed evidence is lacking. To test this, data were collected on *A. tridactylum* head and body size, as well as on bite-marks inflicted by conspecifics. *Amphiuma tridactylum* is sexually dimorphic in several characters. There was no sex difference in body length, but males had heavier bodies than females of the same body length. Larger males had wider and longer heads than larger females, but whether any of these sexually dimorphic characters are attributable to differences in diet is unknown because diet data (by sex) are lacking. There was no difference in the number of bite-marks between males and females, and juveniles also possessed bite-marks, suggesting that the biting is not necessarily related to courtship or other reproductive activities. In addition, fresh bite-marks were present on individuals during months well outside of the breeding season. Biting was observed in the field and lab to occur by both sexes on both sexes, during feeding-frenzy type foraging. Thus, biting is likely related to foraging rather than to courtship. The sexually dimorphic characters remain unexplained, pending sex-specific diet data, but there is no evidence that they are related to male–male combat or to courtship.**

SEXUAL dimorphism is widespread among vertebrates, and may be attributable to sexual selection (Darwin, 1874; Vitt and Cooper, 1985; Shine, 1988), differences in ecology between the sexes (Shine, 1989; Anderson and Vitt, 1990), or both (Sandercock, 2001; Pearson et al., 2002). Cases of sexual selection are well documented and are often attributed to the sexual difference in reproductive roles, including courtship (Hedrick and Temeles, 1989). Ecological causes can include sexual differences in factors such as habitat use, predation (Reimchen and Nosil, 2004), and diet. However, unambiguous evidence of ecological causes is difficult to produce and often involves focusing on the relationship between the mouthparts and food item size or shape (Temeles et al., 2000). Because sexual selection is often more testable and parsimonious, it is often a more easily accepted explanation for observed sexual dimorphism (Shine, 1989).

Among amphibians, females are often larger than males, presumably due to fecundity selection; males, however, are more often larger than females when the males engage in male–male combat, particularly in larger species (Shine, 1979; but see Halliday and Verrell, 1986). The large aquatic salamander *Amphiuma tridactylum* is listed by Shine (1979) as having male biased sexual size dimorphism as well as male–male combat, citing Cagle (1948). However, inspection of Cagle's paper (1948) reveals no indication of sexual dimorphism. In fact, Cagle (1948:482) indicates that body size distributions are similar among sexes. Cagle (1948) did indicate that males collected during the breeding season (which he considered January through April) had numerous scars from bites and some had deep lacerations up to 2 mm in depth. One individual had 20 bite-marks, and five possessed deep lacerations. The descriptions seemed to infer that the bite-marks were the result of male–male combat, but Cagle did not state that combat had been observed, whether females had been checked for bite-marks, nor whether females may have inflicted bite-marks onto males.

Male–male combat usually consists of some sort of wrestling match and generally does not result in injury (Shine, 1979). The implied aggression for *A. tridactylum* is in conflict with the only direct observation of presumed courtship, which did not describe male–male combat or any other form of aggression (Baker et al., 1947). The account describes two females nudging a male on each of several consecutive nights in July. The male was said to choose between the two females, the unchosen female left the area, and the remaining pair attempted copulation but were interrupted by a passing motorboat. ''The pair returned the following evening and moved into a position so that the cloacas were in contact . . . Early the next morning a cloacal smear was made from the female and motile spermatozoa were found'' (Baker et al., 1947:88). In an earlier study of mating, Baker (1937:212) reported no ''no rubbing motions nor other obvious courtship procedure and no definite positions assumed by male and female in their burrows''.

Regarding the Baker et al. (1947) report, the July copulation report is not necessarily at odds with the January–April reproductive season in Louisiana (Wilson, 1941, 1942; Cagle, 1948; Sever et al., 1996), as the timing of reproductive cycles has not been studied in *A. tridactylum* from Tennessee. However, there was no indication that the female's cloaca was checked for spermatozoa prior to their observation, which is important given that females can store sperm for months (Kreeger, 1942; Sever et al., 1996). Aside

[1] Department of Biological Sciences, Southeastern Louisiana University, Hammond, Louisiana 70402; E-mail: cfontenot@selu.edu. Send reprint requests to this address.
[2] Department of Biological Sciences, Towson University, Towson, Maryland 21252; E-mail: rseigel@towson.edu.

from Baker et al. (1947), there are no other observations of courtship and mating in *Amphiuma*. Cagle (1948) reported that *A. tridactylum* were ''unusually active'' following heavy rain, but his observations were not conclusively of copulation or courtship. Specific attempts to observe courtship in *Amphiuma* usually involved construction of a semi-natural enclosure, but have been unsuccessful, (Baker, 1937; Sever et al., 1996; Fontenot, 1999).

