KEEPING AND BREEDING THE

MALAGASY FLAT-TAILED TORTOISE

PYXIS PLANICAUDA (GRANDIDIER, 1867)

by

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Project report

EHAP-Project

Exchange of Experience on Keeping and Rearing *Pyxis planicauda*

prepared for the

Bundesamt für Naturschutz BfN, Bonn

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Basel, February 2018

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DISTRIBUTION

While it was originally assumed that the Malagasy Flat-tailed Tortoise *Pyxis planicauda* (Grandidier, 1867) (Malagasy: Kapidolo) occurs exclusively in the forest of Andranomena (terra typica) (BOUR 1981, 1986) later studies reported additional occurrences and extended the distribution area to the neighboring Kirindy and Amborompotsy forests where *P. planicauda* inhabits the forested areas but is absent from the grasslands in the east (KUCHLING AND BLOXAM 1988). All three forest areas are located in the Menabe region, on the west coast of Madagascar. Here *P. planicauda* inhabits a fragmented tropical dry forest habitat bordered by the rivers Tsiribihina in the north and Morondava in the south (KUCHLING AND BLOXAM 1988, GLAW AND VENCES 2007, Fig. 1). Another small population was discovered north of the Tsiribihina River (BEHLER et al. 1993, BLOXAM et al. 1993, RAKOTOMBOLOLONA 1998, GOETZ et al. 2003). The total distributional range of *P. planicauda* is estimated to measure approximately 500 km^2 (RAXWORTHY AND NUSSBAUM 2000), is sheltered from rain by the western mountain slopes, and does not exceed altitudes of 100 m a.s.l. (above sea level) (KUCHLING AND BLOXAM 1988) (Fig. 1). KUCHLING (1989) regards the current distribution area of *P. planicauda* as a relic occurrence. The characteristic deciduous tropical dry forest is considered one of Madagascar's most threatened ecosystems and its conservation has become one of the main priorities of local nature conservation (KUCHLING 1989, GANTHORN et al. 2001, YOUNG et al. 2008). In 1990 less than 3 % of the original area remained (SMITH 1997). This serious habitat loss is mainly attributed to slash-and-burn agriculture (e.g. for sugar cane cultivation) and grazing, logging for charcoal production, road construction, and petroleum extraction (KUCHLING AND BLOXAM 1988, TIDD et al. 2001, GOETZ et al. 2003, GIBSON AND BULEY 2004, BONIN et al. 2006, YOUNG et al. 2008).

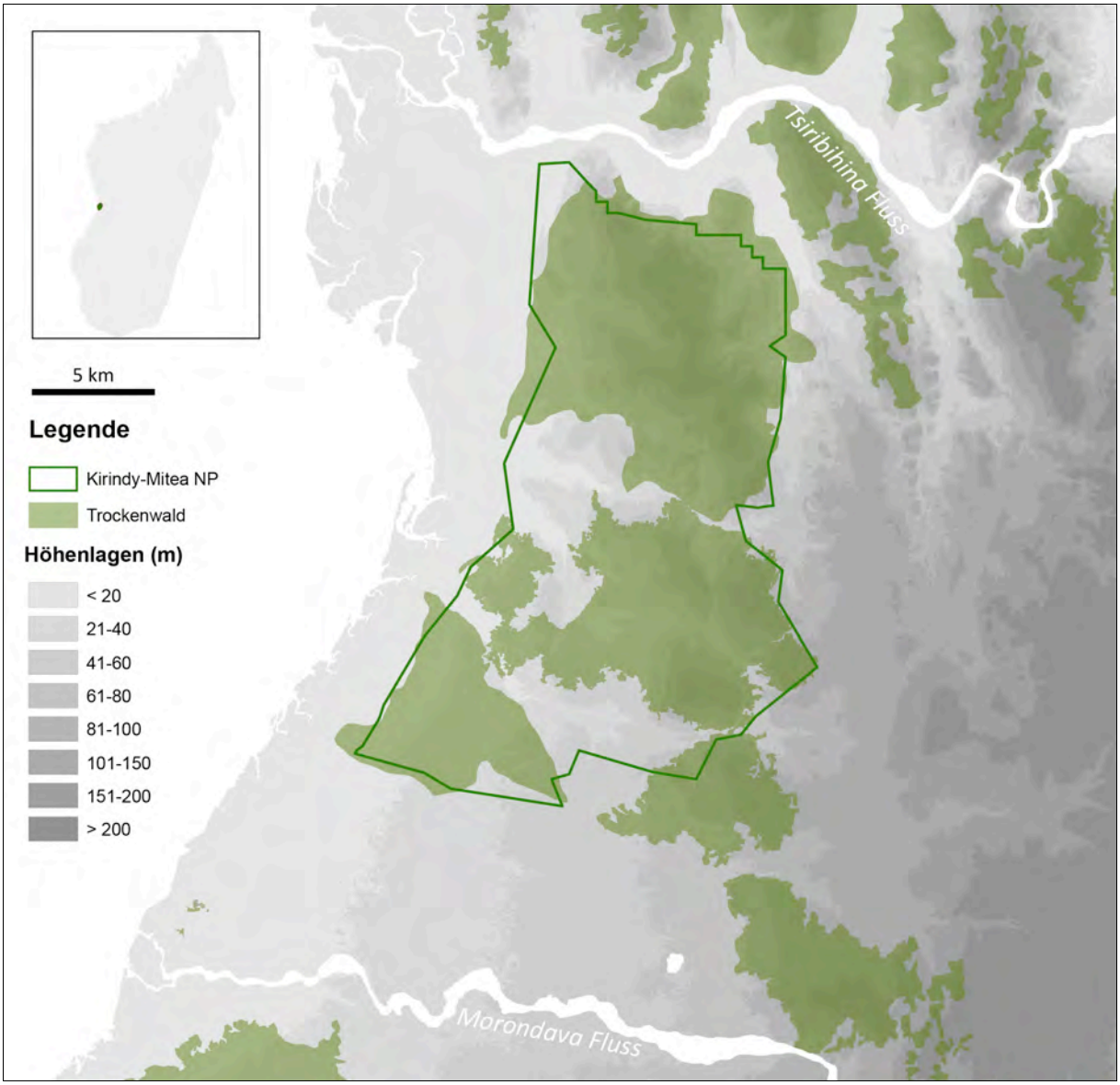

Fig. 1 Distributional range of *Pyxis planicauda* in the Menabe region on the west coast of Madagascar. Altitude from JARVIS et al. 2008, protected areas from IUCN UNEP-WCMC (2016).

