EAZA Reptile Taxon Advisory Group

EAZA Best Practice Guidelines for the Asian giant forest tortoise (*Manouria emys*)

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Preamble

Right from the very beginning, it has been the concern of EAZA member institutions to encourage and promote the highest possible standards for husbandry of zoo and aquarium animals. For this reason, quite early on, EAZA developed the “Minimum Standards for the Accommodation and Care of Animals in Zoos and Aquaria”. These standards lay down general principles of animal keeping, to which the members of EAZA feel themselves committed. Beyond this, some countries have defined regulatory minimum standards for the keeping of individual species regarding the size and furnishings of enclosures etc., which, according to the opinion of authors, should definitely be fulfilled before allowing such animals to be kept within the area of the jurisdiction of those countries. These minimum standards are intended to determine the borderline of acceptable animal welfare. It is not permitted to fall short of these standards. How difficult it is to determine the standards, however, can be seen in the fact that minimum standards vary from country to country. Above and beyond, specialists of the EAZA EEPs and TAGs have undertaken the considerable task of laying down guidelines for keeping individual animal species. Whilst some aspects of husbandry reported in the guidelines will define minimum standards, in general, these guidelines are not to be understood as minimum requirements; they represent guidelines for best practice. As such, the Guidelines for keeping animals intend rather to describe the desirable design of enclosures and prerequisites for animal keeping that are, according to the present state of knowledge, considered as being optimal for each species. They intend above all to indicate how enclosures should be designed and what conditions should be fulfilled for the optimal care of individual species.

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Section 1. Biology and field data

1.1 Taxonomy

Order: Testudines

Family: Testudinidae

Genus: *Manouria*

Species: ***Manouria emys*** (Schlegel & Müller, 1840)

There are two recognised subspecies of *Manouria emys*,

- *M. e. emys* (Schlegel & Müller, 1840)
- *M. e. phayrei* (Blyth, 1853)

The species was first described by Schlegel and Müller in 1840 as *Testudo emys* (fig. 1) and the subspecies *T. e. phayrei* was first described by Blyth in 1853 as *Testudo phayrei*. A few scientific synonyms exist, previously the species was named *Geochelone nutapundi, Testudo emys* and *Manuria emys*. Older literature may refer to the species by one of these synonyms.

*Fig. 1: illustration of "*Testudo emys*" from the first description of the species by Schlegel & Müller (1840).*

Common names

The English language knows various names for *Manouria emys* and its subspecies. These include references to the species distribution, colour, habitat and size.

- Asian/Burmese brown/black tortoise
- Asian/Burmese forest tortoise
- Asian/Burmese mountain tortoise
- Asian/Burmese Giant Tortoise

M. e. emys is often more generally referred to as ''Asian'', "Sundaic", ''forest'' ''southern'' or ''brown'' whereas *M. e. phayrei* is known as ''Burmese'', ''mountain'', ''northern'' or ''black'' tortoise (Schaffer & Morgan 2002).

The subspecies names may be abbreviated to MEE for *Manouria emys emys* and MEP for *Manouria emys phayrei*.

A less common English name for the species is the six-legged tortoise, referring to the large spurs near the tortoise's rear legs resembling two additional legs (Stanford *et al*., 2015).

1.2 Morphology

Manouria emys is a tropical tortoise species, the largest in Asia. It is considered to be one of the oldest tortoise lineages, as it is both genetically and in appearance one of the most primitive tortoise species (Pritchard, 1979).

M. e. emys can reach a straight carapace length of up to 50 cm and weigh 20 kg, while *M. e. phayrei* can grow larger and reach a straight carapace length of 60 cm and a weight of up to 37 kg (Nutaphand, 1979).

The tortoises are equipped with massive tubercular scales on their forelimbs, which may aid in stabilisation on the rugged terrains that they prefer to inhabit (McKeown 1990).

Although the subspecies are colloquially referred to as brown (*M. e. emys*) or black (*M. e. phayrei*), their coloration is very similar. Coloration is usually solid and can vary from light and dark brown to dark grey or almost black. Individuals, especially hatchlings and juveniles, may have a lighter shaded colouration in the centre of the scutes.

Figure 2: carapace and plastron of a subadult M. emys emys*. Photo credit: Wildlands Adventure Zoo Emmen.*

The subspecies can be best differentiated by the pectoral plastral scutes. One or both usually touch at the middle line of the plastron in *M. e. phayrei*, but never reach the midline and do not meet in *M. e. emys* (Fig. 3 and 4). Much individual variation occurs however, and the pectoral scute arrangement typical of *M. e. emys* have been found in individual *M. e. phayrei* several hundred kilometres north of the recognized overlap zone of the two subspecies (Choudhury 1996, Das 1990, Kundu *et al.,* 2013, 2018).

Figure 3: selection of plastrons of M. emys*, left; showing plastron typical of* M. e. emys*, centre; showing shared characteristics, right; showing plastron typical of* M. e. phayrei*. Photo credit: Nathan Haislip.*

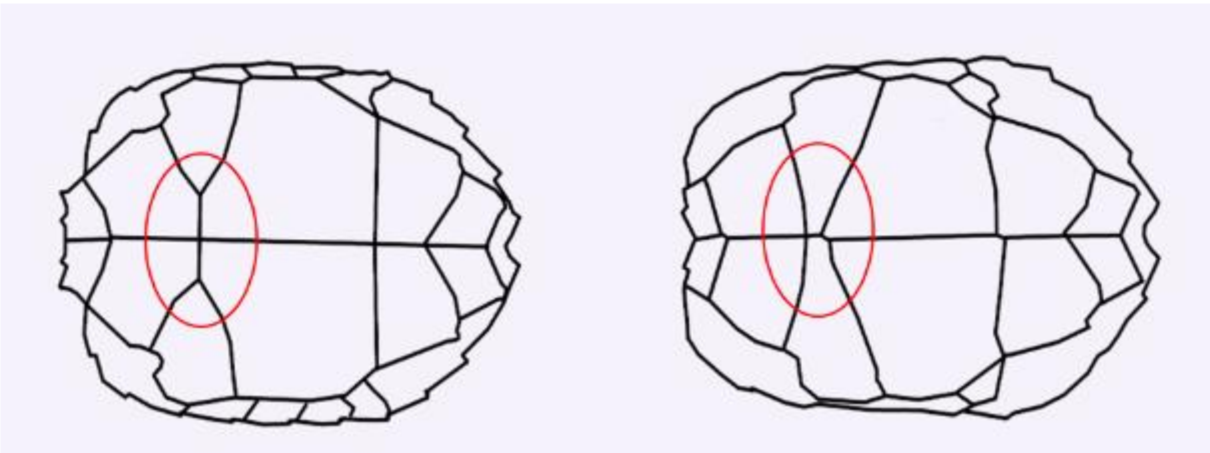

Figure 4: schematic drawing of plastrons with characteristics of M. e. emys *(left) and* M. e. phayrei *(right).*

1.3 Physiology

Physiological characteristics, such as heart rate, breathing rate and internal temperatures, of any reptile and tortoise vary a lot as reptiles and tortoises are ectotherms and are greatly influenced by their environment.

Because of these factors, physiology of *M. emys*, or any reptile, is difficult to establish or describe. No research is known to have been conducted in any European language on the physiology of *M. emys*.

1.4 Longevity

Longevity of *M. e. phayrei* has been recorded in literature of being up to 20 years. This is, however, likely to be much longer as in most other tortoise species, an individual could possibly live much longer, even exceeding 100 years (Slavens & Slavens, 2000). It is assumed that longevity for both subspecies should be similar.

1.5 Conservation status, zoogeography and ecology

1.5.1 Distribution

M. emys occurs over a wide range in Southeast Asia, from Bangladesh and northeastern India through mid-elevation hills of western Myanmar, and western and southern Thailand through the Malay peninsula and onto the Indonesian islands of Sumatra and Borneo (Iverson, 1992; Platt *et al.*, 2018; fig 5).

The subspecies *M. e. emys* inhabits the Malay peninsula south of the Phang-nga-Surat Gap and parts of the islands of Borneo and Sumatra (Fig. 5). Subspecies *M. e. phayrei* occurs from Peninsular Thailand northwards through Myanmar to the north-eastern Indian and eastern Bangladeshi hill tracts.

The distribution of these two subspecies is separated at the tectonic side fault area, the Surat Thani gap, but intergradation is known to occur in southern Thailand where the distribution of the two subspecies meets (Schaffer & Morgan 2006). Different haplotypes within subspecies have been suggested by Kundu *et al.,* (2017) based on two mitochondrial markers.

The trade in live tortoises for the pet trade and for human consumption has led to individual tortoises having been found in far-away locations like Vietnam and China (Smith 1931, Choudhury *et al.*, 2000).

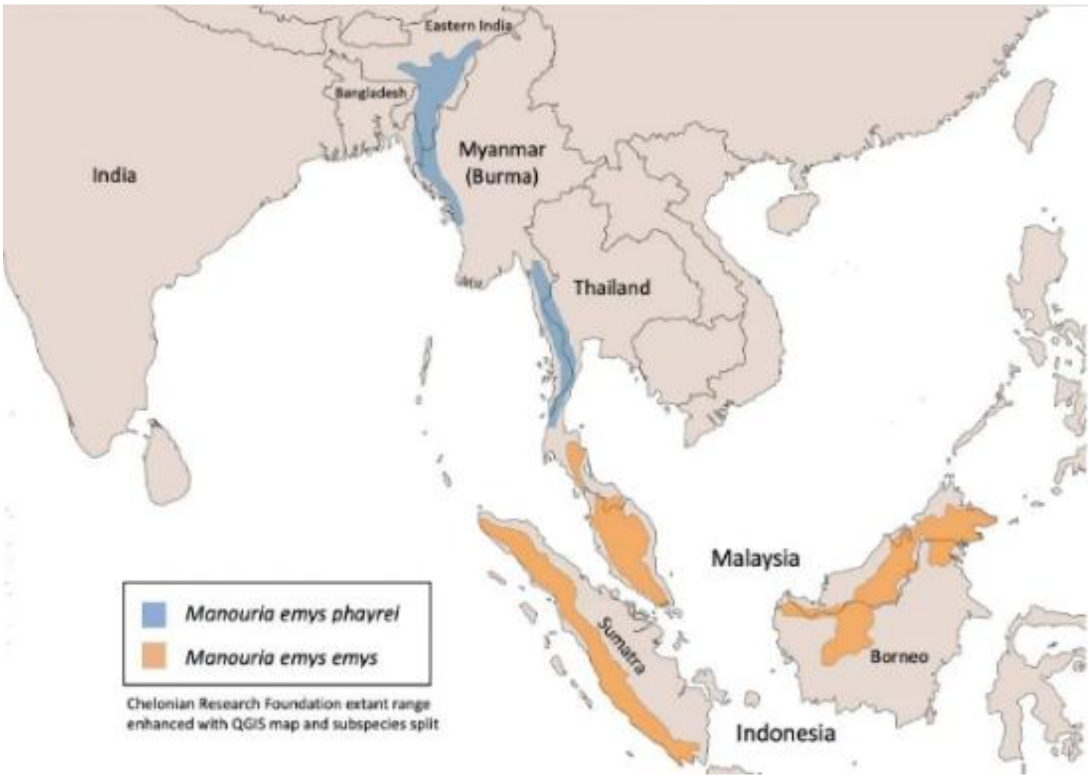

Figure 5: Distribution of M. emys *and its subspecies, based on research from Stanford et al., 2015. (image credit: Bailey, 2020)*

1.5.2. Habitat

The Asian giant forest tortoise inhabits temperate, tropical and subtropical evergreen forest habitats, typically in low/mid elevation at altitudes of 600-1,500 meters above sea level (Nutaphand, 1979). The species usually remains under canopy cover during most of the day (Høybye-Mortensen, 2004).

Population densities are highest in mountainous or densely forested areas, as habitats much disturbed by human activity are unsuitable to support a strong population (Høybye-Mortensen, 2004). Harapan *et al.* (2025) followed 3 tortoises in Sumatra using radio-telemetry and found them to have a mean home range of 27.5 (±28.8 SD) hectares (±28.8) but with a high variation in size (3 to 59.6 ha). Høybye-Mortensen (2004) performed a similar study in Borneo with similar results (15.3 ± 11.9 ha).

The tortoises showed a consistent preference for primary rainforests with forested, hilly or mountainous areas, where they inhabit steep slopes at high elevations and near rivers. Figures 6 and 7 show examples of this habitat.
Important microhabitats of the species include small streams and puddles, swamps, evergreen forest and bamboo forest (Høybye-Mortensen, 2004). Harapan *et al.* (2025) speculate that the animals' choice of hilly areas near rivers may help them in thermoregulation as these areas are typically covered with dense vegetation, which may provide a cooler microhabitat.

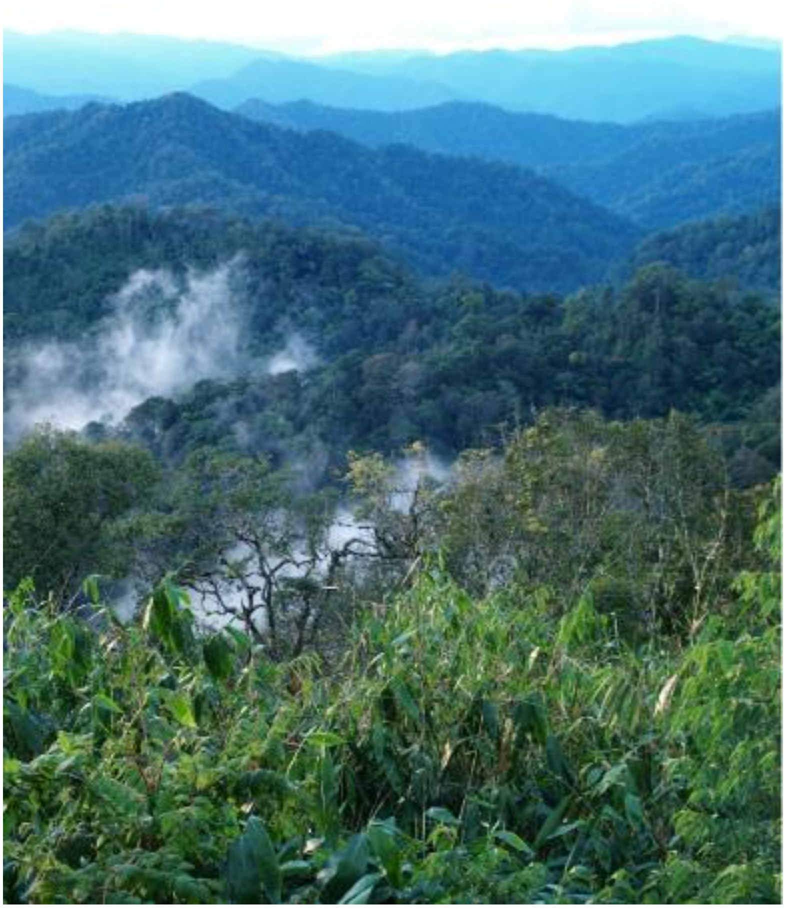

Figure 6: Example of M. e. phayrei *habitat in Kaeng Krachan National Park, Thailand (from Stanford* et al.*, 2015).*

Figure 7: Example of M. e. phayrei *habitat in Kaeng Krachan National Park, Thailand. By C. Stanford (Stanford* et al.*, 2015).*

1.5.3 Climate

As the species has a wide distribution, the climate in which *Manouria emys* occurs may vary considerably.

