Original article

Diet, female reproduction and conservation of Jagor's water snake, *Enhydris jagorii* in Bung Ka Loh wetland, Uttaradit province, Thailand

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ARTICLE INFO

ABSTRACT

Jagor's water snake (*Enhydris jagorii*) is a freshwater snake that is endemic to the Chao Phraya-Ta Chin basin, Thailand. However, habitat change and destruction are the main threats to this snake, where a large area of the wetland has been rapidly transformed into urban and agricultural areas. Moreover, uncontrolled fishing seriously threatens the remaining population of this snake. In order to protect this species, information on its natural history is required. This study was conducted in the Bung Ka Loh wetland during October, 2010 to August, 2014 when 108 specimens of this species were collected. Analysis of the stomach contents revealed that it is piscivorous, with cyprinids being the dominant prey. Prey items were usually less than 10% of the snake body mass and multiple prey items were occasionally found. No significant difference in diet was noted between the sexes. In addition, predation on this snake by *Cylindrophis ruffus* was first recorded in this study. The smallest gravid female collected had a snout-vent length of 34.0 cm. The clutch size and mass ranged from 1 to 28 embryos and 3.1–123.0 g, respectively, and both of these quantities increased significantly with increased female size. Reproduction was possibly seasonal and occurred in the rainy season. A preliminary study of other wetlands in the central plain of Thailand failed to detect the existence of this species. Accordingly, the conservation status of this species should be changed from Data Deficient to Critically Endangered.

Introduction

Oriental-Australian rear-fanged water snakes belong to the Family Homalopsidae and these aquatic and semi-aquatic snakes can be found along a northeast–southwest axis from the Indus River in Pakistan across India and Southeast Asia eastward to Micronesia and Queensland, Australia (Gyi, 1970; Mattison, 2007; Murphy, 2007; Murphy and Voris, 2014). Thailand is centrally located within this distribution and not surprisingly, since freshwater resources are widespread in Thailand, the aquatic fauna and flora are diverse, including divergent species of freshwater snakes, with 16 species in 10 genera of aquatic and semi-aquatic snakes in the family Homalopsidae out of 53 species in 27 genera worldwide (Vidthayanon et al., 1997; Karns et al., 2010; Ito and Barfod, 2014).

This group of snakes lives in freshwater habitats such as canals, streams, rivers, ponds and lakes and is usually associated with mud substrates (Gyi, 1970; Cox, 1991; Karns et al., 2005; Murphy and Voris, 2005; Murphy, 2007; Pongcharoen, 2008; Karns et al., 2010; Cox et al., 2012; Murphy and Voris, 2014). These snakes are viviparous (Murphy, 2007; Voris et al., 2008) and are distinguished from other kinds by a suite of shared anatomical characteristics related to their aquatic habitats including: crescent-shaped valvular nostrils, dorsally oriented small eyes, a watertight seal on the mouth and slightly compressed tails in some species (Voris and Murphy, 2002; Murphy, 2007; Pongcharoen, 2008; Karns et al., 2010). These opisthoglyphous freshwater snakes feed mainly on aquatic vertebrates such as fish and amphibians, often comprising a significant proportion of the vertebrate biomass; hence, they are important to the trophic dynamics of wetlands in Southeast Asia (Murphy, 2007; Voris and Murphy, 2002; Pongcharoen, 2008). Although, some aspects on the anatomy, taxonomy and geographic distribution of these snakes have been studied in a few widely

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distributed and well known species, the study of their natural history is very limited and consists primarily of ecological notes and anecdotes (Murphy et al., 1999). However, some freshwater snakes, including *Enhydris jagorii*, are extremely restricted in distribution and are endemic to specific drainage systems and their natural history is poorly known.

