

HOMING AND EGG DISCRIMINATION IN THE WESTERN SLIMY SALAMANDER, *PLETHODON ALBAGULA* (CAUDATA: PLETHODONTIDAE)

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Abstract.— In some species of vertebrates, egg brooding is a costly form of parental care. Therefore, misdirection of parental care can significantly lower a female's fitness. Because of the maternal investment and increased survivorship to offspring from egg guarding, a brooding female should home to her nest site after being displaced a short distance and discriminate between her own eggs and eggs from other females. In this study, we experimentally tested, in the field, alternative hypotheses concerning homing ability and egg discrimination in a population of nesting Western Slimy Salamanders (*Plethodon albagula*). Experimental results demonstrated that displaced brooding females would return to their nest but can only identify their egg clutch based on its location in that original nesting site.

Key Words. Egg Discrimination; Homing; Parental Care; *Plethodon albagula*; Western Slimy Salamander

INTRODUCTION

Parental investment theory predicts that parental care can be a significant investment for an individual (Trivers 1972). Parental care, which is any form of parental behavior that may increase the fitness of the parent's offspring, includes nest preparation, egg production, and supervision of eggs or young (Clutton-Brock 1991). Although parental investment improves offspring survivorship, it can also impact the parent's ability to produce future offspring (Trivers 1972). In some species of vertebrates, egg brooding is a costly form of parental care (Ng and Wilbur 1995).

Female terrestrial plethodontid salamanders oviposit clutches in moist, protected terrestrial sites where they brood the eggs for several months (Nussbaum 1985). Egg brooding increases egg survival by reducing predation, yolk layering, fungal infection, and desiccation (Highton and Savage 1961, Snyder 1971, Forester 1984). Egg guarding, however, can be a costly investment for females. Females may suffer metabolic costs, as well as injury or death, when defending egg clutches from predators (Jaeger and Forester 1993). Female Allegheny Mountain Dusky Salamander (*Desmognathus ochrophaeus*) apparently fast during the brooding pe-

riod (Forester 1981); whereas, the Eastern Red-backed Salamander (*Plethodon cinereus*) feeds opportunistically throughout incubation (Ng and Wilbur 1995). Energy consumption by a brooding female while defending egg clutches may reduce her ability to reproduce the following year (Jaeger and Forester 1993). Therefore, misdirection of parental care can significantly lower a female's fitness (Waldman 1988). This costly misdirection of parental care is avoidable if a female can home to her nest site after being displaced a short distance (Trivers 1972).

Homing behavior includes any movement to a spatially restricted area that is known to the animal (Papi 1992). This includes incidences of homing from short distances after displacement from a nest site and long-term nest site fidelity year after year. Homing to specific sites for reproduction, nutrition, shelter, and hibernation occurs in adults of at least 50 species of urodeles and anurans (Sinsch 1992). Homing in plethodontids is known from direct observation (Madison 1969) and experimental displacement (Barthalmus and Bellis 1972). The non-migratory Northern Dusky Salamander (*Desmognathus fuscus*) returned to its original capture sector after translocations to upstream, downstream, and non-stream locations (Barthalmus and Bellis 1972). Many species of Pletho-

dontidae are known to return to capture sites (Madison 1969, Kleeberger and Werner 1982, Jaeger et al. 1993) including nest sites (Snyder 1971, Forester 1979, Peterson 2000) upon removal.

Different cues or sensory mechanisms are used for homing (Sinsch 1992). Olfactory/chemosensory responses appear to be the major mechanism for *D. fuscus* and the Red-cheeked Salamander (*Plethodon jordani*) (Madison 1969, 1972, Barthalmus and Bellis 1972), but not in *P. cinereus* (Jaeger et al. 1993). The sensory mechanism required for clutch recognition are important aspects of homing in plethodontids, allowing females to distinguish their eggs from unattended clutches (Forester 1979). However, female plethodontids may brood more developmentally-advanced clutches of conspecifics instead of their own. Olfaction was essential for clutch recognition by *D. ochrophaeus*, but is supported by visual and tactile reinforcement (Forester 1979). Displaced brooding female *P. cinereus* also recognized their nest site location by its structure and habitat features (landmarks) (Peterson 2000). Species whose egg clutches are highly susceptible to displacement from the nesting site, such as *D. ochrophaeus*, are under higher selective pressure to evolve clutch and egg discrimination and nest site recognition (Forester 1977, 1979). Terrestrial plethodontids such as *Plethodon albagula* undergo direct development in a terrestrial setting guarded by brooding females (Pough et al. 1998, Trauth et al. 2004, Trauth et al. 2006) and thus have a lower chance for clutch displacement.

