EAZA Reptile Taxon Advisory Group

Best Practice Guidelines for the Ploughshare tortoise or Angonoka (*Astrochelys yniphora*)

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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. Above and 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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Cover and back photo: A group of semi-adult *Astrochelys yniphora* at Ampijoroa, Madagascar.
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Content (Click to move to page)

Introduction

These guidelines are designed for the captive husbandry and breeding of Ploughshare tortoises (*Astrochelys yniphora*) in temperate countries and indoor enclosures. Although certain chapters are of course universally relevant, these guidelines do not necessarily guide or represent how the species should be or is being kept e.g. in breeding stations in Madagascar or in any other country where suitable outdoor access is available throughout the year and suitable natural climates exist. The husbandry procedures at Durrell Conservation Trust's Chelonian Captive Breeding Centre in Ampijoroa, Madagascar, are guided by a separate, in-house protocol (Goetz et al. 2014).

Section 1. Biology and field data

1.1 Taxonomy

- Order: Testudines
- Family: Testudinidae
- Genus: *Astrochelys*
- Species: *Astrochelys yniphora* (VAILLANT, 1885)

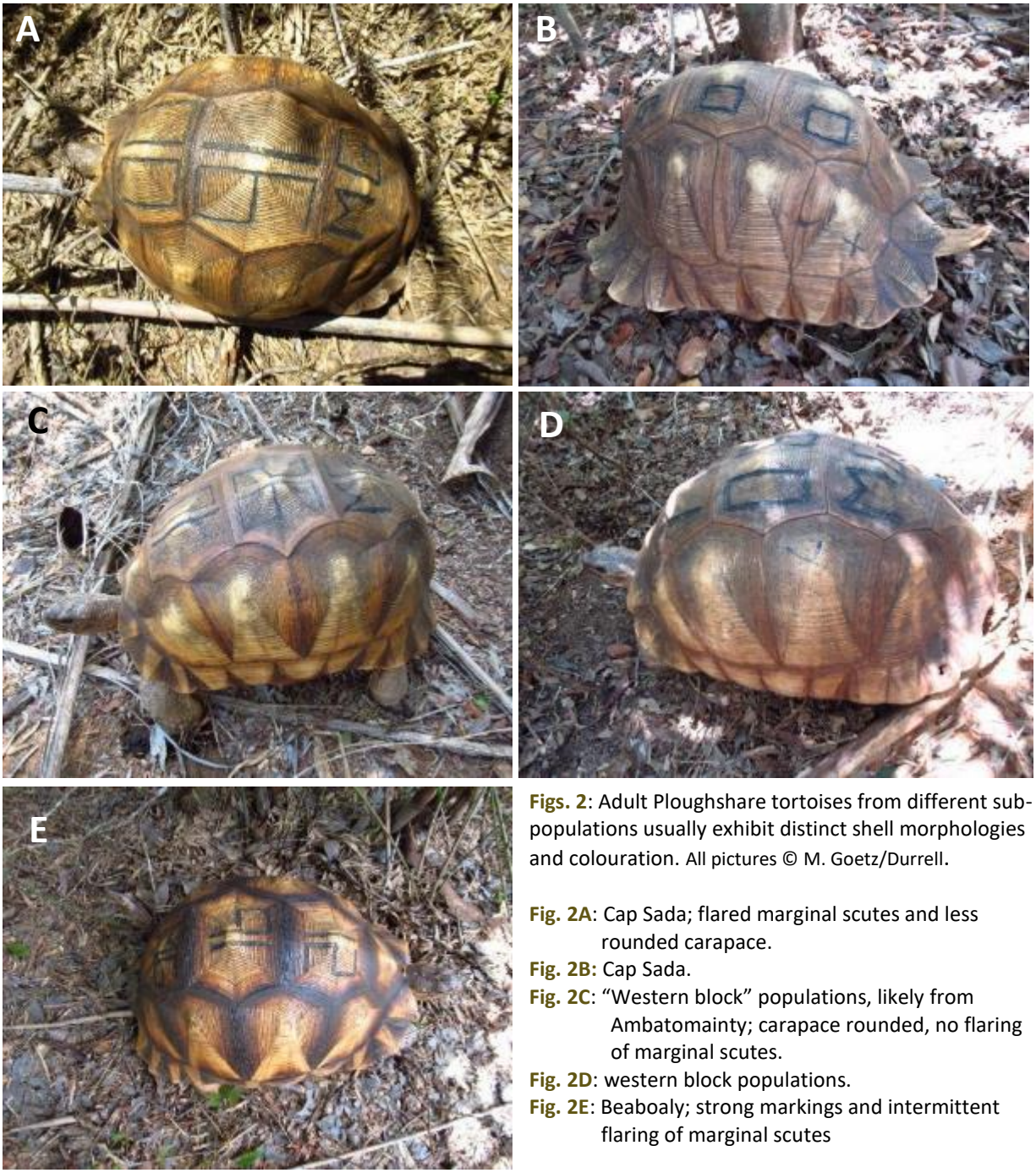
Common names: Ploughshare tortoise (English); Angonoka (Malagasy); Tortue à soc (French); Schnabelbrustschildkröte (German).

1.2 Morphology

The Ploughshare tortoise is the largest extant endemic tortoise in Madagascar. It derives its English, French and German vernacular name from an extended gular projection created by the fusion of two scutes at the anterior ventral section of the carapace. The appearance of this elongated scute is reminiscent of the share of a plough. It is particularly elongated in adult males (Fig. 1A) where it is used during bouts of combat, when adult males attempt to push and overturn each other, prior to mating attempts. The Ploughshare tortoise has a high domed carapace, golden yellow in young and juveniles with strongly demarcated growth rings, which can be counted to age young tortoises up to about 10-20 years of age. The scute margins are reddish to dark brown to black in these younger animals (Fig. 1C). Older animals lose the distinct growth rings as they age and become worn, and the carapace becomes a uniform tan-yellow in colour (Fig. 1B).

Fig. 1A: large adult male Ploughshare tortoise with prominent gular scute. © A. Mandibihasina/Durrell.
Fig. 1B: large adult female Ploughshare tortoise. © A. Mandibihasina/Durrell.
Fig. 1C: group of juvenile Ploughshare tortoises, about 10 years old. © M. Goetz/Durrell.

Adult straight carapace length is described as being 250–486 mm (Juvik et al., 1997; Smith et al., 1999; Pedrono & Markwell, 2001) with a mean adult weight of 8.8 and 10.3kg for females and males respectively; although adult males can achieve maximal weights of over 20kg and a straight carapace length of 52cm (Mandimbihasina & Currylow, 2014).
As the Bay of Baly and the Andranomavo River are natural barriers dividing the Ploughshare populations into East and West (Fig. 4), a very low level of gene flow has been detected between sub-populations with differentiation existing between the eastern and western sub-populations (see 1.4.1. Distribution). Adult tortoise shell morphologies differ slightly between sub-populations and a trained eye is able to allocate most adult animals to a respective sub-population. Some examples are given in Figs. 2A – 2E.



Figs. 2: Adult Ploughshare tortoises from different sub-populations usually exhibit distinct shell morphologies and colouration. All pictures © M. Goetz/Durrell.

Fig. 2A: Cap Sada; flared marginal scutes and less rounded carapace.
Fig. 2B: Cap Sada.
Fig. 2C: "Western block" populations, likely from Ambatomainty; carapace rounded, no flaring of marginal scutes.
Fig. 2D: western block populations.
Fig. 2E: Beabolay; strong markings and intermittent flaring of marginal scutes

1.3 Physiology

There are very few data on physiological aspects of either wild or captive Ploughshare tortoises other than the reproductive data listed below.

One study by Currylow et al. (2017) indicates that adult Ploughshare tortoises show an elevated body condition index (BCI) in captivity with significantly lower values in wild animals whereas juveniles/sub-adult animals exhibit a lower BCI in captivity compared to wild individuals. The paper studies stress hormones in wild animals and compares them to animals both in captivity in a breeding station in Madagascar, and in a zoo setting in the USA. Male and female reproductive hormone cycles are also compared and related to environmental factors and activity patterns (https://doi.org/10.1371/journal).

Lopez et al. (2017) sampled 172 captive and 40 wild Angonoka to determine haematological and biochemical reference intervals which differed between adults and juveniles and between captive and wild animals. These may serve as benchmarks for clinical assessment and conservation of this critically endangered species although further research is required to assess effects of seasonality and reproductive cycles (https://tinyurl.com/yxle8m9r).

1.4 Longevity

Due to the scarcity of the Ploughshare tortoise in captive collections and the general difficulty of conducting research on this species in the wild, its longevity is yet unknown.
The Ploughshare tortoise's sister taxon, the Radiated tortoise (*Astrochelys radiata*), regularly lives beyond the age of 100 (Randriamahazo et al. 2007), possibly even up to 188 years (Robb & Turbott, 1917).
Biologically and ecologically similar to this very close relative, *A. yniphora* is believed to be able to live beyond 100 years as well. The oldest age recorded is 78 years for a male alive at Durrell Wildlife Conservation Trust's chelonian breeding centre in Madagascar in 2019 (Goetz, 2019).

1.5 Conservation status, zoogeography and ecology

1.5.1 Distribution

The distribution of the Ploughshare tortoise is restricted to an area of 16,000 hectares (160 km^2) of mixed bamboo scrub, shrub thickets and palm savannah around Baly Bay on the west coast of Madagascar (Fig. 4), at altitudes varying from 0-90m (e.g. Smith et al., 1999; Andrianandrasana, 2000; Andrianandrasana/Durrell Wildlife Conservation Trust, unpubl. data).
Until very recently, five remaining fragmented sub-populations exist within the confines of Baly Bay National Park: Cap Sada and Beheta in the east of Baly Bay and Ambatomainty, Betainalika and Beaboaly west of the bay (see Fig. 4).
Ploughshare tortoises had been extirpated from Beaboaly in the late 1960s and early 1970s following centuries of collection for use by sailors and export to the Comoros and repeated extensive bush fires (Andrianandrasana, 2000). Between 2006 and 2015 Beaboaly was used as reintroduction site for tortoises bred and head-started at Durrell Wildlife Conservation Trust's chelonian breeding centre in Ankarafantsika National Park until releases had to be stopped due to extensive poaching pressures (see below). The base for Durrell Wildlife Conservation Trust's Baly Bay field station the site is now used to safeguard the last remaining wild Ploughshare tortoises in a large and heavily guarded fenced-in natural enclosure. Reintroductions will likely continue to take place once the safety of the site has been fully established and the guarded area can be extended further.

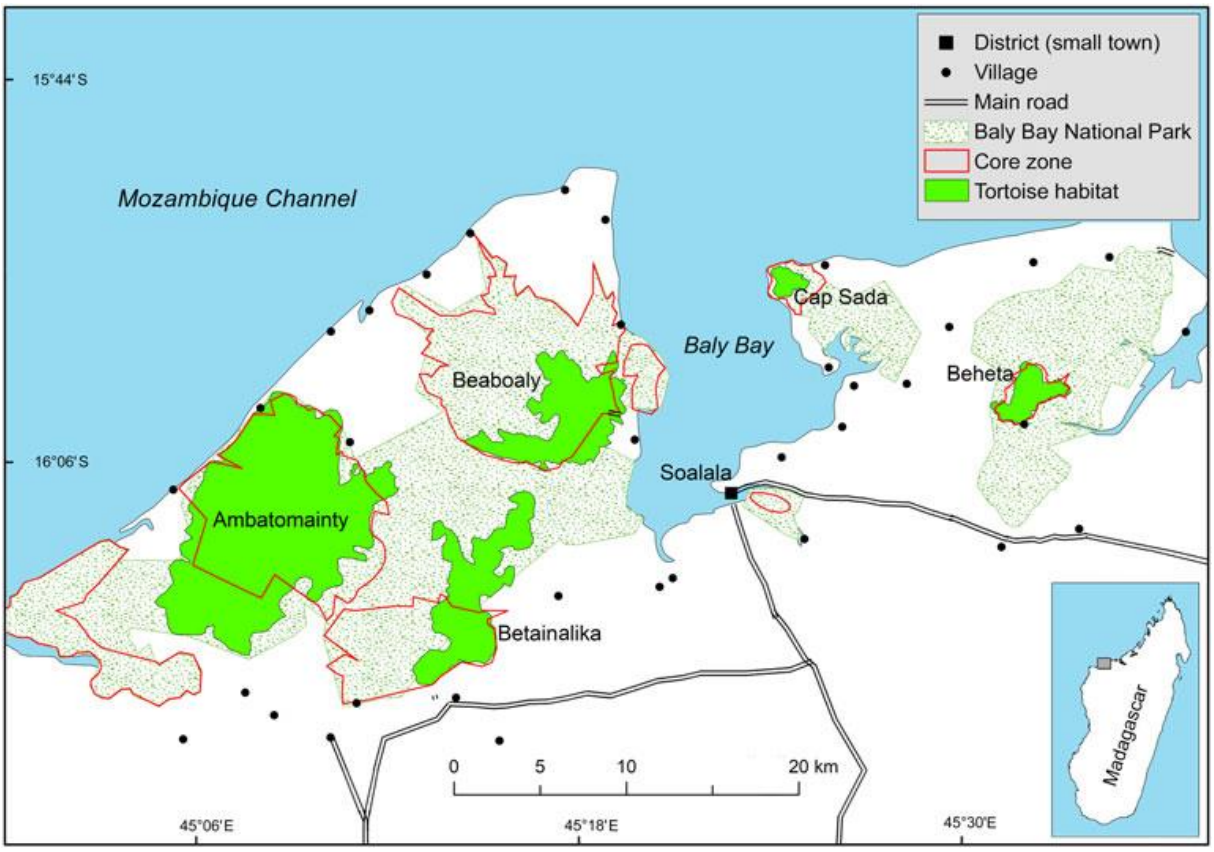


Fig. 4: Ploughshare tortoise sub-population distribution in the wild in Baly Bay National Park, western Madagascar, pre-2015. © Durrell Wildlife Conservation Trust.

1.5.2. Habitat

The Ploughshare tortoise inhabits a mixed habitat of bamboo thicket (Figs. 5E and 5F), sandy palm savannah (Fig. 5A and 5B), and dense scrub-shrub (Figs. 5C, 5D, 5G and 5H). The bamboo habitat is dominated by *Perrierbambos madagascariensis* which is endemic to the northwest region of Madagascar, and the palm savannah by emergent palm trees *Erythrophleum couminga*, *Hyphaene shatan*, and *Hymenodictyon leandrii.* The understory of the savannah is dominated by herbaceous *Mundulea*, and *Croton* spp. and tall grasses *Heteropogon contortus* and *Aristida rufescens* (Andrianandrasana, 2000). The scrub-shrub is dominated by ericaceous *Terminalia boivinii* and *Bauhinia pervilleii* (Smith et al., 1999).

The mix of habitats used by the tortoises provides refugia from predators including man (bamboo and scrub-shrub), nesting sites (savannah) and food (shrub) in what is otherwise an extremely arid and inhospitable environment. Savannah (Fig. 5A and 5B) and the bamboo thicket (Fig. 5E and 5F) are a fire-dependent transitional habitat. Natural fires do not appear to be a major threat to the Ploughshare tortoise as they generally occur during the wet season (Juvik et al., 1981) and are less intense than anthropogenic fires set during the dry season (Smith et al., 1999).