E. jagorii (Fig. 1A) is an excellent example of an extremely restricted homalopsid species in Thailand with an essentially unknown life history. This freshwater snake was reported as an endemic species found only in the Chao Phraya-Ta Chin basin in the central plain of Thailand, with the holotype being described from the specimen collected in the vicinity of Bangkok by Peters in 1863 (Peters, 1863). Since then, more records on the potential distribution of this snake have only been documented from within this region (Nabhitabhata and Chan-ard, 2005; Murphy, 2007; Cox et al., 2012). However, no specific locality was provided until Karns et al. (2010) added the new distribution of this freshwater snake in the Bung Ka Loh wetland located in northern Thailand and close to the Nan River, a tributary of the Chao Phraya River (Fig. 1B). Like other wetlands in this region, this wetland has been substantially affected by numerous kinds of human activities that pose serious threats to the population of aquatic life forms. In addition to habitat change and destruction, the remaining *E. jagorii* population is also threatened by extensive fishing with narrow-meshed gill nets that drown many snakes. In order to protect *E. jagorii*, appropriate policies and procedures on conservation and management are urgently required. However, the basic information on the natural history of *E. jagorii* is virtually unknown. Hence, this study aimed to reveal some information on the natural history of *E. jagorii*, such as its diet and reproduction. Furthermore, the current distribution of *E. jagorii* was investigated following a preliminary survey of the wetlands across the central plain region of Thailand. Due to the previous lack of information on the distribution of this species, the international and national conservation status listings (shown as Data Deficient in International Union for Conservation of Nature, 2014) are underestimated and the proper conservation status is suggested along with potential conservation measures.

Materials and methods

Study area and specimen collection

The diet and female reproduction of *E. jagorii* was investigated in the Bung Ka Loh wetland (Fig. 1B) during October 2010 to August,
2012. This wetland is located close to the downtown Muang district, Uttaradit province in the northern part of the central plain of Thailand. The wetland covers an area of about 10.5 km^2 with a water depth of 2—3 m at the center and is also situated close to the Nan River which flows into the Chao Phraya basin. It is a natural freshwater wetland, where the water level is increased by running surface water inflow from the Nan River in the rainy season and the water is then stored for multiple purposes, such as fisheries, plantations, agriculture, raising livestock and hunting. The wetland has a very high biodiversity of plant and animal species, including at least four species of freshwater homalopsid snakes: the rainbow water snake (*Enhydris enhydris*), the Bocourt's water snake (*Subsessor bocourti*), the puff-faced water snake (*Homalopsis mereljcoxi*) and *E. jagorii* (Karns et al., 2010). Snake specimens in this study were obtained from locals involved in fishing whose traps occasionally caught snakes. For this reason, local fishers living around the wetland were contacted to collect the specimens monthly. Multiple mesh sizes of gill nets and funnel traps were used and set mostly in shallow water with a dense vegetation of aquatic and riparian plants (Fig. 2). Traps were placed in the late afternoon and regularly checked for fish and snakes in the early morning. Snake specimens were observed, identified and separated well before continuing with other procedures. Specimens were subjected to sex determination, measurement and weighing using a measurement tape and a digital balance to the nearest 1 mm and 1 g, respectively. Dead specimens were kept in 95% alcohol for preservation and were later deposited in the collection of the Chulalongkorn University Museum of Zoology.

