INFESTATION OF A NATURALLY INCUBATED NEST OF THE ALLIGATOR SNAPPING TURTLE (*MACROCHELYS TEMMINCKII*) BY THE PHORID FLY *MEGASELIA SCALARIS*

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ABSTRACT—Larvae of the phorid fly *Megaselia scalaris* were in eggs in a naturally incubated nest of an alligator snapping turtle (*Macrochelys temminckii*). It appears that these larvae contributed to failure of this nest. Infestation by phorid larvae has been reported previously for eggs and nests of multiple species of turtles, but has not been reported from *M. temminckii*.

RESUMEN—Larvas del díptero *Megaselia scalaris* (Phoridae) fueron encontradas en los huevos de un nido de incubación natural de la tortuga caimán (*Macrochelys temminckii*). Parece que las larvas contribuyeron al fracaso del nido. Infestación de huevos y nidos de varias especies de tortugas con larvas de moscas (Phoridae) se ha reportado anteriormente, pero no en *M. temminckii*.

Infestation of turtle nests by flies is a common phenomenon, with dipteran larvae reported from nests or eggs of multiple species of turtles (e.g., McGowan et al., 2001; Hall and Parmenter, 2008). Two dipteran families commonly reported in nests are Sacrophagidae and Phoridae. Larvae of Phoridae have been reported in nests or eggs of the green sea turtle (Chelonia mydas; Fowler, 1979; McGowan et al., 2001), hawksbill sea turtle (Eretmochelys imbricata; Bjorndal et al., 1985), loggerhead sea turtle (Caretta caretta; Broderick and Hancock, 1997; McGowan et al., 2001), eastern box turtle (Terrapene carolina; Ewing, 1933), Meso-American slider (Trachemys venusta; Moll and Legler, 1971), and painted wood turtle (Rhinoclemmys pulcherrima; Acuña-Mesén and Hanson, 1990).

The phorid most commonly identified from nests of turtles has been *Megaselia scalaris*, a species in which the larvae feed on a broad spectrum of decaying organic matter (Disney, 2008). *Megaselia scalaris* is known as a scavenger, facultative predator, parasitoid, and parasite (Disney, 2008), so it can be unclear whether larvae of *M. scalaris* in nests of turtles are scavengers or parasitoids (Broderick and Hancock, 1997). This already confusing situation is further complicated by indications that *M. scalaris* is capable of attacking both developing and nondeveloping eggs (Acuña-Mesén and Hanson, 1990). It has been suggested that larvae of *M. scalaris* feed primarily on dead or weakened hatchlings (Fowler, 1979) and that adult *M. scalaris* may be attracted to odors associated with rotting nonviable turtle eggs (Acuña-Mesén and Hanson, 1990; Broderick and Hancock, 1997; Saumure et al., 2006). However, Moll and Legler (1971) reported that adult *M. scalaris* entered turtle eggs through tears made by pipping hatchlings and oviposited on eyes and yolk sacs of hatchlings, which subsequently died.

First-instar larvae of M. scalaris are small enough to enter rigid-shelled eggs through pores in the eggshell (Acuña-Mesén and Hanson, 1990). There is no information about the ability of such larvae to enter hardexpansible eggs, such as those of alligator snapping turtles (Macrochelys temminckii; Ewert, 1979). Wolff (2007) reported that phorids will deposit eggs on healthy chelonian eggs, so flies that initially are attracted to decaying eggs also may take advantage of normally developing eggs present in the nest. Some members of Phoridae are able to burrow ≤ 1 m into soil as adults (Disney, 1994), which means that turtle eggs potentially are vulnerable to infestation at any time during development (Broderick and Hancock, 1997).

Research on nesting ecology of M. temminckii has been ongoing since 2002 at Black Bayou Lake National Wildlife Refuge, near Monroe, Ouachita Parish, Louisiana. Nesting by M. temminckii at the refuge was concentrated primarily along a railroad causeway, where nests suffered high levels of depredation by raccoons, Procyon lotor (Woosley, 2005). To find nests before they were destroyed, surveys were performed daily during nesting season in April and May, and all intact or partially intact nests were covered with a predator excluder. We found a total of 11 nests in 2008, three of which were depredated by mammals before we found them; the other 8 were intact or partially intact. For all intact (n = 5), and two partially intact nests, the nest-cavity was carefully excavated and the clutch was divided in two. One-half of the clutch was returned carefully to the nest and reburied in a manner similar to arrangement of the original nest, and the other one-half was taken back to the lab for incubation. Eggs left in the nest were covered with a predator excluder, which consisted of a ca. 1-m square piece of 1.3-cm-mesh hardware cloth staked to the ground, and were left to incubate naturally. Nests were monitored, but left undisturbed until well after estimated date of hatching based upon hatching in the lab and data from previous field seasons. Two nests were lost during incubation to mammalian predators despite use of predator excluders, which resulted in a total of five nests that were monitored through the end of incubation.