The species occurs in the following Köppen classified climates: tropical (Af), tropical monsoon (Am), tropical savanna (As/Aw) and humid subtropical (Cwa; Beck *et al.,* 2018).

The following graphs (Fig. 8-10) give an impression of the general climatic conditions of regions within the distribution range of *M. emys*. These graphs may not represent the exact climatic conditions in the (micro)habitats of *M. emys*; rather, they are meant to provide a general overview.

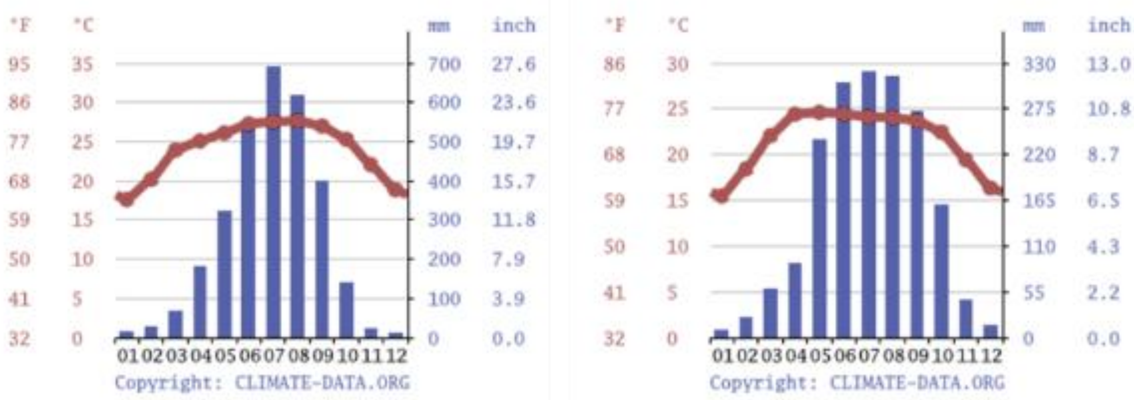

Figure 8: 2 climate charts depicting mean monthly temperature and precipitation in the northern part of the distribution of M. e. phayrei*: Guwahati (left) and Aizawl (right), India.*

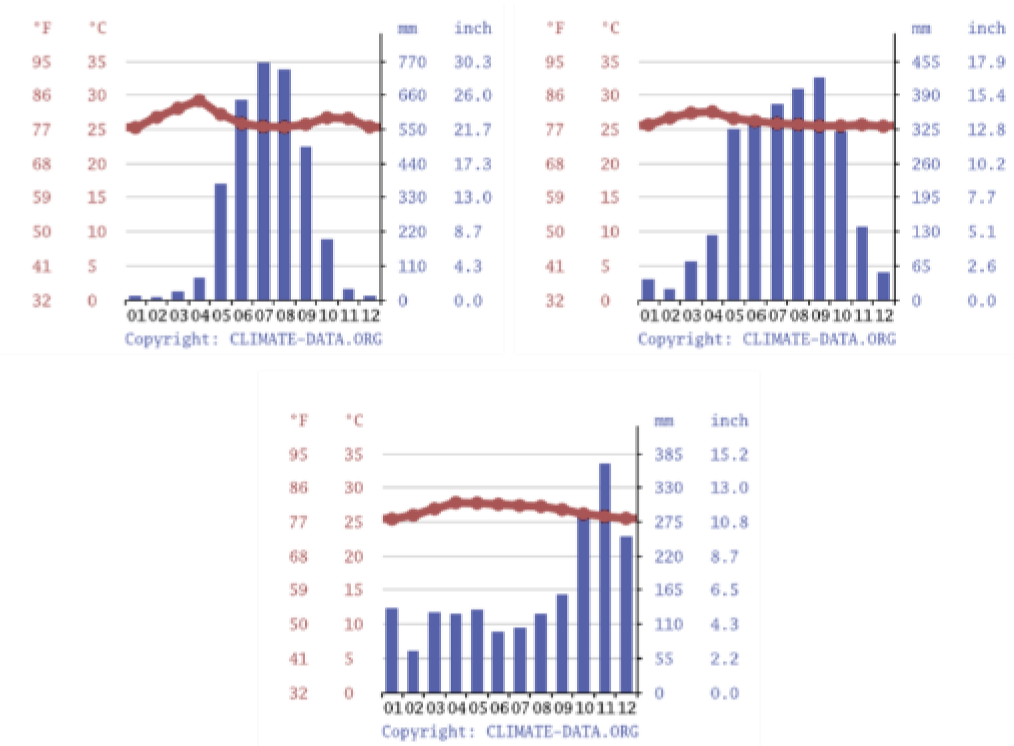

Figure 9: 3 climate charts depicting mean monthly temperature and precipitation in the central part of the distribution of M. e. phayrei*: Mawlamyine, Myanmar (top left), Ranog province, shared: Thailand (top right), and* M. e. emys*: Nakhon Si Thammarat province, Thailand (lower).*

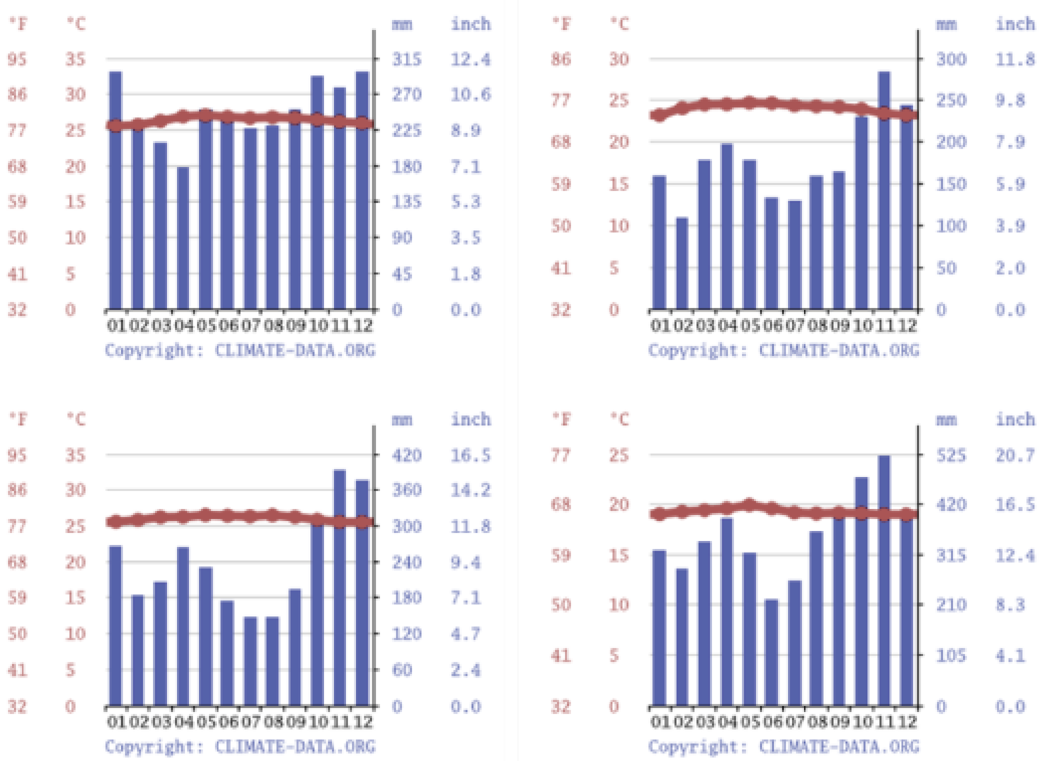

Figure 10: 4 climate charts depicting mean monthly temperature and precipitation in the Southern part of the distribution of M. e. emys: Bentong, Malaysia (top left), Sandakan, Borneo, Malaysia (top right), Pontianak, Borneo, Indonesia (lower left), Bukittinggi, Sumatra, Indonesia (lower right)

M. e. emys experiences tropical average lows of ~20°C and highs of ~30°C throughout the year. As *M. e. phayrei* occurs more northerly and can experience noticeable seasonal differences, this subspecies likely experiences a larger range of climatic conditions. Based on record lows below 0°C in the Indian Assam province, at the northern end of their natural range, it is assumed that wild *M. e. phayrei* occasionally experiences subzero temperatures. The species has been observed to become inactive or go into a state akin to brumation once temperatures drop below 15°C.

Due to the normal heavy natural rainfall conditions of up to around two meters annually (Emer, 2007), both *M. e. emys* and *M. e. phayrei* are used to conditions with high humidity. In his research on *M. e. emys*, Høybye-Mortensen (2004) found that daytime humidity measurements at the forest floor rarely fell below 90%.

1.5.4 Population

Manouria emys is generally considered rare across its range. Populations are largest in areas of primary or secondary forests with little human disturbance but exact figures are unknown. The species' global population is thought to be decreasing, and estimates by the International Union for Conservation of Nature (IUCN) picture a reduction of 80% over the past 3 generations (Choudhury *et al.*, 2019).

1.5.5 Conservation status

1.5.5.1 Conservation and Main Threats

The IUCN Red List assessment states that *Manouria emys* is in severe decline. Based on habitat loss rates alone, half the area of suitable lowland and mid-elevation evergreen forest has been degraded and lost in the past half century throughout its range. In addition, the species has suffered long-term subsistence collection and intensive commercial exploitation for East Asian consumption trade in recent years. Overall, the species has declined by at least 80% in the past three generations (135 years). This decline is expected to continue as turtle exploitation through most of its range remains beyond effective control, subsistence collection and poaching occurs widely even in some protected areas, and forest loss continues across much of its remaining range (Choudhury *et al.,* 2019). "Even today one of the biggest threats on turtles/tortoises in India is consumption, I would say nearly 70%. The local people would also locally trade it or gift it, also for consumption and sometimes pet keeping" (Sushmita Kar pers. comm. 2024). This species is particularly threatened from Jhum cultivation and hunting (Ahmed & Das 2010).

Manouria emys qualifies for listing as Critically Endangered under criteria A2cd+4cd. (Choudhury *et al.,* 2019).

The species is included in CITES Appendix II with all Testudinae spp., allowing international commercial trade in the species provided such trade is not detrimental to the species, and subject to national trade legislation. It is also included in Schedule IV of the Indian Wildlife (Protection) Act of 1972 (amended); it warrants revision to Schedule I. The species is protected under the Wildlife Conservation Act (2010) in Malaysia and trade is regulated. *M. emys* is protected under domestic legislation in Myanmar and under the Wildlife Preservation and Protection Act (1992) in Thailand. It is expected to be included under Government Decree No. 7 in Indonesia soon; in 2017 Indonesian quotas were set at 50 animals, under 20 cm, for the pet trade. The Asian Giant Tortoise is fully protected by regulations in Indonesia, so international trade of the Asian Giant Tortoise from the wild is not allowed (Based on Law No. 5 of 1990) (Choudhury *et al.,* 2019). The import of wild caught animals from Indonesia into the EU is prohibited by the Council Regulation (EC) No 338/97, update January 6th 2025, see European Commission (2025).

1.5.5.2 International Trade History

Johann von Fischer (1884) mentions that this species (known only from Sumatra at the time) is rarely seen in the trade in Europe, and that the animals are "mollycoddle": they need warmth and eat more worms and insects than other tortoises.

It is clear that these tortoises have been traded within Asia already early in the 20th century, since Smith (1931) mentions that specimens found in Vietnam and China may have been imported. For India, the trade until 1999 has been reviewed by Choudhury (2000).

This species (along with all other tortoises) was included in CITES Appendix II already at the first conference of the Parties in 1976. Queries in the CITES trade database (UNEP-WCMC 2024) did not reveal any legal exports of living animals from India or Myanmar after 1975.

In the first years of the CITES trade database, which analyses the trade since 1975, *Manouria emys* was exported in numbers mainly from Thailand, and from 1983 onwards also from Malaysia. As soon as the source codes were available in 1990, one can see that wild caught animals were exported mainly from Malaysia and Indonesia. While Malaysia did not export wild-caught tortoises from 2010 onwards, Indonesia continues exporting wild-caught tortoises until today. The import of wild-caught *Manouria emys* from Indonesia into the EU is continuously prohibited since 1997 (European Commission 1997).

Captive-born or captive-bred animals were exported from the USA and European countries from about 1990 onwards, as the first exports of animals labelled as being captive-born or captive-bred, from Indonesia, are given in this database in 1999. The last legal imports from Indonesia into any EU country took place in 2017: six captive born, live animals were exported to the Czech Republic, and the last legal transfer from Indonesia to the US took place in 2022 – the exporter labelled them as being captive-bred, and the importer assigned the source code as 'wild-caught'. Currently, there is quite a lively trade of captive-bred tortoises from European countries to East Asia (UNEP-WCMC 2024).

Illegal trade with this species is continuing. Already in 2008, TRAFFIC revealed a continuous illegal trade of *Manouria emys* from Indonesia to the countries of East Asia (Shepherd & Nijman 2008). A total of at least 507 live specimens, in seven shipments, were reported as seized from illegal trade during the period 2000-2015 (CITES 2016), with the biggest seizure in Hong Kong in 2001, when 57 live *Manouria emys* were found in a shipping container; the container arrived from Singapore (which has no wild population of *M. emys*), and was destined for China. While travelling China in 2012 and 2013, *Manouria emys* were described being offered at a food market in Guangzhou (Kar *et al.*, 2025). The Wildlife Trade Database (TRAFFIC International 2025) lists several cases of *Manouria emys* confiscations in Indonesia, Thailand, Malaysia in the past ten years, and even outside their natural range in Vietnam two tortoises were found in 2021. In 2024 ten *Manouria emys*, which were going to be smuggled to Singapore and Malaysia, were confiscated on Riau Island, Indonesia (Sahputra 2024). Not all the tortoises confiscated alive are meant to be kept as pets, some of them were sold for slaughtering (Kar *et al.*, 2025).

1.5.6 Conservation actions

Currently, there are some conservation actions in place that support the preservation of *Manouria emys*.

The species occurs in a number of protected areas across Southeast Asia, but large parts of the species distribution do not enjoy a special protection status (Choudhury *et al.*, 2019).