Diet

Stomach contents were obtained from 3 live and 17 dead specimens of *E. jagorii*. Live specimens with diets were gently forced to regurgitate their stomach contents by hand whereas dead specimens were examined using dissection. Prey items from the stomach were immediately identified and weighed to the nearest 0.1 g using a digital balance. Intact prey items were identified to the species level, whereas partially digested prey items were identified to at least the family level. Almost completely digested fish with fin rays, scales and bones were classified as unidentifiable fish. Partially digested prey items were estimated for their weight by comparison with intact conspecific specimens (Voris and Murphy, 2002). The prey type, proportion of prey types and the
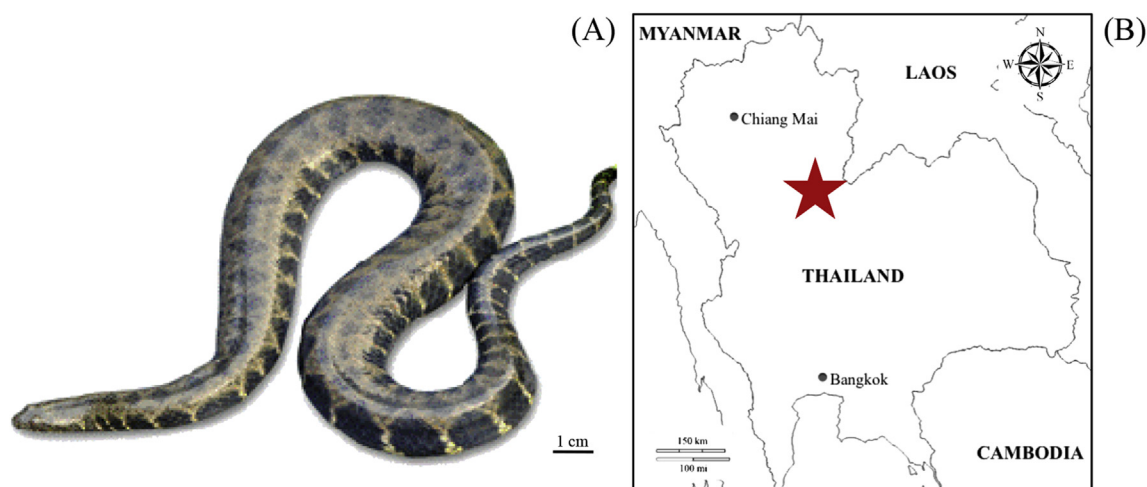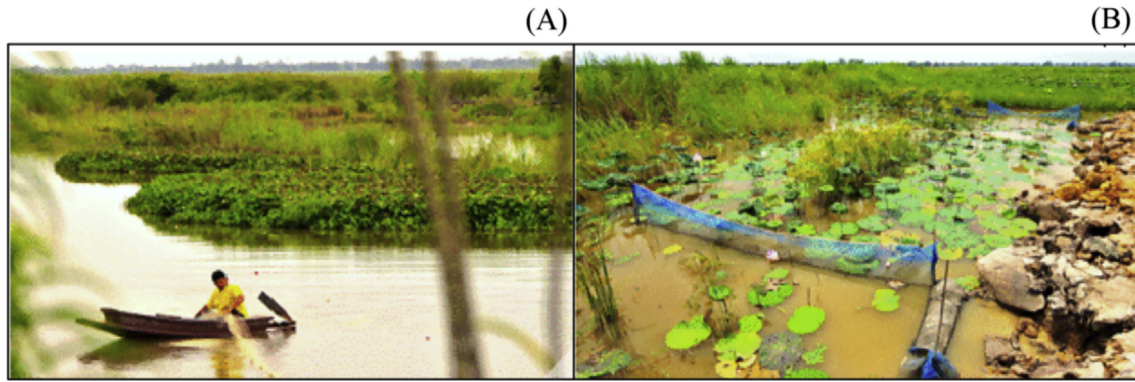

Fig. 1. (A) Male Jagor's water snake (*E. jagorii*); (B) star shows location of Bung Ka Loh wetland in northern part of the central plain of Thailand.

Fig. 2. Fish traps set by local fishermen inside Bung Ka Loh wetland, Uttaradit province, Thailand: (A) gill nets of varying mesh sizes were used all over the wetlands; (B) funnel traps were set with line defenders along the edge of the wetland.

prey:snake mass ratio were compared between male and female snakes using the Mann–Whitney U-test.

Female reproduction

Both live and dead gravid females were used to assess the female reproductive season and clutch sizes. Some live gravid females were kept in captivity until they gave birth and then the neonates of each female, after counting and weighing, along with the female were released at the study site. Dead gravid females were dissected at the abdominal cavity and the number of follicles and vitellogenic eggs, clutch mass, clutch size and the stage of the embryos were recorded. A mature female was defined as being equal to or greater than the snout-vent length (SVL) of the smallest female that contained enlarged vitellogenic follicles, oviducal eggs or exhibited thickened and muscular oviducts indicating the postpartum period (Karns et al., 2010; Voris et al., 2012). For assessing any seasonality in reproduction, the number of gravid females with small follicles in their ovary, developing embryos and postpartum gravid females in each month were recorded. The developmental stage of the embryos was categorized following Zehr (1962) with four periods of visible, developing stages categorized. The first period was considered as the early developing stage of embryos (10th–20th stage), the second as the middle developing stage of embryos (21st–30th stage), the third as the late developing stage of embryos (31st–36th stage) and the last as the full-term stage of embryos (37th stage). The relative clutch mass (RCM) was calculated as the ratio of the clutch mass to the maternal mass (excluding the clutch mass). The relationship between female size traits, SVL, tail length (TL) and body mass (BM), with the clutch mass and clutch size were examined using Pearson's correlation. The data on the daily precipitation, air temperature and relative humidity throughout the study period were collected from the Meteorological Department located downtown in Uttaradit. Correlations between female reproduction (clutch size and mass) and these physical factors were also examined using Pearson's test.