Habitat use by the tortoises varies somewhat seasonally, primarily by females leaving the denser areas of bamboo and scrub to nest in open un-vegetated areas during the wet season (Smith et al., 1999) (Fig. 10).

Angonokas very rarely eat bamboo leaves if another plant material is available and it is suggested that the habitat currently occupied does not necessarily correspond to that which is optimal but that where agents of decline (mainly bushfires and human collectors) have been least evident. Therefore, it might be that the current distribution of the Ploughshare tortoise in this kind of habitat represents only a relic of their historic distribution rather than an ecological need of the tortoises.

Fig. 5A: Open sand savannah at Baly Bay. © P. Krizan/ Durrell.

Fig. 5B: Palm savannah at Baly Bay (wet season). © P. Krizan/Durrell.

Fig. 5C: Dense scrub-shrub dry forest at Baly Bay (wet season). © M. Goetz/Durrell.

Fig. 5D: Dense scrub-shrub dry forest at Baly Bay (dry season). © P. Krizan/Durrell.

Fig. 5E: Bamboo thicket at Baly Bay (wet season). © M. Goetz/Durrell.

Fig. 5F: Bamboo thicket at Baly Bay (dry season). © P. Krizan/Durrell.

Fig. 5G: Grassy forest edge at Baly Bay (wet season). © M. Goetz/Durrell.

Fig. 5H: Grassy forest edge at Baly Bay (dry season). © M. Goetz/Durrell.

Fig. 5J: Overview of the dry forest habitat at Baly Bay (wet season). © M. Goetz/Durrell.

Fig. 5K: Overview of the dry forest habitat at Baly Bay (dry season). © P. Krizan/Durrell.

1.5.3 Climate

Baly Bay is one of the most arid regions of Madagascar, with a very distinct dry and wet season (Fig. 6). The wet season is considered to be from November to March, but varies from year to year and is perceived to have shortened considerably over the last 10-20 years annual rainfall averages less than 1090 mm with nearly all of it falling in January and February whereas previously rains might have started as early as mid-November (E. Bekarany, pers. comm.). According to Andrianandrasana (2000) and Mandimbihasana (2004), the mean annual temperature is 27.8°C and ranges from lows of 19.5°C at night in July to 34.4°C during the day in March.

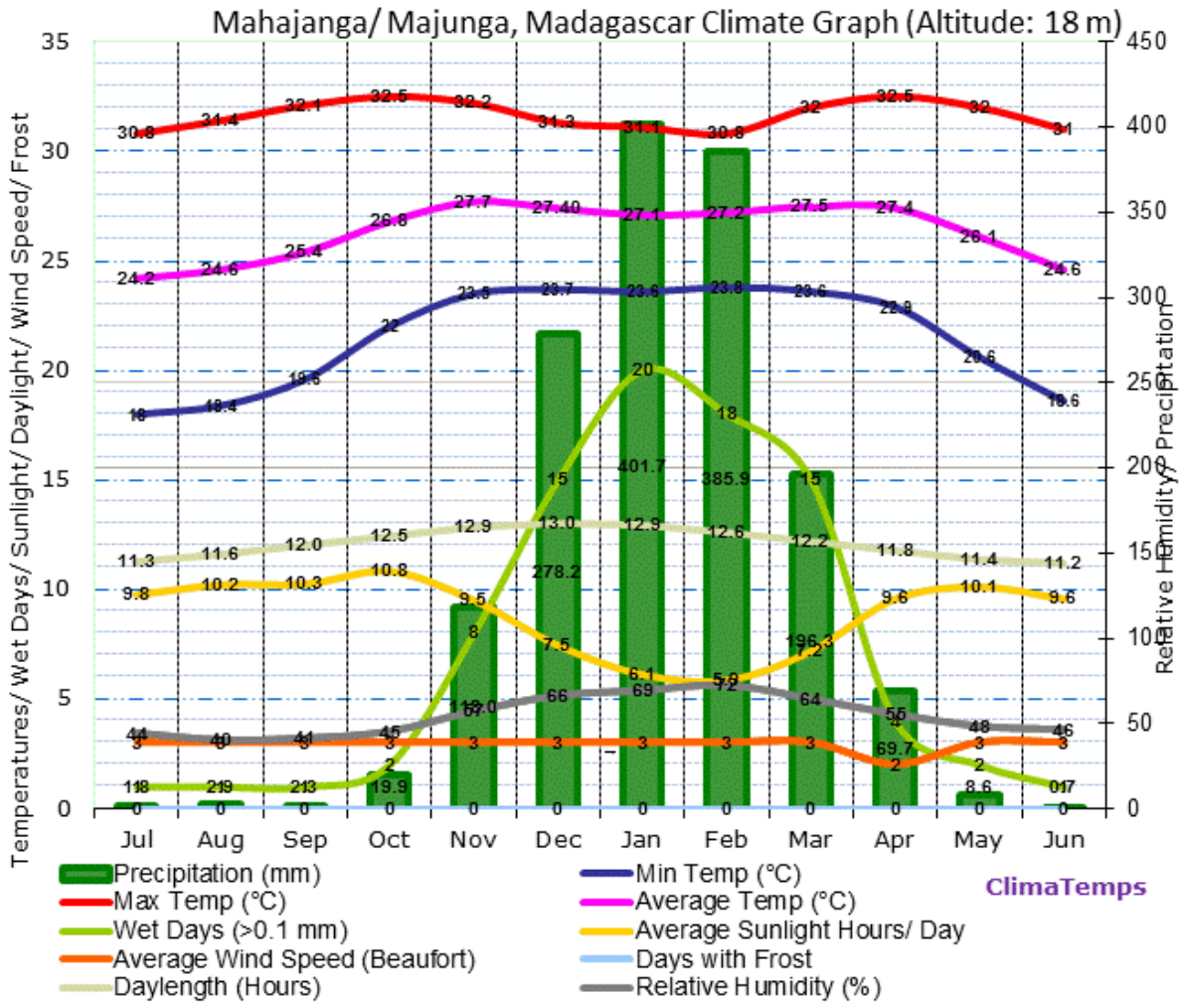


Fig. 6: Climate graph for the Mahajanga region, Madagascar. © http://www.mahajanga.climatemps.com.

Recordings of a temperature data logger placed in a semi-shaded area 45cm above ground at Beaboaly in Baly Bay National Park in 2008 show a mean annual temperature of 26.3°C and ranges from lows of 12.8°C at night in July to absolute daytime maxima of 40.9°C around October (Turtle Conservancy, unpubl. data; Figs. 7) in that year.

Another temperature data logger was placed in a shaded area amongst high bamboo thickets 50cm above ground at Baly Bay National Park in 2018. It shows a mean annual temperature of 26.7°C and ranges from lows of 12.5°C at night in July to absolute daytime maxima of 43.5°C around November (Durrell Wildlife Conservation Trust, unpubl. data; Figs. 8 and 9) in that year.

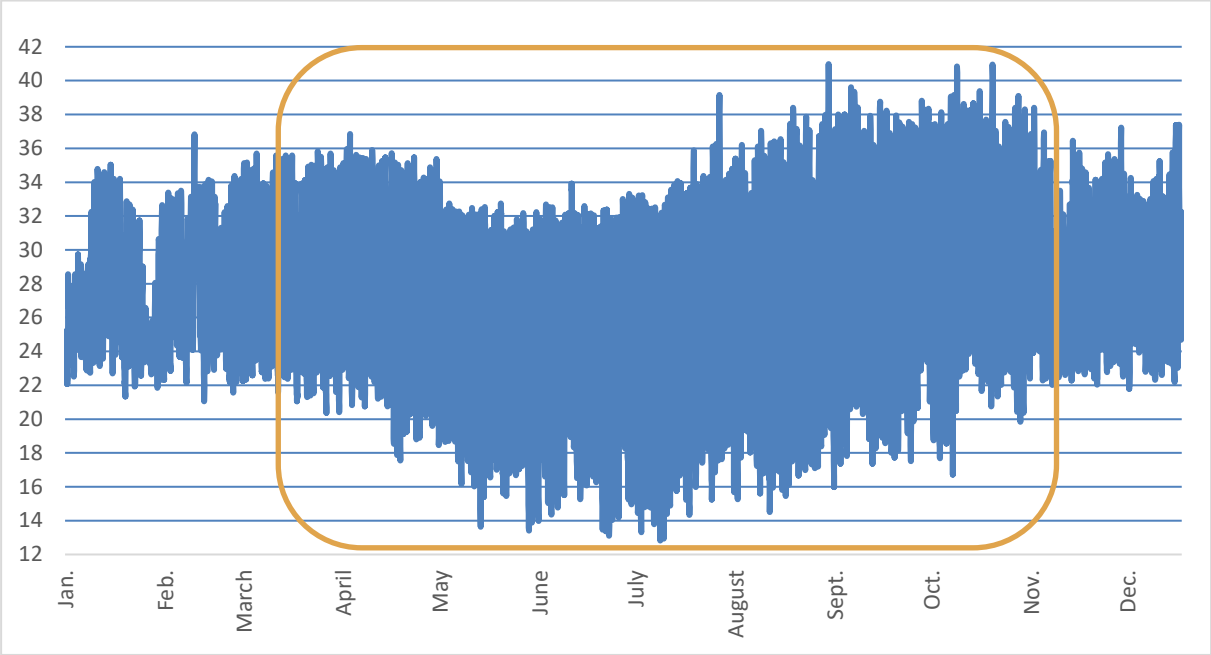


Fig. 7A: Temperatures recorded at Baly Bay in 2008 (formatted Jan-Dec). Dry season circled.
© M. Goetz/Durrell. Data courtesy of Turtle Conservancy.

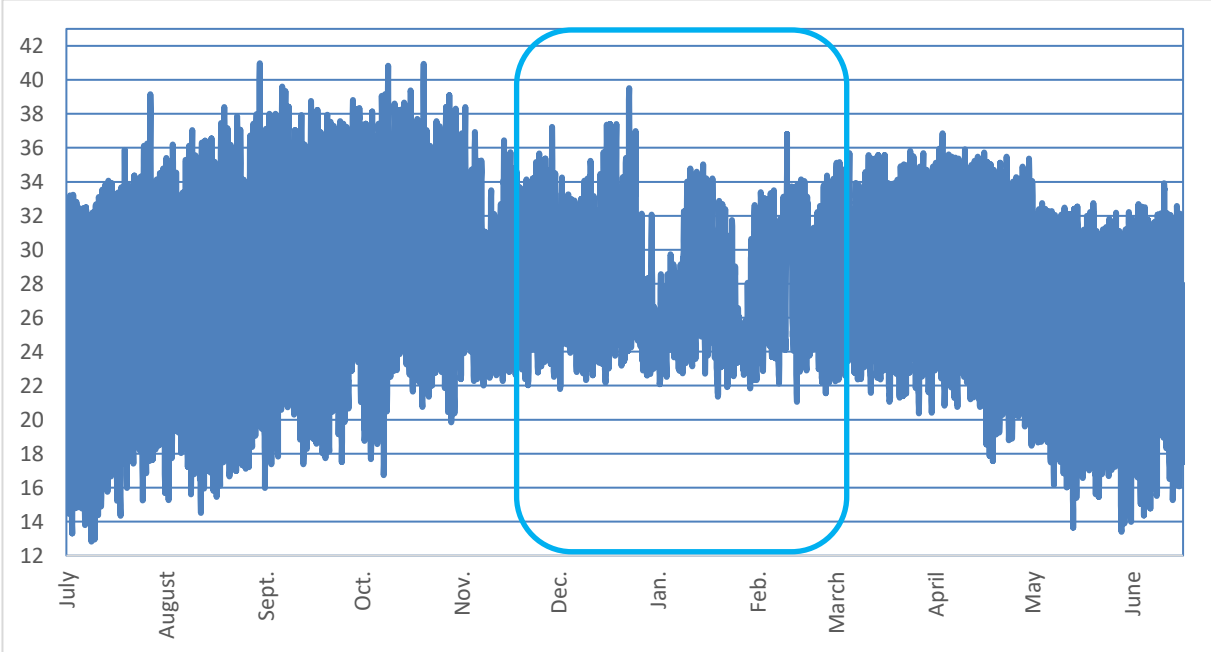


Fig. 7B: Temperatures recorded at Baly Bay in 2008 (formatted July-June). Wet season circled.
© M. Goetz/Durrell. Data courtesy of Turtle Conservancy.

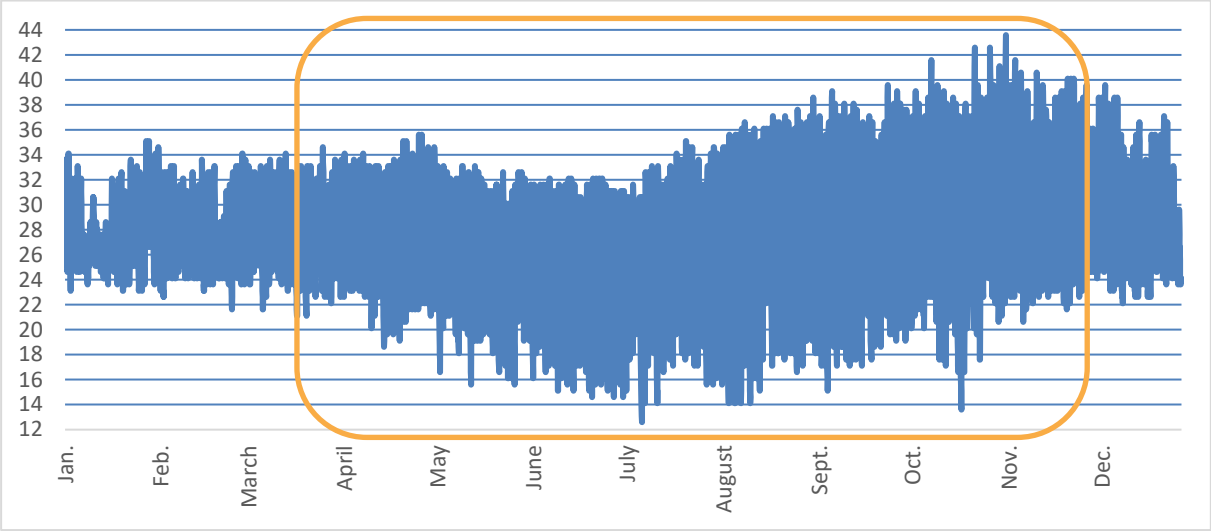


Fig. 8A: Temperatures recorded at Baly Bay in 2018 (formatted Jan-Dec). Dry season circled.
© M. Goetz/Durrell.