Pyxis planicauda inhabits and area with scattered trees, dense undergrowth of four to eight meters in height, and loose sandy soils (KUCHLING AND BLOXAM 1988, GLAW AND VENCES 2007). The tortoises use the leave litter, which is available all year round, as daytime retreats (KUCHLING AND BLOXAM 1988, GLAW AND VENCES 2007, LEUTERITZ et al. 2013, Fig. 2). *Pyxis planicauda* lives in the relatively humid microclimate of the forest floor (KUCHLING 1989) and is closely adapted to this microhabitat.

Fig. 2 Top and center) Habitat of *Pyxis planicauda* in the Kirindy National Park. **Bottom)** Young *Pyxis planicauda* in its natural habitat. Pictures taken by F. S. Eckhardt.

CLIMATE

The climate in the region of the Kirindi National Park is „hot semi-arid" and seasonal with long dry periods of seven to eight months (KUCHLING AND BLOXAM 1988, KUCHLING 1989, Fig. 3). During the cool dry season between March and November, many of the trees (e.g. the majestic Baobabs) lose their foliage and all water bodies fall dry (Fig. 4). During this time, the tortoises bury themselves into the leaf litter, or the substrate, or retreat to holes in the ground where they stay dormant (estivation) (KUCHLING AND BLOXAM 1988, GLAW AND VENCES 2007, PEDRONO 2008, LEUTERITZ et al. 2013). During the hot rainy season (December - February) the forest turns green and all reptiles and amphibians are active. During this time numerous temporary water bodies and streams exist (MISLIN 2006, MISLIN AND HERZOG 2011). BRETZ (pers. comm.) reports that *P. planicauda* are occasionally found drowned in cattle drinking troughs (excavated hollows or pits) in areas cleared for grazing. Some authors report that *P. planicauda* is active at dusk and spends most of the day in hiding during the rainy season (DURRELL et al. 1989, RAKOTOMBOLOLONA 1998, GIBSON AND BULEY 2004), while KUCHLING AND BLOXAM (1988) describe the species as active during the day. The daily activity pattern is strongly dependent on precipitation (KUCHLING AND BLOXAM 1988, YOUNG et al. 2008). Individuals show site fidelity and can often be tracked down over several years in the same retreat sites ECKHARDT (pers. comm.).

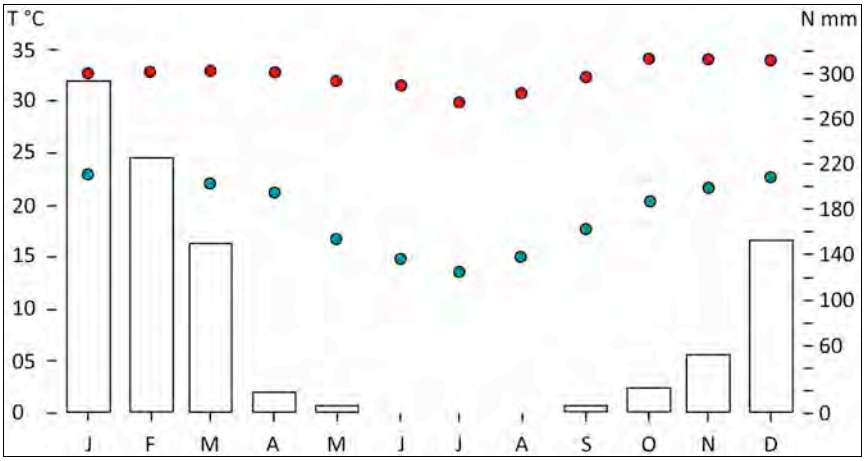

Fig. 3 Climate diagram for the Kirindy National Park on the west coast of Madagascar. Bars represent the precipitation in mm; dots refer to the maximum and minimum temperatures.

Fig. 4 Road dissecting the Kirindy National Park on the west coast of Madagascar in the rainy season (left) and during the dry season (right). Pictures by F. S. Eckhardt (left) and W. Doganowski (right).

POPULATION DENSITY

The population of *P. planicauda* was estimated to comprise less than 10,000 individuals (LEUTERITZ et al. 2013) with a decreasing trend (RAXWORTHY AND NUSSBAUM 2000). A study carried out in Kirindy in 1991 found 6.75 tortoises per square kilometer, while a later survey carried out in 1996 found only 0.6 *P. planicauda* per square kilometer (BLOXAM et al. 1996) with a recapture rate of 83 %. Further studies in the center of the distribution range of *P. planicauda* reported 50 tortoises per km^2 (DURBIN AND RANDRIAMANANPISOA 2000), 200-600 individuals per km^2 (DURBIN AND RANDRIAMANANPISOA 2000), and 100 animals per km^2 (KUCHLING in litt. 2001). YOUNG et al. (2008) calculated significantly higher population densities using a "distance sampling method". However, with only 29 individuals, the data basis of this study was too small to provide valid results.

Moreover, the "distance sampling method" often overestimates the actual population sizes and is therefore criticized (RIOUX PAQUETTE et al. 2009).

NUTRITION

In the wild *Pyxis planicauda* feeds on fruits of *Breonia perrieri*, a shrub of the family Rosaceae, fruits of *Aleanthus greveanus* (KUCHLING AND BLOXAM 1988, GLAW AND VENCES 1994, GIBSON AND BULEY 2004), mushrooms which represent the first available food source at the beginning of the rainy season (BLOXAM pers. obs. in GIBSON AND BULEY 2004) and blossoms (GOETZ et al. 2003). HUNZIKER (1981) reports that over 200 tree species were found in the dry forests of Madagascar which develop fruits at different times. According to HUNZIKER (1981) *Pyxis planicauda* was reported to mainly feed on such fallen fruits which have high fiber contents and on seeds which are abundant during the rainy season. In the wild tortoises were also observed eating leaves, buds, and shoots of low bushes (HUNZIKER 1981, KUCHLING AND BLOXAM 1988, KUCHLING 1989).

