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The discovery of a breeding population of Japanese pond turtles at the northern limit of the Pacific Ocean side of Japan

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Abstract

The Japanese pond turtle (*Mauremys japonica*) is endemic to Japan, inhabiting Honshu, Shikoku, Kyushu, and smaller islands. The ecology and distribution of *M. japonica* have been well studied in western Japan. In contrast, much less studies of this species have been performed in the Kanto and Hokuriku regions and its northern limit is not clear. Here, we report the discovery of a local population of *M. japonica* in a river in the Ibaraki Prefecture, representing the northern limit of its range on the Pacific Ocean side of Japan. This investigation revealed the inhabitation of over 50 turtles aged 0–10 years in the river. The predicted growth curves of both male and female turtles were comparable to those in other river and wetland populations previously reported, indicating that this population reproducing and growing normally. We also discuss future conservation strategies for this population.

Key words: Ibaraki Prefecture; Kanto region; *Mauremys japonica*; Population structure; von Bertalanffy growth curve

Introduction

The Japanese pond turtle (*Mauremys japonica*) is endemic to the Japanese main islands, Honshu, Shikoku, and Kyushu and the adjacent islands (Yasukawa et al. 2008). Multiple anthropogenic factors have contributed to the turtle's decline or disappearance, including habitat destruction (e.g., river alteration), over-harvesting for commercial use, competition with alien freshwater turtles (red-eared slider, *Trachemys scripta elegans*; Reeves' pond turtle, *Mauremys reevesii*), reproductive interference and gene pollution through hybridization caused by related alien species (*M. reevesii*), predation by alien raccoons (*Procyon lotor*) in Japan (Yasukawa et al. 2008; Suzuki et al. 2014; Kosuge and Kobayashi 2015; Ogano et al. 2015a; Kagayama et al. 2020).

Mauremys japonica inhabits a wide area from Kagoshima Prefecture (Tanegashima Island) at its southern limit to Ibaraki Prefecture in the Kanto region and Niigata Prefecture in the Hokuriku region (Yasukawa et al. 2008; Suzuki and Hikida 2011; Kagayama et al. 2020). Although large-scale evaluations of the current status of *M. japonica* have been conducted primarily in western Japan (Taniguchi et al. 2015), there have been only a few studies in the Kanto (e.g., Tempaku et al. 2009; Ogano et al. 2015b; Kagayama et al. 2017) and Hokuriku regions (e.g., Suzuki and Hikida 2011). Consequently, the northern limit range of *M. japonica* has not yet been determined.

Few surveys of freshwater turtle distribution have been conducted in the Ibaraki Prefecture. In

addition, fewer reports indicate that alien turtle species (e.g., *M. reevesii* including hybrids; *T. s. elegans*) have been established (e.g., Hayase 2010; Ogano 2021). Although Ibaraki Prefecture is considered the northern limit of *M. japonica* on the Pacific Ocean side, distribution records are limited (Yasukawa et al. 2008) and the current status is poorly understood. Specifically, there are distribution records from the cities of Mito, Omitama, Hokota, and Namegata (Ibaraki Prefectural Government 2016), but no studies evaluating their abundance or population structure. Therefore, the status of *M. japonica* in the Red Data Book remains Data Deficient (DD) because no breeding populations have been documented in Ibaraki Prefecture to date (Ibaraki Prefectural Government 2016).

This study aimed to determine the northern limit of the distribution of *M. japonica* on the Pacific Ocean side of Japan. Additionally, we evaluated the population structure and predicted the growth pattern of a study population, and discussed directions for future conservation strategies.

Materials and Methods

Study area

Rivers flowing into Lake Kitaura and Lake Kasumigaura in Ibaraki Prefecture, which are considered to be the northern limit of the Pacific Ocean side in the natural distribution range of *M. japonica* (Yasukawa et al. 2008), were selected as the study rivers. This study was conducted on a river (approximately 1 km) in the upland region of Namegata City, Ibaraki Prefecture, Japan, from 2018 to 2021 (Fig. 1). The name, precise latitude, and longitude of the river where *M. japonica* was found are withheld in the interest of conservation. The river is surrounded by paddy fields that serve as feeding grounds for *M. japonica* in irrigation periods (Yabe 1992; Yasukawa et al. 2008). During the study period, the riverbank was not modified with concrete for flood management, but by 2022, the riverbanks of certain reaches had been altered with concrete. In the latter study period (December 2021), road construction around the river began, resulting in the destruction and fragmentation of paddy fields owing to river alteration.