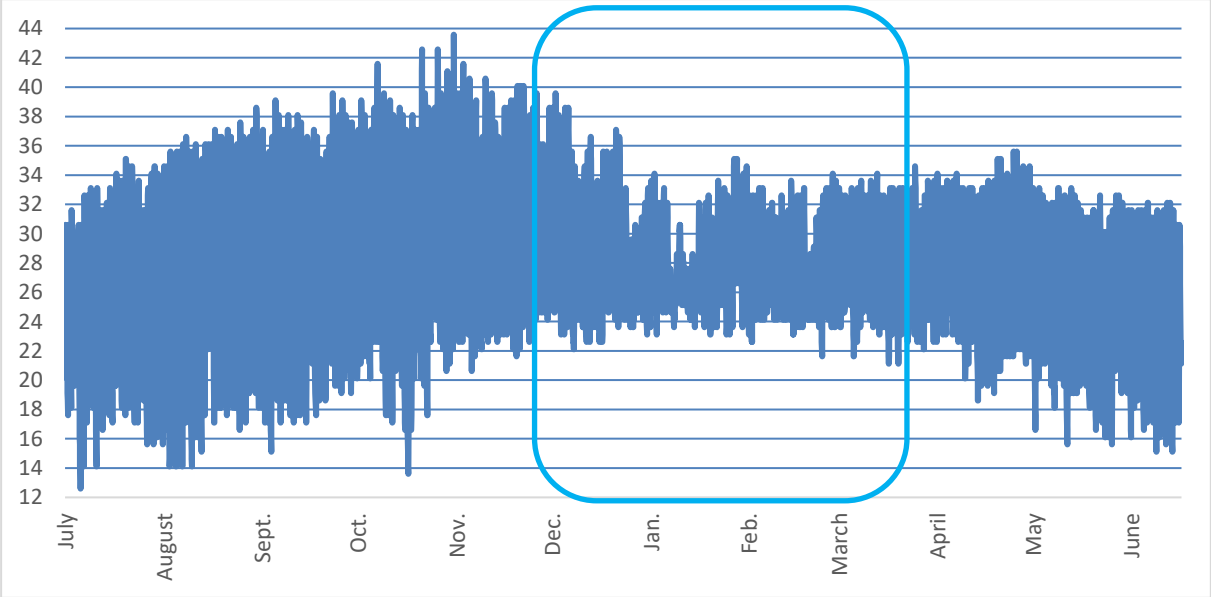


Fig. 8B: Temperatures recorded at Baly Bay in 2018 (formatted July-June). Wet season circled.
© M. Goetz/Durrell.

Fig. 9: Shaded location of the temperature data loggers mentioned on page 13, placed at Baly Bay in 2018. Left: entrance to location in forest. Right: Data logger tied to small tree. © M. Goetz/Durrell.

1.5.4 Population

The Ploughshare tortoise was already considered to be on the verge of extinction by the time research was first carried out on its status and distribution in the 1970s (Juvik & Blanc, 1974; Juvik et al., 1991). Until 2010, the wild population consisted of around 950 ± 375 animals within five remaining fragmented sub-populations. All sub-populations were restricted to the confines of Baly Bay National Park, which was created in 1997. The Bay of Baly divides these into "Eastern" and "Western" groups (Fig. 4).

In the East, Cape Sada is a peninsula on the north-eastern corner of Baly Bay and is the most well studied and accessible wild site, with an area of suitable Ploughshare habitat of approximately 234ha. The easternmost population of Beheta, 15 km southeast of Cape Sada near Marambitsy Bay, consists of circa 688ha of suitable habitat.

Three further subpopulation sites lie to the west of Baly Bay. The largest with an area of 10,644ha is called Ambatomainty-Andranolava. It is adjacent to the Mozambique Channel and divided into three parts by two small rivers, the Andranolava in the south and the Antsahavaky in the north. The second large western population of Betainalika (2,908ha) lies further inland, to the southeast of Ambatomainty.

The last sub-population is a reintroduced population of captive-hatched and head-started tortoises that have been released at Beaboaly, an area of approximately 1,400ha, north of Betainalika and northeast of Ambatomainty. Prior to these reintroductions, Ploughshare tortoises had been extirpated from this area following centuries of collection for export to the Comoros and use by sailors, and repeated extensive bush fires in the late 1960s and early 1970s (Andrianandrasana, 2000).

Smaller fragments (<150ha) of suitable habitat exist in the east, just outside of the National Park, at Ambikobanty-Ankatsakabe, Maroaboaly, and Akijinjaly. Ankatsakabe was known to have a small population of Ploughshares in 1995 (Smith, 1999a) but no tortoises, tracks or faeces have been found at any of these sites, despite repeated surveys by Durrell Wildlife Conservation Trust in the last ten years.

The Bay of Baly and the Andranomavo River are natural barriers dividing the Ploughshare populations into East and West. A very low level of gene flow has been detected between sub-populations with differentiation existing between Cape Sada, Beheta and the western subpopulations. However, these do not seem to warrant classification at the sub-specific level (Mandimbihasina et al., 2004; Mandimbihasina et al., in prep) although adult tortoise shell morphologies differ slightly between sub-populations and a trained eye is able to allocate most adult animals to a respective sub-population (Figs. 2). Gene flow occurs among western sub-populations, albeit at low levels.

Patches of forest and savannah exist between Betainalika and Ambatomainty at distances from 3-9km, providing potential corridors for movement between the major remaining western fragments and there is seasonal connectivity between sites as rivers become dry during the tortoise mating season (September-November).

Cape Sada and Beheta, however, appear to be sufficiently isolated from each other to suppress gene flow and create distinct genetic clusters (Mandimbihasina et al., in prep.) from each other and from the west. Whether this separation is man-made through historical habitat degradation or a natural phenomenon inherent in the species' natural distribution is unknown – the separation of the distinct populations goes too far back in time that no conclusions can be made.

Since 2010, substantial numbers of tortoises were poached annually, mainly for the illegal pet trade in South-east Asia and China, reducing the overall population to around 400-600 animals by 2015 (Mandimbihasina et al. 2018). Between 2015 and 2017, collection for the illegal pet trade increased manifold, most likely driven by a sudden, significant increase in prices paid.

Within 18 months, all existing sub-populations were targeted and apparently emptied completely, largely driven by an about 10-fold increase in prices paid to local poachers. The eastern sub-

populations of Sada and Beheta are now very likely (at least functionally) extinct; Betainalika and Ambatomainty in the west seem reduced to probably no more than 100 animals in total although arriving at a confident census of such small numbers in the given habitat there is extremely difficult.

1.5.5 Conservation status

IUCN Red-List status: Critically Endangered A4ad; B2ab(v); C1; E (Leuteritz & Pedrono, 2008).

Direct *in-situ* conservation efforts have been underway for over 30 years through Durrell Wildlife Conservation Trust's Madagascar programme in conjunction with the Government of Madagascar and support from a range of international partners. Baly Bay National Park was established in 1998 for the entire known range of the species. Recovery efforts in the park include on-going population assessments, comprehensive field research and community relations that include developments of sustainable livelihoods for villagers and the employment of Forest Guardians who communicate suspicious activity to the authorities and participate in field research. A threat is arising in the form of a Chinese iron mining company that plans to construct a road directly through the Park to an envisioned deep sea harbour in the bay of Baly.
Recovery of the species requires not only in *situ-work* to protect natural habitat, but also conservation breeding in assurance colonies and reintroduction. Durrell Wildlife Conservation Trust has established a successful captive breeding program in Madagascar in 1986. This program was expanded to a reintroduction program in 2006. Early successes of the reintroduction program included the annual discovery of several wild-hatched offspring of captive-born, reintroduced adults since 2012. Reintroductions had to be stopped in 2017 due to the aforementioned spike in poaching and current efforts include to establish both, a second breeding centre and a heavily guarded, large fenced enclosure at the reintroduction site for the safekeeping of the last remaining wild animals. This is important as Baly Bay National Park was specifically established for the protection of the Ploughshare tortoise and the removal of all specimen from the wild into guarded captivity would likely result in the decommissioning of the national park and the opening up of this very unique ecosystem to iron ore mining.

A currently (2019) relatively small population of Ploughshare tortoises is managed in zoos worldwide; these are animals confiscated by authorities and although a few specimen are repatriated to Madagascar under bilateral agreements, biosecurity concerns largely prevent the incorporation of such animals into the breeding programme in Madagascar. Therefore, these animals form a separate *ex-situ* sub-population under studbook management; the regional collection plans of both, EAZA and AZA identify the species' *ex-situ* population primarily as an Ark/Insurance population (Goetz et al. 2019) and a Conservation Ark (AZA, 2016) with genetic management; additional roles include veterinary research and public education through exhibit messaging.
The AZA-SSP is managing the American Region population (Gibbons, 2013) while the EAZA-EEP is doing the same for the European region with the additional aim of providing genetic population management and breeding advice to Durrell Wildlife Conservation Trust's captive breeding and release programme in Madagascar (Goetz, 2015).
A WAZA-International Studbook serves as an overall database of all officially and legally held Angonoka worldwide (Goetz, 2016). Given the overall status of the species, the need to use any available individuals to establish and form a managed breeding programme is clearly important. Keeping track of individuals already kept and acting as a first point of contact to allocate confiscated animals to form sustainable breeding groups in experienced institutions is the main goal of the ISB.

1.6 Diet and feeding

Observations in the wild and captivity suggest the Anganoka is an opportunistic herbivore that will feed on a wide range of plant material from herbs, forbs and shrubs as well as grasses. They will also eat dead leaves and other plant material fallen from shrubs and trees (Juvik et al., 1981; Smith et al., 1999b; Smith, 1999; Rakotosalama, 2009). Ploughshare tortoises are known to feed on dried carnivore faeces and African bush pig droppings (Smith 1999).
Juvenile Ploughshare tortoises rarely feed during the dry season and often undergo an aestivation period. Adults will continue to move and feed, now mainly on dry leaves, but at much-reduced rates than during the wet season (Smith, 1999; Mandimbihasina, pers obs.).
It is planned to investigate the full diet in the wild in more detail in the near future and the results will feed into the next version of these guidelines.

1.7 Reproduction

1.7.1 Age of sexual maturity

In captivity in Madagascar, female Ploughshare tortoises mature at 15-20 years of age, males at around 20-25 years (E. Bekarany, pers. comm.). Data from the wild or from animals reared indoors are not yet available.

1.7.2 Clutch and offspring size

Data from the wild show a mean clutch size of 3.2 eggs (range = 1-7 eggs) and a mean clutch frequency of 2.1 (range = 1-4 clutches). Eggs are spherical, 30-36 mm in diameter with a mean egg mass of 36.2g (range = 20-54g) (Pedrono et al., 2001; Pedrono, 2008; Bekarany, unpublished data). Both clutch mass and clutch frequency are positively correlated with maternal body mass until a certain level after which a further increase in maternal body mass tends to result in increased clutch size (Pedrono, 2008).

1.7.3 Nesting and incubation

Females tend to migrate out of the bamboo shrub or dry forest where they might have their home territory to lay eggs in more open areas like grasslands, savannah or open bamboo stands (Figs. 5A and 5B).
The nesting period stretches from January until June where nests in the first and last month are usually fewer and smaller (Bourou et al., 2001). Eggs will usually undergo a diapause with cooler temperatures from June until September triggering development when temperatures rise again. This means that no matter when a clutch was laid, hatching will occur when the first rains start - usually in November/December but with a possible extension until February. Successful nests have been laid in captivity in Madagascar as late as July (Bourou et al., 2001; Bekarany, 2011). This also means that eggs which are laid very late can, in some cases, not undergo a diapause but develop and hatch directly, especially when incubated artificially in a warm incubator. The incubation period is therefore variable and varies in the wild from 197-281 days with a mean incubation period of 237 days (Smith, 1999; Pedrono et al., 2001). Artificial incubation in captivity in Madagascar can shorten incubation significantly to 144-178 days (Bekarany, 2012).

Fig. 10: Open savannah at Baly Bay. The local ranger is following Angankoa tracks to a nest a few meters away.
© Durrell Wildlife Conservation Trust.

Fig. 11: Ploughshare tortoise nest discovered in a more shaded area at Baly Bay.
© H. Rakotosalama / Durrell.

1.8 General and seasonal behaviour

Ploughshare tortoise activity is of course climate-driven and animals are most active in the morning until 10am and, less consistently, after 4pm in the afternoon. They usually remain hidden in the dense bamboo thickets during the hotter hours of the day where they can form a deep tunnel labyrinth, which is hardly accessible to other higher life forms (Reid, 1988; Smith 1999; Mandimbihasina, pers com; M. Goetz, pers obs).

Not surprisingly, activity patterns vary depending on the seasons: as mentioned above, juvenile Ploughshare tortoises usually undergo an aestivation period during the dry season in the wild while adults continue to move and feed at much reduced rates.

Adult males become most active after the first heavy rainfall of the season when their home range shifts significantly from about 7 ha in the dry season to around 20 ha in the wet season (Smith et al. 1999b). During this time, they search out other males for ritualised combats where the name-giving plastral protrusion is used to flip opponents over. The winner of these combats is then searching for females and is possibly receiving elevated hormone levels, which might trigger female acceptance

pre-mating and allows for successful fertilisation: attempts of mixing single males with receptive females in captivity only very rarely results in successful mating or fertilisation (E. Bekarany, pers. comm.).
Adult females are most active in January-May when they search for nest sites. Home ranges for adult females also increase significantly from about 4 ha in the dry season to 12 ha in the wet season (Smith et al. 1999b).

1.8.1 Predation

Small juvenile Ploughshare tortoises (<20 cm carapace length) will presumably be eaten by natural predators such as Madagascar buzzard *Buteo brachypterus*, Common tenrec *Tenrec ecaudatus* and Malagasy civet *Fossa fossana*, and by introduced species such as Ship rat *Rattus rattus*, Indian palm civet *Viverricula indica* and the African bush pig *Potamochoerus larvatus*. Rats but especially pigs also predate the eggs in underground nests. First-year mortality is presumed to be high and documented to be size-dependent with 41 of 152 marked individuals recaptured a year post-hatch during an intensive study by O'Brien et al. (2005). The causes for this mortality were presumed to be due to predation and/or survival in a harsh arid environment. Of the potential predators, the bush pig has the highest potential to impact Ploughshare populations. No studies have examined the effect of bush pigs on juvenile recruitment but Pedrono et al. (2001) reported a 2.8 % predation rate by bush pigs on Angonoka nests (N=71) in Cape Sada and Ambatomainty.
There are no suspected natural predators on Ploughshare tortoises once they have reached >20 cm, which occurs by 8-10 years of age.

Section 2. Captive management

2.1 Enclosure

A variety of enclosure types are suitable for Ploughshare tortoises, ranging from tortoise table-style open-top enclosures for juveniles (Fig. 12) to large, planted exhibits (Fig. 13A, 13B) with or without outdoor access or greenhouses.

Fig. 12: Open-top tortoise-table style enclosure for rearing juvenile Ploughshare tortoises behind the scenes at Jersey Zoo. © M. Goetz/Durrell.

Fig. 13A: Planted exhibit for sub-adult Ploughshare tortoises at Jersey Zoo. © M. Goetz/Durrell.

Fig. 13B: Planted exhibit for juvenile and sub-adult Ploughshare tortoises at Rotterdam Zoo.
© M. de Boer/Diergaarde Blijdorp.

2.1.1 Substrate and furnishing

The main considerations for deciding on enclosure types will be tortoise size, options for climate control and security from theft and rodent incursions. The more natural light reaching the enclosure, the better.

2.1.1 Dimensions and Boundaries

Due to their size and seasonally active nature, Angonokas need comparatively large enclosures and >10m^2 are recommended for a single adult animal. A strong factor in deciding on enclosure size will be the ability to provide an appropriate thermal gradient and sufficient structural elements (see below).