Ex-situ, the species is included in both an EAZA EEP and an AZA Species Survival Programme (SSP). Both In-situ and ex-situ assurance colonies exist (Chodhury *et al.*, 2019). From these assurance colonies, some captive bred-individuals have been released in Bangladesh (Leeflang & Rahman, 2022). CCA (Creative Conservation Alliance) is a nature conservation organization active in Bangladesh. This organization has overseen the reintroduction programme in Bangladesh in cooperation with TSA India. Their website can be found at https://www.conservationalliance.org/. Their activity is mainly focused on conservation and nature development in Bangladesh (Fig. 11).

In North India, Nagaland Zoological Park has an ex-situ breeding colony, and in 2022 already ten captive bred *Manouria emys phayrei* have been released (Kar 2022). TSA (Turtle Survival Alliance; https://turtlesurvival.org/) is an organisation participating worldwide in both in- and ex-situ conservation of *M. emys*. They are helping to coordinate and support local projects and ex-situ holders and breeders, and TSA India had partially sponsored this project. In 2025 the tortoise project in Nagaland is being enhanced, involving the local communities in the area, and supported by several different local and international organisations (Kar *et al.,* 2025).

In Myanmar, an assurance colony was being set up at the Rakhine Yoma Elephant Sanctuary with Turtle Survival Alliance Myanmar support, but before the first tortoises could be released, the country has descended into civil war, and sustaining the turtle and tortoise conservation projects in this country is becoming increasingly challenging (Platt *et al.,* 2024).

Figure 11: Captive Manouria emys *in Bangladesh. Photo credit: Turtle Survival Alliance India.*

1.6 Diet and nutrition

Manouria emys are generally herbivores, frugivores and fungivores. Their diet in the wild has been reported to include substantial amounts of fallen fruits, a moderate quantity of leaves and flowers and even some plant shoots (Harapan *et al.*, 2025; Nutaphand, 1979). In addition to plant material, tortoises may also opportunistically forage on animal matter, including some invertebrates and frogs (Humphrey & Bain 1990). They have a preference for strong-scented and brightly coloured food items (Harapan *et al.*, 2025).

Lambert & Howes (1994) observed a radio-tagged female feeding on 19 occasions. The diet consisted predominantly of green leaves of understory plants and mushrooms, as well as some seedlings, sometimes plant roots, and on one occasion fallen figs (*Ficus punctata*).

Høybye-Mortensen (2004) provides a detailed list of plant species he observed *M. emys* consuming in the wild (table 1). In this research, it was found that 68% of feeding occurred on *Alocasia* species (elephant's ear). The exact quantity of plant material consumed was not measured. It is noted that this list may be an incomplete representation of the diet of *M. emys* during periods of a few months (March-August and January-march) - the items in the diet could differ between seasons.

Family	Species or genus name (Common name)	Part(s) eaten	Number of collections/ observations
Fungi	Unidentified	Whole mushroom	2
Melostomataceae	*Clidemia hirta* (Koster's curse)	Shoots	1
Araceae	*Alocasia* sp. *scabriuscula* group (Taro)	Leafs and petioles	6
	Alocasia sp. (Taro) group or species unidentified	Leafs and petioles	2
	Alocasia sarawakensis (Taro)	Leafs and petioles	7
Begoniaceae	*Begonia* sp.	Shoots	1
Marantaceae	*Phrynium* sp.	Shoots	1
Woodsiaceae	*Diplazium esculentum* (vegetable fern)	Flowers	1
Zingiberaceae	*Etlingera coccinea* (wild ginger)	Flowers	1

Table 1: Plant and fungi consumed by M. emys *as observed by Høybye-Mortensen (2004).*

Harapan *et al.* (2025) list 40 species of plants from understory vegetation across 20 families, including ferns, monocots and dicots that they recorded tortoises feeding on. Plants consumed were primarily tree species, but forbs were also consumed. Moraceae, Araceae and Meliaceae were well represented in tortoise diets.

Tortoises play crucial yet often underappreciated roles in tropical ecosystems, particularly in seed dispersal. They may disperse keystone figs and large-seeded plants over distances of up to 1.9 km and they are able to swallow seeds as large as those of *Durio* and *Artocarpus* (>2 × 4 cm; Harapan *et al.*, 2025).

1.7 Reproduction

In the wild, breeding occurs roughly in the wet season: it has been observed to be in April for *M. e. phayrei*, and between March and July for *M. e. emys*. Reproduction has been observed in captivity year-round if environmental conditions are suitable (Morgan & Schaffer, 2006; Haislip, pers. comm.). Mating behaviour was described in captivity and illustrated in detail by McKeown *et al.*, 1990 (Fig. 12). Much of the below text is based on this study. Courtship of *M. emys* can generally be described as similar to other tortoise species (Holte, 1998).

Head bobbing is a common means of communication, so this often precedes mating. Males display fixation behaviour during courtship, in which they will usually fully extend their necks and keep their gaze on the female. The male then follows the female around (trailing). In attempting to mount, males may employ biting to force cooperation from the female, which may in extreme cases lead to injuries to the female. Once the female halts, the male will attempt to mount the female and try to copulate. After successfully mating, or even without successful mating or the presence of a male, the female may develop eggs, build a nest and lay eggs when conditions are suitable. Females may, like in other chelonian species, store spermatocytes for several years, which may be advantageous if population densities are low (Jiménez-Franco *et al.*, 2020).

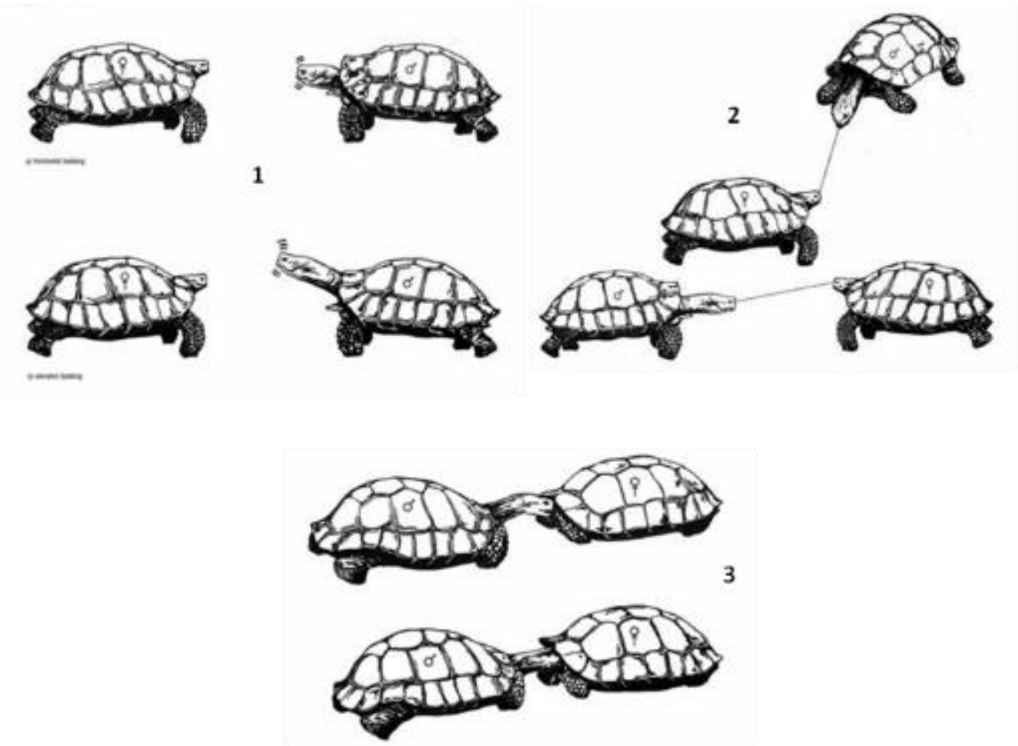

Figure 12: depiction of courtship behaviour of M. emys*, Behaviours depicted are 1: head bobbing, 2: trailing, 3: biting female. Adapted from McKeown* et al. *1990.*

1.7.1 Age of sexual maturity

Subadult tortoises may make their first attempts at reproducing despite not being fertile yet and still growing. Sexual behaviour may be shown at an age of 8 to ten years, but the tortoises are often not fully fertile at this age. Individual tortoises may reach sexual maturity at 10 years (Haislip, pers. comm), but usually both sexes are about 15 years old when the first fertile eggs are laid (Fahz 2010). According to Morgan (2022), females may start laying eggs when they are around 18 kg in weight. Generation length in the wild is estimated at around 45 years, three times the age of maturity

(Chodhury, 2019), but this could be significantly shorter in captivity, where offspring survival may be greater and conditions may favour more rapid growth.

1.7.2 Clutch and offspring size

M. e. emys clutches on average consist of 35 eggs, and clutch size can range from 15-51 (n=24; McKeown *et al.*, 1990). *M. e. phayrei* usually produces bigger clutches, commonly around 60 and up to 74, with a record clutch size of > 100 eggs (Holtmann 2011, Stanford *et al.,* 2015, Kar & Singh, unpublished data). Clutch size depends on the size of the female. The first clutches of a female after reaching sexual maturity are small, and often the eggs are infertile (Bailey 2020).

Eggs are spherical or slightly elongated, have a diameter of 41-54 mm and weight between 46-80 grams. Hatchlings in McKeown's research (*M. e. emys*) ranged from 50-55 g in weight and have a straight carapace length of 51-55 mm.

1.7.3 Nesting and parental care

The genus *Manouria* is unique among chelonian species in their ability to build nest mounds consisting of decaying plant material.

Females construct a nest mound by collecting leaf litter, using their hind legs, from a radius of up to 10 m. This mound is often positioned in a corner or against a solid barrier, which presumably allows for easier construction. A mound is usually 30-100 cm tall with a diameter of up to 3 metres.

There have been observations where multiple captive females have cooperated and built a joint nest mound (Haislip, pers. comm.). Incidental nest mound building and defence also have been recorded in a proven adult male *Manouria e. emys* who was housed solitarily. The cause of this remains unknown (Stumpel, pers. comm.), but the male maintained and defended the nest for multiple months.

Also unique compared to other chelonian species, *M. emys* shows a degree of parental care. The nest is maintained and defended against potential threats by the female for 3 to 23 days after oviposition (McKeown 1999). The nest is maintained by the female by continually adding new material on top of the existing nest mound, possibly to further help regulate the nest's internal temperature.
Females defend their nest mound by either sitting directly on top of the nest in a defensive posture protecting the eggs, or by advancing towards the intruder to attempt to chase it away. The female may employ behaviours which include pushing and biting to remove the threat (McKeown *et al.,* 1991).

The defensive behaviour is possibly meant to protect the nest in the critical first phase where the eggs are more easily detected by predators through smell. After some days or weeks, often after a rain shower, the nest may have become more difficult to locate by predators, and the female may then leave the nest.

Temperatures inside a nest mound in the wild have been recorded by Høybye-Mortensen (2004; figure 13). During the research, a previously encountered nest mound that was destroyed by a predator was reconstructed and temperature loggers were placed inside. Temperature was measured with 20 min. intervals for 9 days. The temperature in the egg chamber remained constant around 28-28,5°C. Heavy rainfall on July 15th caused the temperature in the egg chamber to drop approximately two degrees.

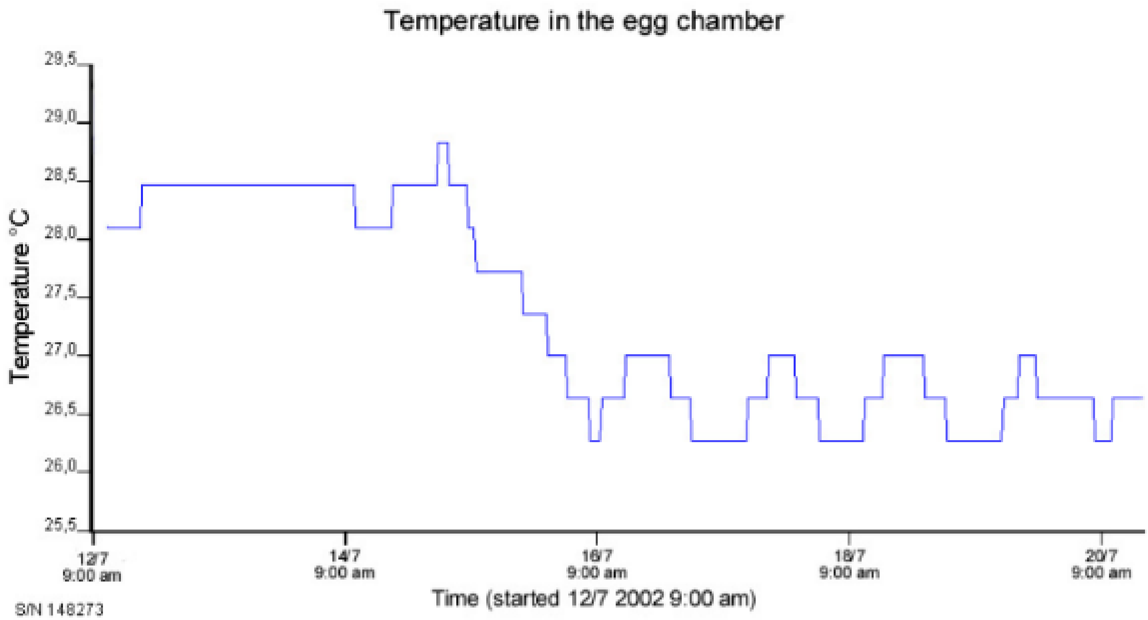

Figure 13: Temperature measurements in the egg chamber of a reconstructed nest (Høybye-Mortensen, 2004).

Convergent evolution

The nesting and nest defence behaviours of *M. emys* are similar to other animal species such as alligators (*Alligator* spp.*;* Deitz & Hines, 1980) and megapode birds (Megapodiidae) such as the Australian brush-turkey (*Alectura lathami;* Vleck *et al.,* 1984). These behaviours were conceived independently in each species, so these shared behaviours are a nice example of convergent evolution. Convergent evolution is a phenomenon in which different species develop similar traits or behaviours in response to similar environmental pressures, despite not sharing a common ancestor (Maloy & Hughes, 2013).

1.8 General and seasonal behaviour

1.8.1 Social behaviour

Though in the wild *M. emys* is usually solitary, their social behaviour is quite complex with elaborate dominance and courtship displays (as described in 1.7). Head bobbing appears to be a common means of communication in the species.

Female on female mounting or ''mating'' has been observed, and this is most likely a display of dominance (Haislip, pers. comm.). Males may compete over reproductive rights. In the wild this behaviour may be less common than in captivity, as in the wild it is less likely that two males meet in proximity of a female, but they may also show intraspecific aggression without the presence of a female. This very much depends on the characters of the individual animals which are sometimes clearly different (Petrás, pers. comm.).