Behavioral studies involving homing and egg determination in plethodontids have typically focused on members of *Desmognathus* and smaller bodied *Plethodon*, chiefly *P. cinereus*. Despite their abundance and diversity within woodlands (Burton and Likens 1975a, Hocking and Babbitt 2014, Semlitsch et al. 2014, Milanovich and Peterman 2016), importance to forest ecosystems (Davic and Welsh 2004), and differences in life history traits from smaller bodied *Plethodon*, little information exists about homing and egg discrimination in larger bodied *Plethodon* (see Mathis 1995 for review), especially those of the widespread Slimy Salamander species complex. The purpose of this study was to test alternative hypotheses concerning homing ability and egg discrimination in an aggregation of nesting females of *P. albagula*, to fill in missing gaps in our understanding of parental investment in this species. We hypothesized that female *P. albagula* can recognize their eggs and home to their nests when relocated. We predicted that control females would remain at their own nest sites, and displaced females would return (or home) to their own nest sites, and that females will be able to discern between their eggs and those of other individuals.

MATERIALS AND METHODS

Plethodon albagula was the subject of this study. It is a large woodland salamander found in the Interior Highlands of Arkansas, Missouri and Oklahoma, and parts of Texas (Highton et al. 1989, Conant and Collins 1998, Trauth et al. 2004). Female *P. albagula* brood their eggs cryptically under rocks, logs, or in underground nesting chambers where brooding behavior cannot normally be directly observed. However, a unique opportunity to observe brooding females exists at an abandoned mine shaft in Garland County, Arkansas (Trauth et al. 2004, Trauth et al. 2006, Ford 2008). The shaft is a straight tunnel, approximately 2 m high and 1.5 m wide, extending approximately 149 m horizontally into a hillside, and

was excavated in 1880 and abandoned by 1890. Female *P. albagula* migrate into the mine during late summer (July-August), oviposit in August-October, and brood their eggs until hatching in December. Females and young often overwinter in the mine, gradually dispersing into the environment December - January. Females lay their clutches in depressions and cavities in the shaft wall formed when the mine was excavated. Most of the nest sites are readily visible and accessible to humans. Because females can be observed without disturbance before, after and throughout the brooding period, this site provides a unique opportunity for studying the reproductive ecology of *P. albagula*.

Experiment 1d (Homing): During the first year of the field study (16-17 November 2001), we tested the homing ability of females with a manipulative experiment following methods of Peterson (2000). We randomly selected 28 females and randomly assigned them to a control ($N = 14$) and experimental group ($N = 14$). Control animals were removed from, and then returned to, their egg clutch to control for clutch abandonment resulting from handling and/or nest disturbance (Fig. 1). Experimental females were randomly displaced 1 m to the left or right of the nest site. We returned to each nest site 24 hours later to determine if females returned or abandoned their nests. Logistic regression was used to test significance ($\alpha = 0.05$) between the experimental and control responses.

Experiment 2 (Egg Recognition): In year two, (2-3 November 2002) we tested for brooding females' ability to recognize their own eggs following Peterson (2000). We randomly selected 39 females for use in the experiment. They were assigned to a control group ($N = 13$), experimental groups ($N = 13$), or for use as an egg donor. The control animals were removed from their nests and released on the floor directly below their nests (Fig. 1). Their clutches were removed from their egg stalks and immediately returned to the ledge of the original nest site. Next, 13 females were assigned to the experimental group and another 13 were used as egg clutch donors. Females in these two groups were selected to ensure that the clutches of egg donors and experimental females had similar sized egg clutches (± 2 eggs), egg diameters (± 0.64 mm), and developmental stages to ensure that females were keying in on their offspring rather than confounding clutch characteristics.

Both the experimental and donor females were removed from their nest sites and placed in Gladware® housing chambers lined with damp filter paper. Then, we removed their clutches from the egg stalks. The egg clutch of the experimental female was placed 1 m to the right or left of the nesting site (chosen randomly). Then, the clutch from the donor female was placed on the ledge at the experimental female's nest site. The donor female was held in captivity for the duration of each trial (24 hours) to prevent female-female aggression from confounding results. Now, the test female was released to the shaft floor at an equal distance from both egg clutches.