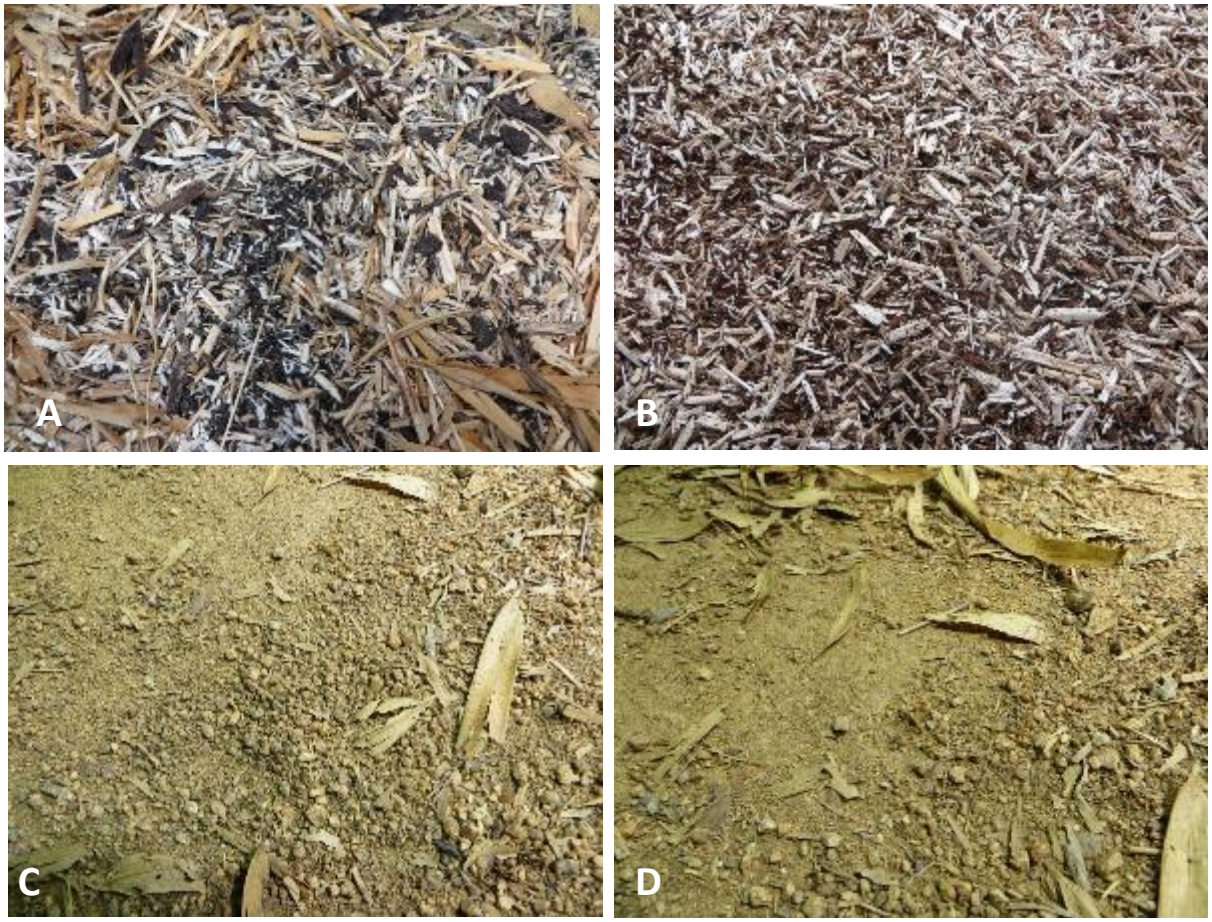
As enclosure boundaries or to separate one enclosure into several plots, the best option are wooden planks, tree limbs (Fig. 14A), railway sleepers or wooden posts (Fig. 14B). Especially when housing large animals, concrete walls or mesh fences have the drawback that they are often abrasive to the tortoises' shell when they patrol the perimeter and often scrape along any structures present. Ploughshare tortoises are poor climbers and a boundary height equalling the carapace height will suffice.

Fig. 14A: A simple log as effective enclosure barrier for sub-adult tortoises. **Fig. 14B**: upright wooden posts as a "transparent" enclosure barrier for large/adult tortoises. © M. Goetz/Durrell.

2.1.2 Substrate and furnishing

The natural substrate in the distribution range of the tortoise consists of a usually very sandy and therefore well-draining Laterite soil covered with a thin layer of leaf litter (Figs. 5). In captivity, various substrates are suitable. Care should be taken that they are relatively non-dusty, that accidentally swallowed particles are unproblematic and that the substrate is able to maintain humidity well without becoming soggy or starting to rot. Of course, it needs to be easy to clean and to exchange when needed.

For these reasons, we consider pure sand or bark chips as not suitable, especially for smaller individuals. Good experience has been made with a sand-clay mix (Fig. 15C and 15D) which comes very close to the substrate in Baly Bay. Due to its unnatural look better suited for off-show enclosures are hemp-based bedding materials for horses (e.g. Aubiose™) mixed with potting soil at a ratio of 2:1 or 3:1 for better retention of humidity (Fig. 15A and 15B).



Two possible options for enclosure substrates. **Fig. 15A and 15B:** 1:1 mix of plant potting soil and shredded hemp (Aubiose™). **Fig. 15C and 15D:** a mix of sandy clay/loam and fine gravel with a topping of dry bamboo leaves works well and provides a natural look in exhibits. © M. Goetz/Durrell.

If mature animals are housed, a suitable egg-laying site needs to be provided, even if no males are present. Females might still develop eggs and need to be able to deposit them to avoid problems with egg retention. In such a case, a substrate needs to be chosen, which is firm and will hold an egg chamber while digging. Females tend to prefer rather firm soils with a dry surface over loose or wet ones, i.e. it seems more relevant that the egg chamber maintains form during digging rather than the substrate being easy to dig in. Further necessary is a substrate depth of more than the length of the largest female's back legs and provision of a suitable temperature achieved with strong lighting, e.g.

a 150W halogen flood light. The natural provision of heat at the nesting area should be from above and heating of the ground by heat mats or underfloor heating is not encouraged. Females prefer to lay next to a structure so a well-placed log or large tuft of grass with roots can be helpful.

Essential furnishings include shelters or areas where the tortoises can feel secure; this means in particular security from above where most natural predators would approach from and is another avenue to reduce any form of potential constant low-level stress. As solitary animals from a highly structures habitat, groups of animals need a highly structured enclosure with plenty of visual barriers and hiding places for each animal so that low-level stress through constant contact is avoided.

Of course, the most naturalistic way of providing such shelter would be live plants (especially bamboo; Figs. 13) but plenty other options exist, chiefly depending on the purpose of the enclosure and the size of the animals. A simple option for smaller animals is depicted in Fig. 12: several bundles of dried bamboo twigs hung together upside-down create a naturalistic “bamboo thicket” which is easy to check and to replace. Other options include e.g. halved, large plant pots, cardboard boxes or “caves” constructed from e.g. logs. Even a simple board of any material attached to a corner of the enclosure will attract the animals to wedge themselves underneath during rest.
If several tortoises are kept together, the enclosure should be well structured to provide sufficient visual barriers. Even tortoises that live together without any obvious aggressive or hierarchical interactions can suffer from constant low-level stress and might be suppressed simply by the permanent presence of other individuals. The options to create such visual barriers of course very much depend on the size of the animals.

2.2 Environmental parameters

Ploughshare tortoises should be kept under environmental parameters mimicking the natural climate and seasons found in the North-west of Madagascar. Emulating seasonality seems especially important for retaining a long-term annual rhythm to help with appropriate stimuli to initiate breeding. Seasonality including a hot and dry season with limited food provision is important as well for growing juveniles, which are prone to too rapid growth when maintained year-round under constant conditions simulating the active season. Plastron deformities, a weak shell through too rapid growth and, in extreme cases, organ defects might be the result, which can lead to pathologies, prevent successful reproduction and can shorten the life expectancy.

Table 1A provides a cumulative overview of climatic data recorded at Baly Bay in 2008, which can be used to guide temperatures and other climatic factors in enclosures. Please note that the temperatures in the table would be ambient room temperatures, not maximums under basking lamps, which of course will always exceed the values in the table.

Table 1B gives the same values but in a reversed seasonality. This appears better suited for husbandry setups in the Northern hemisphere: the wet season can thus coincide with greater food variety in the Northern summer months and the quite low temperatures at night during the simulated dry season might be easier achieved or at least achieved more economically in the Northern winter months.

Season	Wet			Dry							Wet	
Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Day High Temp	32°C 90°F	31°C 88°F	34°C 93°F	35°C 95°F	33°C 92°F	32°C 89°F	33°C 91°F	35°C 95°F	37°C 99°F	38°C 100°F	36°C 97°F	34°C 94°F
Night Low Temp	23°C 74°F	23°C 74°F	23°C 73°F	22°C 71°F	19°C 66°F	17°C 62°F	15°C 59°F	17°C 63°F	19°C 66°F	21°C 69°F	23°C 73°F	23°C 74°F
Day Low Humidity	67%	74%	59%	49%	45%	40%	33%	32%	33%	31%	44%	59%
Night High Humidity	96%	97%	95%	93%	92%	90%	89%	91%	92%	89%	93%	94%
Precipitation (mm)	437	353	241	51	10	2.5	1	2.5	2.5	22.9	117	244

Table 1A: Climatic data recorded at Baly Bay, Madagascar in 2008. Values represent the median of daily minimums/maximums for the month. For actual values see Figs 7 and 8. © Turtle Conservancy.

Season	Dry			Wet					Dry			
Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Day High Temp	35°C 95°F	37°C 99°F	38°C 100°F	36°C 97°F	34°C 94°F	32°C 90°F	31°C 88°F	34°C 93°F	35°C 95°F	33°C 92°F	32°C 89°F	33°C 91°F
Night Low Temp	17°C 63°F	19°C 66°F	21°C 69°F	23°C 73°F	23°C 74°F	23°C 74°F	23°C 74°F	23°C 73°F	22°C 71°F	19°C 66°F	17°C 62°F	15°C 59°F
Day Low Humidity	32%	33%	31%	44%	59%	67%	74%	59%	49%	45%	40%	33%
Night High Humidity	91%	92%	89%	93%	94%	96%	97%	95%	93%	92%	90%	89%
Precipitation (mm)	2.5	2.5	22.9	117	244	437	353	241	51	10	2.5	1

Table 1B: Combined Baly Bay climate data of median daily minimums and maximums arranged to provide a reversed seasonality more suitable for indoor husbandry in the Northern hemisphere. The values are considered a guidance for set points for climate systems for indoor husbandry of Ploughshare tortoises. © M.Goetz/Durrell; data courtesy of Turtle Conservancy.

The above tables were used to develop a simple husbandry sheet to manage the climate seasonality around Ploughshare tortoises at Jersey Zoo. Table 2 below shows this arrangement, which can be used as a template to manage climates in other institutions. In this table, the "seasonal humidity" parameters named "dry", "humid" and "wet" are here explained in more detail:

DRY: the enclosure is not sprayed at all and the substrate remains completely dry at all times; any humidifiers or foggers are operating only at night. No food with high water content, i.e. fruit, vegetables, flowers or succulent greens are given; fresh greens are wilted for a day before being fed out.

HUMID: the enclosure is sprayed only lightly once per day; any humidifier or foggers are operating day and night but the substrate remains completely dry. No foods with high water content are given. Overall, this is the same as the dry season, but with elevated air humidity day and night and allowance for more fresh greens in the food.

WET: any humidifier or foggers are operating day and night; heavy sprays are given twice per day so that most parts of the substrate remain humid at all times. Any food is given, including fresh, succulent greens, flowers, etc.

Month	Room ambient temperature ˚C (night / day)	Seasonal humidity	Basking lights timer
January	17 /31	dry	8am–6pm
February	20 /33	dry	8am–6pm
March	22 / 34	dry	8am–5pm
April	24 / 32	humid	8am–5pm
May	25 / 31	humid/wet	9am–5pm
June	26 / 30	wet	9am–5pm
July	25 / 29	wet	10am–5pm
August	24 / 28	wet	9am–5pm
September	22 / 28	wet/humid	9am–5pm
October	20 / 29	humid	8am–5pm
November	18 / 29	Dry	8am–5pm
December	16 / 30	Dry	8am–6pm

Table 2: Ploughshare tortoise indoor husbandry climate sheet used at Jersey Zoo.

Fig. 16 below shows the temperatures over the curse of one year in an exhibit enclosure where the seasonality outlined in Table 2 has been applied. The blue graph shows the ambient air temperature 30cm off the ground and overlaid in brown data from a temperature data logger attached to the marginal scutes of a tortoise (see Fig. 18) within this enclosure at sporadic times. The graphs show the daily range of temperatures, data were logged every 30 minutes.
Expectedly, as can be seen, the tortoises will increase their day temperatures through basking in the cool season but will use mainly the ambient temperature and stay somewhat cooler when ambient temperatures rise during the hot period. Data loggers were attached to several animals of different sizes and they all show the same data.

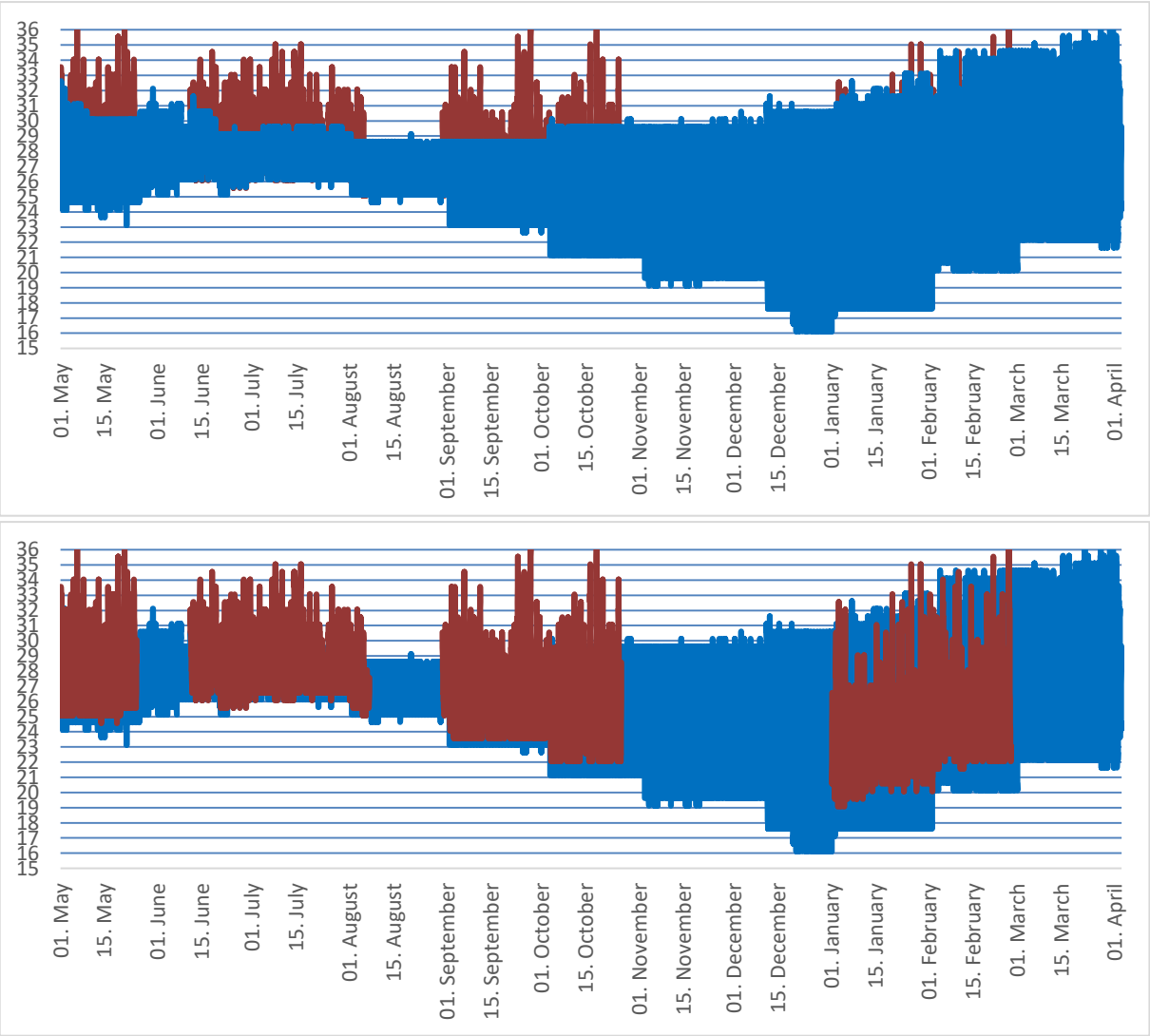


Fig. 16: Enclosure ambient air temperature (blue) in a Jersey Zoo exhibit using the seasonal temperature regime outlined in Table 2 during one year. Over-/underlain in brown corresponding external carapace temperatures of Ploughshare tortoises in this enclosure. © Durrell Wildlife Conservation Trust.