1.8.2 Activity

Individuals have been recorded to travel up to 300 metres in a single day, though distance travelled in a day is usually less than 200 metres. On some occasion, an individual may spend the entire day without moving from a location (Lambert & Howes, 1994).

M. emys is crepuscular, meaning that the species is most active around sunrise and sunset. This way they avoid the higher temperatures around mid-day and the cooler temperatures at night. *M. emys* may have some semi-aquatic tendencies (Stanford *et al.*, 2015, Natchev *et al.,* 2015). There is strong evidence for individuals having a preference for (soaking in) water. Høybye-Mortensen (2004) found that 7% of all individuals were found in water in his study area in Borneo, whereas 20% were found closer than 50 metres to water and the remaining majority was found at a distance of more than 50 metres from water. Soaking behaviour may be linked to getting rid of ticks and to thermoregulation.

Harapan *et al.* (2025) found that the animals he studied in Sumatra appeared more active during the day (77.2%) than at night (22.8%), but with many variations in their small sample size. In 61.5% of the collected GPS data, the tortoises were 'encamped' (resting, hidden) and 38.8% of data were classified as exploratory behaviour.

During the dry season (November to February) in its northern range, some individuals of *M. e. phayrei* become inactive for a period of up to two months, buried in litter or under fallen trees, in a brumation-like state. This behaviour is not universal among individuals in the same range. Due to the tropical climate in its more southern range, *M. e. emys* is not known to brumate. In captivity, individuals of both subspecies have been observed going into a brumation-like state during winter months when temperatures reach below 15°C (Haislip, pers. comm.).

Just like is the case in all tortoise species, juveniles are more rarely spotted in the wild than adults. This may indicate that juveniles are generally wearier and shier, likely because they are more vulnerable to predators (Høybye-Mortensen, 2004).

Predation

It can be presumed that egg-eating predators present in the range of *M. emys* will attempt to predate on its eggs. These may include monitor lizards (*Varanus* spp.), mongoose (*Herpestes* spp.), civets (Viverridae), wild pigs (*Sus* spp.) and rats (*Rattus* spp.). It can be presumed that these predators are also capable of preying on hatchlings. In addition, hatchlings may be vulnerable to raptor species (Alderton, 1988). Juvenile *M. emys* could also be vulnerable to those same predators. Their vulnerability may vary with their size: resistance to predators increases with size.

There is evidence that sun bears (*Helarctos malayanus*) have consumed *M. emys* (Wong *et al.,* 2002), indicating that these powerful bears may be capable of preying on either juvenile or adult tortoises. The other bear species in the region, the Sloth bear (*Melursus ursinus*) and Asian black bear (*Ursus thibetanus*) may be capable of the same. Big cats (*Panthera* spp.) may also be capable of preying on juvenile or even adult tortoises.

1.9 Educational value

M. emys may not be colourful or have other physical features that set them apart from other large tortoise species. Despite this, the species has characteristics which make it interesting and give it a high educational value. Their unique nesting behaviour is certainly of interest. It is, along with its sister species *Manouria impressa*, the only chelonian species which shows a very significant degree of parental care as it is the only species which constructs and maintain very conspicuous nest mounds. These facts can provide an interesting opportunity to educate on evolution and the adaptation of animals to their environment. Another interesting feature is that *Manouria emys* is a very inquisitive and rather tame species of tortoise, which can be target trained easily which can be shown to an audience.

Manouria emys, along with most other chelonian species from Asia, is under threat of extinction. IUCN classifies the species as Critically Endangered. The top 25 most endangered chelonian species are all native to Southeast Asia and China: this situation is referred to as the Asian turtle crisis, and this may be used as a good example of species exploitation and habitat loss. The plight of turtles in Asia is exacerbated by several factors. Political upheavals in the region have led some national and local governments to deprioritize nature conservation efforts. Additionally, challenges in oversight and enforcement of existing rules and regulations, compounded by difficult terrains and unstable political situations, create loopholes that make poaching a relatively low-risk activity compared to other regions. Economic hardships further incentivize poaching, as individuals seek alternative sources of income. The lucrative market for chelonians is particularly fuelled by the demand in Chinese traditional medicine, which drives up prices for poached turtles. Another major market where turtles are illegally traded is the regional pet trade. Especially rare, colourful and exotic-looking species and individuals may fetch high prices, even in international markets (Sevin, 2023).
Moreover, increased habitat loss due to rapid urbanization, agricultural expansion and deforestation further threatens turtle populations. Destruction and pollution of crucial habitats, such as wetlands, rivers and forests, diminishes the available space for turtles to thrive, pushing them closer to extinction.
These conservation challenges present a unique opportunity for educational initiatives to highlight the crucial role that institutions such as zoos and aquariums play in the conservation of critically endangered species like *M. emys*. By raising awareness and initiating or sponsoring conservation efforts, zoological institutions can help mitigate the threats facing these species and inspire action to safeguard their future (see also chapter 1.5.5).

An example of educational signage is presented in Appendix 3.

Section 2. Captive management

2.1 Enclosure

Generally, *M. emys* can be kept indoors as well as outdoors in a temperate (European) climate. A suitably sized heated shelter should always be provided if you plan on keeping individuals mainly outside. In warm climates, animals may be kept outside year-round without specific measures for thermoregulation. Figures 13 - 17 display various examples of enclosures for the species.

Enclosures can generally be classed into two categories: A) greenhouse-type enclosures and B) large outdoor enclosures which usually include separate indoor shelters/enclosures (Fig. 14). Indoor terraria may be used for young individuals (Brinkman, 2023).

The choice between these two types is largely made based on climate. Holders located in colder climates (such as northern and western Europe) may choose to keep tortoises solely in indoor enclosures because the local climate may only allow for *M. emys* to be outside for a few months of the year. Institutions located in warmer climates may find it more practical to provide a mainly outside enclosure (see 2.2 for further details on environmental conditions).

Figure 14: example of an outdoor enclosure in South Carolina, USA. (Photo credit: Turtle Survival Alliance)

2.1.1 Dimensions and Boundaries

2.1.1.1 Enclosure size

The majority of enclosures in EAZA zoos are in the 20-40m^2 range, usually for a group of between 3 to 7 adults.

Various minimal recommendations have been published: Pinson (2020) recommends an enclosure of 4 by 8 times the straight carapace length (SCL). For one large, fully grown, adult *M. emys* this would result in an enclosure of at least 11.5m^2. Other sources mention smaller dimensions (3 x 6 times SCL; Berlin.de, 1997). These dimensions do not necessarily reflect the best practice in keeping the species. In order for the keeper to provide an enclosure that allows the species to display its full range of natural behaviours, a minimum enclosure size of 20 m^2 for a single adult animal and an extra 5 m^2 for every extra individual present should be considered.

It is recommended to provide the largest enclosure possible: larger is always better. A larger enclosure provides more options for choice for the animals. Under crowded conditions the tortoises may exhibit signs of stress.

Space requirements are different for younger animals compared to adults, as younger (and thus smaller) animals naturally require less space and are more sedentary than adults. Monitoring hatchling and juvenile development is also easier in smaller enclosures, as is monitoring of environmental parameters such as humidity and temperature. Hatchlings and yearlings may be kept in groups of up to about 10 in enclosures sized 1 x 0.5 meters; single small juveniles may be kept in the same sized enclosures (1 x 0.5m; based on Haislip, pers. comm.). Enclosure size should be increased as the individual grows, up to the recommended minimal size for adults above.

2.1.1.2 Barriers

M. emys are fair climbers which should be kept in mind when designing an enclosure. It is recommended to construct low barriers with an overhang to prevent individuals from climbing out. Enclosure walls may also need to be more substantial than for similar-sized species due to their nest building capacity, which is usually done in corners or against barriers. A nest can be up to 60 centimetres high, so a barrier of around 60 centimetres high will usually suffice, provided no nests are built nearby.

M. emys is not a species that digs burrows, although digging in loose soil or natural debris is part of the species' natural behaviour. Provided the substrate is solid enough so that tortoises cannot slip under the barrier, constructing the barrier deeper into the ground is not necessary.

Barriers should be made of non-abrasive materials to prevent tortoises from damaging their shells. Barriers made of wood, smooth rockwork, solid glass and/or plastics are recommended. In addition, barriers should be sturdily constructed. *M. emys* are strong tortoises that may break through weak barriers.

2.1.2 Substrate and furnishing

2.1.2.1 Substrate

Loose, moist substrate is recommended to help with humidity requirements and nest-building. In artificial settings, mulch and humus substrates are often used. Regular topsoil and bio-flooring may also be used. Adding leaf litter to the substrate is strongly recommended to reflect the tortoises' natural habitat.

Substrate should be deep enough to hold sufficient moisture and to allow the tortoises to dig and bury themselves in, which is especially important for juveniles.

2.1.2.2 Furnishing

Furnishings should reflect the natural habitat and should provide options for the tortoises to display their natural behaviours. Figures 15-18 illustrate some examples.

A water basin or dish must be provided, large enough for the tortoises to sit in. A water basin is a good way of providing enrichment and offering drinking water. It also allows tortoises to self-regulate humidity and temperature. Addition of a mud pool could provide extra enrichment, but care must be taken that there is not a buildup of dirt or associated pathogens, and that the tortoises cannot cool down as a result of sitting in a (cool) pool for too long.

For nest building, in addition to the standard substrate, a sufficient amount of dry leaves and natural debris must be provided.

The enclosure should preferably be heavily planted to mimic the tortoise's natural environment. If this is not entirely possible, then sufficient shady areas and cover must be available inside the enclosure. This is both to help tortoises avoid the heat of direct sunlight and to make them feel more secure and less exposed. Providing edible foliage is also an excellent and behaviourally interesting way of offering food.

Figure 15: example of furnishings in an M. emys *enclosure (Photo credit: Ethan Parrott of Marwell zoo, United Kingdom).*

Figure 16: example of furnishings in an M. emys *enclosure (Photo credit: Ethan Parrott of Marwell zoo, United Kingdom).*

Figure 17: example of furnishings in an M. emys *enclosure (Photo credit: Ethan Parrott of Marwell zoo, United Kingdom).*

Figure 18: example of furnishings in an M. emys *enclosure (Photo credit: Ethan Parrott of Marwell zoo, United Kingdom).*

2.2 Environmental parameters

M. emys should be kept in environmental conditions similar to their natural habitat of moist, warm tropical broadleaf forest.

2.2.1 Lighting and heating

For effective and suitable lighting and heating of enclosures for reptiles, and especially tortoises, it is considered essential that the principal thoughts and precautions laid out in Baines *et al.* (2016), Highfield (2015) and Muryn (2018) are considered as the baseline for any further thoughts. These guidelines will be updated to reflect new significant knowledge on lighting and heating for reptiles as our understanding and technology develop. With the above fundamental thoughts in mind, it is apparent that lighting and heating for *Manouria emys* tortoises should principally involve:

- A variation in shaded and moderately bright areas with a balanced, close-to-natural spectrum;

- UV radiation within the correct limits, i.e. suitable and safe UV-A and UV-B levels;

- a temperature hotspot which is much larger than the largest animal;

- a suitable gradient of light, heat and UV-B radiation throughout the enclosure.

Compared to other (tropical) chelonian species, *M. emys* can be remarkably cold-tolerant, which is especially true for subspecies *M. e. phayrei*. The species is known to withstand a temperature range of 13-30°C. *M. emys* should not be kept at a temperature below 10°C or above 35°C for an extended period of time. Ambient daytime temperature should be kept at around 18-25°C, whilst night-time temperature may drop to 15°C (Pinson, 2020). Yearlings should preferably not be kept at temperatures below 18°C.

In the wild, *M. emys* spends most of its time under the forest canopy, so the species is not a typical basking species. Although active basking behaviour is not as pronounced in *Manouria emys* as it is in many other tortoise species, it is recommended to provide different temperature zones in the enclosure to allow individuals to self-regulate their body temperature (thermoregulate). Temperature hotspots should be around 26-29°C (Pinson, 2020). These temperatures can be locally achieved by the use of heat-emitting lamps, ceramic emitters, infrared panels or heating mats. Tortoises will preferentially raise their body temperature by moving towards areas with the highest brightness of light, which may not necessarily be the warmest areas. Therefore, non-luminous heaters which only radiate infrared-C are only to be used for raising ambient temperatures in the area, and not to provide heat for a basking area.

It would be highly beneficial if the enclosure could benefit from natural light, e.g. through a skylight or if the enclosure is located in a greenhouse. If this is not available, and to compensate for lower light levels and a shortened photoperiod in the higher latitudes in winter, metal halide (MH/HQI) lamps are recommended as they emit the most balanced spectrum of any affordable artificial light source.

To reflect the light intensity under the forest canopy, the enclosure needs to be amply shaded from the sun. Nonetheless, UVB exposure is vital to the health of all tortoise species. The natural preference of *M. emys* aligns with a classification in either zone 1 or zone 2 of the Ferguson UV zones (Baines *et al.*, 2016; Fig. 18). This corresponds to a UV Index (UVI) gradient of 0.1 – 1.0 throughout the enclosure with one-off maximums (e.g. in the basking area) of up to UVI 3.0 (Baines *et al.*, 2016). The UVI the animals are exposed to must be measured regularly using a UVI meter (e.g. Solarmeter 6.5, Solar Light Company LLC, Glenside, USA), at the level of the animals, because most lamps emit less UV irradiation as they age. I case UVB emission cannot be measured regularly, the lamps should be exchanged every 6-12 months.

The best method to apply to fulfil the lighting requirements of *M. emys* is the so-called 'shade' method, in which there is availability of low-level UVB irradiation over a larger surface area (as opposed to the 'sunbeam method' which lights and heats a small area with a higher-intensity UVB irradiation). This 'shade method' implies that an enclosure is illuminated by UVB fluorescent tubes or by direct, unfiltered sunlight. T5 fluorescent tubes (recommended brands are Arcadia and ZooMed) are probably the most versatile and adaptable method to achieve adequate and stable/long-lasting UV-B (and UV-A) radiation in enclosures. The vast range of options T5 tubes provide and their very consistent and durable UV-B output often make them the superior and more cost-efficient option. Depending on enclosure and animal size and needs, dozens of combinations in length, UV-B strength, attachment height and number of tubes used together with or without reflectors are possible - from a single 60cm fluorescent tube used without reflector on a slight vertical angle for a UV-B gradient in a hatchling enclosure to creating basking zones several m2 in size for a group of adults when several panels of multiple T5 tubes are used in combination.