After 24 hours, we examined each nest site and recorded whether females were present or absent at their clutch or the nest site, and whether nest predation had occurred. After the experiment, we returned the clutches to the ledge of their respective nests and released the donor females back at their original nest sites. Logistic regression was used to test significance ($\alpha = 0.05$) between treatments.

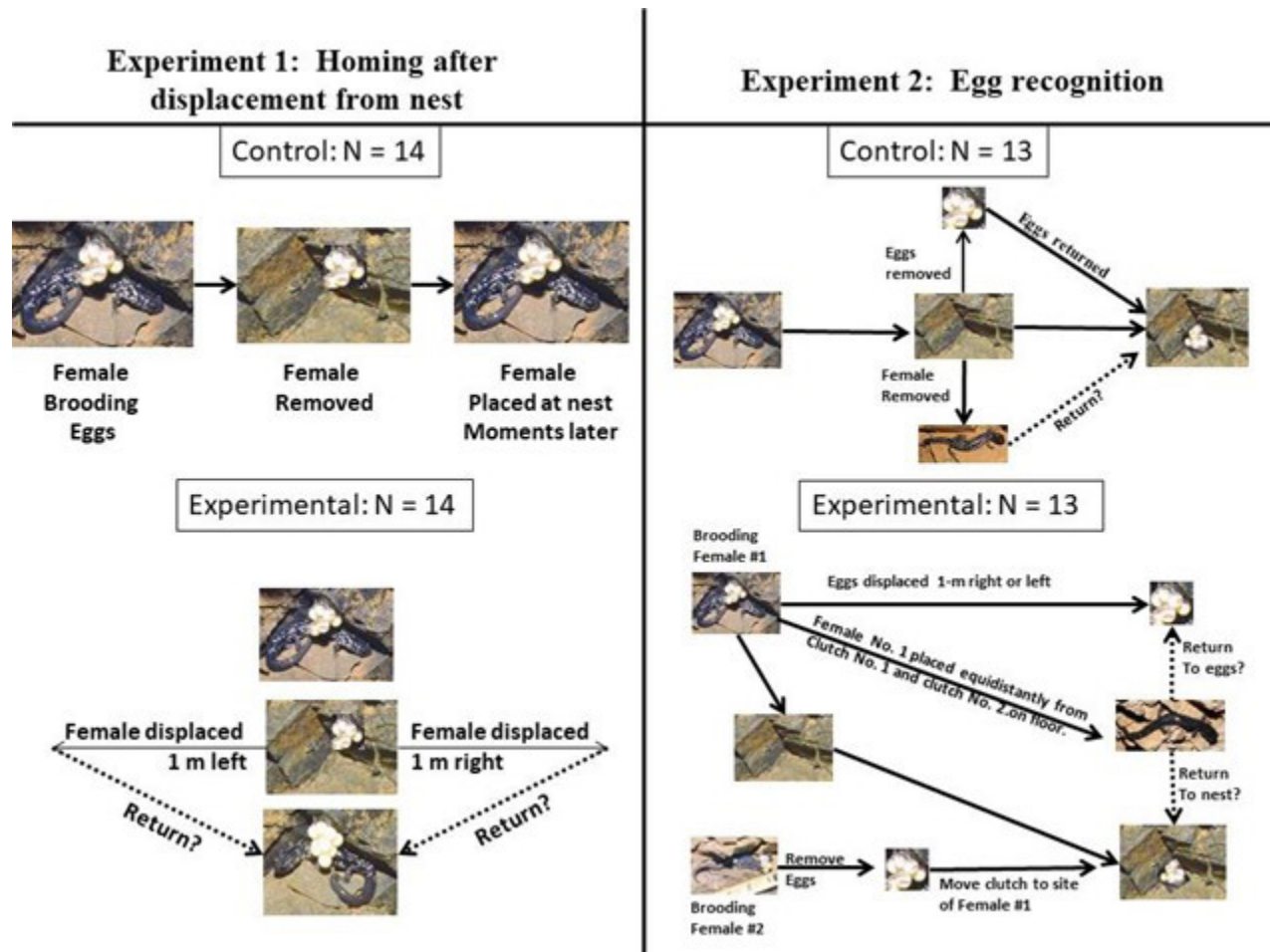


Figure 1. Experimental design for testing homing (left) and egg recognition (right) by female Western Slimy Salamanders (*Plethodon albagula*) in a field setting.

