To study sex ratios and their significance among turtle species, a series of conditions and methods were employed. Sex Chromosomes and Sex Determination in turtles consistently emerged as a focal point. Feminizing turtle species were noted for their ability to influence sex ratios.

In a controlled environment, housing consisted of two 8.4 m × 0.8 m indoor raceways, with the raceways following the protocol outlined by Ettling et al. (2013, Herpetol. Rev. 44:605–610). Water temperature varied with stream flow. Raceways received natural light and were subjected to species-specific decontamination treatments (e.g., saline solution treatment) prior to being introduced into the raceways.

Each night, the three larger individuals placed into one raceway and the two smaller individuals placed in the other raceway. The physical and chemical conditions of the raceways were set to mimic those of the source stream. Raceways contained natural cobble substrate, slate and ceramic cover tiles, and reverse-osmosis water reconstituted to match the water chemistry of the source stream (Ettling et al. 2013, Herpetol. Rev. 44:605–610). Water temperature varied with ambient temperature and ranged between 18–20°C. A pumping system created a current through each raceway, simulating stream flow. Raceways received natural light and were subjected to natural light cycles through large windows within the building. Raceways were stocked with prey (crayfish, small fish) and eDNA samples were subjected to species-specific decontamination treatments (e.g., saline solution treatment) prior to being introduced into the raceways following the protocol outlined by Ettling et al. (2013, op. cit.). Following a two-week acclimation period, after which all C. a. alleganiensis began feeding and pursuing prey, observations were conducted 1–2 nights per week during weekends from 4 June 2016 to 17 July 2016 from dusk (ca. 2045–2130 h) to 0030–0100 h. Raceways were inspected in the dark via a headlamp with red light, which appears to cause little or no disturbance to animals (e.g., salamander activity). The sex-ratio disparity of the endangered Houston toad (Bufo houstonensis) was measured using a sampling protocol to identify and distinguish individuals.

In laboratory conditions, competitive mate searching in Bufo bufo varied with respect to the presence of conspecific males. However, few data appear to have been published concerning nocturnal emergence and behavioral differences among size classes of C. a. alleganiensis.

As part of a conservation-driven ex situ study, we collected four wild Cryptobranchus alleganiensis alleganiensis on 19 May 2016 from a tributary of the Susquehanna River in Pennsylvania (stream name and exact locality details are withheld due to conservation concerns) and placed them into captivity at the U.S. Fish and Wildlife Northeast Fisheries Center in Lamar, Pennsylvania. Individuals had total lengths (TL) of 29.7, 35.7, 42.0, and 45.0 cm. Housing consisted of two 0.8 m × 0.8 m indoor raceways, with the two larger individuals placed into one raceway and the two smaller individuals placed in the other raceway. The physical and chemical conditions of the raceways were set to mimic those of the source stream. Raceways contained natural cobble substrate, slate and ceramic cover tiles, and reverse-osmosis water reconstituted to match the water chemistry of the source stream (Ettling et al. 2013, Herpetol. Rev. 44:605–610). Water temperature varied with ambient temperature and ranged between 18–20°C. A pumping system created a current through each raceway, simulating stream flow. Raceways received natural light and were subjected to natural light cycles through large windows within the building. Raceways were stocked with prey (crayfish, small fish) and C. a. alleganiensis were also presented with earthworms (Lumbricus) weekly to ensure that they could eat ad libitum. Prey organisms were subjected to species-specific decontamination treatments (e.g., saline solution treatment) prior to being introduced into the raceways following the protocol outlined by Ettling et al. (2013, op. cit.). Following a two-week acclimation period, after which all C. a. alleganiensis began feeding and pursuing prey, observations were conducted 1–2 nights per week during weekends from 4 June 2016 to 17 July 2016 from dusk (ca. 2045–2130 h) to 0030–0100 h. Raceways were inspected in the dark via a headlamp with red light, which appears to cause little or no disturbance to C. a. alleganiensis (Reese 1906. Biol. Bull. 11:93–99; Nickerson 1977. Proc. Am. Assoc. Zool. Parks Aquac. 1977–1978:396–399). Activity in C. a. alleganiensis was noted, and individuals were distinguished visually by size and unique natural spotting patterns.