To determine whether sexual size dimorphism exists in *A. tridactylum*, we collected data on body size (SVL), head width, head length, tail length, and body mass on animals from southern Louisiana. The number and severity of bite-marks were also collected to address the issue of male–male combat indirectly and test the following predictions. If bite-marks are associated with courtship, they should only be inflicted on adults during the breeding/courtship season. If bite-marks are the result of courtship aggression by males, only adult females should possess them. If bite-marks are the result of male–male combat, only adult males should possess them.

## MATERIALS AND METHODS

*Amphiuma tridactylum* were collected in Evangeline and Lafayette Parishes, Louisiana using methods described by Fontenot (1999). Sex and juvenile status (no gonad development) were confirmed by dissection. This also confirmed that individuals with a total length of < 33 cm (=25 cm snout–vent length (SVL)) were indeed juveniles (Cagle, 1948), which were not used in tests for sex differences. Juveniles were, however, included in tests involving bite-marks because this would allow us to distinguish a courtship related activity. Each animal was weighed to the nearest 0.1 g on a digital top-loading balance, head width and head length measured to the nearest 0.1 mm with digital calipers, SVL and total length measured to the nearest mm with a tape measure, and the number and condition of bite-marks recorded. Bite-marks were considered those of *A. tridactylum* because *Amphiuma* possess two unique rows of teeth on the upper jaw, one (vomeropalatine) within the other (premaxillary), plus a single row of mandibular teeth, and the bite-marks bear close resemblance to these; *A. tridactylum* was the only *Amphiuma* species occurring at the collecting site. The age of bite-marks could not be determined with the exception of those that were through the dermis and into underlying tissue. Because such cuts on captive animals healed completely in 3–4 weeks and left only a scar and depression in the skin, cuts that were apparently fresh with no sign of healing were assumed to have occurred within a week.

Because most individuals were collected between January and May and since samples from other months would best determine whether fresh bite-marks occurred outside the courtship/breeding season, our field data set was supplemented with bite-mark data from 48 (23 male, 13 female, 12 juvenile) preserved specimens (see Material Examined).

Body size variables were log transformed to meet assumptions of parametric tests. Sex difference in SVL was tested by ANOVA, with sex as the independent variable and SVL as the dependent variable. Because there was no significant difference, data were pooled for remaining analyses. To account for the affect of SVL on other morphometric variables, SVL was used as the covariate in a series of three ANCOVAs, with SVL*sex as a second independent variable

to test for differences in slope. Head width, head length, and mass were each used as a single dependent variable in the four separate tests. When there was no difference in slope, the interaction term (SVL*sex) was dropped from the model and the analysis re-run. Sex difference in the presence of bite-marks was tested by ANOVA, with sex as the independent variable and number of bite-marks as the dependent variable. The affect of body size on number of bite-marks was tested by regression analysis with SVL as the independent variable and bite-marks as the dependent variable. Means are given with $\pm$ 1 SE.

## RESULTS

A total of 152 *A. tridactylum* (80 males, 59 females, and 13 juveniles) was collected and used in morphometric analyses. Males (mean SVL = 55.5 $\pm$ 1.1 cm, range = 21–81 cm) were very similar in body size to females (mean SVL = 54.5 $\pm$ 1.3 cm, range = 25–82 cm). An ANOVA showed no statistical difference in SVL ($F_{1,137}$ = 0.34, $P$ = 0.559) between sexes, and in all of the subsequent ANCOVA models, most of the variation was explained by SVL.