RESULTS

In 2001, most displaced females (12/14 [85.7%]) returned to, and control females (13/14 [92.9%]) remained with, their own nest sites 24 hours post-handling. There was no significant difference between the two groups ($G = 0.383$, $df = 1$, $P = 0.536$).

In 2002, after 24 hours, most experimental (9/13 [69.2%]) and control (12/13 [92.3%]) females returned to their own nest sites. There was no significant difference between the groups ($G = 2.357$, $df = 1$, $P = 0.125$). All experimental returnees were observed brooding the opposing salamander's egg clutches in their own nest site. Neither control nor experimental females moved to egg clutches that were displaced from the nest site. There was no significant difference between the number of control and experimental females present at their own nest sites versus present at other nest sites ($G = 2.357$, $df = 1$, $P = 0.125$).

DISCUSSION

Parental investment theory predicts a significant investment for females that care for their young (Trivers 1972). Fitness can be significantly lowered due to misdirected parental care. Therefore, evolution of a mechanism for identifying one's own egg clutch is assumed to avoid these fitness costs (Trivers 1972).

This species appears to use the nest site as a proxy for

egg clutch recognition. This mechanism for egg recognition likely evolved because in most settings, females are isolated in underground abodes where egg displacement is much less likely due to the nest site's containment. The environment and number of nest sites in the immediate area can influence the intensity of natural selection on egg discrimination (Peterson 2000). However, the salamanders in our study nested on rock faces, which are more open areas. In this setting, egg clutches are probably dislodged occasionally while fending off nest predation by neighboring females (Milanovich et al. 2007, Ford 2008) and other organisms (Milanovich et al. 2005). On our first visit to the mine, we found an egg clutch at the base of the wall with no female present. Further, members of the *P. glutinosus* complex are known to inhabit (Camp and Jensen 2007) and nest (Gunter 1958, Hines et al. 2004) on rock faces in naturally occurring caves. Observations of *P. albagula* occupation (Briggler and Prather 2006) and nesting in caves also exist, although occupancy appears rare during the winter brooding season (Briggler and Prather 2006). So, there should be selective pressure to recognize displaced egg clutches.

Unfortunately, we did not test the influence of directional displacement, nor did we test if females would return to their empty nest site versus their own displaced clutch. It is possible that the behavioral mechanism for egg recognition requires a behavioral trigger to elicit

search behavior for displaced clutches. Further, the most likely place to search for eggs is probably below the nest, rather than to either side. So, even if they had searched, they may have given up and accepted the replacement clutch. In hindsight, it seems probable that a return to a vacant nest site may elicit searching as it does in some birds (personal observation RJ). This missing gap in our research would reveal conclusively if egg recognition was restricted to the nest site proxy. However, this study site has been closed due to risks of white nose syndrome to resident bats.

Because females were able to relocate their nest sites after displacement, some form of egg discrimination, either direct or indirect, took place within this population. Egg discrimination has been documented in *Desmognathus* (Forester 1983) and other plethodontids have homed to specific nest sites (e.g., Green Salamander, *Aneides aeneus* (Williams and Gordon 1961), Holbrook's Southern Dusky Salamander, *Desmognathus auriculatus* (Rose 1966), and the Ocoee Salamander, *Desmognathus ocoee* (Forester 1979)). Forester (1974, 1979, 1986) and Forester et al. (1983) found female *Desmognathus* were able to locate or home to their own eggs when displaced and when presented with other female's eggs. However, Peterson (2000) suggested female *P. cinereus* indirectly recognized their own eggs by use of territorial nest sites and suggests the differences between egg recognition abilities may be due to the difference in selective pressures between semi-aquatic and terrestrial plethodontids.

Our data coincide with Peterson (2000) and fail to support the hypothesis that females discriminate between their eggs and those of a conspecific. After returning to their original nest sites, test females began to guard the unfamiliar egg clutches. The current study lacks evidence that *P. albagula* can recognize their own clutches. This supports findings with other *Plethodon* sp. where females depend on chemical or environmental cues to locate their nest sites as opposed to their egg clutches after displacement.