Distribution and conservation status

The distribution of *E. jagorii* was evaluated from a preliminary survey of selected wetlands inside the Chao Phraya basin, Thailand. The wetlands were initially selected by considering the potential suitability of the habitat for the freshwater homalopsid snake, based on having an area of at least 3 km^2 with dense vegetation in the water body and along the edge of the wetland. The area of each wetland was estimated using satellite pictures and the Polygon Measurement program in Google Earth (https://earth.google.com/;

software version 7.1.2.2014). In this study, 18 wetlands were surveyed and local fishers were interviewed, using photographs and live specimens of *E. jagorii* to ascertain if they had trapped any specimens in their region. Furthermore, the conservation status of *E. jagorii* was considered at the international and national levels. The current status was obtained from the International Union for Conservation of Nature red data list of threatened species (International Union for Conservation of Nature, 2014), the appendices of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES; CITES, 2013) and the Office of Natural Resources and Environmental Policy and Planning of Thailand (Nabhitabhata and Chan-ard, 2005). From the obtained survey data, the proper conservation status of *E. jagorii* was then determined following the categories of each organization.

Results and discussion

Diet

From 108 collected specimens, 5 males and 15 females of *E. jagorii* specimens had detectable prey items in their stomachs. For these 20 snakes, a total of 22 prey items, all fish, were found (Table 1). Of the 22 prey specimens, 17 items (77.3%) were identified to at least the family level while 5 (22.7%) were unidentifiable fish. Of the 15 females with prey items, 13 had a single prey item and 2 (11.8%) had 2 prey items. Thirteen of these 17 prey fish were identified to at least the family level, and were from 6 families, with the Cyprinidae (24%) and Anabantidae (23.5%) being the dominant prey types and the Channidae (11.8%), Bagridae (5.9%), Nandidae (5.9%) and Osphronemidae (5.9%) as minor (Fig. 3). Nevertheless, four prey items were unidentifiable fish (fish bones and rays). The mean prey mass was 14.0 ± 12.1 g (range 0.8–41.0 g) with a mean prey:female snake mass ratio of 6.5 ± 6.8% (range 0.31–24.9%). For the five males with prey items, only one prey item per male *E. jagorii* was found in the stomach, of which one was unidentifiable to the family level and the other four were three cyprinids and one osphronemid. The mean prey mass per snake was 6.38 ± 2.24 g (range 5.0–9.7 g) with a mean prey:male snake mass ratio of 6.38 ± 5.13% (range 1.9–13.4%).

Thus, for both male and female *E. jagorii* specimens with prey, the highest proportion of prey type was cyprinid fish (31.8%), with a mean prey mass of 12.2 ± 11.0 g (range 0.8–41.0 g) and a mean prey:snake mass ratio of 6.8 ± 6.3%. The largest and the smallest prey items and the highest and lowest prey:snake mass were found in females, but the sample size was too small and skewed to allow any meaningful interpretation. Likewise, the absence of any significant differences in the prey types, prey mass and prey:snake
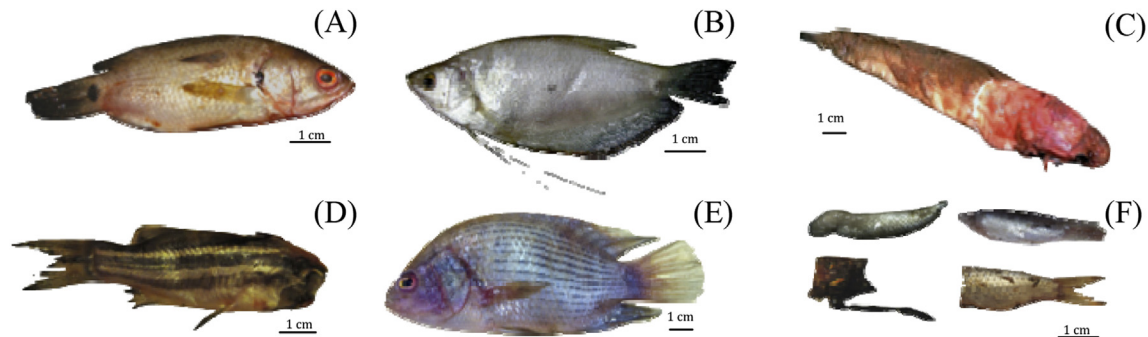
Table 1
Prey items from the stomach contents of 15 females and 5 males of *E. jagorii* specimens collected in Bung Ka Loh wetland, Uttaradit province, Thailand during October 2010 to August 2012.

