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Female Reproduction in the Rainbow Water Snake, *Enhydris enhydris* (Serpentes, Colubridae, Homalopsinae)

JOHN C. MURPHY[1], HAROLD K. VORIS[1]*, BRYAN L. STUART[1,2] AND STEVEN G. PLATT[2]

[1] *Division of Amphibians and Reptiles, Field Museum of Natural History, 1400 South Lake Shore Drive, Chicago, IL 60605, USA*
[2] *Wildlife Conservation Society, P.O. Box 1620, Phnom Penh, CAMBODIA*

ABSTRACT.–One hundred and thirteen female *Enhydris enhydris* from three locations (Cambodia, Myanmar, Thailand) were examined for eggs and embryos. In the sample of 32 gravid females from Cambodia the mean snout-vent length was 564 mm and the clutch size ranged from six to 39 with a mean of 20.3. Eight gravid females from Myanmar had an average snout-vent length of 428 mm and the clutch size ranged from six to 11 with a mean of 7.8. In the sample of 18 gravid females from Thailand the mean snout-vent length was 426 mm and the clutch size ranged from five to 16 with a mean of 8.1. On average the gravid female snakes in the Cambodian sample were larger in body size, clutch size, and relative clutch mass than the female snakes from the Myanmar and Thailand localities. Female mass was significantly correlated with clutch size. Females synchronize reproduction within populations and in Cambodia it appears that there are two seasons when snakes reproduce. A comparison of *E. enhydris* clutch sizes at Tonle Sap, Cambodia in 1971 and 2000 suggests that clutch size has increased markedly, which may be an indirect result of human fishing activities.

KEY WORDS: *Enhydris enhydris*; Homalopsinae; aquatic snake reproduction; Cambodia; Myanmar; Thailand; Tonle Sap

INTRODUCTION

The Australasian rear-fanged water snake clade Homalopsinae includes approximately 35 species that are viviparous, morphologically and ecologically diverse, and widely distributed. *Enhydris enhydris* (Schnedier, 1799), the rainbow water snake, ranges from northeast India and Myanmar eastward into peninsular Indochina, and through Indonesia as far east as Sulawesi. It is piscivorous, highly aquatic, reaches spectacularly dense populations (Murphy et al., 1999; Voris and Murphy, 2002), and is commercially exploited (Stuart et al., 2000). However, published comments on its reproduction are largely anecdotal. This study describes aspects of female reproduction for *E. enhydris* from Tonle Sap, Cambodia and compares that sample with those from Myanmar, and Thailand.

* Corresponding author.
E-mail: hvoris@fieldmuseum.org

MATERIALS AND METHODS

We examined 52 female *Enhydris enhydris* purchased alive or freshly dead from fishermen and markets near Siem Reap and Kampong Chhnang (13°16′21″N, 103°49′22″E and 12°16′08″N, 104°40′50″E) on the shores of Tonle Sap Great Lake, Cambodia in August 1999, and in June and August 2000. Another 33 females studied were collected by us at Lake Songkhla, Thailand (7°23′00″N, 100°26′00″E) in June-July 1996, June-July 1997, and November 2000. One additional gravid female was examined that was collected from Paknampo (15°43′00″N, 100°09′00″E) in central Thailand in June 1949. We examined 23 females from Myanmar, comprising 7 from Myaungmya, Irrawaddy (16°36′00″N, 94°56′00″E) collected in January 1960, and 16 from Twante (16°43′00″N, 95°56′00″E) collected in December 1963. Specimens collected by us were fixed in 10% buffered formalin for several days and then transferred to 70% ethanol. The museum numbers of the gravid specimens examined are listed in Appendix I.

Prior to fixing in formalin, snout-vent lengths (SVL) of the dead or euthanized females were measured to the nearest millimeter, weighed to the nearest gram, and examined for ova and embryos. Ova and embryos were removed from the carcasses, labeled, and stored in 10% buffered formalin separately from the females. At Field Museum the preserved female snakes were drained, patted dry and weighed to the nearest gram. The preserved eggs were counted, drained and patted dry, measured for greatest diameter to the nearest 0.1 mm, and weighed to the nearest 0.1 g. Yolk plugs were not counted as ova, but were included in the total clutch mass. The analysis presented here on snake and egg mass is based on preserved specimens. When visible, the diameters of the coiled embryos were measured to the nearest 0.1 mm and then staged following Zehr (1962). Relative clutch mass (RCM) was computed as the ratio of clutch mass to female mass.