The two largest C. a. alleganiensis consistently emerged at dusk (ca. 2045–2130 h) every night during observations. The 35.7-cm TL individual typically emerged at dusk, but emerged approximately 30 minutes after dusk (ca. 2115–2145 h) on 4 June 2016 and 9 July 2016. The smallest individual consistently emerged 60–120 minutes after dusk (ca. 2200–2330 h). Differences in behavior and habitat use after emergence were also observed. Each night, the three larger C. a. alleganiensis actively crawled around the raceway on top of substrate and were also observed swimming within and near the top of the water column. Larger

**Literature Cited**


**HERPETOCULTURE NOTES**

**SALAMANDERS — CAUDATA**

**CRYPTOBRANCHUS ALLEGANIENSIS ALLEGANIENSIS (Eastern Hellbender). TEMPORAL AND SPATIAL NICHE PARTITIONING.** Cryptobranchus alleganiensis, large, fully aquatic salamanders native to the eastern United States, are typically nocturnally active (Nickerson and Mays 1973. The Hellbenders: North American Giant Salamanders, Milwaukee Public Museum Press, Milwaukee, Wisconsin. 106 pp.; but see Humphries 2007. Southeast. Nat. 6:135–140). In laboratory conditions, C. alleganiensis have been reported to have an activity peak corresponding to approximately 2–2.5 h after dark (Noeske and Nickerson 1979. Copeia 1979:92–95). However, few data appear to have been published concerning nocturnal emergence and behavioral differences among size classes of C. alleganiensis.

As part of a conservation-driven ex situ study, we collected four wild Cryptobranchus alleganiensis alleganiensis on 19 May 2016 from a tributary of the Susquehanna River in Pennsylvania (stream name and exact locality details are withheld due to conservation concerns) and placed them into captivity at the U.S. Fish and Wildlife Northeast Fisheries Center in Lamar, Pennsylvania. Individuals had total lengths (TL) of 29.7, 35.7, 42.0, and 45.0 cm. Housing consisted of two 0.8 m × 0.8 m indoor raceways, with the two larger individuals placed into one raceway and the two smaller individuals placed in the other raceway. The physical and chemical conditions of the raceways were set up to mimic those of the source stream. Raceways contained natural cobble substrate, slate and ceramic cover tiles, and reverse-osmosis water reconstituted to match the water chemistry of the source stream (Ettling et al. 2013, Herpetol. Rev. 44:605–610). Water temperature varied with ambient temperature and ranged between 18–20°C. A pumping system created a current through each raceway, simulating stream flow. Raceways received natural light and were subjected to natural light cycles through large windows within the building. Raceways were stocked with prey (crayfish, small fish) and C. a. alleganiensis were also presented with earthworms (Lumbricus) weekly to ensure that they could eat ad libitum. Prey organisms were subjected to species-specific decontamination treatments (e.g., saline solution treatment) prior to being introduced into the raceways following the protocol outlined by Ettling et al. (2013, op. cit.). Following a two-week acclimation period, after which all C. a. alleganiensis began feeding and pursuing prey, observations were conducted 1–2 nights per week during weekends from 4 June 2016 to 17 July 2016 from dusk (ca. 2045–2130 h) to 0030–0100 h. Raceways were inspected in the dark via a headlamp with red light, which appears to cause little or no disturbance to animals (e.g., salamander activity). The sex-ratio disparity of the endangered Houston toad (Bufo houstonensis) was measured using a sampling protocol to identify and distinguish individuals.

The two largest C. a. alleganiensis consistently emerged at dusk (ca. 2045–2130 h) every night during observations. The 35.7-cm TL individual typically emerged at dusk, but emerged approximately 30 minutes after dusk (ca. 2115–2145 h) on 4 June 2016 and 9 July 2016. The smallest individual consistently emerged 60–120 minutes after dusk (ca. 2200–2330 h). Differences in behavior and habitat use after emergence were also observed. Each night, the three larger C. a. alleganiensis actively crawled around the raceway on top of substrate and were also observed swimming within and near the top of the water column. Larger
individuals would also retreat at intervals (ca. 20–60 minutes) under cover tiles and remain motionless with heads, necks, and occasionally the forelimbs protruding. When active, the smallest *C. a. alleganiensis* was observed to weave through interstices within the cobble substrate and rarely crawled on top of the substrate. This individual was observed to swim on only one occasion on 18 June 2016 at ca. 0015 h. In this case, the larger conspecific within the raceway was observed to enter a cover tile and the smaller individual immediately exited the cover tile and swam ca. 5–8 seconds (travelling approximately 2 m) and subsequently retreated within the interstices of cobble substrate.