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 principle 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 principle thoughts in mind, it is apparent that heating for Ploughshare tortoises should principally involve

- very strong/bright light with a balanced, close-to-natural spectrum and with the highest levels of brightness and heat in the basking zone.
- UV radiation within the correct limits, i.e. suitable and safe UV-A and UV-B levels.
- basking-zone heat provided mainly as IR-A (also termed Near Infra-Red, NIR), i.e. not using ceramic heaters or other non-luminous heated bodies to heat the basking zone.
- a basking zone which is much larger than the largest animal.
- a suitable gradient of light, heat and UV-B radiation throughout the enclosure.

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 highly recommended as they emit the most balanced spectrum of any affordable artificial light source. This is the case for both, as ambient lighting and as the means for providing adequate light levels at the basking zone. The basking zone might need additional heating if animals are larger and MH spot lamps are insufficient to create a zone large and/or hot enough.

UV-A radiation can penetrate glass and is therefore sufficiently provided by MH lamps or through natural light entering a window. For UV-B radiation, a gradient must be available with a UV-B Index (UVI) of 0 in the hiding areas towards a maximum UVI of around 4-8 in the basking zone. The lower range of this maximum (UVI 4-5) is provided for juveniles, the higher range (up to a UVI of 6-8) for adults. Areas outside the basking zone, but not in full "shade" like the retreats or hiding/resting areas would be, will benefit if a UVI of 2-4 is still available.

Please note that UVI levels measured in the natural habitat in Baly Bay are of course reaching much higher values, with a UVI of up to 15 in the mid-day sun under a clear sky (M. Goetz, pers. obs.). However, it is rare for tortoises to bask for extended periods (or, in fact, at all) in those circumstances; moreover, the UV-B spectrum from artificial light sources is not quite the same as from natural sunlight with an artificially created UVI of, say, 7 being equally biologically potent than a UVI of ~10 created by sunlight (Baines et al. 2016).

A bank of 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. Using MH spots for UV-B radiation makes only little sense in enclosures larger than a size used for hatchlings and their UV-B output and longevity is inherently unpredictable. Therefore, MH lamps are highly recommended for their light intensity, relatively good spectrum and heat output but not as a (sole) UV-B radiation source. 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 m^2 in size for a group of adults when several panels of multiple T5 tubes are used in combination.

Fig. 17 provides an example of such a combined lamp setup with Figs. 18 giving an overview of the same basking space in the enclosure. Note the relatively uniform and large (i.e. much larger than two tortoises sitting next to each other) basking area created by several metal halide lamps with the main UV-B radiation and extra illumination being concentrated in this same area via a multi-fluorescent tube array.

General ambient enclosure lighting by 2x 400W metal halide floods (here: Iwasaki MT 400 DL/BH).

Basking area lighting and UV-B radiation provisioning by a multi-fluorescent tube array (here: 4x Arcadia 14% T5 Dragon Lamp 54W). Height ~120cm.

Basking area lighting and heating by 2x 150W metal halide lamps (here: Lucky Reptile Bright Sun ULTRA Desert 150 W). Height ~60cm.

Attachment site of the temperature data loggers mentioned on page 26 and Fig 16.

Fig. 17: Example of a combined lamp setup to provide visible light, ultraviolet radiation and heat. © M. Goetz/Durrell.

Figs. 18A and 18B: Overview of the hot area in an Angonoka exhibit enclosure emphasizing the size of the immediate basking area(s) compared to tortoise sizes. © M. Goetz/ Durrell.

2.2.2 Humidity

The required changes in humidity (see 2.2. Environmental parameters) can be achieved through various methods, mainly depending on the size and type of enclosure. Spraying the enclosure more or less frequently depending on the season is a good way to simulate the rainy season and heighten air humidity. However, the resulting increase in air humidity is usually short-lived, especially if artificial heating or air-conditioning is provided. Since a constantly wet substrate can over time cause lesions on the plastron, increasing air humidity permanently while keeping the substrate relatively dry requires technical solutions. Good results have been achieved both, with ultrasonic foggers for smaller juvenile enclosures and evaporation room humidifiers suitable for a larger room or exhibit.

2.3 Water, diet and feeding

2.3.1 Basic diet

As is recommended for most tortoises, especially from arid regions, Ploughshare tortoises need to be provided with a diet very high in fibre, high in calcium and low in protein and sugar. A second principle is again true for most herbivore reptiles as well as for the Ploughshare tortoise: variety is key and no single type of food plant should be considered “good” or “bad” for the tortoises by itself – it always depends on how much of the overall food this particular type of plant consists of. The vast majority of food plants can be considered “good” or “suitable” if they are mixed with many others at every feed. Conversely, most food plants should be considered “bad” food if they are given exclusively and then over-deliver certain nutrients over time while others are lacking.
The basis of the food should be hay, ideally in the form of cobs/pellets consisting of a good variety of herbaceous hay; a highly recommended European brand is Agrobs Pre Alpin™ Testudo.

At Jersey Zoo, the species is fed three times per week. During the wet season, a large variety of fresh food plants is cut and the occasional vegetable (e.g. grated carrots mixed in once every one or two weeks) is mixed with the hay cobs at a ratio of approx. 1:1. Occasionally, extra “wet season food” like hibiscus flowers, *Opuntia* cactus pads/fruit/flowers are added as a nutritious treat.
During the dry season, only hay cobs and other forms of hay or dry leaves are given three days per week to aid in providing seasonality, to prevent too fast growth in juveniles and to avoid obesity in larger animals.

Please see Appendix 1 for size-weight ratios of captive Angonoka at Ampijoroa, Madagascar, to help assess the effects any nutritional regime has on the growth rate and body condition of your Ploughshare tortoises.

Pieces of cuttlefish bone should be provided *ad lib* in the enclosure, which largely negates the need for supplementing calcium by dusting the food. As the tortoises are supplemented with UV-B lighting (see 2.2.1), adequate to high levels of vitamin D_3 should be available to the tortoises. Involuntary ingestion of high amounts of calcium through the normal diet might, therefore, carry the risk of hypercalcemia, whereas the provided pieces of cuttlefish bone allow the animals to regulate their calcium intake themselves at safe levels. Gnawing at the cuttlefish bone also helps to trim beaks and prevents them from overgrowing.

2.3.2 Water

Although unproven, in the wild Angonoka are likely not able to find drinking water for extended periods during the dry season, which is likely a leading factor for aestivation in juvenile animals. In captivity, the species can be observed drinking throughout the year, even when a dry period is simulated. A clean, shallow bowl with fresh water needs to be provided at all times; it is especially needed as air humidity in captivity is generally lower than in the wild and often further reduced by air conditioning and heating/lighting systems in the enclosures (Highfield, 2015), leading to generally faster dehydration, which the animals need to be able to compensate.

2.4 Social structure

Ploughshare tortoises generally show a docile nature and aggressive interactions are very rare outside of the mating season or unless males are kept together. Nevertheless, even amongst juveniles, a hierarchy is established and subordinate, usually smaller animals will at times suffer from delayed access to food and possibly from some levels of low-level stress. Therefore, it seems prudent to a) not keep too large a group of animals together and b) only house animals of similar size in one enclosure, which includes the periodical sorting of juvenile animals by size as they grow.
Having said that, we found at Jersey Zoo that four juveniles of three different sizes are able to live together permanently when provided with enough space, plenty of visual barriers and separate food dishes in different positions throughout the enclosure (Figs. 12, 13, 17 and 18).
Adult/mature tortoises will benefit greatly from a separation of sexes, both for the avoidance of stress and for successful reproduction. Males must be kept separate and only introduced to each other briefly at the beginning of the mating season for ritualised combat (see 2.5).

2.4.1 Sharing enclosure with other species

Due to the very high risk of disease transmission to this very rare species, it is generally not advised to house Angonoka with other chelonian species! When mature animals are kept, a communal enclosure with radiated tortoises (*Astrochelys radiata*) must especially be avoided, as the two species are able to hybridise and to store sperm for several years. Captive-bred radiated tortoises can reach maturity by the age of 8-12 years (M. Goetz, pers. obs.).
A mixed enclosure with suitably disease- and parasite-free Malagasy lizard species able to tolerate the same climatic conditions (e.g. *Furcifer oustaleti* or *Oplurus* spp.) poses no problems.

2.5 Breeding

2.5.1 Pre-mating combat and mating

Adult males become most active after the first heavy rainfall of the wet season when their home range shifts significantly from 6.6 ha in the dry season to 21.1 ha in the wet season (Smith et al. 1999b). During this time, they search out other males to combat with and females to mate with (Fig.

19). It is generally thought that Ploughshare tortoise males have a need to perform a ritualised combat to successfully mate, although the exact mechanism is not fully understood; it is likely that through these combats, and more so through winning combat, suitable hormone levels are reached which facilitate mating and actual fertilisation. It has certainly proven to be true at the breeding station in Ampijoroa, Madagascar (E. Bekarany, pers. comm.; Durrell Wildlife Conservation Trust, unpubl. data). The evolutionary development of a morphological particularity used in these combats, the extended gular scute in males, suggests that these combats are indeed of importance in this species.

In Durrell Wildlife Conservation Trust's breeding station in Ampijoroa, Madagascar, adult animals are kept in same-sex groups in large enclosures throughout most of the year. In September, temporary barriers are placed within the enclosure of the males so that each male is kept on its own for at least one month. Combat sessions then begin in early October: the temporary barriers are removed between two pre-determined males for one or two hours, allowing these males to interact and fight. The barriers are then replaced. This is repeated again four to five days later and this rota continues until about mid-November. Staff observe each combat session to ensure that no animals are injured, to ensure that the most aggressive and least aggressive males are not mixed together, and to note down which male wins and which loses. The winning male involved in a particular combat will then be used immediately for breeding.

After each combat, a pre-selected female is placed with each male in his isolated part of the larger male enclosure. The female is kept with the male for the rest of the day and until the following morning after which the female is removed and placed back into the adult female group enclosure. As these mating sessions follow each combat session, this occurs every four to five days throughout October until mid-November. Once a pair copulates, they continue to be placed together every 4-5 days after the male combat session, until the end of the mating season. If pairs would be left together continuously, they would stop copulating. If a male and female are placed together three consecutive times without copulating the pairing will be changed according to the studbook recommendations.

Picture 19: Ploughshare tortoise mating at the Ampijoroa breeding station in Madagascar (the brick is supposed to help in balancing the male and preventing disengagement of the two animals if the male would lose balance and topple back).
© L. Woolaver/Durrell.

2.5.2 Egg Laying

An adult female Ploughshare can lay 1- 3 nests per year with 1-7 eggs per nest (Pedrono et al., 2001; Durrell Wildlife Conservation Trust, unpubl. data). Mean clutch size in the wild is 3.2 eggs (Pedrono et al., 2001). Incubation periods in the wild vary from 197-281 days with a mean incubation period of 237 days (Smith 1999; Pedrono et al., 2001).

Artificial incubation in captivity can shorten this incubation period significantly to a period of 144-178 days (Bekarany, 2012). Data on clutch and egg sizes and fertility rates in comparison between captive and wild animals can be found in Bourou et al. (2001).
Eggs are spherical, ping-pong ball-shaped, 30-35 mm in diameter with a mean egg mass of 36.2 g (Pedrono et al., 2001). Eggs are generally laid in excavated nests underground at depths of 9-15 cm (Durrell Wildlife Conservation Trust, unpubl. data) which are then filled back in by the female.

2.5.3 Incubation

Temperature-dependent sex determination (TSD)

Although not experimentally proven, it seems fact that the Ploughshare tortoise exhibits TSD. It does, of course, make great sense due to the taxonomic and phylogenetic position of the species but it was also conclusively observed at Durrell Wildlife Conservation Trust's breeding station in Ampijoroa, Madagascar. The pivotal temperature, the upper and lower transitional limits as well as the sensitive time period are yet unknown.
For the Angonoka's close relative, the radiated tortoise, a preliminary but yet unproven estimate is that the pivotal temperature lies between 28.0 and 28.9°C and the upper limit of the transitional range of temperatures (i.e., above which only females are produced) is between 28.9 and 30.0°C (Kuchling et al., 2013).

Incubation substrate

Whether to use substrate to incubate hard-shelled tortoise eggs or to incubate these eggs freely in the incubator is largely a question of habit and personal preferences rather than of actual needs for the eggs. Some people will incubate hard-shelled tortoise eggs only without substrate; some others will insist that incubation on substrate in closed boxes is the best method. Both work perfectly well, although the method with substrate tends to be safer in cases where humidity in an incubator cannot be easily controlled and in case of eggs exploding. The substrate method does usually require slightly more work, though, especially if annual dry/humid cycles are employed.
Usually, one method works better in one situation while the other is more applicable in another. The choice is very much dependant on local circumstances, personal preferences and experiences, but especially on the equipment used.

Natural and semi-natural incubation data

While we have relatively good data on clutch, egg and hatchling data (see above), hardly any information at all exists for incubation parameters of *A. yniphora* in the wild. The reason is mainly the scarcity of the animals in a largely inaccessible habitat making the close observation of gravid females dependant on luck, but also the logistical challenges of deploying data loggers reliably. Only two wild nests have so far yielded success in providing us with internal nest temperatures (Figs. 21 and 23). To supplement these data while more efforts are underway to locate wild nests for temperature recordings, Figs. 25 and 27 show nest temperatures from semi-natural (i.e. not artificially incubated) eggs at Durrell Wildlife Conservation Trust's captive breeding station in Ampijoroa, Madagascar. Please note though, that Ampijoroa is about 120km inland from Baly Bay with a slightly different climate through its inland position and different vegetation structure.
With egg chambers only ~15cm underground, daily temperatures can vary by a good 10˚C and juveniles hatched apparently successfully from nests experiencing maximum temperatures of nearly 42˚C during the latter stages of incubation.

Fig. 20, left: nest discovered by observing a female digging in June 2015 in a shaded area at Baly Bay. Nest contained five eggs of which four had hatched by the time it was checked in mid-December. The topmost egg was ~15cm below the ground surface.
© H. Rakotosalama/Durrell

Fig. 21, below: temperature data of a data logger placed in shaded nest (Fig. 20) between June and December 2015.
© Durrell Wildlife Conservation Trust.