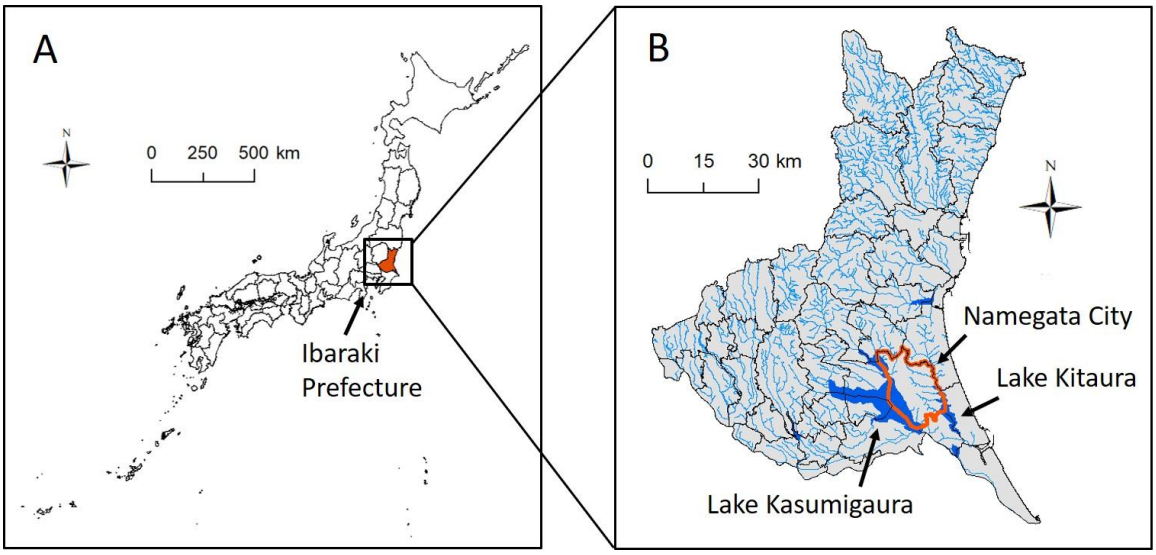

Fig. 1. Map showing Japan (A) and Ibaraki Prefecture (B). Blue lines indicate rivers.

Turtle Identification Method

We identified *M. japonica* and *M. reevesii* by morphological characteristics (such as head color, head and neck pattern, carapace and plastron color, number of carapace keels, the existence of a line pattern on the four limbs, serrated carapace, and iris color) as described by Yabe (1994), Yasukawa et al. (2008) and Lovich et al. (2011). Individuals that had both morphological characteristics of *M. japonica* and *M. reevesii* and/or showed at least one intermediate characteristic were identified as putative hybrids, as per Kosuge et al. (2003), Kato et al. (2010) and Suzuki et al. (2014). For the identification of *T. s. elegans*, we followed the criteria described by Ernst and Lovich (2009), which included a wide red postorbital stripe, narrow chin stripes, a transverse yellow stripe on each pleural, and a plastral pattern of one large dark blotch or ocellus on every scute.

Capture-recapture procedure

Capture-recapture techniques were conducted 17 times from March 2018 to December 2021 (except for Jun., Jul., Aug., and Nov.) in a river. During 16 of these capture studies, almost all the turtles were caught by hand by a single person. Moreover, some turtles were captured using traps (72 cm×44 cm×55 cm) with a 1.7 m baglike passage (one survey using three traps), which enabled the trapped turtles to breathe (following Kagayama et al. 2021) in September 2018. Traps were installed in the afternoon and checked the following morning. All captured turtles were marked for identification using the approach outlined by Kobayashi (2008). Sex was determined based on the relative position of the cloaca (situated anterior to the rear edge of the carapace in females and posterior in males, according to methods by Yabe 1989). Turtle age was estimated by counting the annuli on the plastron scutes (Sexton 1959). Strait carapace length (Kobayashi et al., 2010) was measured using digital calipers (Digital caliper 150 mm, 19975, Shinwa Sokutei; Absolute Digimatic caliper 200 mm, CD-20APX, Mitutoyo; Absolute Digimatic caliper 300 mm, 500-153 CD-30C, Mitutoyo).