REPRODUCTION

Literature references for the maximum carapace length of adult specimens vary between 13.0 cm (GIBSON AND BULEY 2004), 13.7 cm (ERNST et al. 2000), 14.8 cm (PEDRONO 2008) and 16.0 cm (HERSCHE AND LIEBEL 2003). With a maximum of 14.0 cm (HERSCHE AND LIEBEL 2003) males remain smaller and weigh about 300-450 g (BLOXAM AND HAYES 1991, HERSCHE AND LIEBEL 2003), while adult females weigh about 475-670 g (KUCHLING AND BLOXAM 1988, HERSCHE AND LIEBEL 2003). In addition, males have a distinctly longer tail and a concave shaped plastron (TABAKA 2003). The Durrell Wildlife Breeding Center reports that females become sexually mature at about ten years, while GIBSON AND BULEY (2004) report that sexual maturity is reached at eight to 12 years.

According to KUCHLING (1989), sexes are distinguishable at about ten to 12 years (Fig. 5). KUCHLING AND BLOXAM (1988) report a linear increase in size and weight for both sexes over the first 15 to 16 years.

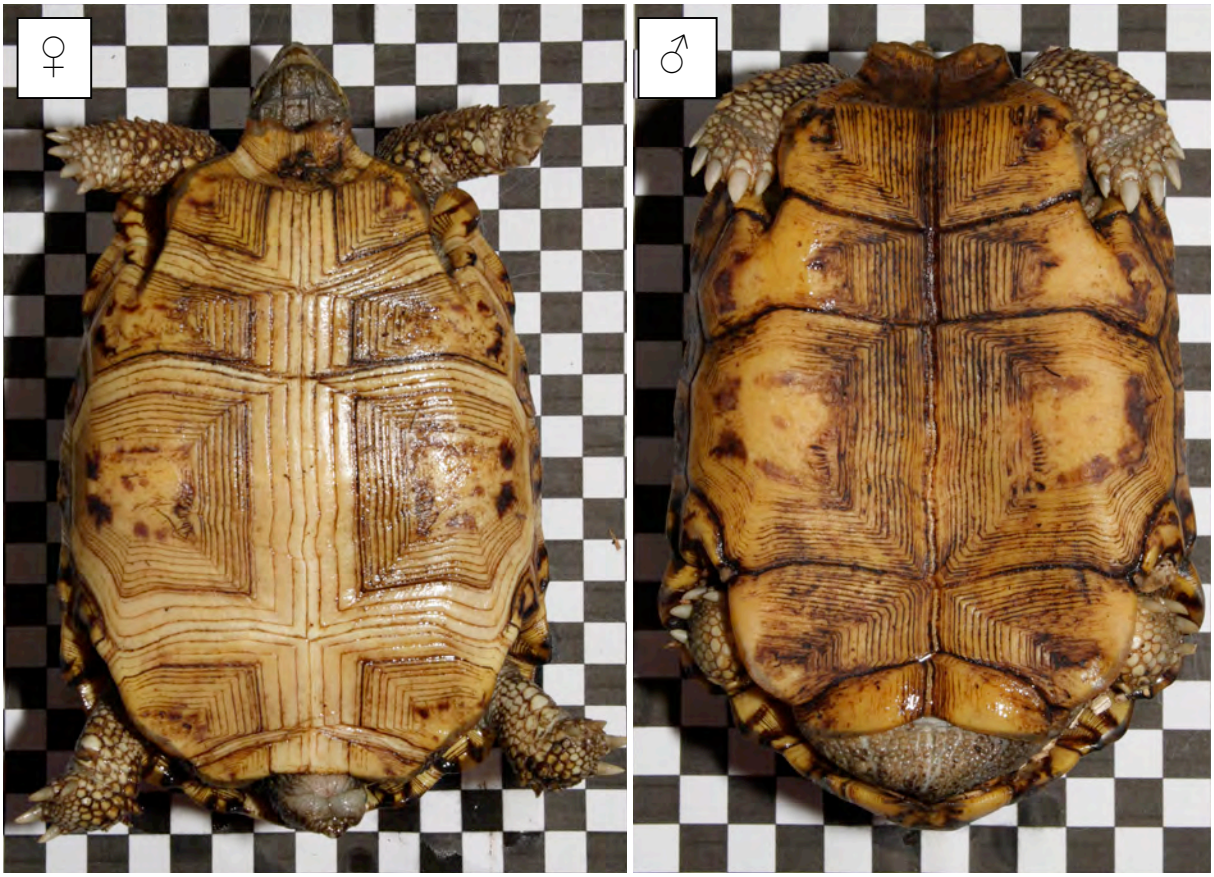

Fig. 5 Adult pair of *Pyxis planicauda.* **Left)** Female; **Right)** Male.

The generation time in which the number of individuals in a population doubles is estimated to be at least 25 years (LEUTERITZ et al. 2013). Mating of the skittish animals was observed only rarely and except for KUCHLING (1989) is limited to observations from captive husbandry (GIBSON AND BULEY 2004). According to these observations, the mating season is restricted to the first half of the rainy season between January and early March (KUCHLING AND BLOXAM 1988, KUCHLING 1989, GLAW AND VENCES 2007). Before attempting to mount, males butt their anterior carapace rims against the females shell while frantically circling the female several times (GIBSON AND BULEY 2004).

Receptive females remain stationary while mounting and mating occurs, while unreceptive females move continuously, making mounting and mating attempts impossible (GIBSON AND BULEY 2004). Towards the end of the rainy season females lay one to three single eggs (PRITCHARD 1979, GOETZ et al. 2003, PEDRONO 2008). These eggs measure 25-30 mm x 33-36 mm and weigh 15-20 g (PRITCHARD 1979). It has not yet been scientifically demonstrated whether *P. planicauda* exhibits sex chromosomes or whether sex determination is driven by one of three temperature-dependent sex determination mechanisms (KUCHLING pers. comm.). However, a temperature-dependent gender fixation is suspected (PEARSON 2013). Incubation times in the wild were reported to vary between 250 and 340 days (RAZANDRIMAMILAFINIARIVO et al. 2000, LEUTERITZ et al. 2013).