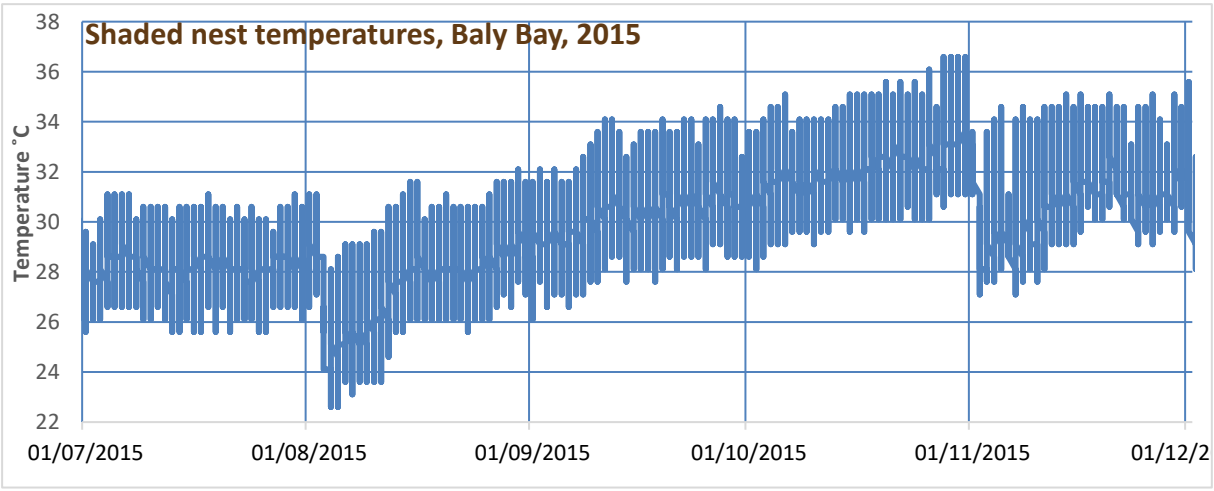


Fig. 22, left: approximate nest location in June 2015 in an open area at Baly Bay. Nest contained five eggs of which all had hatched by the time it was checked in mid-December. The topmost egg was ~15cm below the ground surface.
© Durrell Wildlife Conservation Trust.

Fig. 23, below: temperature data of a data logger placed in full sun nest (Fig. 22) between June and December 2015.
© Durrell Wildlife Conservation Trust.

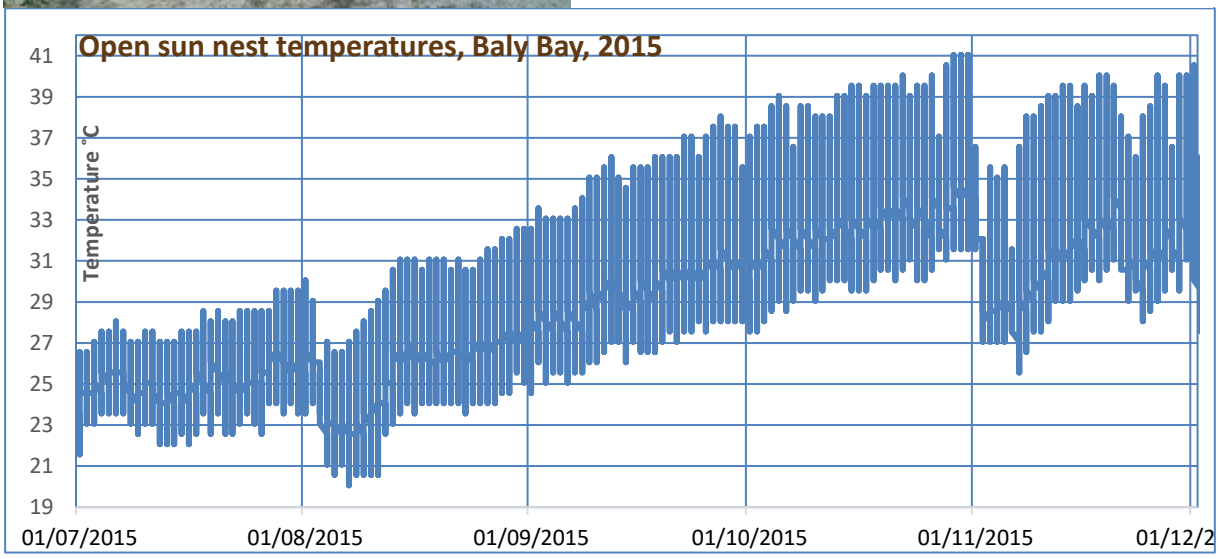


Fig. 24, left: nest in May 2015 in a sunny area in captive breeding enclosure at Ampijoroa. The topmost egg was ~12cm below the ground surface.
© M. Goetz/Durrell.

Fig. 25, below: temperature data of a data logger placed in sunny nest (Fig. 24) between July and December 2015.
© Durrell Wildlife Conservation Trust.

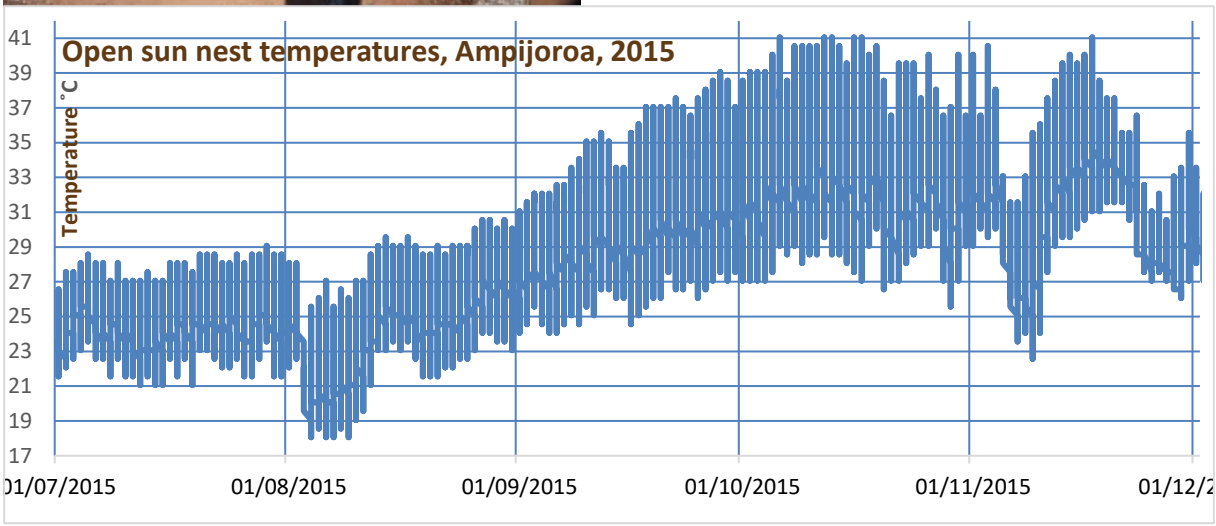
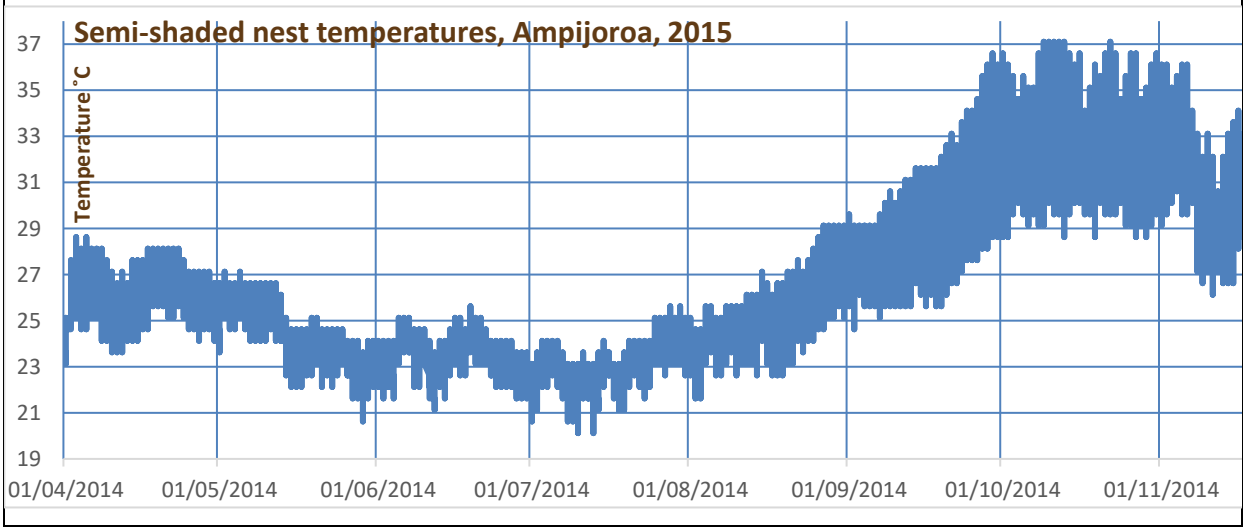


Fig. 26, left: nest in March 2014 in a semi-shaded area in captive breeding enclosure at Ampijoroa. The topmost egg was ~14cm below the ground surface.
© M. Goetz/Durrell.

Fig. 27, below: temperature data of a data logger placed in semi-shaded nest (Fig. 26) between April and December 2014.
© Durrell Wildlife Conservation Trust.



Diapause

With a marked wet and dry season in north-western Madagascar and with the Ploughshare tortoise's variable but often long incubation period, it seems a fact that Angonoka eggs, as is true for other Malagasy tortoise species, undergo a diapause in the wild (see wild nest temperatures in Figs. 21, 23, 25 and 27).

As a diapause is an evolutionary adaptation, it can be argued that it might be beneficial for egg development, as has been shown e.g. for *Pyxis planicauda* (Mislin, 2017; W. Zowickian, unpubl. data; Durrell Wildlife Conservation Trust, unpubl. data).

Angonkoa eggs laid early in the season incubate for longer than eggs laid later in the year: eggs laid between January and June will all hatch in November-December. This points to some flexibility and means that, in principle, eggs can develop successfully with and without diapause. This is also the case e.g. with *A. radiata*, where the incubation period can range between (without diapause) 70 and (with diapause(s)) 565 days (Durrell et al., 1989; Iaderosa et al., 1990; Highfield, 1996; H.J. Bidmon, pers. comm.; Durrell Wildlife Conservation Trust, unpubl. data). Further, good hatching results were recorded in Durrell Wildlife Conservation Trust's captive breeding station in Ampijoroa, Madagascar, from non-cooled artificially incubated eggs in 2010, 2011 and 2013 (Durrell Wildlife Conservation Trust, unpubl. data).

The benefit of incubating without diapause is the shorter and therefore safer incubation period. However, the disadvantage is that it would be unclear whether any undeveloped eggs are due to the missing diapause cue or for other reasons, e.g. infertility. Current thinking for the Angonoka and radiated tortoise is that eggs laid early in the season, i.e. before the cooler, drier months tend to have a better hatch rate after undergoing a diapause while eggs laid later, when temperatures and humidity levels rise, do not necessarily benefit. However, in captivity, with an often not very natural seasonal cycling (i.e. encompassing all natural cues which contribute to seasonality in the tortoises), it is probably best advised to use a diapause in all cases as no eggs will take harm from it whereas some eggs might not hatch if not cooled.

A simple diapause regime employed for various Malagasy tortoise species incubates eggs one month at around 27-30˚C, then cools the eggs for one month to around 18-20˚C before warming again to the actual incubation temperatures desired for the remaining incubation. Of course, care should be taken to check eggs by candling for any development before the cooling phase. If a development would be detected, the egg would then not undergo the cooling.

If no development ensues within a month or so after the eggs were transferred to the actual, final incubation temperature, another, second cooling phase might be attempted. Although most eggs not developing fail due to not being fertilised, a few Angonoka eggs, but also *A. radiata* and *P. planicauda* eggs have started to develop only after a second or even multiple cool phases/diapause cues (Durrell Wildlife Conservation Trust, unpubl. data).

2.5.4 Hatching

Hatching in the wild depends on the seasonal rains and can occur from November to February while the main period extends from mid-November until the end of the year. Hatchlings leave the nest somewhat synchronously over a period of 1-3 days, and weigh 11.0-35.0 g (mean 24.1 g) at hatching with carapace lengths of 35.3-50.2 mm (mean 44.7 mm) (Pedrono et al., 2001). In captivity, however, artificially incubated eggs from a single clutch do not necessarily hatch together but can vary by weeks (Durrell Wildlife Conservation Trust, unpubl. data), suggesting that in natural nests juveniles don't necessarily hatch together but can remain in the nest chamber after hatching until suitable weather/soil conditions allow all hatchlings to emerge somewhat simultaneously.

2.5.5 Development and care of young

The basic husbandry requirements for hatchlings and small juveniles are the same as for larger animals. However, care needs to be taken that

a) lower max. basking temperatures are provided especially in smaller enclosures, or that basking areas are suitably reduced in area

b) plenty of low retreats are present for the tortoises to wedge themselves under
c) that ambient air humidity is kept at higher thresholds and that especially the air in/around retreats are kept consistently humid
d) that growth is carefully monitored and the feeding regime adjusted to ensure a smooth growth. To that effect, please see Appendix 1 for a "Jackson's ratio" plotted for *Astrochelys yniphora* from animals kept and bred at Durrell Wildlife Conservation Trust's breeding station in Ampijoroa, Madagascar, over the past decade. This graph should allow for an easy way of assessing whether an individual is within accepted ranges.

2.5.6 Population management

Since the virtual loss of the species from the wild due to poaching after 2017, the captive population at Durrell Wildlife Conservation Trust's breeding centre in Madagascar is holding well over 90% of the effective population size of this species (read: population which can be used for reintroduction into the wild). Therefore, proactive and careful genetic and demographic management through the EAZA EEP and a forthcoming Long Term Management Plan is needed to secure this population, and therefore the species in Madagascar, 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 recruiting new holders) will be required to deliver the EAZA contributions to the *ex-situ* management roles selected for *Astrochelys yniphora* in the EAZA Regional Collection Plan for Chelonians (Goetz et al. 2019). While careful management of genetics and space are currently a priority for the Madagascar breeding centre, any attempt for successful breeding in the small zoo population is highly encouraged.

2.6 Handling

2.6.1 Catching/General handling

Generally, Ploughshare tortoises will not need to be "caught" as even their maximum speed allows for simply picking them up without employing any specific catching method.
However, tortoises should only be picked up when absolutely necessary: handling is very often accompanied by the voiding of the bladder which introduces additional stress on the body especially in small animals through loss of hydration. It can also be misleading in the calculation of body weights.

2.6.2 Restraining

If an animal needs to be restrained e.g. for taking blood, for radiography or examining the mouth, the physiology and morphology of the animal needs to be taken into consideration. More than one person is usually needed to forcibly extend either limbs or neck of animals >10 years 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 incurable fractures, especially in captive reared animals with potentially poorly calcified bones (Barbon, pers. comm.). While near impossible in adult animals, the forcible extension of limbs or head/neck puts an enormous amount of physical and emotional stress on the animal and is potentially damaging to tendons, skin and bones; if an animal with full intestinal content is turned upside down, it can lead to usually fatal gastric torsions.