Prey type		Prey mass (g)	%Weight	Sex	SVL[a] (cm)	Mass (g)
Family	Species					
Anabantidae	*Anabas testudineus*	25.00	4.50	F	60.00	561.00
Anabantidae	*Anabas testudineus*	4.70	1.90	F	48.30	248.00
Anabantidae	*Anabas testudineus*	4.00	1.30	F	52.80	320.00
Anabantidae	*Anabas testudineus*	2.60	0.80			
Bagridae	*Mystus mysticetus*	16.00	10.70	F	40.00	150.00
Channidae	*Channa striata*	41.00	12.90	F	51.00	318.00
Channidae	*Channa striata*	15.00	3.30	F	55.00	456.00
Nandidae	*Pristolepis fasciatus*	32.20	6.30	F	60.00	511.00
Osphronemidae	*Trichopodus trichopterus*	0.80	0.30	F	50.50	254.00
Cyprinidae	–	6.50	2.80	F	46.40	236.00
Cyprinidae	–	10.00	6.60	F	36.70	152.00
Cyprinidae	–	12.00	7.90			
Cyprinidae	–	12.70	24.90	F	30.50	51.00
Unidentifiable fish	–	–	–	F	50.10	310.00
Unidentifiable fish	–	–	–	F	51.50	387.00
Unidentifiable fish	–	–	–	F	56.00	423.00
Unidentifiable fish	–	–	–	F	48.10	263.00
Osphronemidae	*Trichopodus trichopterus*	9.70	5.90	M	42.00	165.00
Cyprinidae	–	5.70	13.30	M	27.00	43.00
Cyprinidae	–	5.00	10.60	M	29.80	47.00
Cyprinidae	–	5.00	1.90	M	50.00	267.00
Unidentifiable fish	–	–	–	M	47.20	222.00

[a] Snout-vent length.

Fig. 3. Prey types found inside the digestive system of Jagor's water snake, *E. jagorii* collected within the Bung Ka Loh wetland: (A) climbing perch, *Anabas testudineus*, Family Anabantidae; (B) three spotted gourami, *Trichopodus trichopterus*, Family Osphronemidae; (C) common snakehead, *Channa striata*, Family Channidae; (D) striped catfish, *Mytus mysticetus*, Family Bagridae; (E) banded leaf fish, *Pristolepis fasciatus*, Family Nandidae; (F) partially digested fish, Family Cyprinidae.

mass ratio between males and females is tentative due to the low sample size. The relative population sizes of the different prey species in this habitat are not available, although cyprinids were very common, and so any potential for prey selection by species as well as size rather than simple availability cannot be alluded to. Interestingly, the predation of *E. jagorii* was first recorded here, where a male *E. jagorii* (161 g) was found as a prey item inside the stomach of a female red tailed pipe snake, *Cylindrophis ruffus* (family Cylindrophiidae), of 279 g, giving a large prey:predator mass proportion of 57.7%.

Female reproduction

Reproductive biology

Of the 54 collected female specimens of *E. jagorii*, 36 specimens (66.7%) were gravid females. The smallest gravid female recorded in this study had an SVL of 34.0 cm. The SVL, TL, neck girth (NG), body girth (BG) and BM of gravid females collected within the Bung Ka Loh wetland are summarized in Table 2.

The mean clutch size was 11 ± 9 embryos (range 1–28; n = 18) with a mean clutch mass of 56.3 ± 50.6 g (range 3.1–123.0 g; n = 14) and RCM of 0.18 ± 0.14 (range 0.03–0.52; n = 14). In addition, the mean fat body weight of gravid females was 25.6 ± 11.6 g (range 10.2–47.7 g). There was a significant relationship between the size of gravid females and their clutch size, where the clutch size was significantly related to the SVL (r = 0.575, $p = 0.013$), TL (r = 0.607, $p = 0.036$) and BM (r = 0.526, $p = 0.025$) (Fig. 4A–C), while the clutch mass was significantly related to the SVL (r = 0.595, $p = 0.019$) and BM (r = 0.533, $p = 0.041$) (Fig. 4B–D).