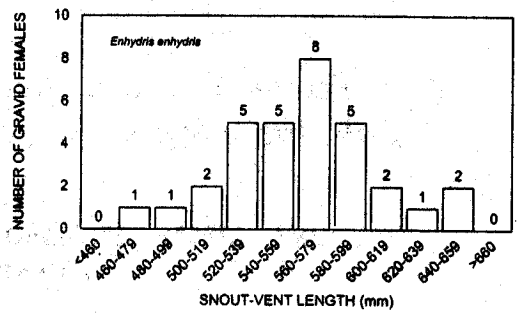

FIGURE 1. Histogram showing the size (snout-vent length) distribution of gravid female *E. enhydris* collected from Tonle Sap, Cambodia. The distribution does not differ significantly from a normal distribution.

RESULTS

Cambodia.–In our sample of 52 female *E. enhydris*, 32 (62%) had oviducal eggs. The 32 gravid females in this sample ranged from 475 mm to 647 mm SVL, and the distribution of body size in the sample is near normal (Fig. 1). The mean SVL of gravid females was 564 mm (SD = 4.08). The smallest non-gravid female in the sample was 389 mm while the largest non-gravid female was 572 mm SVL. The mean clutch size was 20.3 (range = 6-39, SD = 7.0) and the mean clutch mass was 34.6 g (range = 7.3-80.1 g, SD = 14.61). The SVL and mass of the females (minus their ova) were significantly correlated ($r = 0.52$, $p = 0.003$) but the SVL and clutch size were not significantly correlated ($p = 0.40$). Nor were the SVL and clutch mass significantly correlated ($p = 0.36$). However, female mass (less ova) and clutch size were significantly correlated ($r = 0.43$, $p = 0.017$) (Fig. 2). The relative clutch mass (RCM) for this population had a mean of 0.20 (range = 0.04-0.35, SD = 0.82). Embryos in specimens collected in June were in Zehr's stage 7 (gastrula), while those collected in early August were stages 15-35 (amnion just closed, to nearly full term). One specimen contained embryos in different stages; most were in stage 34, but one much smaller embryo was in stage 26.

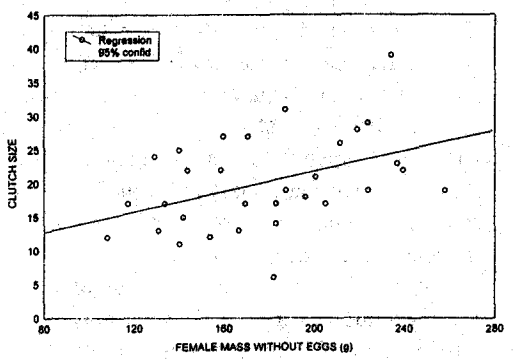

FIGURE 2. Scatter plot, regression, and 95 percent confidence levels of the mass of 32 female *E. enhydris* (after eggs removed) from Tonle Sap, Cambodia versus the clutch size. The correlation is significant (clutch size = 6.678+ .0759 x mass, r = 0.435, p< .05).

Myanmar.–Of the 16 female snakes collected from Twante in December, eight (50%) contained oviducal eggs. The eight gravid females ranged in size from 298 mm SVL to 443 mm SVL with a mean SVL of 428 mm (SD = 2.6). The smallest non-gravid female was 298 mm SVL and the largest was 443 mm SVL. The mean clutch size was 7.85 (range = 6-11, SD = 2.4). The mean clutch mass was 7.8 g (range = 3.4-19.3 g, SD = 5.9). The mean RCM was 0.11 (range = 0.06-0.23, SD = 0.06). Embryos were not visible in any of the eggs. The collection of seven females of similar body size made at Myaungmya, Irrawaddy, Myanmar in January contained no gravid females.

Thailand.–Of the 33 female *E. enhydris* examined from Lake Songkhla, 18 (54%) had oviducal eggs. The mean SVL for the 18 gravid females was 426 mm (range = 375-509 mm, SD = 3.5). The smallest non-gravid female was 275 mm and the largest was 514 mm. The mean clutch size was 8.1 (range = 5-16, SD = 2.5). The mean clutch mass was 9.55 g (range = 5.1-17.6 g, SD = 3.19). The mean RCM was 0.12 (range = 0.06-0.23, SD = 0.04). Embryo stages represented in the 1996 sample ranged from stage 10 to 24, and stages for those collected in 1997 ranged from 7 to 34. One female (FMNH 257397) contained embryos that varied from stages 25 to 30.