For procedures which require the extension of neck and limbs, it is therefore highly recommended to lightly sedate or fully anesthetise animals for a brief period of time, which has no lasting effects.

Training animals to extend limbs and neck while being stationary and using desensitisation training to allow injections or to draw blood takes time but is usually very successful and is considered the best possible option for examinations, routine interventions and taking samples.

2.6.3 Individual identification and marking

Microchipping / PIT-tagging

As a CITES Appendix 1 species, all Ploughshare tortoises should be micro-chipped, as is a common standard, subcutaneously at the left dorsal thigh area using ISO/TROVAN compatible passive integrated transponders (PIT-tags). Other areas on the animals have been trialled but are not as effective in retaining the microchips (Durrell Wildlife Conservation Trust, unpubl. data); especially the area ventral to the tail and parallel to the plastron is relatively often used but is prone to either losing the microchip into the coelom and/or can be even less reliably read once the tortoise is larger and tissue layers and plastron thicken.

With the left hind leg extended and held with the left hand, the needle should be inserted proximal to the knee joint in a point on the dorsolateral (i.e. on the upper side) surface of the thigh (Figs. 28). The needle should be directed dorsocraneally (i.e. slightly upwards and towards the head of the animal) under the skin, across the thigh and into the fold of skin present in that area. The incision should be closed with a small drop of tissue glue and the leg held until the glue is completely dry. Gently blowing at the glue site quickens the drying process. No bleeding should occur but if so, this can be contained by pressure with cotton wool and closure of the wound with tissue glue.

All tortoises above 100mm carapace length can be implanted with TROVAN/ISO standard microchips. For animals of 100-200mm carapace length, 7mm microchips need to be used.

For animals above 200mm carapace length, either 7mm or 8mm microchips can be used but whenever possible, preference should be given to smaller implants, i.e. the 7mm microchips.

Figs. 28: the dorsolateral aspect of the left thigh is the standard location for sub-cutaneous insertion of implantable microchips for identification.
© M. Goetz/Durrell.

Animals below 100mm carapace length must be marked with alternative methods e.g. gluing microchips on the animals' shell with epoxy resin or using photographic identification. Standardised photographs are also recognised by some authorities (e.g. in Germany and Switzerland) for CITES documentation purposes.

Tattooing

Tattooing the plastron of small juveniles has been trialled and offers another alternative (Fig. 29). In these trials the smallest size for tattooing has been determined to be 65mm (Woolaver et al., 2016).

However, these tattoos fade within a couple of years (E. Bekarany pers. comm.; M. Goetz, pers. obs.) and animals will have to be regularly re-marked; therefore, identification should be changed to PIT-tags once an animal has reached 10cm carapace length.

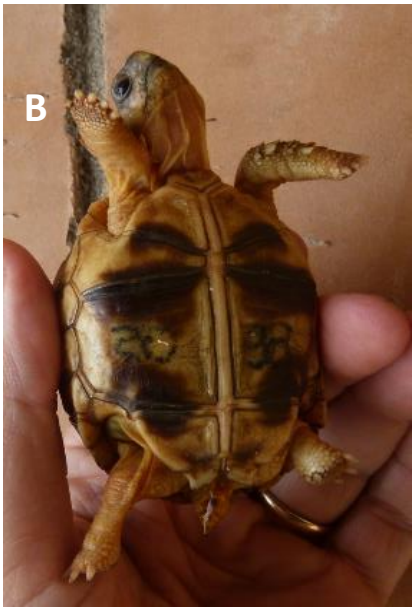


Fig. 29A: tattooing a small juvenile Angonoka on the plastron.
Fig. 29B: a small juvenile Angonoka showing a plastral tattoo.
© L. Woolaver/Durrell.

Engraving

Engraving the carapace is a third method of permanently marking Ploughshare tortoises which was extensively employed in Madagascar from 2015 onwards. The main purpose was to deface the animals' beautiful shell to lower their value and discourage poachers from taking these animals, the secondary benefit being an easy way of identifying individuals without the need for special equipment or training both in the field and during confiscations.

However, while this initially seemed to have the intended results, the seemingly insatiable demand for the species on the illegal pet market and the exorbitant prices paid from 2017 did not stop poachers (or buyers) from taking even engraved animals. Also, the engraved figures do fade through new shell growth within about two years in sub-adult animals and even in adults after three to four years so that animals will have to be re-engraved permanently. It is still a good method to semi-secure animals and for easy identification of large cohorts.

Fig. 30A: Engraving an adult tortoise in Madagascar. **Fig. 29B**: the resulting visible pattern about one year past engraving. © E. Bekarany/Durrell.

Animals larger than 10cm carapace length (weighing ~200g) can be engraved using a battery-powered rotary (Dremel™) tool using a #107 round bur engraving cutter or #100 or #191 round bur high speed cutter as appropriate for the size of the animal and desired width of the inscription (Fig. 30A). The Dremel™ tool is always operated at the highest possible RPM speed setting as this allows rapid engraving without producing too much heat. Lower speeds increase contact time and can lead

to burning of the keratin. The Dremel™ tool is held like a pencil between thumb and forefinger. The bur always needs to be moved within 2 seconds of contact in any given location. Light pressure is applied in a series of shallow passes back and forth to cut approximately 3mm deep into the keratin. For easy restraint, the tortoise should be elevated on a pedestal or small bucket.

2.6.4 Sexing

The Ploughshare tortoise is sexually dimorphic when adult. Males are typically larger and heavier than females and have a longer tail, a longer gular scute, a pronounced concave plastron, a smaller anal notch, and a wider anal fork.
However, these features are only expressed strongly enough for visual differentiation of sexes once an animal reached maturity. For juvenile and semi-adult animals, the only reliable sexing method is by endoscopy as detailed in Kuchling & Lopez (2000), Kuchling et al. (2013) and Kuchling (2015). Durrell Wildlife Conservation Trust has had good results with endoscopic sex determination of dozens of animals aged 5-12 years. Animals below the age of five years should not undergo an endoscopic examination as the interpretation of gonads in animals that small and young seems too unreliable: in many cases, five-year-old juveniles are not yet fully differentiated and some can even still show both, male and female gonads (Kuchling et al., 2013; G. Kuchling, pers. comm.; M. Goetz, pers. obs.). Therefore, anaesthesia and a surgical intervention, which is rather significant for animals of that size, seems unjustified for sexing purposes at this age.

Although this is only empirically but not yet experimentally proven, the Angonoka exhibits temperature-dependent sex determination (TSD) during egg incubation (e.g. Ewert et al., 2004; Miyagawa et al., 2018). Hence, this species does very likely not possess sex chromosomes and therefore sex determination via chromosomal analysis is not possible.
To be able to adjust incubation parameters for the management of sex ratios in captive populations more readily, a technique of sexing animals at younger ages should ideally be developed so that the outcome of an incubation regime (the sex of an individual) can be discovered earlier than five+ years after hatching. Assessing sex hormones in amniotic fluid or blood samples could be trialled.

Fig. 31: Dr. Gerald Kuchling determining the sex of an about 10-year-old tortoise via endoscopy at Ampijoroa, Madagascar. © M. Goetz/Durrell.

Fig. 32: entry site for the endoscope on the left groin. © M. Goetz/Durrell.

2.6.5 Transportation

Ploughshare tortoises 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 the death of animals.

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

Tortoises should be kept in an enclosure that resembles their natural habitat as closely as possible; there is little additional scope for behavioural enrichment other than daily and seasonal changes to climate, food and various resting places with different temperatures and humidity levels as outlined above. To introduce more variation on a daily basis, feeding times can be varied and food items

made harder to reach by distributing these in various places although care has to be taken to still feed in the first half of the day to allow sufficient time for digestion under optimum body temperatures.

2.8 Veterinary considerations

It is advisable to regularly weigh and measure tortoises to assess growth trends and body condition as a proxy for general health monitoring.
Health monitoring might be aided by the haematological and biochemical reference intervals in Lopez et al. (2017). Blood for blood chemistry analysis is best drawn from the jugular vein as samples from venepuncture of the subcarapacial vein are regularly diluted with lymph.

As with all captive tortoise species, most veterinary problems can be avoided by providing optimum husbandry parameters and isolation from animals of other species possibly carrying transmittable disease agents, incl. parasites. It is important to note that pathogens are not only spread from and between taxonomically close specimen (i.e. from tortoise to tortoise) but also via fomites, keepers and by food (e.g. plant leaves) and taxonomically distant animals, e.g. ranavirus from amphibians (e.g. Marschang 2011; Marschang 2016; Marschang et al. 2016).
The most important veterinary problems currently known are listed in the following.
Please find a standardised post-mortem and sample protocol in Appendix 2.

Herpes viruses
One of the most important (read: well-known) viruses found in captive tortoise are herpes viruses. Symptoms include swollen eyes, respiratory distress, and oropharyngeal plaques/stomatitis although an outbreak of the disease is usually associated with prolonged stress and/or suboptimal husbandry conditions (e.g. Marschang 2011).
It is possible to detect herpes viruses from the tongue of a tortoise by virus isolation and Polymerase Chain Reaction (PCR) or by performing antibody assays in clinically healthy carrier individuals.

Mycoplasma
This organism can cause upper-respiratory infections in tortoises. Signs include lethargy, weight loss, nasal discharge and failure to thrive (e.g. Jacobson et al. 2014). Diagnosis can be made by PCR testing of an oropharyngeal swab or (better) through nasal flushing and treatment of the disease is possible. Treated animals might not lose the infection but will become asymptomatic carriers. As in herpes virus, stress or suboptimal conditions usually induce the development of clinical disease.

“Juvenile wasting syndrome” or “shell weakness syndrome”: Picornavirus (formerly “Virus-X”) and Hexamitosis
The complex problem commonly summarised as “juvenile wasting syndrome” or “shell weakness syndrome” in tortoise husbandry circles has also been affecting juvenile Ploughshare tortoises in the breeding facility in Madagascar. Symptoms are usually a rapid onset of weakening of the shell (i.e. both, the plastron and carapace) in animals up to 1.5 years of age, in many cases coupled with incomplete digestion. After 1-4 months, the affected animal becomes weak, stops feeding and dies not long after. By that time, the plastron has often become paper-thin.
Two pathogens seem often to be involved in the syndrome (see below). However, it is important to note that the onset of these symptoms can also be driven purely by inadequate husbandry, nutrition and/or environmental parameters and in the apparent absence of the below pathogens (i.e. negative tests); therefore, applying best possible animal husbandry and ruling out unfavourable environmental/husbandry factors is essential before relying on the tests alone.

Hexamites (specifically *Hexamita parva*) are thought to be able to contribute to the syndrome especially by affecting the kidneys (e.g. Zwart & Truyen, 1975). In living tortoises, Hexamites are usually only detectable in fresh urine samples. Detection can best be done through suspension in a

solution of 0.9% sodium chloride. Standard flotation solutions for parasite analysis are hypertonic and will kill many protozoans through osmotic shock. Hexamites can be only detected and distinguished from other flagellates through their characteristic movements. The movement is very fast, arrow-like straight, like a torpedo. Other flagellates show more twitching/trembling (=> Trichomonads) or stationary (=> Giardia) movements.
While there is no proof that Hexamites alone are causing the syndrome through "nephro-hexamitis", any signs of Hexamites need immediate attention, e.g. through treatment with Metronidazole: once clinical signs can be observed, the kidneys are usually already beyond reparation (Mutschmann, pers. comm.).

Picornaviruses (formerly known as "Virus X"), specifically a Torchivirus named *Tortoise Picornavirus* (ToPV) have been another strong suspect of causing juvenile wasting syndromes, mainly in European tortoise species (especially *Testudo graeca*). However, *Geochelone elegans* and African species like *Stigmochelys pardalis* can also be affected while most other tortoises and even *Terrapene* sp. can carry the virus (Marschang 2011; Heuser et al. 2014; Marschang 2016; Marschang et al. 2016a).
ToPV has recently been proven to cause symptoms consistent with shell weakness syndrome (Paries et al. 2019).
It seems important to test all suspect cases in Ploughshare tortoises and other tortoise species kept in the same facility, not least to learn more about this syndrome in Angonokas.
Picornavirus can be detected by PCR and the most suitable tissues are kidneys and the nerves serving the tongue of the animal. It seems important to note that the virus is hard to detect and that only very fresh samples lead to conclusive results. Therefore, kidneys and the whole tongue should be frozen without being fixed and immediately sent to a suitable lab making sure the tissue will not defrost on the way. Another but less reliable option are pharyngeal swabs (Paries et al. 2019; S. Blahak, pers. comm.; R. Marschang pers. comm.).

2.9 Specific problems / safety

The rarity of the Ploughshare tortoise and the high demand on the illegal/black pet market make this species vulnerable to theft, especially since tortoises are a relatively easy animal to steal, conceal and transport. It is therefore suggested that suitable measures are taken to secure the animals and/or their exhibits and that animals are microchipped AND identifiable by regularly updated photographic ID cards at all times.

As with all reptiles, it is sensible to bear in mind that chelonians are also potential carriers of *Salmonella* sp. which can be pathogenic to humans; therefore, good hygiene/hand washing procedures after or wearing gloves during handling or enclosure maintenance is recommended.

2.10 Recommended research

Research needs pertaining to the *ex-situ* population in 2019 include

- Genetic analysis of most of the captive population to determine the relationship status of all potential and actual founder animals is currently underway. This is important as all (potential) founders are wild animals whose relationship status is unknown. It is well possible that a portion of those animals are actually siblings, collected together shortly after hatching although their "wild" status would make them unrelated by default in any studbook analysis software.
- Pending funding, it is planned to monitor feeding in a natural, fenced-in setting at Baly Bay National Park and to analyse the nutritional content of ingested food plants. This would not only give the first assessment of natural foods in this species but also allow for a better understanding of the species' nutritional needs in captivity.
- Veterinary research to determine the cause of juvenile wasting syndrome in hatchlings at the Madagascar breeding station is underway. PCR analysis is being carried out for Picornavirus but further funding is needed to extend the analysis incorporating more pathogens.

- A very long-term but rewarding project would be an experimental approach to determine the pivotal temperature in the TSD system of Ploughshare tortoises.