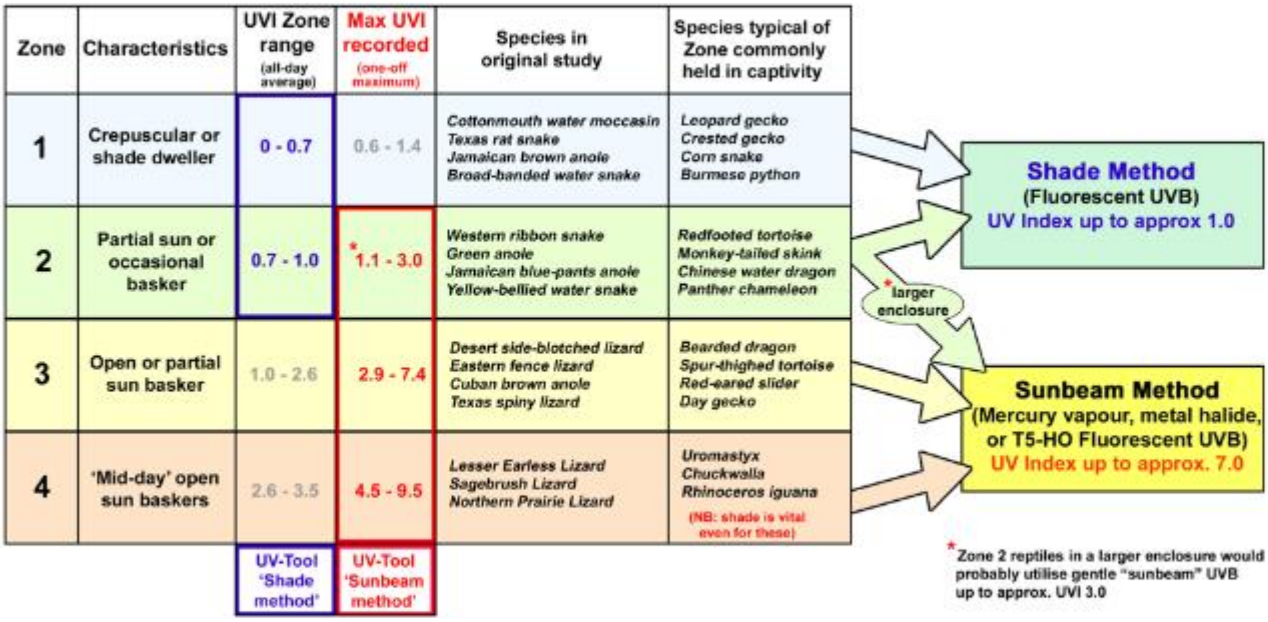

Figure 19: UV index estimates based upon the Ferguson zones (Baines et al.*, 2016).*

2.2.2 Humidity

M. emys is a rainforest species, so it prefers high environmental humidity. Air humidity must be kept at a minimum of 60%, and ideally around 80-99%. High humidity can be accomplished by misting the enclosure often or by keeping the substrate humid. If providing high humidity in the entire enclosure is unpractical, then providing humidity hotspots in the enclosure in the form of moist substrate or humid shelters is a good alternative. Having a water pool or mud wallow in an enclosure also gives individuals the option of regulating their humidity (Stanford *et al.*, 2015).
Hatchlings and juveniles need consistently higher humidity while they are developing, because insufficient levels of humidity will inevitably lead to carapacial deformities (see chapter 2.8.1). Closed-chamber techniques, in which the animals are raised in closed terraria at very high substrate and air humidity, have proven successful in preventing abnormal growth.

The different *Manouria emys* populations experience different climatic conditions. While the annual cycle of most *Manouria emys phrayrei* habitats comprises wet (monsoon) and dry conditions, most *Manouria e. emys* habitats lie in tropical climate regions without a dry period (see above: 1.5.3 Climate). Depending on the geographical origin of the founder animals a dry period may be beneficial to the health of the tortoises.

2.3 Water, diet and feeding

Feeding is preferably done on a clean flat surface, in order to prevent tortoises from ingesting inedible substrate.

2.3.1 Basic diet

Diet in captivity should reflect the diet in the wild. It should consist of mostly leafy greens and high fibre items. The leafy greens should preferably be similar to items eaten in the wild, but some more easily available substitutes are also suitable (see table 2). Soft and palatable hays, such as timothy or alfalfa hay, are suitable to be fed as high fibre feeds. Various kinds of vegetable may also be included. Fruits can be included in the diet, although this should be done in moderation as fruits contain high levels of sugars and generally low levels of essential minerals. Fruits that are used for human consumption are generally very different, with significantly higher sugar levels and less fibre, compared to fruits that tortoises would find on the forest floor. Fruits should not be fed as more than 10% of the daily diet, and in such a way that the animals can't eat selectively. Eggenschwiler (2003) recommends to feed only a very high-fibre strictly plant-based diet containing a large proportion of grass and he kept the animals in good health and breeding for many years, although this diet does not entirely reflect the natural diet.

Manouria emys is presumed to only eat animal protein opportunistically in the wild. Therefore, animal protein is not suitable to be fed in large quantities. Alternatively, protein should come mainly from vegetable matter and fungi. Mushrooms are a favourite food of *M. emys*. Feeding commercial tortoise pellets should be done with caution because there is a wide range of pellets available with very different nutritional values, many of which do not align with the natural diet of this species. If used, pellets low in proteins and carbohydrates and high in fibre (such as those consisting of high-fibre pressed plant matter) are the preferred options.

Table 3 lists food items that may be included in the diet (Brinkman, 2023). Please note that these are examples, and other food items may also be suitable.

Category	Item name (Common name)	Part(s) eaten	notes
Fungi	White and brown champignons (*Agaricus bisporus*)	Whole mushroom	Fed 1-2 per week
	Chanterelles (*Cantharellus cibarius*)	Whole mushroom	
	King trumpet and oyster mushrooms (*Pleurotus ostreatus* and *P. eryngii*)	Whole mushroom	
	Shiitake mushrooms (*Lentinula edodes*)	Whole mushroom	
	Wine Cap Mushroom (*Stropharia rugoso-annulata)*	Whole mushroom	
Fruits	Mango (*Mangifera indica*)	Chopped fruit	Fed once per week
	Papaya (*Carica papaya*)	Whole fruit	
	Mulberry (*Morus alba*)	Whole fruit	
	Low-sugar berries (blueberry *Vaccinium corymbosum*, raspberry, blackberry *Rubus* sp., strawberry *Fragaria x ananassa*)	Whole fruit	
Category	**Item name (Common name)**	**Part(s) eaten**	**notes**
Vegetables	Carrots	Whole vegetable	Fed 1-2 times per week
	Sweet potato	Whole vegetable	
	Squash	Whole vegetable	
Greens/ leaves	Elephant ear *(Alocasia* sp.*)*	Stem and leaves	Large part of diet
	Banana plant *(Musa* sp.*)*	Stem and leaves	
	Papaya *(Carica papaya)*	Leaves	
	Hibiscus sp.	Leaves and flowers	
	Fruit tree (*Prunus* sp.)	Leaves	
	Plantain *(Plantago* sp.*)*	Whole plant	
	Dandelion (*Taraxacum* sp.)	Whole plant	

	Bindweed (*Convulvulus* sp.)	Whole plant	
	Spineless cactus (*Opuntia* sp.)	Whole plant	
	Bramble *(Rubus* sp.*)*	Leaves	
	Stinging nettles *(Urtica* sp.*)*	Whole plant	
	Bamboo (Bambusoideae)	Shoots and leaves	
Dry matter	Timothy hay (*Phleum pratense*)	Hay	Large part of diet
	Orchard grass hay	Hay	
	Alfalfa/lucerne hay (*Medicago sativa*)	Hay	
Meat/animal matter	Garden worms (*Lumbricus* sp.)	Whole	Fed with moderation, once per week in small amounts
	Snails and slugs	Whole	
	Mouse (*Mus musculus*)	Whole, newborn	
	Fish	Whole or cut	
	Feeder insects	Dead or cold-stunned; gut-loaded	
Pellets	Commercial tortoise pellets or grass pellets for horses	Pellets	Depending on nutrient composition; do not feed excessive protein, i.e. no pellets for tropical tortoises

Table 2: suitable diet items for M. emys (Brinkman, 2023)

2.3.2 Supplements

Calcium supplements are common supplements for chelonians and other reptiles. As a general recommendation, an amount of calcium should be provided equal to 1% of the dry weight of the regular diet. It is furthermore recommended to provide ample calcium in the diet of females to help with egg production. This may be done by providing a source of calcium, such as cuttlefish bone, separate from the normal diet so that females can regulate their calcium intake.

A balanced, varied diet and the provision of UV-B lighting as detailed above should not necessitate the addition of other supplements.

It is recommended to provide ample calcium in the diet of females to help with egg production. This should be done by providing a source of calcium separate from the normal diet so that females can self-regulate their calcium intake. Cuttlefish bones (sepia), snail shells and egg shells are examples of appropriate calcium sources.

2.3.3 Water

Tortoises must have an *ad libitum* supply of clean water available in their enclosure. A good way to provide both water, enrichment and to help regulate humidity is to include a water basin or dish in the enclosure.
Water basins or dishes should be deep enough to allow tortoises to have a good soak but not too deep so that tortoise either cannot get out or risk drowning. It is recommended to not construct a water pool deeper than the carapace height of the tortoises. The edges of the pool should also not be too steep as to hinder tortoises in getting out, and they should provide adequate grip.
Tortoises have a tendency to defecate in the water, so frequent cleaning is necessary. Adding a drain opening to the water basin allows for easier cleaning.

2.4 Social structure

2.4.1 Group keeping

Although *M. emys* is generally considered to be a solitary species, this does not mean that it could not live in an enclosure that is shares with conspecifics.

Presence of males may cause stress to females, and males may not tolerate each other's presence. This is very much dependent on the characters of individual animals. It is recommended to keep some males separated from females outside of mating periods, and to supervise the situation when multiple males (temporarily or permanently) share an enclosure with females. Male combat may lead to individuals being injured by biting or to individuals being turned upside-down. Individuals turned upside down may overheat or have compromised blood flow which may eventually lead to complications or even death. It must be noted, however, that there are multiple instances known of males that co-habited peacefully without issues.

Nesting females may be separated to allow for other inhabitants to not disturb the nesting process or the other inhabitants may be removed from the enclosure so as to not disturb the female.

It is recommended to separate the different age groups, or individuals differing significantly in size, into different enclosures (see 2.5.4 for further details).

2.4.2 Mixed-species exhibits

Due to the generally friendly and sturdy nature of *M. emys*, the species has been combined with various different other species of birds, reptiles and mammals by numerous holders (Table 3).

Most small to medium-sized birds usually rarely cross paths or interact with the tortoises. *M. emys* has also been cohabited with various other small or medium-sized reptile and chelonian species. The main concern with these combinations is that environmental conditions should be suitable for both species, and that the large size of *Manouria emys* may inadvertently damage the other animals. Combinations with mammal species have also been tried successfully. Most successful mammal species combinations are those with mammals that do not share the same area in the enclosure as the tortoises and so do not interact with the tortoises often.

Occasional negative interactions have been noted with medium sized ground dwelling (tropical) birds (crowned pigeon; *Goura victoria*) and medium-sized ground dwelling mammals (mouse deer; *Tragulus javanicus*). For further details please see table 3 below. Care should be taken when co-habiting *M. emys* with either these or behaviourally/ecologically similar species.

Compatibility may depend on the tortoise's size and age: young or small individuals are more vulnerable than larger adults. This should be kept in mind when planning for small tortoises. It is not recommended to mix hatchlings and yearlings with other animal species in a mixed exhibit.
In addition, care should be taken not to include egg eating animals in enclosures with (nesting

female) *M. emys*. This is both a possible threat to eggs and defensive females may injure those predators.

Table 3 lists known species combinations with *M. emys,* along with information about the compatibility of those combinations (Brinkman, 2023). This information was gathered through the questionnaire. New species combinations should always be well-monitored so that possible conflicts or incompatibility are identified early.

Class	Other species	Location/zoo	Notes	Successful
Birds	Small (tropical) birds, including but not limited to: crested wood partridge, Sclater's crowned pigeon, white-naped pheasant pigeon, Nicobar pigeon, Brazilian tanager, Asian glossy starlings, and Fischer's turaco.	Cologne Zoo, Marwell Zoo.	Generally no problems.	Yes
	Victoria crowned pigeon (*Goura victoria*)	Zoo Leipzig	One instance of *M. emys* being attacked several times by pigeon and subsequently separated.	No
	Violet turaco (*Tauraco violaceus*)	Camperdown Wildlife Centre	No problems	Yes
	Pink-necked green pigeon (*Treron vernans*)	Zoo Leipzig	No problems	Yes
	Wrinkled hornbill (R*habdotorrhinus corrugatus*)	Zoo Leipzig	No problems	Yes
	Sandhill cranes (*Antigone canadensis*)	Santa Fe College Teaching Zoo	No problems	Yes
Reptiles	Sailfin lizard (*Hydrosaurus* sp.)	Loveland Living Planet Aquarium	One instance of being bitten by *M. emys*	Generally, yes
	False gharial (*Tomistoma schlegelii*)	Mandai Wildlife Group	Spacious enclosure, no problems	Yes
	Green tree monitors (*Varanus prasinus*)	Loveland Living Planet Aquarium	No problems	Yes
	Elongated tortoise (*Indotestudo elongata*)	Taipei Zoo, Nordens Ark, Mandai Wildlife Group	No problems	Yes
	African spurred tortoise (*Centrochelys sulcata*)	Camperdown Wildlife Centre	Habitat requirements too different	No
	Yellow pond turtle (*Mauremys mutica*)	Taipei Zoo	No problems	Yes
	Chinese stripe-necked turtle (*Mauremys sinensis*)	Taipei Zoo	No problems	Yes
	Radiated tortoise (*Astrochelys radiata*)	San Antonio Zoo	No problems but habitat requirements are very different	Yes
Mammals	Muntjac (*Muntiacus* sp.)	Santa Fe College Teaching Zoo	*M. emys* eats from muntjac diet	Yes
	Long nosed potoroo (*Potorous tridactylus*)	Camperdown Wildlife Centre	No problems	Yes
	Java mouse-deer (*Tragulus javanicus*)	Marwell Zoo	Bitten by *M. emys* in the past	No
	Two-toed sloth (*Choloepus* sp.)	Marwell Zoo	Little interaction between species, no problems	Yes

	White handed gibbon (*Hylobates lar*)	Omaha's Henry Doorly Zoo	Little interaction between species, no problems	Yes

Table 3: current and historic mixed species combination with M. emys *(Brinkman, 2023).*

2.5 Breeding

2.5.1 Pre-mating combat and mating

Pre-mating male-male combat is thought by some to stimulate breeding (Haislip, pers. comm.). Introducing two males in an enclosure should always be supervised as male-male combat may lead to injury or even death an individual if they are turned on their back for a prolonged period. The mating process itself may incidentally lead to injuries to the female. Keeping a close watch on possible injuries to females is recommended, especially around the areas near the legs from biting and on the carapace with may show abrasions due to excessively vigorous or frequent mating attempts.