The first nest in 2008 was found on 26 April and contained a clutch of 30 eggs. Of these, 15 eggs were replaced in the nest and were not disturbed again until excavation of all nests of M. temminckii in the field was undertaken on 5 December. As of that date, there had been no emergence of hatchlings from the nest and the nest did not fall victim to above-ground depredation. When the nest was excavated, eggs were placed into resealable plastic bags and returned to the lab where they were refrigerated until they were dissected in an attempt to determine cause of failure of the nest. When the first egg was examined, two small holes were noted in the eggshell. These small holes were measured, along with similar holes (an average of 1.3 holes/egg, n = 17) in other eggs, and averaged 1.45 by 1.60 mm (n = 8). On the inside of the eggshell, many empty fly puparia were noted, as well as remains of what appeared to be a fully developed hatchling, including bones, scutes, and nearly intact tail. The portion of the eggshell containing the two small holes was preserved in alcohol, along with some of the puparia. Similar observations were made regarding four additional eggs. In addition, two eggs were as described above, but also had fly puparia on the outside of the eggshell. Three other eggs had small holes, remains of hatchlings, and internal puparia, but the eggs were invaginated so that the egg had a bowl-shaped appearance. This could have been the result of dehydration of the egg caused by feeding of larval flies (Acuña-Mesén and Hanson, 1990). One egg had small holes and fly puparia, but no remains of a hatchling. This egg presumably was infertile, or experienced early embryonic death, and was entirely consumed by fly larvae. Three eggs had large tears in the eggshell, such as would be caused by a pipping hatchling, as well as remains of hatchlings and fly puparia. The 15th egg was not recovered from the nest, although there were several pieces of eggshell from another egg. A total of 14 adult flies were recovered from inside five of the eggs and the flies were identified as M. scalaris; six voucher specimens were deposited in the Louisiana State Arthropod Museum at Louisiana State University (LSAM 0154233-0154238).

It appears that phorid flies played a significant role in failure of this nest of M. temminckii. The fact that no living M. scalaris was recovered, coupled with the fact that empty puparia persist long after the adult fly has emerged (Disney, 2008) would be consistent with infestation of this nest at pipping, as described by Moll and Legler (1971), and indicates that the flies had completed their life cycle long before the nest was excavated. Pipping in M. temminckii is asynchronous, so a plausible scenario is one in which flies were attracted to the first eggs that pipped, and then proceeded to lay eggs not only on the pipped hatchlings, but also on the remainder of the clutch. Thus, remaining eggs were infested with fly larvae, which killed embryonic turtles in situ, leaving behind only indigestible scutes, bones, and tails. This could explain why only a few eggs in this clutch appeared to have pipped, yet most eggs contained remains of well-developed hatchlings that seem to have been close to pipping, at ca. stage 25-26 (Yntema, 1968). A similar scenario was described for a sarcophagid fly by Bolton et al. (2008), who suggested that female flies may be attracted by olfactory cues associated with hatching. It seems likely that the first-instar larvae of M. scalaris would be able to enter intact eggs of *M. temminckii*. Eggs of the confamilial eastern snapping turtle (Chelydra serpentina) have the same type of eggshell (Ewert, 1979) and are characterized by arrangement of the mineral layer into groups of shell units, with large numbers of spaces between units where underlying shell membrane is exposed. This is particularly evident after the egg has taken up water and expanded, as would be the case late in incubation (Packard, 1980), and would require only that the larvae penetrate the shell membrane, which might be achieved by use of proteolytic enzymes (Acuña-Mesén and Hanson, 1990). It is still uncertain whether or not fly larvae have detrimental effects on populations of turtles (McGowan et al., 2001) and only one of the five nests we monitored during 2008 appears to have failed due to infestation. This indicates that infestation by larvae of phorid flies is only one potential source of mortality in this population and the full impact of infestation remains unknown. This account provides additional evidence that fly larvae can have disastrous effects on individual nests. This is the first report of infestation by fly larvae in M. temminckii and for the family Chelydridae.

This research was performed under Louisiana State Scientific Collecting Permit LNGP-08-043 and United States Fish and Wildlife Service Special Use Permit 42651-08-04. Funding was provided by the Louisiana Department of Wildlife and Fisheries and United States Fish and Wildlife Service, Division of Federal Aid, through the State Wildlife Grants Program. We thank the staff of Black Bayou Lake National Wildlife Refuge for cooperation and M. Ray for assistance with field work. M. Pardue and R. Fiorillo provided assistance in the lab. Flies were identified as phorids by C. Carlton of the Louisiana State Arthropod Museum at Louisiana State University, V. Bayless facilitated deposition of vouchers in that museum, and B. Brown of the Natural History Museum of Los Angeles County provided identification to species.

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Submitted 7 July 2009. Accepted 12 June 2011. Associate Editor was Paul A. Stone.