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Section 3

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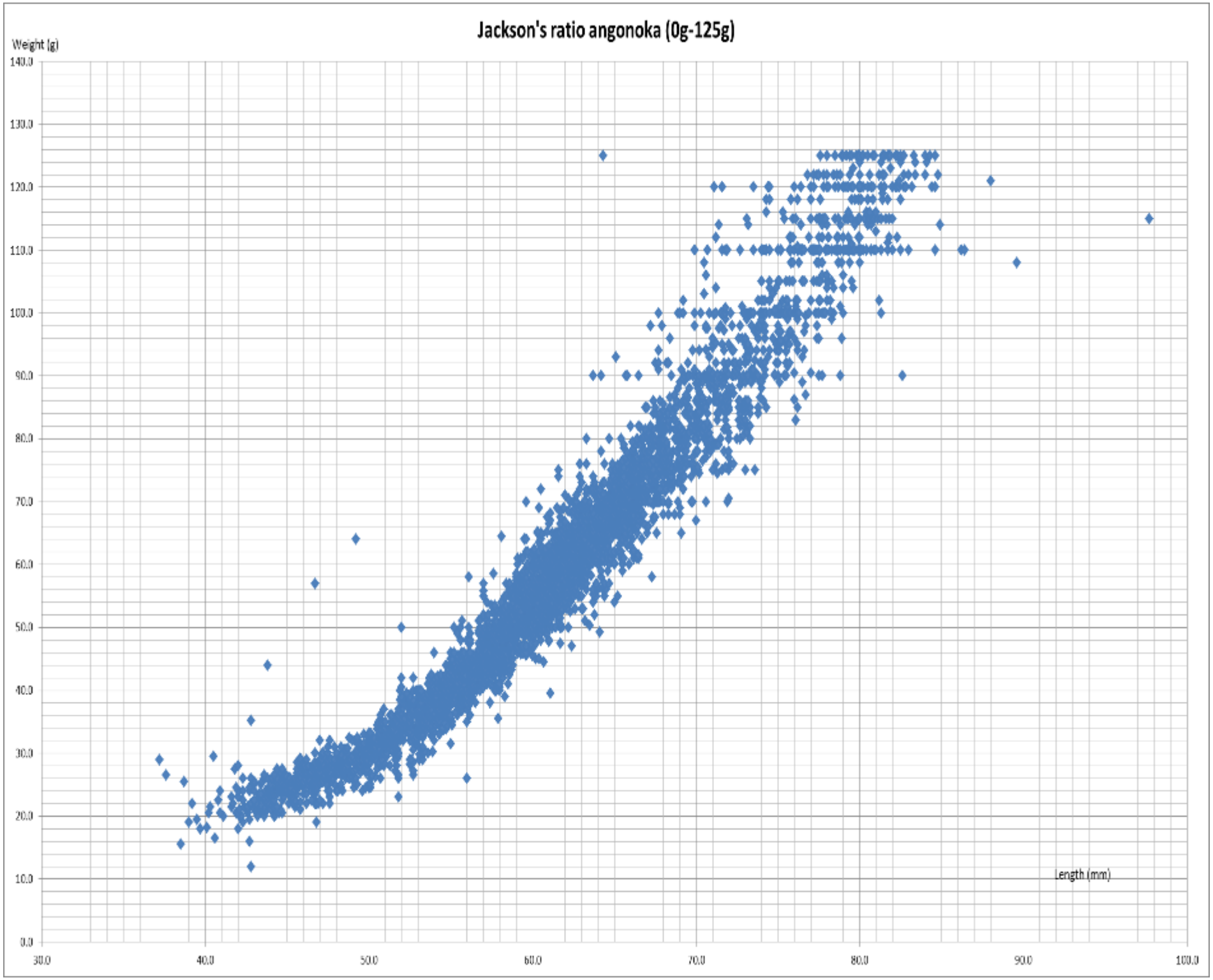
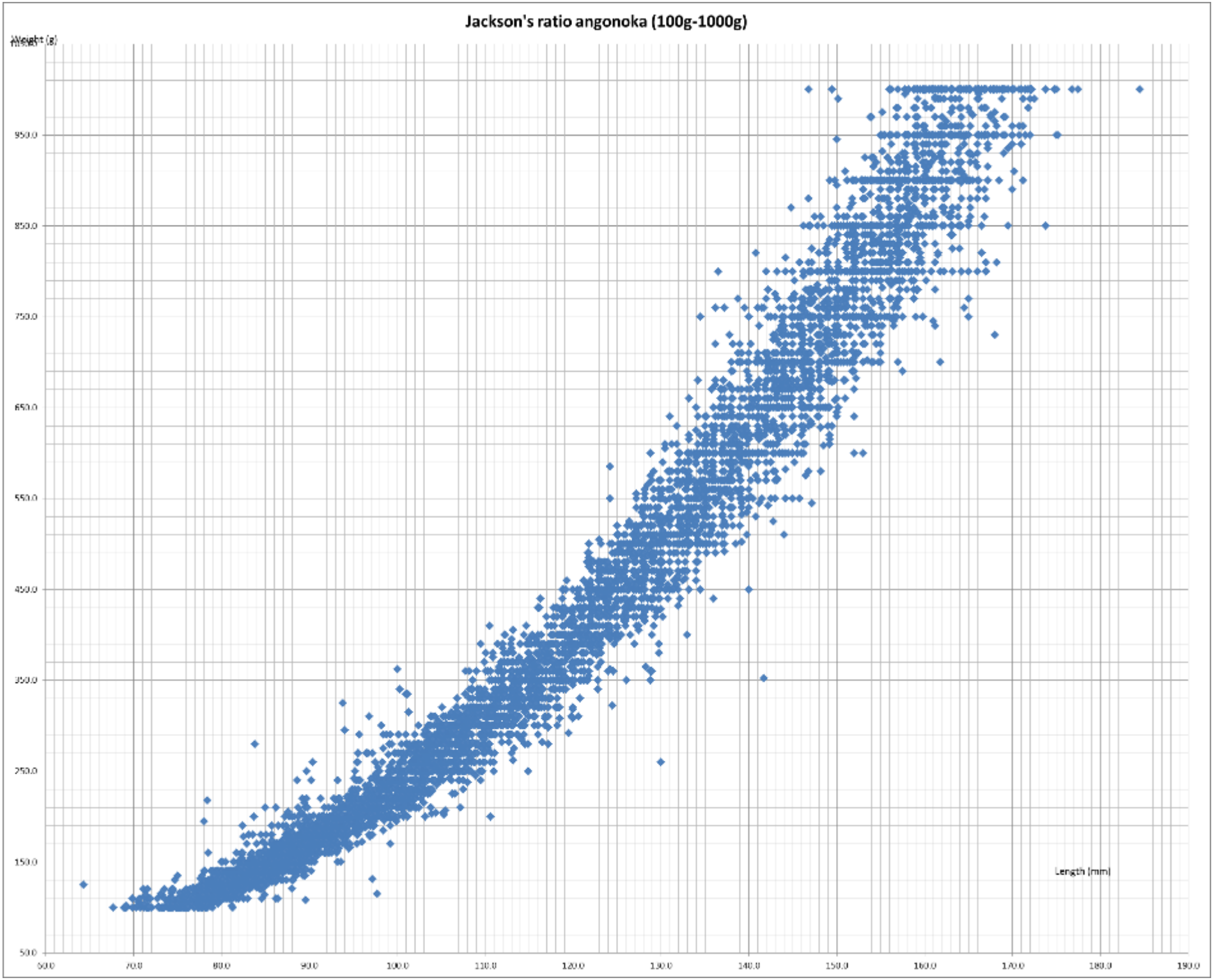
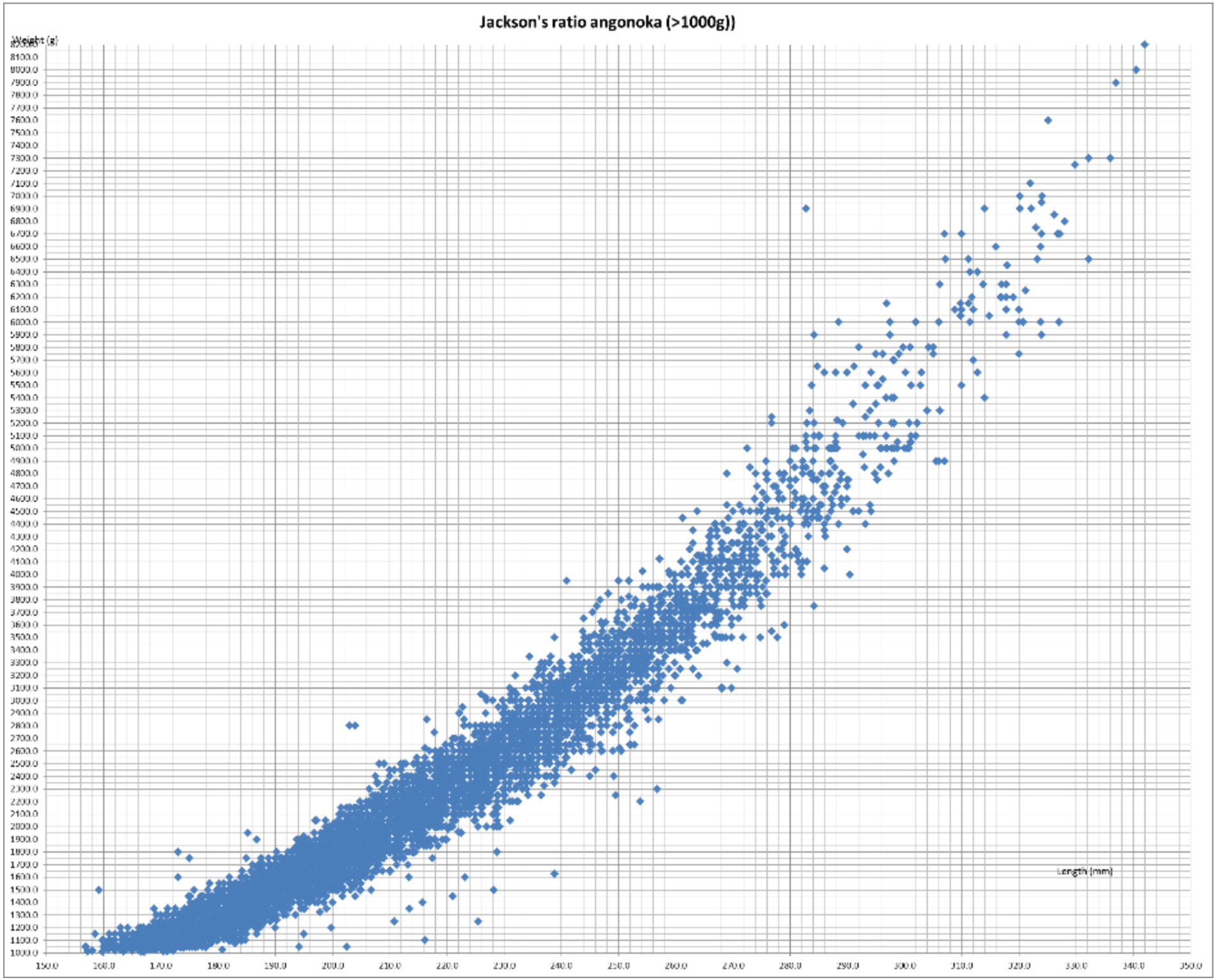
Appendix

Appendix 1

Growth data and size/weight ratio of captive animals in Ampijoroa, Madagascar (data ©Durrell Wildlife Conservation Trust)


Jackson's ratio angonoka (0g-125g)
Weight (g)
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
Length (mm)


Jackson's ratio angonoka (100g-1000g)
Weight (g)
950.0
850.0
750.0
650.0
550.0
450.0
350.0
250.0
150.0
50.0
50.0
70.0
80.0
90.0
100.0
110.0
120.0
130.0
140.0
150.0
160.0
170.0
180.0
190.0
Length (mm)


Jackson's ratio angonoka (>1000g))
Weight (g)
Length (mm)

Appendix 2

Standardised post-mortem and veterinary sample form for Ploughshare tortoises (developed by Javier Lopez, LdoVet, MSc, Dipl. ECZM (ZHM), MRCVS)

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

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	**Notes**

CLINICAL EXAMINATION

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	**Notes**

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:

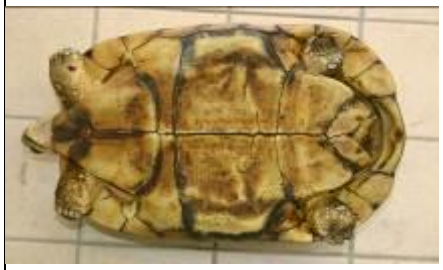
6) Nasal washes

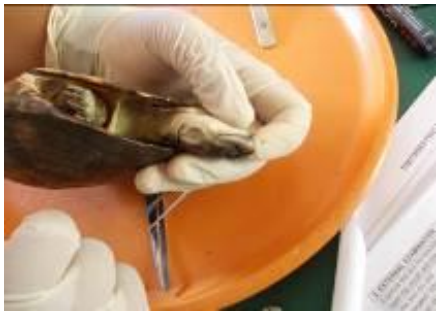
Date:

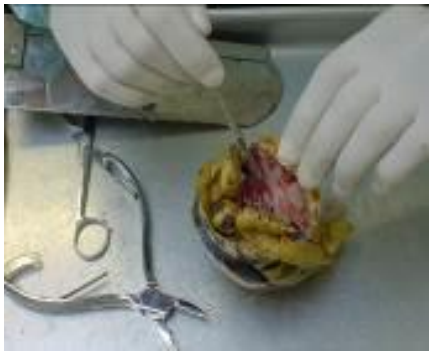
Number:

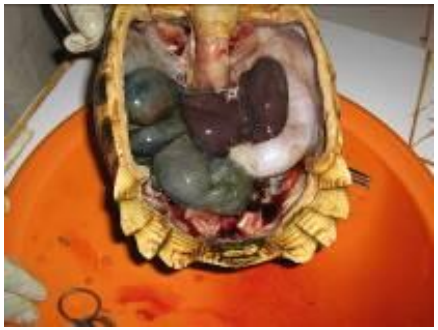
Identification:

Tortoise post-mortem examination sheet

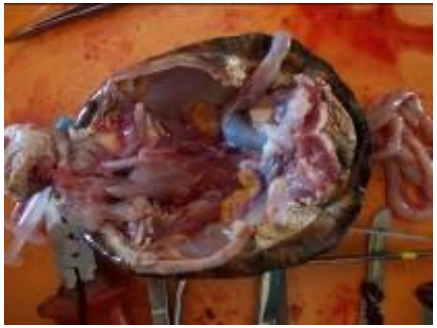
1- PREPARATION		Standard pictures to take	standard samples
Read microchip. Take morphometric measures. Take ventral, dorsal, frontal and caudal pictures			
Give details of circumstances of death and clinical history. Use the back of this sheet if necessary.	Weight:…….g Carapace length:..…….mm Clinical 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.			
Is the <u>skin</u> 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 <u>lesions</u>? Any lesion in <u>oral cavity</u> or <u>eyes</u>? Any contents in oral cavity? Any contents in the nose? What is the colour of the <u>mucosa</u>? Examine the left and right <u>tympanic membranes</u>. Examine <u>cloaca</u> and <u>tail</u>.	Eyes: concave – convex - dehydrated – wet – shiny – dry	 	Any lesions observed, take bacteriological swab or frozen, and in formalin 10%. Any parasites in Ethanol 70% or Formalin 5%

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**			
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	 	Free fluid frozen + x2 air dried smears + one bacteriology swab + frozen. Fat body frozen and in formalin 10%

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.			
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?			x2 Heart blood smears Liver and heart in formalin 10% Liver and heart frozen Spleen frozen and in formalin 10%

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

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 <u>stomach</u> contents (quantity and quality). Describe the GIT wall: Is it thick and fleshy or thin like a membrane? Any parasites? Any areas of <u>haemorrhage</u>? Is the <u>small intestine</u> or <u>large intestine</u> 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.			
Any observations?			Half of the head in formalin 10% and half of the head frozen
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.

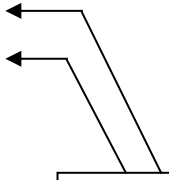
Sample	Frozen	Fixed in formalin	Smear	Fixed in alcohol	Bacteriology swab	
Skin and muscle (for DNA)		////////////	////////////	////////////	////////////	
Fat body			////////////	////////////	////////////	
Whole tongue (for Picornavirus PCR)		////////////	////////////	////////////	////////////	
Heart 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.