Reproductive cycle

No specimens were collected in November 2010, March 2011 and during December 2011 to May 2012, but in the remaining 15 months of this survey period, 49 females with small follicles in the ovary were found, with the highest monthly number being eight specimens in May, 2011, while the lowest monthly number of collected gravid females with small follicles was single specimens in December, 2010 and in January, February and April, 2011 (Table 3). For gravid females with developing embryos, 36 specimens were collected over the same study period. The highest and lowest monthly number of collected gravid females were eight specimens in May, 2011 and one specimen in October and

Table 2
Size of gravid *E. jagorii* collected within the Bung Ka Loh wetland, Uttaradit province, Thailand. Data are shown as mean ± SD and the range (minimum–maximum).

SVL[a] (cm)	TL[b] (cm)	Neck girth (cm)	Body girth (cm)	Body mass (g)	TL:SVL
50.73 ± 7.07 (34.0–65.0)	13.34 ± 1.83 (10.0–16.1)	4.35 ± 0.89 (3.0–6.7)	10.36 ± 1.97 (6.5–14.5)	345.25 ± 154.93 (94.0–707.0)	0.26 ± 0.02 (0.23–0.30)

[a] Snout-vent length.
[b] Tail length.

Fig. 4. Regression line and 95% confidence limits (dotted lines) of clutch size (A, C) and clutch mass (B, D) versus the SVL (A, D) and body mass (B, C) of gravid *E. jagorii* females collected in Bung Ka Loh wetland, Uttaradit province, Thailand. All correlations are significant ($p < 0.05$).

December, 2010, in February, April and September, 2011 and in July, 2012, respectively. With respect to the four categories of developing embryos found during the study period, 16 gravid females contained embryos in the early developing stage in 6 of the 15 months (December, 2010, May and September to November, 2011 and August, 2012), with the highest monthly number being five females in November, 2011 (Table 3). Two gravid females containing embryos in the middle development stage were collected—one each in February and April, 2011—whilst only one female with embryos in the late development stage was collected (May, 2011). Lastly, 15

Table 3
Number of collected females, gravid females and gravid females with embryos in each developmental stage of *E. jagorii* collected from Bung Ka Loh wetland, Uttaradit province, Thailand. Samples were collected each month from October, 2010 to August, 2012.

Number	2010			2011												2012							
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.
Collected females	4	–	2	1	1	–	1	8	3	3	2	4	6	5	–	–	–	–	–	–	4	2	8
Gravid females	1	–	1	–	1	–	1	8	3	3	–	1	3	5	–	–	–	–	–	–	4	1	4
Gravid females with embryos in each developmental stage																							
Early	–	–	1	–	–	–	–	2	–	–	–	1	3	5	–	–	–	–	–	–	–	–	4
Middle	–	–	–	–	1	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Late	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Full-term	–	–	–	–	–	–	–	5	3	3	–	–	–	–	–	–	–	–	–	–	4	–	–
Postpartum	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–

gravid females with full-term stage embryos were collected but only in 4 of the 15 months (May to July, 2011 and June, 2012). In addition, two gravid females at the postpartum stage were found—one each in October, 2010 and July, 2012. Nevertheless, small follicles were also found inside the ovaries of these gravid females.

Correlation between female reproduction and physical factors

The precipitation, air temperature and relative humidity of this wetland were obtained from the nearest weather station in Muang district, Uttaradit province. The monthly precipitation level was used to determine the season at the study site. During this study period, the rainy season occurred over 13 mth in October, 2010, March to October, 2011 and May to August, 2012, whereas the dry season occurred during 10 mth from November, 2010 to February, 2011 and from November, 2011 to April, 2012. The mean relative humidity was positively correlated with the number of gravid females each month with either small follicles ($r = 0.595$, $p = 0.003$) or developing embryos ($r = 0.418$, $p = 0.047$).