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LITERATURE CITED

- Barthalmus, G.T. and E.D. Bellis. 1972. Home range, homing, and the homing mechanism of the salamander, *Desmognathus fuscus*. *Copeia* 1972: 632-642.
- Briggler, J.T. and J.W. Prather. 2006. Seasonal use and selection of caves by plethodontid salamanders in a karst area of Arkansas. *American Midland Naturalist* 155: 136 – 148.
- Burton, T. M. and G.E. Likens. 1975a. Salamander populations and biomass in the Hubbard Brook Experimental Forest, New Hampshire. *Copeia* 1975: 541-546.
- Burton, T.M. and G.E. Likens. 1975b. Energy flow and nutrient cycling in salamander populations in the Hubbard Brook Experimental Forest, New Hampshire. *Ecology* 56: 1068-1080.
- Camp, C.D. and J.B. Jensen. 2007. Use of twilight zones of caves by plethodontid salamanders. *Copeia* 2007: 594 – 604.
- Clutton-Brock, T.H. 1991. *The Evolution of Parental Care*. Princeton, Princeton University Press.
- Conant, R. and J.T. Collins. 1998. *A Field Guide to Reptiles and Amphibians of Eastern and Central North America*. Boston, Houghton Mifflin Co.
- Davic, R.D. and H.H. Welsh Jr. 2004. On the ecological roles of salamanders. *Annual Review of Ecology, Evolution and Systematics* 35: 405-434.
- Ford, S. 1998. *David Attenborough's Life In Cold Blood*. United Kingdom, BBC Natural History Unit and Animal Planet.
- Forester, D.C. 1974. Parental care in *Desmognathus ochrophaeus* Cope (Urodela, Plethodontidae): a behavioral study. Ph.D. Dissertation, Raleigh, North Carolina State University.
- Forester, D.C. 1977. Comments on the female reproductive cycle and philopatry in *Desmognathus ochrophaeus* (Amphibia, Urodela, Plethodontidae). *Journal of Herpetology* 11: 311-316.
- Forester, D.C. 1979. Homing to the nest by female mountain dusky salamanders (*Desmognathus ochrophaeus*) with comments on the sensory modalities essential to clutch recognition. *Herpetologica* 35: 330-335.
- Forester, D.C. 1981. Parental care in the salamander, *Desmognathus ochrophaeus*: female activity pattern and trophic behavior. *Journal of Herpetology* 15: 29-34.
- Forester, D.C. 1984. Brooding behavior by the mountain dusky salamander: can females reduce clutch desiccation? *Herpetologica* 40: 105-109.
- Forester, D.C. 1986. The recognition and use of chemical signals by a nesting salamander. In *Chemical Signals in Vertebrates*, p. 205-219. Duvall, D., D. Muller-Schwarze, Silverstein, R.M., Eds. New York, Plenum Press.
- Forester, D.C., K. Harrison, and L. McCall. 1983. The effects of isolation, the duration of brooding, and non-egg olfactory cues on clutch recognition by the salamander, *Desmognathus ochrophaeus*. *Journal of Herpetology* 17: 308-314.
- Forester, D.C., C.L. Anders, A.M. Struzinski, and J.W. Snodgrass. 2005. Are brooding salamanders able to differentiate the developmental status of their eggs? *Herpetologica* 61: 219-224.
- Gunter, G. 1958. Egg clutches and prehensilism in the slimy salamander. *Herpetologica* 13: 279 – 280.
- Highton, R. and T. Savage. 1961. Functions of the brooding behavior in the female red-backed salamander, *Plethodon cinereus*. *Copeia* 1961: 95-98.
- Highton, R., G.C. Maha, and L.R. Maxson. 1989. Biochemical evolution in the slimy salamanders of the *Plethodon glutinosus* complex in the eastern United States. *University of Illinois Biological Monographs* 57: 1-153.
- Hines, J.G. and D.C. Beckett. 2004. Survey of salamanders in Mississippi limestone caves. *Southeastern Naturalist* 3: 241 – 248.
- Hocking, D.J. and K.J. Babbitt. 2014. Effects of red-backed salamanders on ecosystem functions. *PloS One* 9: e86854.
- Jaeger, R.G. and D.C. Forester. 1993. Social behavior of plethodontid salamanders. *Herpetologica* 49: 163-175.
- Jaeger, R.G., D. Fortune, G. Hill, A. Palen, and G. Risher. 1993. Salamander homing behavior and territorial pheromones: alternative hypotheses. *Journal of Herpetology* 27: 236-239.
- Kleeberger, S.R. and J.K. Werner. 1982. Home ranges and homing behavior of *Plethodon cinereus* in northern Michigan. *Copeia* 1982: 409-415.