THREATS

Pyxis planicauda represents one of the most threatened chelonian species in the world (BEHLER 2000, IUCN/SSC TFTSG 2011). The species is presently classified as critically endangered and listed on the Red List of threatened species of the International Union for Conservation of Nature (IUCN) (IUCN Red List 2016). Although *P. planicauda* was listed on Appendix II of the "Convention on International Trade in Endangered Species of Wild Fauna and Flora" (CITES) since 1977 (UNEP-WCMC 2007) and was upgraded to Appendix I in 2002, the species was only added to the "Red List" as endangered in 1996. It was not until 2008 that the protection status was elevated to the current classification. Although the distribution range of *P. planicauda* overlaps with three protected areas: the Andranomena Reserve (64 km^2), Analabe and the Kirindy-Mitea National Park (811 km^2) (PROTECTED PLANET 2016, Fig. 1.), these also contain plantations, roads, and mining areas and are inadequately patrolled and managed. Massive habitat destruction is considered to represent the main treat for *Pyxis planicauda*. Between 1963 and 1993 alone, a decline of 32 % of dry forest habitat was recorded (TIDD et al. 2001). Since then, deforestation rates have dramatically increased and projections forecast that half of the remaining 730 km^2 will be destroyed by 2040 (TIDD et al. 2001). Consequently, habitat loss is expected to reach more than 70 % (1963 to 2040) (TIDD et al. 2001, HARPER et al. 2007).

Although extensive slash-and-burn agriculture is prohibited by law, this method is still practiced due to the lack of law enforcement (KUCHLING 1989). The vegetation of Madagascar, unlike that of the African mainland, is not fire-resistant (KUCHLING 1989). KUCHLING (1989) suspects that *P. planicauda* has disappeared from the Andranomena forest due to slash-and-burn agriculture and extensive sugar cane cultivation. The consumption of tortoises is prohibited by law, as well as for traditional reasons for the Mahafaly and Antandroy (KUCHLING 1989). However, these prohibitions are widely ignored by other tribes and immigrants. Chelonians are illegally traded in the cities and offered as a delicacy for human consumption (KUCHLING 1989). Until recently, *P. planicauda* was also collected in large numbers for the international pet trade (GOETZ et al. 2003, BONIN et al. 2006). Between 2000 and 2002, about 4000 adult tortoises were collected, which according to estimates, corresponds to about 20-40 % of the total population (GOETZ et al. 2003, BONIN et al. 2006, PEDRONO 2008, LEUTERITZ et al. 2013). Between 1999 and 2000, several thousand wild caught tortoises were traded internationally (GIBSON AND BULEY 2004, MISLIN 2006, MISLIN AND HERZOG 2011, PEARSON 2013). Even though exports for the international pet trade have officially stopped in 2002, tortoises which were illegally exported from Madagascar are still confiscated on a regular basis (NIJMAN et al. 2012). Even indigenous Malagasy people are not allowed to keep tortoises without official permission (KUCHLING 1989). Likely *P. planicauda* has already vanished from some parts of the forest in the southwest of its distribution area due to overcollection and trade (FRIENDS OF ANIMALS, WILDEARTH GUARDIANS 2013). Based on the existing collection and deforestation rates a population model, predicted that *P. planicauda* might already become extinct in 2030 (CBSG 2001). However, since the collection and export of *P. planicauda* is prohibited since 2002, these estimates are no longer entirely accurate. Further hazards are imported predators such as cats, dogs, pigs, and mongooses as well as diseases such as blood parasites which have been observed both in captivity and the wild (PRASCHAG et al. 2010 FRIENDS OF ANIMALS, WILDEARTH GUARDIANS 2013).

PROTECTION SATUS & CONSERVATION BREEDING MEASURES

In addition to the EHAP project, there are conservation breeding projects for *Pyxis planicauda* at the turtle conservation center situated at the “Ampijoroa forest station” in Mahajanga Province, in northwest Madagascar (RAZANDRIMAMILAFINIARIVO et al. 2000), at Jersey Zoo, UK, and at Bronx and Knoxville Zoo, USA (OGLE 2005). The species is considered extremely difficult to keep and the rather complicated conditions required for successful incubation make conservation breeding projects challenging (GIBSON AND BULEY 2004).

STATE OF KNOWLEDGE IN KEEPING AND BREEDING

Pyxis planicauda is rarely maintained in captive husbandry and breeding success is still extremely low. After 25 years of keeping in three renowned zoos (Jersey, Bronx and Knoxville) GIBSON AND BULEY 2004 (2004) report only four hatchlings outside of Madagascar (three in Jersey Zoo and one in Knoxville Zoo). Even the turtle center in Ampijoroa successfully bred and reared only 19 animals between 1995 and 1999 (PEARSON 2013). However, breeding success has heavily increased since (IHLOW AND MISLIN pers. comm.). The international studbook of the "Association of Zoos and Aquariums" (AZA), maintained by Michael Ogle, Zoo Knoxville, USA, contained 160 specimens in 2013, but listed only 24 hatchlings since 1990, most of which died within the first year. Even though the majority of *P. planicauda* specimens kept in the US are in private hands, private owners are unfortunately excluded from participation in the AZA studbook project (PEARSON 2013). Given the small clutch size and the demanding incubation conditions requiring temperature fluctuations make breeding very difficult.

GIBSON AND BULEY 2004 report on 15 years of experiences of keeping *P. planicauda* at the Jersey Zoo and the turtle center in Ampijoroa. The Jersey Zoo started to keep a group of 4.2 *P. planicauda* in 1991, which was supplemented in 1997 with three females. These animals originate from the dry forest in the Kirindy region. At the Jersey Zoo the tortoises are kept in open topped enclosures constructed of fiberglass covered plywood. Males are kept individually in pens measuring 0.5 m x 0.5 m, while groups of females are kept in larger enclosures (4.0 m x 0.5 m) (GIBSON AND BULEY 2004). All tanks have sloping floors and drainage holes.