2.5.2 Egg Laying

Like many other reptile species, female *M. emys* have the ability to store sperm for an extended period of time, potentially for multiple years, until conditions are suitable for egg laying. This should be kept in mind when breeding. Females will lay eggs when environmental conditions are suitable and nesting materials are available. Mimicking natural seasonal fluctuations in humidity and temperature may stimulate reproductive behaviour. *M. e. phayrei* females at Nagaland Zoo in India appear to be stimulated to lay their eggs by both the sounds of monsoon thunderstorms and the associated increase in humidity, which were offered artificially after the animals failed to lay eggs in their already constructed nest mounds after the start of the monsoon was delayed (Koirala, 2024).

Figure 20: exposed eggs in an opened nest mound. Photo credit: Nathan Haislip.

2.5.3 Incubation

Incubation of the eggs of *M. emys* in captivity may be performed in an artificial incubator. Commercial and home-made incubators have been used successfully (Haislip, pers. comm; Brinkman, 2023).

Pivotal sex determining temperature, the temperature at which an equal ratio of male and female offspring hatches, is estimated to be 29.29°C for *M. e. emys* (Emer 2007). Data for *M. e. phayrei* are not as detailed, but Bailey (2020) mentions an incubation temperature of about 26°C for mostly males and 29°C for mostly females, and he recommends 27°C for a mix of males and females with the highest healthy hatch rate. Incubation at cooler temperatures results in a male bias, while higher temperatures result in a female bias. Incubation temperatures should not exceed 32°C or fall below 25.5°C. Air humidity inside the incubators should be kept high, at least 80 to 100%, and substrate should be humid, but never wet. The choice of substrate should not be critical, provided that it is loose, allowing gas exchange for the eggs, and able to hold moisture.

The Incubation period depends on incubation temperature. Incubation takes around 60 to 75 days at higher temperatures (at which more females hatch). At lower temperatures (producing more males), incubation may take up to 100 days or longer. McKeown *et al.* (1991) observed an incubation period of *M. e. emys* in captivity of around 63-84 days and a clutch fertility rate of 78.4-84.4% at an incubation temperature between 25.6-28.9° C. Like in many chelonian species, twin birth from a single egg has been described in this species (Kar, 2024).

2.5.4 Hatching, Development and care of young

The hatching process may take several days. Immediately after hatching, hatchlings should be kept in a clean container without loose substrate or debris. Once the yolk has been fully absorbed and the umbilicus is closed (0-4 days), the hatchling may be moved to an enclosure with a normal soil/leaf litter substrate (Emer, 2007).

High humidity is key for proper development of young animals. To make sure humidity is adequate, it is recommended to soak/bathe hatchlings and yearlings in water regularly, and to raise them in closed-topped terraria. Furthermore, hatchlings and yearlings must not be kept at temperatures below 18°C.

Vulnerable to environmental factors, juvenile tortoises need to be carefully monitored to thrive. Hatchlings and juveniles are naturally rather shy and prefer to remain hidden in foliage or buried in substrate and debris. Providing plenty hiding opportunities in their enclosures is therefore necessary. Hatchlings need high humidity conditions for normal growth. If they are kept too dry, the growth zones at the edges of the carapace shields will appear sunken, and later lead to scute pyramiding.

Hatchlings will start to eat within days of hatching. Hatchling diet should be equal to that of adult animals. Portions may be cut to a smaller appropriate size and hard vegetables may be boiled to help with feeding. Hatchlings and yearlings should be fed at least every other day. They can be fed *ad libitum*, but care should be taken to make sure that they do not receive very high-protein diets which will lead to fast growth. The negative effects of rapid growth are poorly known, but it appears that husbandry errors may lead to more pronounced growth anomalies in rapid-growing animals. This is assumed be due to the absence of the buffering effect of slower growth on temporary errors.

Data on wild growth rates are not available, but it is assumed to be relatively slow. Studies on tortoises of other genera show variable ages to sexual maturity, ranging from about 5 to 15 years (Baard 1995, Germano 1994, Hailey & Lambert 2002, Loehr *et al.,* 2007, Znari *et al.,* 2005).

It can be assumed that *Manouria emys* do not grow faster than other tortoise species. In captivity, the growth rate of *Manouria emys* may be around 2.5-5 cm per year or faster: fig. 20 shows the example of an individual captive female. Growth rate is highly dependent on external factors such as feeding frequency, dietary protein and environmental conditions. Growth rates between individuals may differ significantly. Growth rates also differ depending on age, younger individuals generally have a higher growth rate than older individuals. When reaching sexual maturity, the growth rate slows down. In old animals, growth is almost immeasurable.

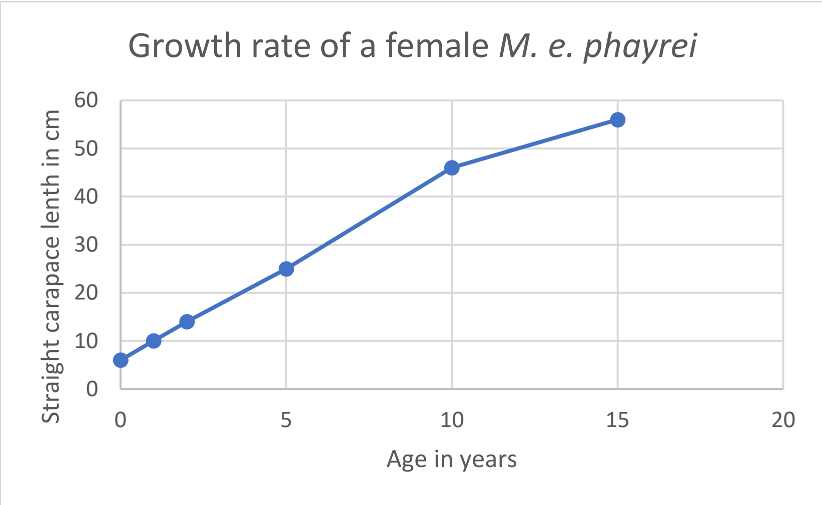

Figure 21: Recorded growth rate of one individual female M. e. phayrei *(from Bailey, 2020).*

2.5.5. Population management

Since the species is listed as critically endangered by IUCN (2018) and with a decreasing population trend, it warrants the careful management of an assurance colony in human care. Therefore, proactive and careful genetic and demographic management through the EAZA EEP is needed to secure the existing zoo population in the long-term. This proactive management extends to the ex-situ zoo population and active coordination along a clear plan among all holders (and possibly recruiting new holders) will be required to deliver the EAZA contributions to the ex-situ management roles selected for *Manouria emys* in the EAZA Regional Collection Plan for Chelonians (Goetz *et al.,* 2019).

EEP animals are encouraged to be housed together to allow for natural breeding behaviour to happen, but eggs should only be incubation to hatching if a recommendation has been issued by the EEP coordinator. For non-recommended breeding pairs, incubation of eggs for a short period to establish the fertility of the parents is encouraged.

2.6 Handling

2.6.1 Catching/General handling

Tortoises should only be picked up when absolutely necessary, to avoid stressing the animal. Handling may be accompanied by voiding of the bladder which induces additional stress on the body especially in small animals through loss of hydration. It may also be misleading in the calculation of body weights.

2.6.2 Restraining

If an animal needs to be restrained e.g. for medical procedures, the physiology and morphology of the animal needs to be taken into consideration. A good technique to immobilise individuals may be by carefully placing the tortoise with their plastron on an appropriately-sized can. If the can is tall enough and placed securely on a flat surface, and the tortoise cannot touch it with its limbs, a tortoise cannot move from it.

More than one person is usually needed to forcibly extend either limbs or neck of larger animals and any such manipulations need to be carried out with care: especially when front limbs are extended by force, the animal's dorsal process of the scapula can be put under substantial strain which might result in trauma. For procedures which require the extension of neck and limbs, it is recommended to lightly sedate or fully anesthetise animals. Training animals to extend limbs and neck while being stationary and using desensitisation training to allow injections or to draw blood is recommended as is often very successful and is considered the best possible option for examinations, routine interventions and taking samples. This is a good option in this usually curious and relatively tame species (Weiss & Wilson 2003).

2.6.3 Individual identification and marking

Microchipping
The application of microchip transponders is a common means of permanently marking animals. Microchipping is an invasive procedure and identification requires the use of a transponder reader.

Tortoises can be fitted with small microchip transponders, and the ideal location for microchipping is in the skin fold directly under the carapace, next to the tail.

Photo identification
Another method of identifying individuals is to have reference photos at hand for keepers to help identify tortoises. The advantage of this method Is that it is non-invasive. This method has been statistically proven to allow identification of individual tortoises in *Testudo* spp. (Bender 2001). This should also be a good method in Manouria sp.

Tattooing, painting or other visible marks
Any number of temporary marks can be applied to tortoises. It usually means painting unique identifiable marks on each tortoise in a collection. It may be done with skin-safe paints/substances such as nail polish, latex paint or acrylic paint. A good location to apply these marks is in the centre of a carapacial scute. The marks will need to be reapplied periodically.

For more permanent marks, tortoise shells may be engraved or clipped. Engraving involves shallow indentations being made in a tortoise's shell using a burr. Clipping involves removing a piece from the edge of a tortoise's shell. The fact that these marks are permanent does not mean they cannot fade over time from either growth or wear to the shell, so these marks may need to be periodically reapplied (Ferner & Plummer 2016). Permanent marks may not be recommended, as they are an invasive method.

2.6.4 Sexing

Sexing individuals can be a challenge for M. emys, particularly for juveniles and sub-adults.

Unlike other tortoises, male *M. emys* often do not have a concave plastron, so this feature cannot always be reliably used in sexing. As mentioned previously, mounting of another individual is also not a sex-specific characteristic.

Knowing that an animal has built a nest mound and laid eggs is normally a reliable way of ascertaining its sex, as is penile protrusion in male animals. Other more or less reliable methods are described below.

The best way to visually identifying sex is by looking at the tail. Males will generally have a longer and overall larger tail than females. The tail of the males extends past the large spurs. Another less conspicuous mark are the anal scutes. In males these scutes are curved and are slightly angled upwards while they are more pointed, flat and straight in females. The angle of the anal scute is also different in males and females. In females the angle is around 90^{O}, in males the angle is usually greater (fig. 21).

For a guaranteed result, laparoscopy can be performed. This invasive procedure involves investigating the reproductive organs using a laparoscope entered into the coelomic cavity of the anesthetised tortoise and it has been proven successful in a variety of species, including *Manouria emys* (Divers *et al.,* 2022). A laparoscopy can be performed on individuals as young as 5 months (Emer 2007) or 107 grams (Divers *et al.,* 2022). A less invasive cystoscopic method was described by Selleri *et al.* (2013).

Figure 22: Difference in tail between male (left) and female (right) (Photo credit: N. Haislip)

Laparoscopy (excerpt from Emer, 2007)

Tortoise sex was determined via laparoscopy when hatchlings reached five months of age. 0.02 mL 2% lidocaine was administered subcutaneously to each tortoise at the incision site. Each was placed in lateral recumbency with the caudal end at a 60° angle to displace the bladder and intestinal tract from the incision site. The right hindlimb was extended and a stab incision was made on the skin in the cranial inguinal area. Blunt dissection, with a 18 curved mosquito hemostat, was then used to enter the coelomic cavity. Each animal was turned upright and the telescope (diameter 2.7 mm) was inserted through the incision to identify the gonads visually. Gonad identification was based on morphology described by Rostal *et al.* (1994); testes were characterized as lobular structures while ovaries were characterized as granular structures with developing follicles.

2.6.5 Transportation

M. emys can be transported like any other tortoises in suitable transport crates, compliant with IATA

Live Animal Regulations (IATA, 2015). The animals should be individually packed in cloth bags of appropriate size and the remaining crate space suitably cushioned with a material that allows enough airflow through the crate, e.g. shredded paper. Transport temperatures should ideally be maintained between 20°C and 25°C. For short periods, temperature minima and maxima of 15°C and 30°C respectively will be tolerated; an extended time at those temperatures or any further diversion up or down might result in significant stress or even death of animals.

Transport by plane (adapted from *Goetz, 2019*)

When shipping commercially by plane, extra precautions need to be considered. Airlines will usually ship live animals in a heated cargo hold; the pilots will be advised on the appropriate temperature the hold will need to be kept in and will engage the hold heating during pre-flight checks. However, the cockpit usually has no thermometer or any continuous influence over the hold heating which means that the cockpit might not be aware of a possible malfunction of the hold heating; in any case, there would not be anything that could be done during the flight to rectify a fault. Therefore, to make sure the animals survive a possible heating failure in the hold, longer shipments by plane, e.g. commercial trans-Atlantic flights, should only be undertaken when outside ambient temperatures on the ground during loading of the crates onto the airplane are >24°C. Monitoring commercial shipments by plane through temperature data loggers enclosed in shipping crates (Goetz *et al.*, unpublished data) indicate that hold temperatures drop by about 1°C/h at cruising altitude if the hold heating is not functioning as intended. Therefore, if the animal crate is loaded at 25°C air temperature, a critical minimum temperature in the transport boxes might only be reached after >10h flight time.

2.7 Behavioural enrichment

Enrichment is providing an animal species with suitably engaging surroundings to assure animal health and welfare. In this, novelty is an important factor, as enrichment is a change in the established routine.

It is always encouraged to write an enrichment plan for any species kept. An enrichment plan is a document which includes keeper instructions to ensure the target species is always provided with items/objects/surroundings to stimulate natural behaviour.

Bartolomé *et al.* (2023) show that there are many ways of enriching reptiles in captivity. There are a few different types or ways of providing of enrichment (Shape of enrichment Inc. N.d.):

- **Environmental,** providing an appropriate environment to allow a species to choose to display various natural behaviours

- **Food related,** providing interesting/novel food, or providing food in interesting/challenging ways.
- **Sensory (tactile, smell and sound),** providing stimulating smells, sounds, colour or surfaces for animals to interact with.
- **Manipulative / Cognitive,** providing cognitive stimulation such as novel objects.
- **Social,** providing appropriate group structure and other enclosure inhabitants.

A good start to provide enrichment for *M. emys* is to have their enclosure resemble their natural habitat to allow them to display their natural behaviours. These methods of enrichment are further described in their respective sections of these guidelines: furnishings (2.1.2.2), substrate (2.1.2.1), and environmental parameters (2.2).

A large part of the natural behaviour of *M. emys* is food-focused. Providing novel and interesting food items or offering food items in interesting and challenging ways can prove beneficial. Planting natural browse in the enclosure could provide good enrichment, though this may not be practical to implement for every holder in their enclosures. Hiding or scattering food are good options to provide a challenge for tortoises, although care must be taken to prevent tortoises from accidentally ingesting substrate.

M. emys can be target-trained. This was done in one case to ease transportation so that the individual could be moved without lifting it. Target training can be both useful for enrichment as well as for overall management.