The additional gravid female examined from Paknampo was 444 mm in SVL, and weighed 81 g without the embryos. It contained 19 near full term embryos, two of which were 171 and 174 mm in total length. Other embryos with their yolks weighed 3.2 to 3.4 g (mean =3.3 g), without the yolks they weighed 2.4 to 2.8 g (mean = 2.6), and the yolks alone weighed 0.6 to 0.9 g (mean =0.8 g). All of these embryos were in Zehr's final stage 37.

DISCUSSION

The body sizes of gravid females of *E. enhydris* in the Tonle Sap, Cambodia sample (SVL 475 to 647 mm and mean of 564 mm) are consistent with those of Saint Girons (1972), who reported five females from Tonle Sap with SVLs of 480 to 627 mm and mean of 552 mm. However, gravid females from Cambodia have larger average body size, clutch size, and RCM than do the samples from Thailand and Myanmar (Fig. 3, Table 1). Minimum body size of gravid females appears to be fairly constant across the range of *E. enhydris*, as the smallest gravid female in a sample from Java reported by Bergman (1955) was 386 mm, a size comparable to the smallest gravid female found in this study, a 371 mm SVL female in the Thailand sample.

The maximum clutch size previously reported for *E. enhydris* was 29 eggs in a female from northeast India (Biswas and Acharyo, 1977). A female in our Cambodian sample contained 39 eggs. Clutch sizes in the samples from Thailand and Myanmar are generally consistent with clutch sizes from Thailand and Myanmar in the literature (see Appendix II).

Fitch (1970) suggested that *E. enhydris* has one, long breeding season. However, Saint Girons and Pfeffer (1971) reported that in a sample of 24 females collected from Tonle Sap, Cambodia throughout the year, oviducal eggs were observed in February, and females were

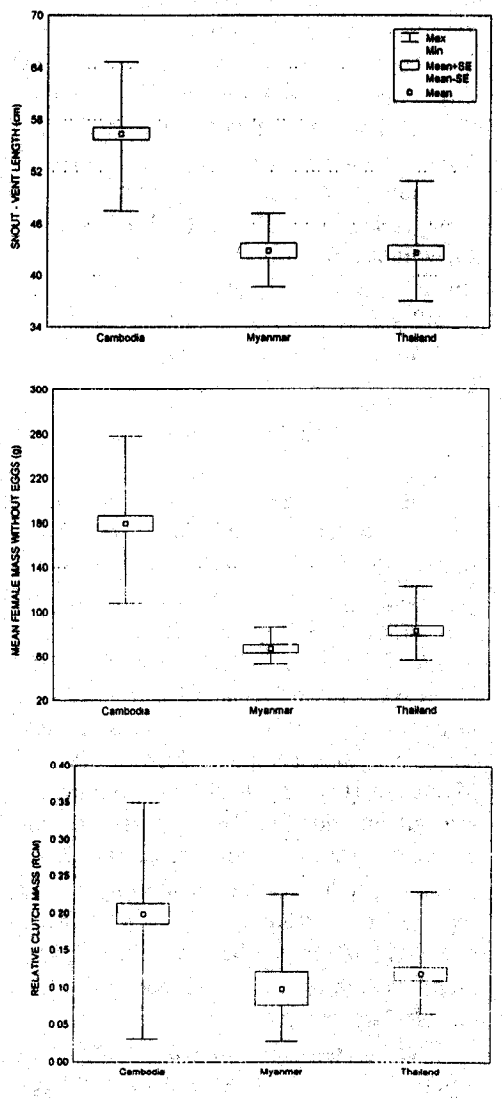

FIGURE 3. Box and whisker plots of snout-vent lengths, female mass, and relative clutch mass (RCM) for samples of gravid female *E. enhydris* from Cambodia (Tonle Sap), Myanmar, and Thailand (Lake Songkhla). The box and whisker plots show the means (small square), standard error of the means (box) and the minimum and maximum values (whiskers).

observed in gestation in October and November. Therefore, they suggested that *E. enhydris* at Tonle Sap produces two clutches per year, one parturition in April-May (the start of the rainy season) and one in November-December (after the end of the rainy season). Embryo stages in our Tonle Sap sample project an earlier parturition date in September or October. Unfortunately we do not have samples from the start of the rainy season and so cannot speculate on a second parturition date. In our Cambodian sample only 62% of the adult females were gravid, leaving open several explanations that are not necessarily exclusive of one another. Some females may not reproduce every year, some may reproduce during one of two reproductive periods each year, or some may reproduce during both periods each year. It does appear that females do synchronize reproduction, as all 10 gravid females collected in Cambodia in June had stage 7 embryos, and 18 of 19 gravid females collected in early August had embryos representing stages 15 to 35. Year-round sampling at Tonle Sap is needed to confirm or reject the suggestion by Saint Girons and Pfeffer (1971) that this population has two reproductive cycles per year.