Mean body mass was 495.1 $\pm$ 30.4 g for males and 404.5 $\pm$ 28.6 g for females. Initial ANCOVA for sex difference in body mass showed no significant difference in slope ($F_{1,132}$ = 0.16, $P$ = 0.687) and this was dropped from the model. Subsequent ANCOVA showed a highly significant SVL effect ($F_{1,133}$ = 1433.06, $P$ < 0.0001), and that males had significantly heavier bodies than females ($F_{1,133}$ = 13.11, $P$ = 0.0004, $R^2$ = 0.92). Pooled sex data had a slope of y = $-7.37$ + 3.33 log SVL, $R^2$ = 0.91.

Mean head width was 30.0 $\pm$ 0.07 mm for males and 28.1 $\pm$ 0.08 mm for females. Initial ANCOVA for sex difference in head width showed no significant difference in slope ($F_{1,132}$ = 2.56, $P$ = 0.112) and this was dropped from the model. Subsequent ANCOVA showed a highly significant SVL effect ($F_{1,133}$ = 1346.46, $P$ < 0.0001), and that males had significantly wider heads than females ($F_{1,133}$ = 11.75, $P$ = 0.0008, $R^2$ = 0.91). Pooled sex data had a slope of y = $-3.47$ + 1.13 log SVL, $R^2$ = 0.90.

Mean head length was 41.2 $\pm$ 0.09 mm for males and 38.4 $\pm$ 0.09 mm for females. Initial ANCOVA for sex difference in head length also showed no significant difference in slope ($F_{1,132}$ = 2.15, $P$ = 0.144) and this was dropped from the model. Subsequent ANCOVA showed a highly significant SVL effect ($F_{1,133}$ = 1263.73, $P$ < 0.0001), and that males had significantly longer heads than females ($F_{1,133}$ = 16.32, $P$ = 0.0009, $R^2$ = 0.91). Pooled sex data had a slope of y = $-2.74$ + 1.02 log SVL, $R^2$ = 0.90.

Bite-marks were found on 88% of males, 81% of females, and 44% of juveniles. These marks were usually visible as white dots on the brown pigmented skin and matched the *A. tridactylum* tooth pattern. Some bite-marks showed the tooth pattern clearly but occurred as a scrape, usually from dorsal to ventral. This condition resulted from twisting by the individual being bitten so that the biter's teeth were dragged across the skin, which we observed in the field and in captivity. Bite-marks on an individual were not necessarily similar in size to its own jaw size, i.e., medium to large individuals often had bites of all sizes, including some that would match a juvenile jaw size. However, no juveniles had large bite-marks.

While some individuals had no bite-marks, some were covered from head to tail. Two adult individuals were essentially covered with bite-marks, and were statistically

identified as outliers with large leverage. Although these exceptional observations were valid, the appropriate course of action is to either incorporate a variable that reflects the extraordinary situation into the model, or delete the outlier data points (Hair et al., 2005). Because the source of influence was unknown, the two outliers were removed and the analysis re-run. There was no difference between adult males and females in number of bite-marks ($F_{1,81}$ = 0.056, $P$ = 0.813). That juveniles had roughly half as many bite-marks of adults is likely because the number of bite-marks was significantly related to SVL ($F_{1,37}$ = 6.79, $P$ = 0.013). Also, the age of an old bite-mark/scar could not be determined, so scars may have been months old. Thus, the scar data are of little use in determining when biting occurs. However, fresh bite-marks likely occurred within a few days before being collected.

In the course of collecting *A. tridactylum* in the field, and in animals kept in the lab before processing we often observed individuals biting each other, apparently indiscriminately, while attempting to capture food. On several occasions, minutes after placing small pieces of beef into an aquarium (200 l) that housed six *A. tridactylum*, all six of the animals (three males, three females) seemed to be in a ''feeding frenzy,'' very alert and moving their heads side to side attempting to locate and ingest the meat, but also biting anything that brushed against them. In many cases animals endured deep lacerations, especially when two animals attempted to swallow the same piece of food. These were especially severe when the biter had a solid bite and began to roll its body (in crocodile fashion) in attempt to tear a piece of meat off. In these laboratory observations, all sex combinations of biting occurred, i.e., male–male, male–female, and female–male. We also observed a similar scenario in the field where individuals were feeding on a fish carcass, but we were not able to collect them to determine sex.