Growth curve estimation

The von Bertalanffy growth curve, frequently used in freshwater turtle growth studies (Lovich et al. 1998; Lindeman 1999), was used to examine growth patterns. The von Bertalanffy equation is as follows.

$$\mathrm{CLest} = CL_{\infty}\,(1 - e^{-\mathrm{k}\,(\mathrm{t}-t_0)})$$

where CLest is the carapace length at an estimated age (t); CL_{∞} written as CL infinity, is the asymptotic length at which growth is zero; t_0 is the hypothetical age at which the organism is zero length; and k is the growth rate. Unsexed individuals (juveniles) were included in the growth analysis for each sex based on the assumption that juvenile turtles of both sexes grow at the same rate. Repeated measurements of individuals were included when they were recaptured in different years, and the data were treated as independent samples. Data from the most recent capture date were used for individuals captured multiple times during the same year. The parameters of the von Bertalanffy growth curve were estimated using the least-squares method and the FSA package. All statistical analyses were conducted using R software (version 3.6.1) (R Core Team 2018).

Results

Species composition

We captured a total of 300 *M. reevesii* (of which 280 were marked), a total of 56 *M. japonica* (of which 50 were marked and one was dead), a total

of two putative hybrids of *M. japonica* and *M. reevesii* (of which two were marked), and a total of six *T. s. elegans* (all were marked). The percentages of species composition based on the total of marked individuals were 82.8% (*M. reevesii*), 14.8% (*M. japonica*), 1.8% (hybrids), and 0.6% (*T. s. elegans*), respectively.

Population structure of *Mauremys japonica*

The size composition and age structure of *M. japonica* are shown in Fig. 2. The mean carapace length of females, males, and juveniles of *M. japonica* were 121.68 ± 34.37 mm (70.58－186.65 mm, N = 18), 105.49 ± 12.06 mm (80.09－121.26 mm, N = 30), and 57.60 ± 14.39 mm (35.96－73.84 mm, N = 8), respectively, with various body sizes (Fig. 2A). In addition, individuals of *M. japonica* were captured at various ages ranging from 0 to over 10 years (Fig. 2B).