Embryo stages in the Thailand sample project a parturition date in July or August, which is consistent with the report of a birth in Thailand in July (Taylor, 1965). Females in the sample from Twante, Myanmar were gravid in December but had not yet formed embryos. However, none of the seven females in the sample from Myaungmya, Myanmar were gravid in January. We suggest that this difference is either an artifact of small sample sizes or these two populations are on very different reproductive cycles, despite being separated by a linear-distance of ca. 100 km.

The variation in body size, clutch size, RCM, and reproductive timing across the geographic range of *E. enhydris* may be due to changes in local environmental conditions and food availability. It is possible that over-fishing by humans at Tonle Sap has altered the reproductive ecology of *E. enhydris*. Saint Girons and Pfeffer (1971) reported a litter size of 3 to 15 young for 24 females from Tonle Sap, while our sample of 32 females from 1999 and 2000 had litter sizes ranging from six to 39 and a mean of 20.9. Perhaps an increase in small fish in the lake, due to the removal of

TABLE 1. Statistics on several variables measured on samples of gravid *E. enhydris* from three localities. RCM is relative clutch mass.

		Cambodia (n=32)	Myanmar (n=8)	Thailand (n=18)
Snout-Vent Length	mean	564	428	426
(mm)	range	475-647	387-472	375-509
	SD	4.1	2.6	3.5
Mass	mean	179.0	66.9	81.9
(grams)	range	165.4-194.1	52.9-85.6	55.9-122.3
	SD	39.80	11.35	18.97
Clutch Size	mean	20.3	7.8	8.1
	range	6-39	6-11	5-16
	SD	6.96	2.41	2.48
Clutch Mass	mean	34.6	7.8	9.6
(grams)	range	7.3-80.1	3.4-19.3	5.1-17.6
	SD	0.082	0.063	0.039
RCM	mean	0.199	0.120	0.119
	range	0.03-0.35	0.06-0.22	0.06-0.23
	SD	0.081	0.063	0.039

large fish predators, has allowed the *E. enhydris* population to increase its reproductive output (Stuart et al., 2000). Barron and Andraso (2001) have experimentally demonstrated that an increase in food volume is positively correlated with the number of ovarian follicles that are committed to secondary vitellogenisis in the northern water snake, *Nerodia sipedon*. Dramatically different mean clutch sizes have been reported for different populations across the ranges of several other species of snakes. Fitch (1985) reported means of 17.8 and 30.3 offspring for different populations of the *Boa constrictor* (family Boidae) and means ranging from 4.2 to 24.2 for different populations of the rattlesnake *Crotalus durissus* (family Viperidae).

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LITERATURE CITED

Acharji, M. N. and A. K. Mukherjee. 1966. Report on a collection of snakes from lower Bengal (Reptilia: Ophidia). J. Zool. Soc. India 16:76-81.

Barron, J. N. and G. M. Andraso. 2001. The influence of fall foraging success on follicle number in the northern water snake, *Nerodia sipedon*. J. Herpetol. 35(3):504-507

Biswas, S. and L. N. Acharjyo. 1977. Notes on the ecology and biology of some reptiles occurring in and around Nandankankanan Biological Park, Orissa. Rec. Zool. Sur. India 73:95-109.

Bergman, R. A. M. 1955. L'anatomie de *Enhydris enhydris*. Rivista Biol. Col. 15:1-28.

Campden-Main, S. M. 1970. A Field Guide to the Snakes of South Vietnam. Smithsonian Institution, Washington.

Cantor, T. 1847. Catalogue of reptiles inhabiting the Malayan Peninsula and islands, collected or observed. J. Asiatic Soc. Bengal 16(2): 1-157.

Deuve, J. 1970. Serpents du Laos. Mémoirs O.R.S.T.O.M. No.39.

Fitch, H. S. 1970. Reproductive cycles in lizards and snakes. Mus. Nat. Hist., Univ. Kansas Misc. Publ. (52):1-247.

———— 1985. Variation in clutch and litter size in New World reptiles. Mus. Nat. Hist., Univ. Kansas Misc. Publ. (76):1-76.

Gyi, K. K. 1970. A revision of colubrid snakes of the subfamily Homalopsinae. Univ. Kansas Publ. Mus. Nat. Hist. 20:47-223.

Kopstein, F. 1930. Herpetologische notizen: II. Oologische Beobachtungen an West Javanischen Reptilien. Treubia 11:301-307.