- Madison, D.M. 1969. Homing behaviour of red-cheeked salamander, *Plethodon jordani*. *Animal Behaviour* 17: 25-39.
- Madison, D.M. 1972. Homing orientation in salamanders: a mechanism involving chemical cues. In *Animal Orientation and Navigation*. p. 485-498, Galler, S.R., Schmidt-Koenig, G.J. Jacobs, G.J., Belleville, R.E., Eds. Washington, National Aeronautics and Space Administration.
- Mathis, A., R.G. Jaeger, W.H. Keen, P.K. Ducey, S.C. Walls, B.J. Buchanan. 1995. Aggression and territoriality by salamanders and a comparison with the territorial behaviour of frogs. *In Amphibian Biology*, Vol. 2. Social Behaviour, p. 633-676. Heatwole, H., Sullivan, B.K. (Eds.). Chipping Norton, Surrey Beatty and Sons.
- Milanovich, J.R. and W.E. Peterman. 2016. Revisiting Burton and Likens (1975): Nutrient standing stock and biomass of a terrestrial salamander in the Midwestern United States. *Copeia* 104: 165-171.
- Milanovich, J.R., S.E. Trauth, and D.A. Saugey. 2007. *Plethodon albagula* (Western Slimy Salamander). Brooding defense behavior and oophagy. *Herpetological Review* 38: 67.
- Milanovich, J.R., S.E. Trauth, and M.N. Mary. 2005. *Diadophis punctatus sticktogenys* (Mississippi Ringneck Snake). Diet. *Herpetological Review* 36: 323 – 324.
- Ng, M.Y. and H.M. Wilbur. 1995. The cost of brooding in *Plethodon cinereus*. *Herpetologica* 51: 1-8.
- Nussbaum, R.A. 1985. The evolution of parental care in salamanders. *Miscellaneous Publications of the Museum of Zoology of the University of Michigan* 713: 1-49.
- Papi, F. 1992. General Aspects. *In Animal Homing*. p. 1-18, Papi, F., Ed., New York, Chapman and Hall.
- Peterson, M.G. 2000. Nest, but not egg, fidelity in a terrestrial salamander. *Ethology* 106: 781-794.
- Petranka, J.W., M.E. Eldridge, and K.E. Haley. 1993. Effects of timber harvesting on southern Appalachian salamanders. *Conservation Biology* 7: 363-370.
- Petranka, J.W. and S.S. Murray. 2001. Effectiveness of removal sampling for determining salamander density and biomass: a case study in an Appalachian streamside community. *Journal of Herpetology* 35: 36-44.
- Pough, F.H., R.M. Andrews, J.E. Cadle, M.L. Crump, A.H. Savitzky, and K.D. Wells. 1998. *Herpetology*. Saddle River, Prentice-Hall.
- Rose, F.L. 1966. Homing to nests by the salamander *Desmognathus auriculatus*. *Copeia* 1966: 251 – 253.
- Semlitsch, R., K. O'Donnell, and F. Thompson. 2014. Abundance, biomass production, nutrient content, and the possible role of terrestrial salamanders in Missouri Ozark forest ecosystems. *Canadian Journal of Zoology* 92: 997-1004.
- Sinsch, U. 1992. Amphibians. *In Animal Homing*. p. 213-233 Papi, F. Ed., New York, Chapman and Hall.
- Snyder, D.H. 1971. The function of brooding behavior in the plethodontid salamander, *Aneides aeneus*: a field study. Ph.D. Dissertation. Notre Dame, University of Notre Dame.
- Trauth, S.E., M.L. McCallum, R.R. Jordan, and D.A. Saugey. 2006. Brooding postures and nest site fidelity in the western slimy salamander, *Plethodon albagula* (Caudata: Plethodontidae), from an abandoned mine shaft in Arkansas. *Herpetological Natural History* 9: 141 – 149.
- Trauth, S.E., H.W. Robison, and M.V. Plummer. 2004. *The Amphibians and Reptiles of Arkansas*. Fayetteville, University of Arkansas Press.
- Trivers, R.L. 1972. Parental investment and sexual selection. *In Sexual Selection and the Descent of Man, 1871-1971*. p.136-179, Campbell, B. Ed., Chicago, Aldine.
- Waldman, B. 1988. The ecology of kin recognition. *Annual Review of Ecology and Systematics* 19: 543-571.
- Williams, K.L. and R.E. Gordon. 1961. Natural dispersal of the salamander *Aneides aeneus*. *Copeia* 1961: 353 – 353.