While limited, these observations support hypothesized and observed *in situ* temporal and spatial niche partitioning between large and small *C. a. alleganiensis*. Large, adult *C. alleganiensis* have few predators (Nickerson and Mays 1973, *op. cit*.), but smaller individuals are likely prey items of large fish, reptiles, and larger *C. alleganiensis* (Nickerson and Mays 1973, *op. cit*.; Groves and Williams 2014. Herpetol. Rev. 45:108–109). A later emergence time of smaller *C. a. alleganiensis* may facilitate the avoidance of predators, including larger conspecifics. Use of interstices by smaller individuals may also facilitate predator avoidance as the habitat is less accessible by large predators (Pitt et al., *in press*. Herpetol. Bull.). Differences in behavior and habitat use between larger and smaller hellbenders may also reflect differences in diet. Larger (i.e., sub–adult, adult) *C. alleganiensis* predominantly consume crayfish, but may also consume fish, aquatic invertebrates, amphibians, reptiles, and carrion (Nickerson and Mays 1973, *op. cit*.; Petranka 1998. Salamanders of the United States and Canada. Smithsonian Books, Washington, D.C. 592 pp; Hill 2011. Herpetol. Rev. 42:580), prey items typically found under cover rocks and within the water column. Smaller (i.e., larval, post–larval) *C. a. alleganiensis* consume aquatic insects (e.g., Megaloptera, Ephemeroptera, Diptera; Pitt and Nickerson 2006. Herpetol. Rev. 37:69) and small conspecific and heterospecific salamanders (Hecht-Kardasz and Nickerson 2013. Herpetol. Rev. 44:490) that also use interstitial spaces, thus use of interstices may also be a result of foraging for appropriately sized prey.

Our observations were conducted opportunistically in conjunction with a larger, conservation-driven ecological study, and are thus limited. However, such observational data could readily be collected in zoos already maintaining hellbenders in captivity.

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**TESTUDINES — TURTLES**

**RHINOCLEMMS PUNCTULARIA** (Spot-legged Turtle).

**NESTING.** Little detailed information has been published on the reproductive biology of *Rhinoclemmys punctularia*, though females have been reported to deposit one or two eggs in March and April (Vogt 2008. Amazon Turtles. Gráfica Biblos, Lima, Peru. 104 pp.). *Rhinoclemmys pulcherrima* has been observed and photographed nesting in Costa Rica; although the nest reached the same depth of 10 cm, it was not as large in diameter and did not appear to have a body pit as seen with *R. punctularia* (Monje-Najera et al. 1988. Herpetol. J. 1:308). Monje-Najera and others (1988, *op. cit*.) also claimed that *R. punctularia* does not dig a nest, and instead deposits eggs on the forest floor. In Belize and Mexico, *R. areolata* has been documented to deposit a single egg in leaf litter, but will also dig shallow 5–cm deep single egg nests; a *R. areolata* nest with two eggs was also recorded in Belize (Vogt et al. 2009. In Rhodin et al. [eds.], Conservation Biology of Freshwater Turtles and Tortoises: A Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group. Chelonian Research Monographs No. 5, pp. 022.1–022.7. Chelonian Research Foundation, Lunenburg, Massachusetts). A female *R. areolata* was collected while covering a one egg nest near Villahermosa, Tabasco, Mexico and afterwards dug three more nests at five-day intervals in captivity, depositing one egg in each nest (Perez-Higareda and Smith 1987. Great Basin Nat. 48:263–266). *Rhinoclemmys nasuta* has been reported to lay single egg clutches among the leaf litter in the forest without digging a nest (Carr and Giraldo 2009. *In* Rhodin et al. [eds.].

![Fig. 1. Nesting female Rhinoclemmys punctularia. Note that the female is well within the body pit below the surface to deposit her egg, and manipulating the egg with her left hind foot. The egg was deposited at an angle, near the base of a succulent plant.](image)