Housing *M. emys* with other individuals of the same or other species may prove enriching (see 2.4).

2.8 Veterinary considerations

Health problems are considerably more common in wild-caught individuals than in those that have been bred in, or have been acclimated to, captivity.

It is recommended to perform periodic health checkups on kept individuals. The following text describes some common health problems or diseases that might affect *M. emys*. A post-mortem on all deceased individuals should always be performed. A standardised post-mortem and veterinary sample form is included in appendix 4.

2.8.1 Husbandry-related disease

Metabolic Bone Disease

M. emys, like other chelonian species, is prone to developing metabolic bone disease (MBD), which mainly occurs due to feeding an inappropriate diet (low calcium/high phosphorus) or inadequate UVB exposure, or a combination of both. Signs may include stunted growth, a depressed shell shape, low bone density (soft shells), limb weakness, egg retention, constipation and others (de Boer *et al.*, 2019). Metastatic calcifications due to an improper calcium metabolism may result in kidney failure and gout. Accelerated growth as a result of high dietary starch content has been shown to cause lower bone mineralization as well in *Chelonoidis carbonarius* (Mendoza *et al.,* 2022).

Beak overgrowth

In some cases, an individual's rhamphotheca, or beak, may overgrow and cause the tortoise to have difficulty eating. This may be caused by physical factors such as congenital, MBD-related or traumatic rhamphotheca misalignment, or by an excess of dietary protein and/or the provision of soft foods that do not allow natural beak keratin abrasion. Long rhamphothecas have also been suggested as a secondary male characteristic in *Manouria emys phayrei* (Vic Morgan 2021 in the facebook group

“Manouria”). If the overgrowth is causing difficulty eating, trimming or filing of the rhamphotheca may be warranted.

Pyramidal growth

Wild-raised tortoises of most species have smooth carapaces, while tortoises raised under captive conditions in many cases have carapaces that display so-called ‘pyramiding’ (pyramidal growth syndrome; PGS): scutes that are centrally raised. PGS is generally considered abnormal and an indication of the use of inappropriate husbandry techniques. It can, in more extreme cases, lead to depression of the vertebral column which in term can lead to neurological symptoms.

Abnormal growth as a result of PGS cannot be reversed; it can however be stopped and further growth can continue physiologically. Keepers should be aware of this issue and improve their husbandry as needed.

Various causes for PGS have been researched, with a main focus on diet and environmental humidity. Wiesner & Iben (2002) showed that in African spurred tortoises (*Centrochelys sulcata*), a lower environmental humidity led to more pronounced pyramiding, with higher dietary protein having a positive correlation with the degree of PGS recorded. The higher-protein test group indeed grew faster, which made their pyramiding more pronounced not because of the protein itself but simply because the effects of the lower humidity were clearer due to the higher growth rate. The same applies to a study by Heinrich & Heinrich (2016) that found increased nocturnal high temperatures to be positively correlated to a higher incidence of pyramiding in Leopard tortoises (*Stigmochelys pardalis*) and Spurred Tortoises (*Centrochelys sulcata*): this study, too, did not correct for growth rate. Fife (2014) describes environmental humidity as the only parameter responsible for PGS in red-footed tortoises (*Chelonoidis carbonarius*). Other parameters, such as dietary calcium and phosphorus, have been described as causes for PGS (e.g. Gerlach 2004) but here, the presence of metabolic bone disease indicates general poor husbandry practices, probably including low humidity.

Wild tortoises live in microclimates (e.g. burrows, rock crevices, under vegetation) for at least part of their days, and these shelters expose the tortoises to a higher local environmental humidity than the surrounding habitat may have. Also, in their wild habitat, tortoises grow only during periods of food availability (humid/wet season), and not when there is no food available (dry season). Loehr *et al.* (2007) describe how speckled padlopers (*Homopus signatus*) even shrink during the dry season in South Africa.

Low humidity leads to dehydration of the cells which produce the new shell tissue and keratin in between the existing keratin scutes of a tortoise. This dehydration leads to shrinkage of the growing cells, with depresses them. New growth continues and thus has to connect to the existing, higher, keratin: pyramidal growth is the result (fig. 22).

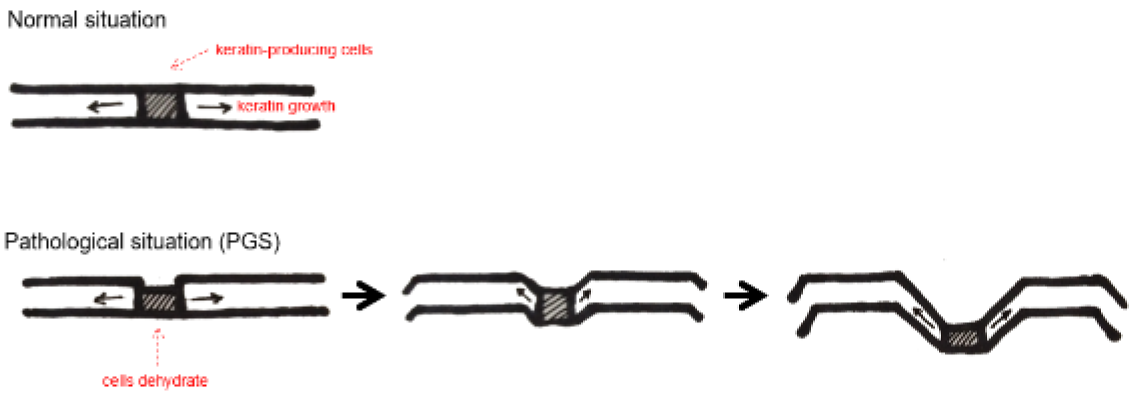

Figure 23: Normal vs. pathological shell formation in tortoises. Picture credit by the authors.

Humidity levels are often underestimated by keepers. All tortoise species, whether from relatively arid or very humid natural habitats, should be exposed to appropriate levels of environmental humidity. This can be easily achieved by making sure that the animals have access to humid microhabitats. Care should be taken not to compromise appropriate ventilation in order to achieve this. Added risks of keeping tortoises too dry are the development of skin issues and the formation of bladder stones. Hatchling and juvenile tortoises are most at risk of developing PGS, but adult tortoises should be cared for in the same way.

Some examples of successful ways in which to maintain adequate humidity levels for proper tortoise growth and health are (a combination of) the following:

- The use of mist-producing devices ("foggers"), although these often only provide short periods of high humidity as the mist quickly evaporates especially at higher temperatures; using these devices at night is therefore recommended;
- Humid hides: making sure that the tortoises rest in humid places in essential. These hides can contain any suitable humid substrate, such as Sphagnum moss, leaves or humidified terrarium soil. Wet sponges on top of ceramic hides have also been used successfully, as have humid hides made of potting soil in which plants have established themselves (Krüger, 2008; fig. 24);
- Closed-chamber rearing: this is a husbandry practice which is more and more practiced especially in the US. It entails the rearing of tortoise hatchlings in what is called "closed chamber rearing", i.e. not using open-top enclosures for the first few years but initially rearing even "dry habitat" species in closed vivariums on loose, slightly damp substrate. In this environment, air humidity is constantly elevated, mimicking the loose humus/leaf litter layers in which many hatchling tortoises spend the initial months or years after hatching;
- Avoid using very hot lamps, as these may dehydrate the tortoise's shell; especially any black-body radiators (such as e.g. ceramic heaters) are never appropriate to heat a basking area as their IR-C radiation superheats the outer keratinous shell layers while not penetrating the shells/skin, preventing heat transfer to subdermal layers and blood vessels. This makes the animals spend excessive time under these heaters and additionally dries and damages the top scutes of the carapace;
- During dry periods that may be created to mimic the natural annual cycle, care should be taken to not feed the tortoises a diet that will cause significant growth. Rather, a fibre-rich and protein-poor diet can be offered that is sufficient for the tortoise to maintain its body condition without growing. The quantity of this diet should differ drastically from what is offered during the humid season.

Fig. 24: A hide made of soil and rooted plants creates adequate humidity for juvenile tortoises (pictured: Testudo graeca*). From Krüger, 2008.*

2.8.2 Infectious diseases

Intestinal parasites

Nugroho *et al.* (2017) describe nematodes found in M*anouria emys* in Indonesia. These and other intestinal parasites such as nematodes, cestodes and flagellates can cause disease in tortoises. Signs of disease may include inappetence and low fecal consistency. The presence of intestinal parasites can be diagnosed by testing fecal samples. Treatment can take place using appropriate antiparasitic drugs. The use of ivermectin or other avermectins must always be avoided as this drug is toxic to chelonians. The presence of some protozoal organisms like *Nyctotherus* sp. Is considered physiological in herbivorous tortoises. Other intestinal flagellates, like flagellates of the genus *Hexamita*, may be pathogenic and should be treated.

Mycoplasma

Mycoplasma is a bacteria-like organism that can cause upper-respiratory infections in *Manouria emys* and other tortoise species. Signs include lethargy, weight loss, nasal discharge and 'failure to thrive'. Diagnosis can be established by PCR testing of an oropharyngeal swab and treatment of the disease is possible. Infected animals should be considered life-long carriers of the disease and asymptomatic carriers are frequently seen. Stress or suboptimal conditions may induce the development of clinical disease.

All animals should be checked for infection with *Mycoplasma*. Positive animals should be considered contagious and should not be kept together with negative animals. Used materials should be separated. Rearing of the offspring of positive animals should be done in isolation, away from positive and negative animals. They should ideally be tested as soon as the size of the animal permits (de Boer *et al.*, 2019).

Testudine intranuclear coccidiosis (TINC)

Testudine intranuclear coccidiosis (TINC) is an emerging and highly contagious disease in chelonians (turtles and tortoises) caused by an intranuclear coccidian parasite that infects host cells within the nucleus, a rare phenomenon for coccidia. Characterized by severe systemic illness including lethargy, weight loss, and oculonasal discharge, diagnosis often requires specialized quantitative PCR (qPCR) rather than standard fecal flotation, as oocysts are difficult to find in feces but can be detected in nasal or cloacal swabs. Transmission is believed to be fecal-oral, although nasal-oral routes are also possible. The disease has not been described in *Manouria emys* yet, but rather in a large number of other tortoises species, including the closely related *Manouria impressa* (Wellehan *et al.* 2022).

Testudinid herpesvirus

A possibly fatal group of viruses found in tortoises is that of the testudinid herpesviruses (ChHV; de Boer *et al.*, 2019; Goetz, 2019). These viruses have not yet been described in captive *M. emys* but they could pose a major risk (Stumpel, 2023). Symptoms may include swollen eyes, respiratory distress and oropharyngeal plaques/stomatitis. It is possible to detect herpesviruses from the tongue and oral cavity of a clinically ill individual by virus isolation and PCR, and clinically healthy carrier individuals may be detected by performing antibody assays. Just like in *Mycoplasma*, stress or suboptimal conditions induce the development of clinical disease. All tortoises of unknown status should be checked for herpesvirus infection before introductions with other tortoises. Positive animals should be considered contagious. Negative and positive animals should not be able to interact, and used materials should be separated. Rearing of the offspring of positive animals should be done in isolation, away from positive and negative animals. They should ideally be tested as soon as the size of the animal permits (de Boer *et al.*, 2019).

2.9 Specific problems and safety

Salmonella spp. carriage appears to be highly prevalent in reptiles. Reptiles should be considered nonclinical carriers of *Salmonella* spp. The organism is intermittently or continuously shed in their faeces and, if ingested by humans, may result in illness (Percipalle *et al.*, 2011). Safety precautions to prevent *Salmonella* infection include:

- Washing hands thoroughly after handling tortoises, including equipment. Be aware of cross-contamination;
- Children, elderly people, pregnant women and immunocompromised people should be extra careful when handling or touching tortoises or their environment.

2.10 Recommended research

Habitat fragmentation in the wild and unknown origins of ex-situ individuals highlight the need for research on the genetic makeup of both the wild population and in kept assurance populations: the study by Kundu *et al.* (2017) suggested haplotypes based on 18 individuals of the different subspecies, but they did not look into within-population variance. Studies looking into the genetic makeup of the populations in captivity and the possible subpopulations in the wild could provide a better insight into the genetic health of the remaining populations.

Some physiological characteristics of *M. emys* are not well documented. Studies looking further into factors such as natural growth rate and preferred optimal body temperature would be beneficial to better understand the biology of *the species*. Research is also needed to establish the pivotal temperature for sex determination in subspecies *M. e. phayrei*.

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Appendices

Appendix 1: List of abbreviations

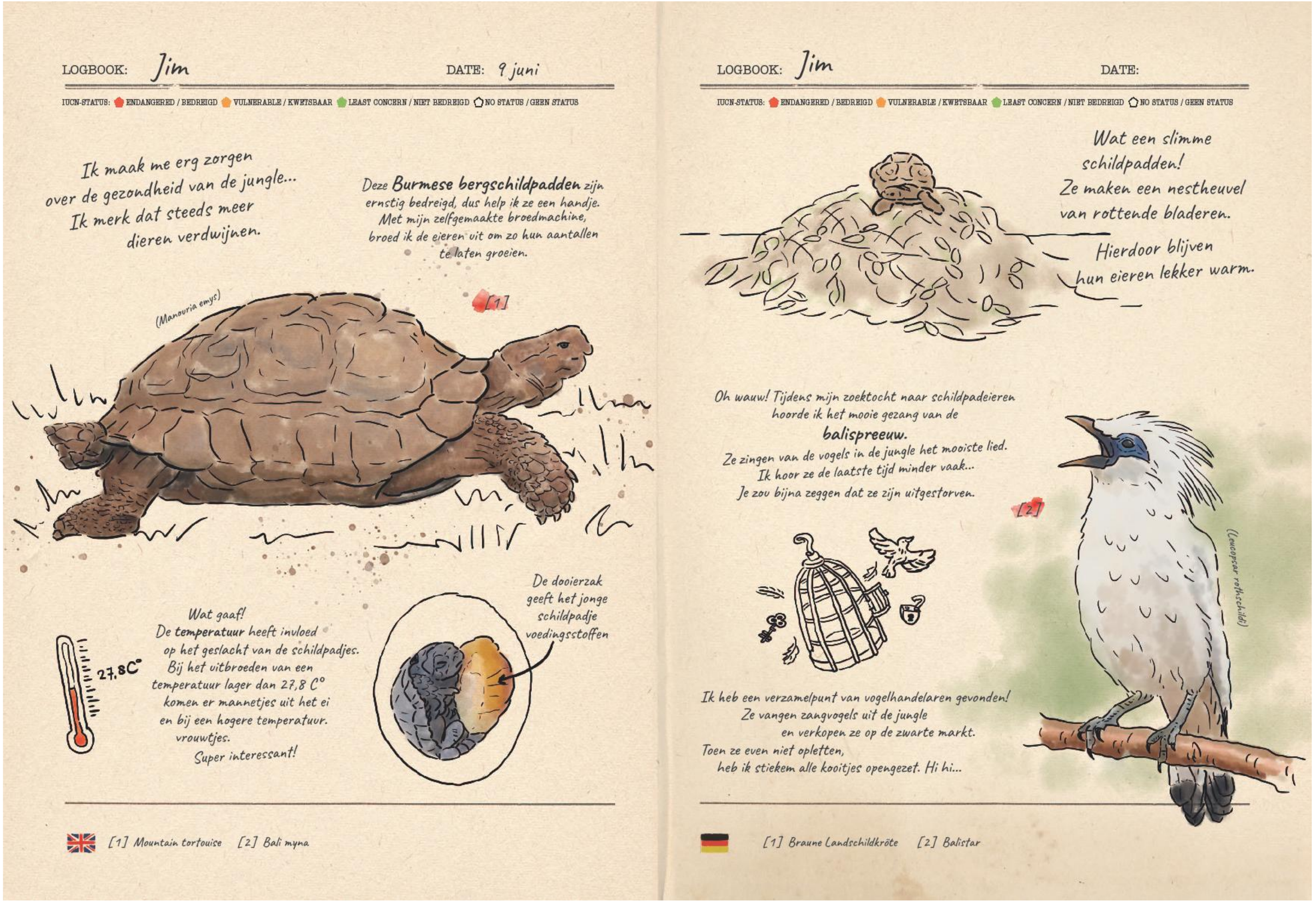
AZA:	Association of Zoos and Aquaria (America)
BPG:	Best practice guidelines
EAZA:	European Association of Zoos and Aquaria
EEP:	European ex-situ programme
IATA:	International Air Transport Association
IUCN:	International Union for Conservation of Nature and Natural Resources
MEE:	*Manouria emys emys*
MEP:	*Manouria emys phayrei*
SCL:	Straight carapace length
SSP:	Species Survival Programme
TSD:	Temperature-dependent sex determination
TSA:	Turtle Survival Alliance