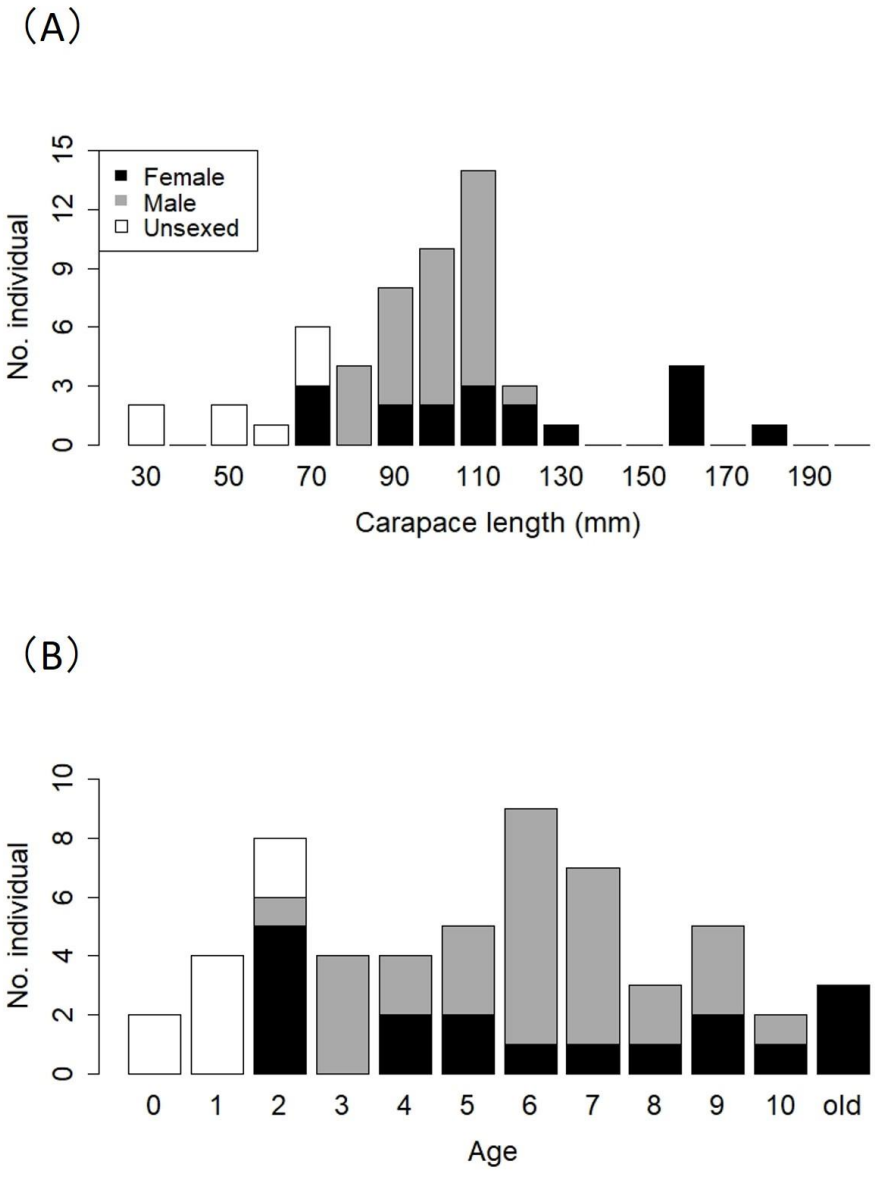

Fig. 2. Size composition (A) and age structure (B) of *Mauremys japonica* (N = 56).

Growth curve projection of *Mauremys japonica*

The parameters of the von Bertalanffy growth model were estimated (Table 1). Based on these parameters (such as CL_∞, t_0, and k), we predicted the growth curves of the turtles (Fig. 3). The growth curves of *M. japonica* populations in this study indicated that both sexes show similar growth patterns up to approximately 2 years of age, but that the growth of males slows down after 2 years of age (Fig. 3). Specifically, females grew larger after two years old.

Discussion

Mauremys japonica has been found in Yamagata Prefecture, which is located farther north than Ibaraki Prefecture (The Nature Conservation Society of Japan 2014). However, this is well outside the natural distribution range of *M. japonica* (Yasukawa et al. 2008), it is considered to be of exotic origin. Our survey is the first study to quantify the existence of a local population of *M. japonica* in Ibaraki Prefecture, which represents the northern limit of the Pacific

Table 1. The estimated parameters of the von Bertalanffy growth curves (95% CI = 95% confidence interval).

Sex	Parameter	Coefficient	95% CI	SE	p value
Female	CL_∞	203.30	144.65 – 261.96	28.35	< 0.001
	K	0.12	0.04 – 0.20	0.04	< 0.01
	t_0	-1.96	-3.31 to 0.60	0.66	< 0.01
Male	CL_∞	125.36	115.12 – 135.60	5.04	< 0.001
	K	0.27	0.18 – 0.36	0.04	< 0.001
	t_0	-0.39	-2.00 to 0.79	0.30	< 0.001

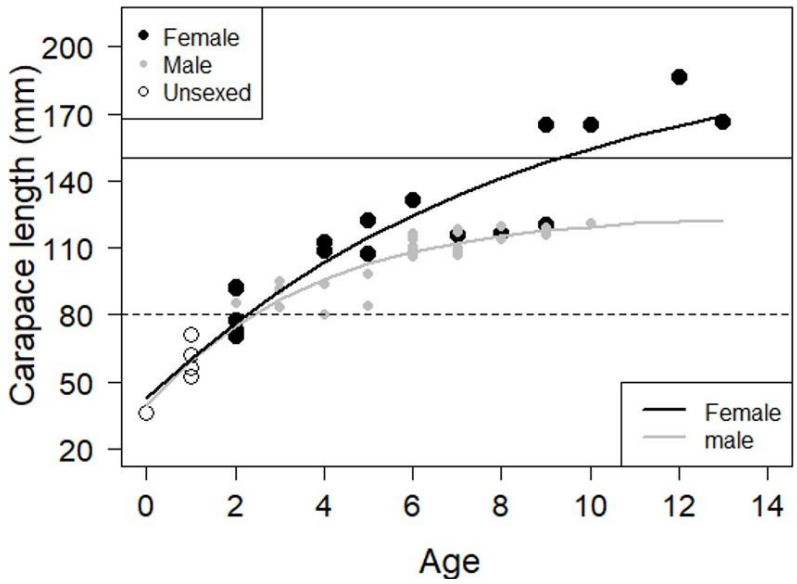

Fig. 3. Relationship between age, carapace length, and the von Bertalanffy growth curves of *Mauremys japonica* in this study river (female model, N = 26; male model, N = 38). Black and grey lines indicate the estimated growth curves of the female and male turtles, respectively. The solid (150 mm) and dotted (80 mm) lines indicate the body sizes of mature adult females and males, respectively, based on the report of Ishihara (1986) and Yabe (1992).