Distribution and conservation status

Eighteen wetlands located inside the Chao Phraya-Ta Chin basin were surveyed for the potential presence of *E. jagorii*. These comprised wetlands in the Ping River (1 site), Wang River (3 sites), Yom River (3 sites), Nan River (6 sites) and Chao Phraya River (5 sites). Most local fishers from the selected wetlands around the Ping, Wang and Yom Rivers did not recognize *E. jagorii*, but a few thought they did. However, at least some of these were subject to misidentification with Bocourt's water snake (*S. bocourti*) and no actual specimens of *E. jagorii* were observed in these wetlands during this study to confirm those reports. For the six wetlands surveyed around the Nan River, *E. jagorii* was recognized by local fishers from photographs and live specimens at the two locations in the Bung Ka Loh (N01) and Bung Mine (N02) wetlands. This was confirmed by observation and specimen collection in the Bung Ka Loh wetland during the study period, but no specimens of *E. jagorii* were observed or collected for confirmation in the Bung Mine wetland (N02) during the study period. In addition, this wetland was seriously disturbed by human activities. None of the of the interviewed local fishersen in the five selected wetlands close to the Chao Phraya River reported knowledge of *E. jagorii* and no specimens were observed or caught in traps inside these selected wetlands. Even within the biodiversity-rich and largest freshwater wetland of the Chao Phraya basin, the Bung Boraphet wetland (C01), no evidence of the existence of *E. jagorii* was found during the study period.

The International Union for Conservation of Nature red list of threatened species (International Union for Conservation of Nature, 2014), contains 39 homalopsid species classified into four categories—Data Deficient (16 species), Least Concern (20 species), Vulnerable (1 species) and Endangered (2 species). Of these, *E. jagorii* was categorized as Data Deficient, a status that refers to an animal when there is inadequate information to make an assessment of its risk of extinction based on its distribution and population status. Moreover, *E. jagorii* is not currently classified in any of the Appendices of CITES (CITES, 2013). At a national level, *E. jagorii* was classified as Data Deficient (Nabhitabhata and Chan-ard, 2005).

Most species of freshwater homalopsid snakes in the genus *Enhydris* have been reported to be mainly or totally piscivorous (Murphy et al., 2002; Voris and Murphy, 2002; Karns et al., 2005; Murphy, 2007; Pongcharoen, 2008; Cox et al., 2012). The stomach contents analysis of the current study suggested that *E. jagorii* is also exclusively piscivorous and feeds on fish of many types. Six families of fish were found as prey types with members of the

Cyprinidae being the main prey type, which could be explained by the abundance of these fish inside this wetland. Prey items were usually small (less than 10% of the snake body mass) and multiple prey items were occasionally found (2 out of 20 specimens had 2 prey items). Unfortunately, a high proportion (22.7% of items) of unidentifiable fish was found in their stomachs, but this is consistent with a high rate of passage of small prey items (Voris et al., 2012). Although significant differences in the diets of male and female freshwater homalopsid snakes have been reported (Karns et al., 2005, 2010; Pongcharoen, 2008), there was no significant difference in the prey types, prey mass and the prey: snake mass between male and female *E. jagorii* in the current study. Furthermore, the two exotic fish species—nilotica (*Oreochormis niloticus;* Ciclidae) and the red bellied pacu (*Piaractus brachypomus;* Characidae)—were accepted as prey by live gravid females of *E. jagorii* in captivity, while guppy (*Poecillia reticulate;* Porciliidae) was also eaten by neonates in captivity (after birth from captive gravid females before releasing in the Bung Ka Loh wetland). Feeding on exotic fish by freshwater homalopsid snakes has been reported previously, where *O. niloticus* was found in the stomach contents of the Cox's masked water snake (*H. mereljcoxi*) collected from the Bung Ka Loh wetland (Karns et al., 2010). In addition, homalopsid snakes have been reported to have a variety of predators, including larger fish, mammals, birds and reptiles, and especially snakes from several different families (Voris and Murphy, 2002; Murphy, 2007; Pongcharoen, 2008). Indeed, snakes including the red tailed pipe snake (*C. ruffus*) were reported to be the most important predators of homalopsid snakes in several different localities (Pongcharoen, 2008; Voris and Murphy, 2002). Consistent with this, in the current study, *C. ruffus* was first recorded as a predator of *E. jagorii* in the Bung Ta Loh wetland.

The endemic existence of *E. jagorii* in the Bung Ka Loh wetland has been reported previously (Karns et al., 2010), but no information on its reproduction was provided. Thus, the first information on the female reproduction of this freshwater snake has been reported in the current study. A significant correlation and positive relationship between the clutch size and clutch mass of gravid females with their size, in terms of the SVL, TL and BM, were found, suggesting that females of this freshwater snake exhibit a significant positive size-fecundity relationship, where larger females reproduce a larger clutch size and clutch mass. Similarly, larger females producing a larger clutch have been reported for other species of freshwater homalopsid snakes (Murphy et al., 2002; Karns et al., 2005; Brooks et al., 2008; Pongcharoen, 2008; Karns et al., 2010).