## DISCUSSION

Statistical analyses showed that large male *A. tridactylum* had significantly wider and longer heads than large females. While there was no sex difference in body length (SVL), males were significantly heavier-bodied than similar females of the same length. Sex differences in such morphological characters are often attributable to sex differences in diet or reproductive roles (Shine, 1989). *Amphiuma tridactylum* seem to eat any animal they can capture or scavenge including crayfish, earthworms, insect larvae, mole crickets, fish, grasshoppers, giant water bugs, ground skinks, spiders, snails (Chaney, 1951), and even small common snapping turtles (Fontenot and Fontenot, 1989). Chaney (1951) suggested that diet was influenced more by locality than by season or size, but did not compare diet among sexes. Thus, whether there are sexual differences in diet or prey size remains unknown.

Our data failed to confirm the prediction that if bite-marks are associated with courtship, they should only be inflicted on adults during the breeding/courtship season. The fact that bite-marks occurred on males, females, and juveniles suggests that biting is not restricted to reproduction. This idea is further supported by the lack of difference in the number of bite-marks between males and females; if biting was part of male–male combat, then only males should have bite-marks. In addition, courtship and mating are believed to occur January through April (Wilson, 1941,

1942; Cagle, 1948; Sever et al., 1996), but fresh bite-marks were present on animals collected in February, March, April, May, September, and November, clearly indicating that bites are not restricted to reproductive activities.

We suggest that the bite-marks are most likely an artifact of feeding. Our observations of *A. tridactylum* in the field and lab biting each other, apparently indiscriminately while attempting to capture food, strongly suggest that biting is random with respect to sex. This also explains why juveniles also possessed bite-marks, roughly half that of adults. Because the number of bite-marks was significantly related to body size (SVL), and body size presumably increases with age, non-lethal bite-marks are to some extent cumulative, depending on how long the marks/scars remain visible. Thus, juveniles have not lived as long as adults and have had less time to accumulate bite-marks. A larger body size also increases the target size, and likelihood of a bite occurring. In addition, bites by a large adult onto a small juvenile could easily be lethal, and cannibalism has been reported (Cagle, 1948). Another potential explanation consistent with the observed pattern of bite-marks is that it is related to territoriality; however, there is no published evidence of territoriality in *Amphiuma*. Territoriality and aggression are well documented in the Plethodontidae (Jaeger and Forester, 1993) but apparently much less common among other families of salamanders (Mathis et al., 1995; Houck and Arnold, 2003). Among fully aquatic salamanders (Sirenidae, Cryptobranchidae, Amphiumidae, Proteidae), evidence of biting exists only for *A. tridactylum* (Cagle, 1948; this study), *A. means* (C. Fontenot, unpubl. data), and *Siren intermedia* (Godley, 1983). Interestingly, whether biting in *S. intermedia* is a courtship or agonistic behavior remains unclear, but is not solely associated with courtship (Fauth and Resetarits, 1999).

None of our three predictions about the relationship between bite-marks and courtship aggression was supported. Given the bite-mark data, biting observations, and the lack of any aggression observed in courtship (Baker et al., 1947), we conclude that biting is associated with feeding ecology rather than reproduction and courtship aggression. Accordingly, the male biased sexually dimorphic characters cannot be attributable to male–male combat due to lack of evidence. However, ecological causes, particularly diet and population density, remain to be tested. Future studies should also focus on differences in prey size rather than just prey type, as variation in prey availability among sites may be confounding.

## MATERIAL EXAMINED

LSUMZ specimen numbers 642, 643, 644, 648, 4158, 4159, 4160, 4161, 4162, 4163, 4167, 4168, 4169, 4170, 4172, 4999, 5000, 5001, 6073, 6836, 6837, 6841, 6842, 6844, 6845, 6846, 6847, 6848, 6849, 6850, 6851, 13554, 17683, 17717, 47537, 53337, 53338, 57058, 57607, 57751, 60931, 60932, 60933, 60937, 60942, 60947, 60948, 60949, 60951, 60954, 60956, 60973, 60974, 60975, 60976, 70387, 70388, 70389, 70390, 73319, 74865, 85688, 85694.

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