The substrate consists of a five centimeter thick drainage layer of volcanic pebbles covered with capillary matting for drainage and a two to three centimeter thick layer of sandy substrate. The substrate is covered with a thick layer of leaves. The females' enclosures feature an area with deeper, slightly dampened sandy soil (10-12 cm) intended for nesting. All pens are structured with roots, logs, different robust plants such as *Ficus*, *Sansevieria*, *Maranta*, *Syngonium* and *Yucca*. In the rainy season ambient temperatures vary between 27-34 °C (day) and 22-26 °C (night). All enclosures are equipped with a warm water irrigation system (20 °C) operating between thirty minutes and four hours a day to maintain a humidity of 70-95 %. During the dry season, temperatures are slowly reduced to 22-28 °C during the day and 14-18 °C during the night, while the irrigation system is switched off. General lighting is provided by fluorescent tubes (ZooMed Reptisun 2.0) and varies seasonally between 14 and 11.5 hours. In addition, all tanks are equipped with a local basking spot, locally reaching 45-55 °C. The large enclosure of the females is additionally fitted with a 275 W (Active UV heat) lamp, while the enclosures for males are equipped with a 60 W lamp each. Water bowls for drinking and bathing are offered permanently but are rarely used. The tortoises prefer to drink water that collects in the leave litter or runs down their head while the irrigation system is operated. At the beginning of the rainy season, the diet consists almost exclusively of mushrooms such as oyster mushrooms (*Pleurotus ostreatus*), shiitake (*Lentinula edodes*), and beefsteak fungus (*Fistulina hepatica*) for about two weeks. Later, dandelion (*Taraxacum officinale*), fruits and vegetables such as tomato, pear, papaya, chicory and pakchoi as well as small amounts of low-fat cat food are accepted. Fruits are sprinkled with a vitamin powder (Nutrobal). Food is provided daily, with leafy vegetables, lettuce, and fruit three to four times a week, mushrooms twice a week, and cat food once or twice a month. For calcium supply cuttlefish bone is permanently available. Sweet potatoes (*Ipomoea batatas*), whose leaves are also eaten, are planted in pots or directly into the enclosure. The feces of other animals are also consumed which may contribute to the development of the intestinal flora of young tortoises (GIBSON AND BULEY 2004). *Pyxis planicauda* is vigilant and careful and it took up to 18 months for the animals to get used to the presence of a keeper and to feed, mate, or engage in combat in his presence. At the beginning of the rainy season, two males are paired for 15-60 minutes for intra-sexual combat. These fights consist of ramming and biting the opponent into the fore- and hind legs.

In addition, the dominant male frequently mounts the inferior one. After, the males are separated and a female is introduced to each male. Mounting by the male is usually preceded by bouts of butting and frantic circling the female (GIBSON AND BULEY 2004). The females remain for two to 24 hours in the enclosure of the males. Even though the tortoises may mate several times, mating activity seems to be highest in the early morning hours. During the rainy season, the animals are introduced for mating three to six times a week as described.

A conservation breeding program, originally for the Malagasy Ploughshare Tortoise (*Astrochelys yniphora*), was established in 1986 at the turtle center in Ampijoroa. In 1988, 2.3 *P. planicauda* were collected in the Amborompotsy forest and brought to Ampijoroa to study the husbandry and breeding conditions and to document the behavior and biology of the species (RAZANDRIMAMILAFINIARIVO et al. 2000). The group was extended in 1997 by 2.2 tortoises from the Analaiva forest. In Ampijoroa the tortoises are kept in 1.5 m x 2.5 m outdoor enclosures separated by 22 centimeter high concrete walls. Natural forest soil and leaf litter form the substrate and the enclosures are planted with natural vegetation, including various low bushes, and shaded by larger trees such as teak (*Tectona grandis*) (RAZANDRIMAMILAFINIARIVO et al. 2000). Since the animals are kept here in a natural climate, the use of artificial light and irrigation technology is not necessary. The tortoises are fed four times a week with various leaves and mushrooms from the forest, but also accept opuntia, banana, mango, papaya and tomatoes (RAZANDRIMAMILAFINIARIVO et al. 2000). Like many other tortoise species, *Pyxis planicauda* was reported to exhibit a bimodal activity pattern with high activity between 6:00 and 10:00 am and 4:00 and 7:00 pm. During this time, the ambient temperatures fluctuate between 20 and 30 °C. In the turtle center in Ampijoroa, tortoises of both sexes are also kept separately and are introduced for mating. Mating attempts were mainly observed in the morning at temperatures between 23 °C and 33.5 °C. During mating, both animals hardly move, except for head-movements. Egg deposition occurs approximately 30-60 days after the first mating between sunset and midnight. The nesting pits are about five centimeters deep and have a diameter of three to four centimeters. Only one single egg is laid. Typically eggs weigh between 17.5 g and 20.5 g, are 34.1 mm to 39.2 mm long, and 28.1 mm to 30.1 mm wide. Between January and the end of March, a female can deposit up to three clutches, 29 to 42 days apart.

Under natural conditions in Ampijoroa incubation periods of 257-343 days were observed (average: 291 days, n = 2, RAZANDRIMAMILAFINIARIVO et al. 2000). The authors report a hatching rate of 37 %. The enclosures for rearing juveniles measure 1.0 m x 0.5 m.

In the Jersey Zoo, tortoises of both sexes are kept separately and introduced for breeding. The presence of further females seemed to distract the male and was therefore omitted (GIBSON AND BULEY 2004). Egg deposition takes place at the end of the rainy season, approximately 30 days after mating (GIBSON AND BULEY 2004). In the evening, the females begin to excavate a five to six centimeters deep nesting pit in. A single egg is placed into the nest. In the following weeks, up to three more clutches are deposited at intervals of three to six weeks (GIBSON AND BULEY 2004). The eggs weigh on average 19.7 g (15.5 g - 22.2 g), and measure 38.0 mm (34.1 mm - 41.6 mm) in length (GIBSON AND BULEY 2004). Since the eggs hatch at the beginning of the following rainy season, a diapause is necessary (GIBSON AND BULEY 2004, RUBY AND SENNEKE 2004). Thus, the incubation time varies considerably depending on the time of egg deposition. The three hatchlings emerged after 213, 262, and 306 days (GIBSON AND BULEY 2004, RUBY AND SENNEKE 2004). For incubation, the eggs are half covered with dry vermiculite and placed into a larger container which is half filled with moist vermiculite and closed with a lid (GIBSON AND BULEY 2004). This tank is stored in the turtle room and opened twice a week to allow air exchange (GIBSON AND BULEY 2004). At the end of the drying period, the vermiculite is moistened and the containers transferred to an incubator set to 30-31 °C (GIBSON AND BULEY 2004). The tortoises remain within the egg for up to one week after the shell has been pipped (GIBSON AND BULEY 2004).