The relationship of the reproductive cycle and physical factors of gravid females of freshwater homalopsid snakes has been reported previously, where both seasonal and continuous reproduction were documented from female specimens collected inside Thailand (Brooks et al., 2008; Pongcharoen, 2008). In the current study, the number of collected gravid females with developing embryos in each developing stage was found to peak in the two rainy season periods (March to October, 2011 and May to August, 2012). The number of collected gravid females with developing embryos in each stage was also significantly related to the relative humidity of this wetland. Moreover, female *E. jagorii* reproduction inside the wetland was potentially seasonal, occurring in the rainy season.

Since 1863, a few reports on the distribution of *E. jagorii* have appeared in the literature, and the holotype was collected from within the vicinity of Bangkok (Peters, 1863; Murphy, 2007; Cox et al., 2012). However, unambiguous localities were not provided. In contrast, the study on semi-aquatic snake communities by Karns et al. (2010) reported the locality of *E. jagorii* in the Bung Ka Loh wetland. In the current study, this wetland was the only one from 18 surveyed wetlands within the Chao Phraya-Ta Chin basin where

reliable evidence of the existence of *E. jagorii* was obtained. Although local fishers at nearby wetlands apparently recognized this snake, no specimen was collected or observed from these wetlands to confirm this and there was some evidence of the misidentification of other species as *E. jagorii*. Therefore, it appears that *E. jagorii* is an endemic species found only within the central plain of Thailand and potentially only in the Bung Ka Loh wetland.

The restricted distribution of *E. jagorii* indicates that the conservation status of this freshwater snake should be re-classified at both the international and national levels. From its distribution, the new status of *E. jagorii* should re-classified following the International Union for Conservation of Nature red list categories and criteria version 3.1: International Union for Conservation of Nature (2001)—based on its geographic range only, due to insufficient data on the population size—as being in criteria B1a, b (i, ii, iii, iv) and c (i, ii, iii) and B2a, b (i, ii, iii, iv) and c (i, ii, iii) of the Critically Endangered status criteria. Hence, the current conservation status at the international and national levels of *E. jagorii* should be Critically Endangered. Although *E. jagorii* is not listed in CITES Appendices I, II or III, there was no evidence of trade of this species during the study period, and so trading is not likely to be a main threat to this freshwater snake at present.

Conservation of E. jagorii in Bung Ka Loh wetland

A large proportion of the wetlands within the central plain of Thailand have become urbanized and converted into agricultural areas, posing a threat to the large number of homalopsid species. Nevertheless, homalopsids have received little attention despite the fact that *E. jagorii* and Chan-ard's water snake (*Enhydris chanardi*) are in need of immediate protection (Murphy, 2007). *E. jagorii* was considered endemic to the wetlands located in the Chao Phraya-Ta Chin basin on the central plain of Thailand, and now specifically to the Bung Ka Loh wetland. However, the area is subject to a large proportion of ongoing habitat change and destruction caused by the local administrative agency, which is likely to be the greatest threat to the survival of this species and indeed all homalopsid snakes (Murphy, 2007). Large areas of the northern part of the Bung Ka Loh wetland have already been transformed into a local university campus, local government offices and a power plant. In addition, one of the main causes of mortality observed in this wetland was due to the trapping of freshwater snakes in small-meshed gill nets used for fishing, resulting in a large proportion of the trapped snakes being found dead. Because of these threats in its potentially last known habitat, *E. jagorii* is potentially at risk of becoming extinct and so conservation and management of this freshwater homalopsid snake is very urgent in terms of implementing both appropriate conservation procedures and policies. To conserve this species, the control of fishing methods and intensity in the wetland as well as the establishment of a protected area are required. Furthermore, captive breeding and reintroduction at alternative suitable locations might be possible following more information on the diet and reproductive cycle of *E. jagorii* along the lines of that obtained from the current study. In addition, data on the population size and dynamics, life history traits and microhabitat of *E. jagorii* are currently unknown but are important for developing conservation and management programs. Therefore, further research on *E. jagorii* should be conducted to obtain more precise and complete information.

Conflict of interest

The main conflicts are habitat change and destruction and incontrolable fishing inside the wetland.

Acknowledgements

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