Appendix 2: Glossary

Straight carapace length:	The shortest distance between the back and the front of the carapace of a tortoise.
Temperature-dependent sex determination:	Sex determination based on temperature of incubation rather than being genetically fixed through the presence of sex chromosomes.
Convergent evolution:	When two or more species or groups of species evolve the same adaptation independently of each other.

Appendix 3: Example of an educational zoo display in Dutch

(Copyright: WILDLANDS Adventure Zoo Emmen).



Appendix 4: Standardised post-mortem and veterinary sample form

developed by Javier Lopez, LdoVet, MSc, Dipl. ECZM (ZHM), MRCVS; included with permission

DIAGNOSIS

A- Anamnesis Problems/ Troubles: Date of first concern: Signs: Evolution: Treatments: **B- Diagnosis** Clinical signs: Postulated diagnosis: Treatments: Prophylaxis:	**C- Sampling** 1) Faecal samples: Yes - No Date: Number: Identification: 2) Ectoparasites: Yes – No Date: Number: Identification: 3) Blood samples: Yes - No Date: Number: Identification: 4) Blood smears: Yes – No Date: Number: Identification: 5) Bacteriology swabs: Yes – No Date: Number: Identification: 6) Nasal washes Date: Number: Identification:

Physical examination form (to be used both for living and dead sps)

Instructions for use:

- **Use one form for each tortoise examined**
- **For all live tortoises, a medical history form must also be completed. This can be downloaded from MEDARKS.**
- **For all dead tortoises a post mortem examination form must also be completed.**
- **Submit all forms to Tana office and Jersey within 24hrs.**
- **If lesions found, always TAKE PICTURES and SAMPLES.**

Animal ID	
Species	
Age	
Sex	
Weight	
Carapace length	
Yniphora-Jackson ratio status	
Is the animal alive or dead?	
If dead: Date (and time) died	
Date and time this form filled in	
Name of person filling in this form	

CLINICAL ASSESSMENT FROM A DISTANCE

	Notes
A- **Behaviour** Alertness when food given? Yes - No – Other Dynamism? Yes – No – Other B- **Posture** Head in a normal posture? Yes – No – Other Limbs in a normal posture: Yes – No – Other Is it walking normally? Yes – No – Other	

CLINICAL EXAMINATION

	Notes
A- **Respiration** Is it breathing normally? Normal - Hard- Quick Respiratory sounds normal? Yes - No – Other Respiratory rate: ..../ minute B- **Head** Any lesion? Yes – No – Other C- **Beak and Jaws** Beak: Normal – Malocclusion- Overgrowth Any fractures? Yes – No – Other D- **Oral cavity** Mucosa: Pink – Pale- Other Lesion: Tongue – Palate – None Any abnormal smell? Yes – No – Other E- **Eyes** Eyelids: Normal – Abnormal – Other Eyes: Shiny – Dry Clear – Opaque Round – Sunken Mucosa: Pink – Pale- Other Any discharge or damage? Yes – No – Other	

	Notes
F- Nares Nares: Humid – Dry • *Before the pressure*: Any discharge? Yes – No If yes: Clear – Opaque White – Yellow – Green – Other • *After the pressure*: Any discharge? Yes - No If yes: Clear – Opaque White – Yellow – Green – Other **G- Tympanic membranes** Abnormal contents? Yes – No - Other Any damage? Yes – No- Other F- **Skin** Skin: Bright - Drab Any lesion? Yes – No – Other Ectoparasites? Yes – No – Other G- **Limbs** Any lesion? Yes – No- Other Any fractures? Yes – No – Other Ectoparasites? Yes – No- Other H- **Cloaca** Cloaca: Clean - Soiled If soiled, which colour? .... Any abnormal contents? Yes – No - Other J- **Carapace** Pyramidal growth: Slight – Obvious – None Any lesion? Yes – No – Other Any fungus? Yes – No – Other K- **Plastron** Shape: Flat – Concave – Convex Any lesion? Yes – No – Other Any fungus? Yes – No – Other	

Tortoise post-mortem examination sheet

1- PREPARATION		Standard pictures to take	standard samples
Read microchip. Take morphometric measures. Take ventral, dorsal, frontal and caudal pictures			
Give details of circumstances of death and clinical history. Use the back of this sheet if necessary.	Weight:..........g Carapace length:............mm Clinical history and details of the circumstance of death:	 	

2- EXTERNAL EXAMINATION			
Examine the skin for wounds, change in colour, ectoparasites, fly eggs or larvae. Manipulate bones to detect fractures. Open the mouth and check its contents, mucosa and tongue, push eyes out and examine them.		 	Any lesions observed, take bacteriological swab or frozen, and in formalin 10%. Any parasites in Ethanol 70% or Formalin 5%
Is the skin dry and dehydrated? What is the colour of the skin? Are there ulcers on the tip of the toes? Is skin sloughing and how: in large sheaths? or in small, brown bits?. Any other lesions? Any lesion in oral cavity or eyes? Any contents in oral cavity? Any contents in the nose? What is the colour of the mucosa? Examine the left and right tympanic membranes. Examine cloaca and tail.	Eyes: concave – convex - dehydrated – wet – shiny – dry		

3- INTERNAL EXAMINATION			
Using saw, open the plastron from both sides and separate it from underlying tissues. **Take pictures**. Examine the coelomic cavity.		 	
Examine the coelomic membrane: clear- dark, Describe all abnormalities: Location, size, orientation, colour. Remove all the legs. Examine the muscles: consistency, colour, any lesions or haemorrhage? Examine fat bodies and how much?	Coelomic membrane: clear – dark Fat bodies:		

4- BODY CAVITY			
Take pictures after opening the coelomic cavity. Collect any free fluid. Observe the lay out and general aspect of all organs without disturbing normal anatomy. Are the organs in the right position? Any abnormalities: large- small-twisted-distended- swollen. **Always take pictures of all abnormalities**			Free fluid frozen + x2 air dried smears + one bacteriology swab + frozen. Fat body frozen and in formalin 10%
Locate the urinary bladder What are its contents? Check the intestines are they enlarged? Distended, full contents? Is there some free liquid inside the coelomic cavity? Quantity, colour. Do smears and measure the total protein of the liquid.	PT: ....g/dl	 	

5- HEART, LIVER AND SPLEEN			
Remove the heart by cutting blood vessels at base. If carcase is fresh, make a blood smear from heart blood. Separate the liver from the intestines and remove. Find the spleen and remove. Examine each of these organs. Take picture of heart, spleen and liver together. Take a photo of the coelomic cavity (to include, GIT, lungs, reproductive organs and kidneys). Cut heart in ½ and liver in several small pieces. Examine the cut surface.			x2 Heart blood smears Liver and heart in formalin 10% Liver and heart frozen Spleen frozen and in formalin 10%
Any lesions in surface of liver, spleen and heart? What colour is the liver and how is the edge? What size is the gall bladder and what are its contents like? How is the spleen?			

6- LUNGS			
Examine glottis. Cut lungs at bronchus and remove. Check thoroughly for nematodes			x1 lung + nematodes in Ethanol 70% lung frozen lung formalin 10%
Any lesions observed? Any parasites? Describe.			

7- KIDNEYS AND REPRODUCTIVE TRACT			
Gently pull GIT caudally while cutting membranes until gastrointestinal tract is reflected caudally, and the coelomic cavity empty except for kidneys and gonads. Take a picture (including GIT outside of coelom). Remove gonads, cut a section to put in formalin 10%. Remove kidneys, examine.			x1 kidney frozen x1 kidney formalin 10% Gonad (section) formalin 10%
What is the size of the gonads? Describe. Is the ovary full of follicles? What is the size and colour of kidneys? Are they symmetrical? Haemorrhage? Any round, white mass?			

8 - GASTROINTESTINAL TRACT (GIT)			
With the GIT still attached to the cloaca, insert a blunt rod into LI to assess patency of lumen Take PIC. Examine the length GIT. Identify any lesions, and adhesions to other organs. Open gastrointestinal tract longitudinally from oesophagus to cloaca. Take picture. Collect bacteriology swab from large intestine contents. Examine contents Remove and preserve all contents in Ethanol 70%.Take picture. Examine mucosa. Examine distal small intestine, large intestine and rectum for lumps, adhesions or fistulae, Take pictures, take small sections of lesion frozen and in formalin 10%. Place parasites in Ethanol 70%. Separate GIT from body by cutting around cloacal opening and place whole (including urinary bladder) in a separate, large pot of formalin 10%.			GIT + Urinary bladder in formalin. GIT contents in Ethanol 70%. LI contents bacteriology swab. From any mass or lesion observed: one piece in formalin 10% and one piece frozen. Any parasites found in a bijoux pot of Ethanol 70%.
Describe the stomach contents (quantity and quality). Describe the GIT wall: Is it thick and fleshy or thin like a membrane? Any parasites? Any areas of haemorrhage? Is the small intestine or large intestine distended? How much (compare to coelomic cavity volume)? Any mass on the intestinal wall? Any adhesions to bladder? Any fistula or rupture?		 	

9- NERVOUS SYSTEM			
Cut the head transversally in two parts and remove the mouth.			Half of the head in formalin 10% and half of the head frozen
Any observations?			
10- FINISH			
Collect section of muscle and place in formalin 10%. Remove one leg, remove muscles and place bones in Ethanol 70%, labelled. Double bag carcass remnants and freeze. Label all pots correctly: ID / SAMPLE / DATE / PRESERVATIVE. Use pencil to write labels and protect with layer of cello tape around pot. Send samples to labs for analysis. Make sure all waste is incinerated and surfaces disinfected to prevent disease spread. Contact the EEP coordinator attaching this form to help with decision on sample analysis.			Muscle in formalin. Leg in alcohol for skeleton-chronology Carcass double bagged for freezing.

Prospector:.. **Date:**...../....../....... **Path No:**.......................................

SPECIMEN DETAILS
Scientific name:.. **Common name**:.. **Sex**: male – female - unknown
ID No.: .... ARKS No.:.................. Chip No.:...................................... Hatched - Arrival:...../...../....
Age: new hatched/ juvenile / young adult / adult / old adult (.....years) Enclosure:..
Found dead:...../...../.... Circumstances of death: ....
Post mortem:...../...../.... State of preservation: good / fair / poor / marked autolysis
Storage since death: fresh / refrigerated / ambient temperature / frozen / fixed with:..............................

Carefully examine the tortoise following the steps laid down in this form. ***Whenever you observe something that you think it may be a lesion or abnormal always: take pictures, take a bacteriology swab, take samples in formalin and frozen***. Describe the lesion as best as you can, including: Location, size (and numbers or percentage of organ involved), shape, consistency, colour, contents.

Check list for samples to be collected during post-mortem examination
(Please tick the boxes to ensure all relevant samples have been collected.

- **Blank cells** indicate REQUIRED samples from all post mortems whether lesions are detected or not.
- **Grey boxes** indicate that samples are only required if a lesion has been observed.
- Hatched boxes mean samples are not required.

Sample	Frozen	Fixed in formalin	Smear	Fixed in alcohol	Bacteriology swab	
Skin and muscle (for DNA)		/////////////	/////////////	/////////////	/////////////	
Fat body			/////////////	/////////////	/////////////	
Whole tongue (for Picornavirus PCR)		/////////////	/////////////	/////////////	/////////////	
Heart blood	/////////////	/////////////		/////////////		
Heart			/////////////	/////////////		
Liver				/////////////		
Spleen				/////////////		
Kidney			/////////////	/////////////		
Gonad			/////////////	/////////////		
Lung			/////////////			
Lung nematodes	/////////////	/////////////	/////////////		/////////////	
Head			/////////////			
GIT + urinary bladder						
GIT contents	/////////////	/////////////	/////////////		/////////////	
GIT nematodes	/////////////	/////////////	/////////////		/////////////	
Thigh muscle			/////////////	/////////////		
Leg			/////////////		/////////////	
Carcass			/////////////		/////////////	
Other organ with abnormalities / lesions:	**Frozen**	**Fixed in formalin**	**Smear**	**Fixed in alcohol**	**Bact. swab**	**Pictures**
1 Coelomic fluid		/////////////		/////////////		
2 Lymph sac fluid		/////////////		/////////////		
3 Urinary bladder contents		/////////////	/////////////			
4 GIT mass or other lesion				/////////////		
5						
6						
7						
8						
9						

Note:
Frozen samples must be always maintained frozen. Thawing and refreezing will damage them.
For fixing tissues in formalin or alcohol use a ratio of 10:1 fixative to tissue by volume and ensure all pieces are a maximum of 10mm cube.

COPROLOGY
Macroscopy:

Colour: ……………………………………….
Consistency: Soft – Medium – Hard
Plants non digested rate: 0 – 2 – 4 (0: None, 2: between 1 or 2, 4: > 3)

Microscopy
Pin worm:
Hookworm:
Cestodes:
Other (adult worms, Larvae…):

Please also do a fresh microscopic examination for motile protozoa, especially Hexamita.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