SENNEKE AND TABAKA (2003) recommend enclosures of 0.6 m x 0.9 m for keeping one individual and 1.2 m x 0.6 m for a pair. If several tortoises are kept together, it is important to structure the enclosure, to provide sufficient hiding places (SENNEKE AND TABAKA 2003). These should be humid and cool. In addition a shallow water dish should be permanently available (SENNEKE AND TABAKA 2003). The authors recommend a radiant heater with temperatures below 32-38 °C and a UV supply. The diet should be rich in fiber and calcium, as well as poor in protein. A mixture of fruits, vegetables, mushrooms and green leaves is recommended and should be enriched with calcium, especially for juveniles and egg-laying females (SENNEKE AND TABAKA 2003).

In 2013 another report on captive husbandry of *P. planicauda* was published (PEARSON 2013). The author keeps his tortoises in outdoor enclosures in Gainesville, Florida, USA between May and November and indoors during the winter months (November - May) (PEARSON 2013). PEARSON (2013) reports frequent mating at the beginning of the rainy season which decreased towards the summer. Although most nesting events were observed between July and September, individuals also laid fertile eggs during the dry season (February) (PEARSON 2013). According to PEARSON (2006), the Knoxville Zoo uses a mixture of 1.5 parts vermiculite and 1 part water for incubation. No moisture is added until the third section of the incubation. In the first stage, the eggs are incubated at room temperature (24-29 °C) for four months and then for two weeks at 20-22 °C followed by another week at room temperature (PEARSON 2006). Finally, the eggs are incubated at 30-31 °C and moistened weekly. Hatchlings typically emerge 106 days after the end of the cooler phase. Overall, the incubation period varies between 175 and 255 days (PEARSON 2006). PEARSON (2013) incubates the eggs in vermiculite dampened with water at a 1:1 ratio. According to the experience of the Knoxville Zoo clutches are incubated for eight to 12 weeks at day temperatures of 31 °C and a night setback to 26 °C in a self-constructed incubator, then the substrate is no longer remoistened and the containers are stored outside the incubator for about six to eight weeks at room temperature (20-24 °C) to simulate a diapause (PEARSON 2013). After an average of 14 days (n = 10, variation 0-32 days) the eggs are candled. If an embryo is visible, the diapause is ended by moistening the substrate and returning the containers into the incubator. Eggs are incubated using the same conditions as at the beginning and remoistened weekly (PEARSON 2013). After the diapause, the hatchlings emerge after an average of 86 days (n = 10, variation 73-97 days). As anomalies were frequent in the beginning, the maximum daytime temperatures were reduced by 0.5 °C in later years while the night-time temperatures were raised to 26.5 °C. In addition, the first incubation phase was shortened to four to seven weeks. Since then, fewer anomalies have been observed (PEARSON 2013). The average incubation period was 240 days (n = 10, variation 213-275 days) and the hatching success was 100 % (PEARSON 2013).

ZOVICKIAN (2003) covers half of the eggs with vermiculite and uses sealed containers and a Hovabator Incubator (G.Q.F. Mfg. Co. Inc., Savannah, GA).

The eggs are incubated at 30.5-31.6 °C for five weeks and then candled. Eggs showing development remain in the incubator while the remaining eggs are stored for another five weeks in a cooler place (18.3-22.3 °C). Subsequently, the eggs are incubated at 30.5-31.6 °C and moistened twice a week (ZOVICKIAN 2003). To ensure that the water has the same temperature, it is stored within the incubator. After five weeks, the eggs are candled again. Eggs that show no development are subjected to another cooling period of five weeks. In some cases, three cooling periods were required to initiate egg developed (ZOVICKIAN 2003).

ZOVICKIAN (2003) reports about another keeper who had to move and thus was forced to store eggs laid between January and March at 18.3-22.2 °C until July before they could be incubated. The hatchlings hatched after 75-82 days of incubation. According to PEARSON (2006) *P. planicauda* hatches very quickly. Hatchlings are transferred to containers with wet paper towels until the yolk sack has been absorbed completely (PEARSON 2006). These containers are fitted with a shallow water dish, a food bowl and a hiding place. PEARSON (2006) observed hatchlings as well as adults to drink from the water dish or directly from the wet ground. Later, juveniles are reared in bigger enclosures with cypress mulch as substrate. Various herbs and vegetables including strawberries, hibiscus flowers, and the commercial turtle food "Mazuri Tortoise Chow" are offered to juveniles and adults alike (PEARSON 2006). Climate charts of the city of Morondava show average minimum temperatures of 14 °C during the cooler dry season and 23 °C during the rainy season. Substrate temperatures recorded with dataloggers in the Kirindy forest and the turtle center in Ampijoroa show that incubation temperatures in the colder phase should be between 25 °C (day) and 17 °C (night) (PEARSON 2013). In the later course of the drying season, the substrate temperatures rise to 30 °C (maximum during the day) and drop down to 21 °C at night. In the Kirindy Forest, night-time lows never fell below 21 °C (RAZANDRIMAMILAFINIARIVO et al. 2000, PEARSON 2013). Apparently, the conditions during the dry season in Madagascar are so extreme that *P. planicauda* has developed a diapause which delays embryonic development. Juveniles hatch only when the conditions become more favorable in the following rainy season, as is also known from the Radiated Tortoise *Geochelone radiata* and the closely related Spider Tortoise *Pyxis arachnoides* (PEARSON 2006). Temperature, humidity, or a combination of both seems to trigger the end of the diapause (PEARSON 2006).