———— 1938. Ein Beitrag zur Eierkunde und zur Fortpflanzung der Malalischen Reptilien. Raffles Bull. Zool. 14:168-174.

Murphy, J. C., H. K. Voris, D. R. Karns, T. Chanard, and K. Suvurat. 1999. The ecology of the water snakes of Ban Tha Hin, Songkhla Province, Thailand. Nat. Hist. Bull. Siam Soc. 47:129-147.

Saint Girons, H. 1972. Les Serpents du Cambodge. Mém. Mus. Nat. d'Hist. Nat. Nou. Ser. A. Zoologie 74:1-170.

Saint Girons, H. and P. Pfeffer. 1971. Le cycle sexuel des serpents du Cambodge. Ann. Sci. Nat. 13:543-572.

———— 1972. Notes sur l'ecologie des serpents du Cambodge. Zool. Med., Rijk. Natuur. Hist. 47:65-86. (English summary)

Smith, M. A. 1914. The snakes of Bangkok. Part 3. J. Nat. Hist. Soc. Siam 1(2):93-104.

Stuart, B. L., J. Smith, K. Davey, P. Din, and S. G. Platt. 2000. Homalopsine watersnakes: The harvest and trade from Tonle Sap, Cambodia. TRAFFIC Bulletin 18(3):115-122.

Taylor, E. H. 1965. The serpents of Thailand and adjacent waters. Univ. Kansas Sci. Bull. 45:609-1096.

Theobald, W. 1868. Catalogue of the reptiles of British Burma embracing the Provinces of Pegu, Martaban, and Tenasserim; with descriptions of new or little-known species. J. Linn. Soc. 10:4-67.

———— 1876. Descriptive Catalogue of the Reptiles of British India. Thacker, Spink, and Co., Calcutta.

Voris, H. K. and J. C. Murphy. 2002. Prey and predators of homalopsine snakes. J. Nat. Hist.: In press.

Wall, F. 1925 [1926]. Notes on snakes collected in Burma in 1924. J. Bombay Nat. Hist. Soc. 30:805-821.

Wall, F. and G. H. Evans. 1901. Notes on Ophidia collected in Burma from May to December, 1899. J. Bombay Nat. Hist. Soc. 13:343-354.

Zehr, D. R. 1962. Stages in the normal development of the common garter snake, *Thamnophis sirtalis sirtalis*. Copeia 1962:322-329.

APPENDIX I

Gravid females of *Enhydris enhydris* examined in this study. Specific localities and geographic coordinates are provided in the text.

Cambodia: FMNH 259315-18, 259361, 259364, 259366-71, 259374-92 (n=32 gravid females). Myanmar: KU 92470, 92473, 92475-77, 92480, 92486, 92491 (n=8 gravid females). Thailand: FMNH 60973, 252538, 252540, 252545, 252564-65, 252571, 252573, 252578-79, 252592, 252594, 252662-63, 252665, 252668-670, 257397 (n=19 gravid females.

APPENDIX II

Summary of the literature on reproduction in *Enhydris enhydris*. Data that could not be traced to a specific locality were omitted.

Copulation date.–November, Burma (Myanmar), Wall and Evans (1901).

Gestation.–August 6, gravid, west Java, Kopstein (1930); April-July, Thailand, Smith (1914); March, Burma (Myanmar), Wall (1912).

Litter Size.–9 young, India, Acharji and Mukherjee (1966); 3-13 young, Java?, Translation of Bergman (1955); 14 young, NE India, Biswas and Acharyo (1977); 29 eggs, NE India, Biswas and Acharyo (1977); 13 young, Con Son, Vietnam, Campden-Main (1970); 11 young, Cantor (1847); 9-11 eggs, Gyi (1970); 4 and 11 young, Java, Kopstein (1930, 1938); 10 embryos, Thailand, Smith (1914); 18 in well development, Thailand, Smith (1914); single still born, last of brood, Thailand, Smith (1914); 11 young, Thailand, Taylor (1965); 6 and 11 eggs, Burma (Myanmar), Theobald (1876); 9 young, Wall (1925).

Parturition Date.–July 3, India, Acharji and Mukherjee (1966); June 14, NE India, Biswas and Acharyo (1977); January 25, NE India, Biswas and Acharyo (1977); April and May with rain, Deuve (1970); July 8, 1959, Thailand, Taylor (1965).

Size of Offspring.–175-198 mm, India, Acharji and Mukherjee (1966); 175-185 mm, NE India, Biswas and Acharyo (1977); neonates 155 mm, Java, Kopstein (1930); 5 females 201-206 mm, Wall (1925); 3 females 185-193 mm, Wall (1925).

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