Ocean side of Japan. Although the presence and the distribution records of *M. japonica* in Ibaraki Prefecture have been described (Yasukawa et al. 2008; Ibaraki Prefectural Government 2016), the population size and breeding status of this species have not been evaluated. The 50 individuals of *M. japonica* captured in this study, including 14 young individuals (0-2 years old individuals) suggests that this population is reproducing normally. In addition, present study indicated that both males and females of *M. japonica* in the study population showed similar growth patterns to other populations in previous studies. Males grow to approximately 125 mm (CL_∞), while females tend to grow approximately 200 mm (CL_∞). For example, similar to the populations inhabiting rivers in the plains and hills of Chiba Prefecture (Kagayama 2020), the hilly population in Mie Prefecture (Yabe 1989), and the marsh population in Fukui Prefecture (Kagayama and Nishibori 2021), both sexes grew similarly up to age 2, but males tended to grow more slowly after the age of 2. Therefore, the population growing normally may represent the northernmost breeding population on the Pacific Ocean side of Japan.

However, the river was dominated by an alien turtle species, *M. reevesii*, which is phylogenetically closely related to *M. japomica*. Due to the hybridization between *M. reevesii* and *M. japon*ica, there is a potential risk of reduced population growth of *M. japonica* due to reproductive interference and the loss of pure *M. japonica* through gene pollution (Suzuki et al. 2014; Kagayama et al. 2020). In addition, resource competition from *M. reevesii*, which shares comparable ecological characteristics, such as dietary preferences (Ueno et al. 2014) may reduce *M. japonica* population size. Until recently, it was thought that *M. japonica* and alien turtles (*M, reevesii* and *T. s. elegans*) tended to have allopatric distributions (e.g., Yabe 2002) due to different environmental preferences. However, Kagayama et al. (2020) argued that the potential suitable habitat of these turtles largely overlapped and their ecological niches were remarkably similar, and that alien turtles displaced *M. japonica* in environments where their distributions overlapped. Therefore, *M. japonica* may be eradicated by the dominant alien, *M. reevesii*, in the future. In the future, we will need to continue a capture-recapture study of *M. japonica* to monitor whether this population will be maintained or become extinct. However, to prevent the extinction of the local population of *M. japonica*, we must eradicate the dominant *M. reevesii*, including hybrids, and conduct genetic analysis on putative *M. japonica* to ensure that the turtle is not a hybrid.

Furthermore, river modification for flood management significantly contributes to the drastic decline of freshwater turtle populations in Japan (e.g., Usuda et al. 2012). Recently, river alteration has been underway in this study river, posing a potential risk of significant decrease in the population of *M. japonica* (Ogano et al. 2015a) in future.

Typically, the local reproductive population of *M. japonica* typically contains a higher number of adult females (e.g., over 150 mm CL, over ten years old), compared to adult males and young individuals. For example, local population in Mie Prefecture (Yabe 1989), Fukui Prefecture (Kagayama and Nishibori 2021), and Chiba Prefecture (Kagayama et al. 2021; Kagayama 2022) contains many adult females compared to adult males and juveniles. This is likely related to the higher survival rate and longer lifespan of large-growing female adults compared to adult males and juveniles (Yabe 1989; Kagayama 2022).

However, there were very few adult females in this population (Fig.2). This may be due to the reproductive interference (e.g., courtship, mating), caused by the dominant *M. reevesii* (particularly in males), which reduces the survival rate of adult females of *M. japonica*. In addition, because *M. japonica* exhibits temperature-dependent sex determination (Okada et al. 2010), this study site, located at the northern limit of the Pacific Ocean side, may likely to produce males than females. Furthermore, although no dead female *M. japonica* (roadkill) were found during the study period, it is expected that the mortality rate of adult females searching for spawning sites may have increased due to road collisions with vehicles (e.g., Kagayama 2019).

The survival rate of young *M. japonica* and their eggs is significantly low due to the presence of numerous native and alien predators (e.g., *Mustela itatsi*, *Felis catus*, *Corvus macrorhynchos japonensis*, *Elaphe quadrivirgata*, *Lithobates catesbeianus*, Kagayama and Ogano 2021) of young individuals and eggs of *M. japonica* (Yabe 2002; Kagayama 2022) resulting in only few individuals reach adulthood. Therefore, although breeding is occurring in this study population, the population may decline in the future due to the low number of adult females compared to other populations.

In this study, we surveyed a population of *M. japonica* in Ibaraki Prefecture. Similar surveys should be conducted in rivers and ponds around the study area to reveal the status of populations of *M. japonica* in Ibaraki Prefecture.

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