The EHAP Project

The EHAP project aims to investigate optimal rearing and husbandry conditions as a contribution to the conservation of the endangered Malagasy Flat-tailed Tortoise *Pyxis planicauda.* The project was initiated in 2007 by Viktor Mislin (Project Leader, Switzerland). In particular, the incubation of eggs in captivity is extremely difficult. After initial success using a diapause, the EHAP project aims to investigate the ideal incubation conditions and answer the question about the mechanism of gender fixation and pivotal temperature. In 2006, the EHAP project was officially launched by signing a cooperation agreement with Professor Dr. Dieter Ebert of the Zoological Institute of the University of Basel and approved by the Federal Agency for Nature Conservation (BfN). This cooperation was required to legally extend the EHAP project to Germany. In 2008 six juveniles were exported to project members in Germany. In 2010 another four tortoises were handed over to project participants (MISLIN et al. 2010). In the meantime, 33 of the 55 hatchlings have been distributed to different project members in Europe. The project participants are obliged to regularly collect data and photographs in order to ensure a complete and consistent documentation of the development of the juveniles. In 2014 some tortoises, of which the sex could already be determined, were exchanged between project participants in order to form suitable breeding groups.

The export of *P. planicauda* from Madagascar has now been completely discontinued, which means that no more animals can be legally bought. Since the small species is in high demand, overexploitation and trade continues nevertheless. Hence, it is of uttermost importance to optimize the breeding success in captivity to meet this demand with captive bred and raised tortoises. This is the only way to reduce illegal collection and trade and ultimately support the protection of *P. planicauda* in Madagascar.

AIMS AND OBJECTIVES

The objectives of the EHAP project are:

1) To optimize the keeping conditions of *Pyxis planicauda* in captivity in order to optimize the reproductive success.

2) To formulate recommendations for keeping and breeding *Pyxis planicauda* in captive husbandry.

3) To study of the optimal incubation methodology. This should increase the hatching rate to almost 100 %.

4) To rear hatchlings under controlled, standardized conditions up to sexual maturity while consistently collecting data to document the hatchlings development. These data facilitate e.g. the compilation of growth curves, etc.

EXPECTED RESULTS

1) Formulation of optimal husbandry conditions
2) Formulation of optimal rearing conditions
3) Formulation of optimal incubation conditions
4) Publication of the results in the form of guidelines for the optimal keeping and breeding of *Pyxis planicauda* in human care

PROJECT SCHEDULE

1) 2008-2013 Rearing of the juveniles handed over in 2007 until they reach sexual maturity
2) From 2014 onwards, the breeding of reared animals for conservation purposes

STOCK OF THE EHAP PROJECT

The entire breeding group, currently consisting of eight adults, is housed by Viktor Mislin (Project Leader, Switzerland). So far 55 hatchlings hatched from which 44 are still live (80 %). The first offspring, hatched in 2003 turned out male and could be introduced with an adult female in 2014. Recently the first hatchlings of this group (group 4) hatched (hatchlings 53, 54, and 55).

COMPOSITION OF THE BREEDING GROUP

Group 1)

-Female white

-Female blue (**2014 deceased**)

-Small male

Group 2)

-Female red

-Female green

-Male (**2016 deceased**)

-Offspring male No. 5 (since August 2016)

Group 3) (obtained from another keeper in 2006)

-Female large (He.gr.)

-Male

-Female small (He.kl.) until 2010

Group 4)

-Female small (He.kl.) since 2014

-Offspring male since 2014

PROJECT LEADER GERMANY

Tortoise 1: since October 2010

Studbook entry: **No. 25** (Documents from the Zoological Institute Basel)

Hatching date: 27.02.2009, Viktor Mislin (Basel)

Parents: Female green, P046 M7

EG-Import documents: E 01158/10

Sex: unknown

Tortoise 2: since October 2010

Studbook entry: **No. 28** (Documents from the Zoological Institute Basel)

Hatching date: 01.04.2010, Viktor Mislin (Basel)

Parents: Female white, male small, P057 M13

EG-Import documents: E 01158/10

Sex: unknown

Tortoise 3: since 2015

Studbook entry: **No. 35** (Documents from the Zoological Institute Basel)

Hatching date: 05.03.2012, Viktor Mislin (Basel)

Parents: Female white, male small, P080 M07, red dot 1st Scute

EG-Import documents: E 00933/14

Sex: unknown

Tortoise 4: since 2015

Studbook entry: **No. 40** (Documents from the Zoological Institute Basel)

Hatching date: 18.04.2012, Viktor Mislin (Basel)

Parents: Female red, Male large, P0082 M07, yellow dot 2nd Scute

EG-Import documents: E 00933/14

Sex: unknown

Tortoise 5: since 2015

Studbook entry: **No. 7** (Documents from the Zoological Institute Basel)

Hatching date: 06.05.2006, Viktor Mislin (Basel)

Parents: Female green, P016 M7

EG-Import documents: E 0681/07

Sex: male

Tortoise 6: since 2015

Studbook entry: **No. 8** (Documents from the Zoological Institute Basel)

Hatching date: 10.05.2006, Viktor Mislin (Basel)

Parents: Female blue, P017 M7

EG-Import documents: E 0681/07

Sex: male

Tortoise 7: since 2015

Studbook entry: **No. 12** (Documents from the Zoological Institute Basel)

Hatching date: 01.03.2007, Viktor Mislin (Basel)

Parents: Female blue, P023 M7

EG-Import documents: E 0681/07

Sex: female

PROJEKT PARTICIPANT 1

Tortoise 1: since 18.10.2010

Studbook entry: **No. 26** (Documents from the Zoological Institute Basel)

Hatching date: 22.03.2009, Viktor Mislin (Basel)

Parents: Female blue, P048 M7

EG-Import documents: E 01159/10

Sex: presumable female

Tortoise 2: since 18.10.2010

Studbook entry: **No. 30** (Documents from the Zoological Institute Basel)

Hatching date: 26.05.2010, Viktor Mislin (Basel)

Parents: Female red, P061 M13

EG-Import documents: E 01159/10

Sex: presumably female

Tortoise 3: since 24.11.2013

Studbook entry: **No. 19** (Documents from the Zoological Institute Basel)

Hatching date: 25.03.2008, Viktor Mislin (Basel)

Parents: Female blue

EG-Import documents: E 00964/13

Sex: female

Tortoise 4: since 24.11.2013

Studbook entry: **No. 16** (Documents from the Zoological Institute Basel)

Hatching date: 26.02.2008, Viktor Mislin (Basel)

Parents: Female blue

EG-Import documents: E 00964/13

Sex: presumably female

Tortoise 5: since 29.11.2014

Studbook entry: **No. 2** (Documents from the Zoological Institute Basel)

Hatching date: 04.05.2015, Viktor Mislin (Basel)

Parents: Female green

EG-Import documents: E 0681/07

Sex: male

PROJEKT PARTICIPANT 2

Tortoise 1: since 26.11.2011 †: 25.08.2014

Studbook entry: **No. 31** (Documents from the Zoological Institute Basel)

Hatching date: 07.03.2011, Viktor Mislin (Basel)

Parents: Female white, P067 M7

EG-Import documents: E 01653/11

Sex: female

Tortoise 2: since 26.11.2011 †:16.08.2014

Studbook entry: **No. 33** (Documents from the Zoological Institute Basel)

Hatching date: 17.04.2011, Viktor Mislin (Basel)

Parents: Female green, P073 M7

EG-Import documents: E 01653/11

Sex: male

Tortoise 3: since 10.05.2013: †: 28.08.2014

Studbook entry: **No. 41** (Documents from the Zoological Institute Basel)

Hatching date: 27.04.2012, Viktor Mislin (Basel)

Parents: Female blue, male small

EG-Import documents: E 00203/13

Sex: male

Tortoise 4: since 10.05.2013

Studbook entry: **No. 42** (Documents from the Zoological Institute Basel)

Hatching date: 02.05.2012, Viktor Mislin (Basel)

Parents: Female green, male large

EG-Import documents: E00203/13

Sex: presumably female

Tortoise 5: since 10.05.2013

Studbook entry: **No. 22** (Documents from the Zoological Institute Basel)

Hatching date: 15.05.2008, Viktor Mislin (Basel)

Parents: Female green, male large

EG-Import documents: E 00474/13

Sex: presumable female

Tortoise 6: since 10.05.2013

Studbook entry: **No. 6** (Documents from the Zoological Institute Basel)

Hatching date: 27.04.2006, Viktor Mislin (Basel)

Parents: Female white, male small

EG-Import documents: E 00474/13

Sex: male

PROJEKT PARTICIPANT 3

Tortoise 1: since 03.05.2013

Studbook entry: **No. 37** (Documents from the Zoological Institute Basel)

Hatching date: 10.03.2012, Viktor Mislin (Basel)

Parents: P0081/M07, female green, male small, red dot 3rd scute

EG-Import documents: E00231/13

Sex: male

Tortoise 2: since 03.05.2013

Studbook entry: **No. 39** (Documents from the Zoological Institute Basel)

Hatching date: 15.04.2012, Viktor Mislin (Basel)

Parents: P0085/M07, female white, male small, yellow dot 1st scute

EG-Import documents: E00231/13

Sex: female

PROJEKT PARTICIPANT 4

Tortoise 1: since 01.11.2014

Studbook entry: **No. 38** (Documents from the Zoological Institute Basel)

Hatching date: 09.04.2012, Viktor Mislin (Basel)

Parents: Female green, male large P0086

EG-Import documents: E 00956/14

Sex: unknown

Tortoise 2: since 01.11.2014

Studbook entry: **No. 43** (Documents from the Zoological Institute Basel)

Hatching date: 07.03.2013, Viktor Mislin (Basel)

Parents: Female blue, male small P0096

EG-Import documents: E 00956/14

Sex: unknown

PROJEKT PARTICIPANT 5

Tortoise 1: since 30.10.2014

Studbook entry: **No. 36** (Documents from the Zoological Institute Basel)

Hatching date: 10.03.2012, Viktor Mislin (Basel)

Parents: Female blue, male small

EG-Import documents: E 000934/14

Sex: unknown

Tortoise 2: since 30.10.2014†: 17.05.2017

Studbook entry: **No. 44** (Documents from the Zoological Institute Basel)

Hatching date: 18.03.2013, Viktor Mislin (Basel)

Parents: Female green, male large

EG-Import documents: E 000934/14

Sex: male

Tortoise 3: since 30.11.2014 (taken over from a former project member)

Studbook entry: **No. 04** (Documents from the Zoological Institute Basel)

Hatching date: 15.08.2009, Viktor Mislin (Basel)

Parents: Female white, male small

EG-Import documents: E 0681/07

Sex: male

Tortoise 4: since 30.11.2014 (taken over from a former project member)

Studbook entry: **No. 09** (Documents from the Zoological Institute Basel)

Hatching date: 23.05.2006, Viktor Mislin (Basel)

Parents: Female blue, male small

EG-Import documents: E 000934/14

Sex: male

PROJEKT PARTICIPANT 6

Tortoise 1: since 15.08.2015 (taken over from a former project member)

Studbook entry: **No. 10** (Documents from the Zoological Institute Basel)

Hatching date: 24.05.2006, Viktor Mislin (Basel)

Parents: Female green, male large

EG-Import documents: 07CH067331

Sex: male

Tortoise 2: since 26.09.2015

Studbook entry: **No. 17** (Documents from the Zoological Institute Basel)

Hatching date: 26.02.2008, Viktor Mislin (Basel)

Parents: Female green, male large

EG-Import documents: E 00946/15

Sex: unknown

Tortoise 3: since 26.09.2015

Studbook entry: **No. 18** (Documents from the Zoological Institute Basel)

Hatching date: 04.03.2008, Viktor Mislin (Basel)

Parents: Female white, male small

EG-Import documents: E 00946/15

Sex: male

Tortoise 4: since 26.09.2015

Studbook entry: **Nr.21** (Documents from the Zoological Institute Basel)

Hatching date: 08.04.2008, Viktor Mislin (Basel)

Parents: Female white, male small

EG-Import documents: E 00946/15

Sex: male

Tortoise 5: since 26.09.2015

Studbook entry: **Nr.32** (Documents from the Zoological Institute Basel)

Hatching date: 18.03.2011, Viktor Mislin (Basel)

Parents: Female green, male large

EG-Import documents: E00946/15

Sex: Presumably female

PROJEKT PARTICIPANT 7

Tortoise 1: since 19.06.2010

Studbook entry: **No. 23** (Documents from the Zoological Institute Basel)

Hatching date: 25.05.2008, Viktor Mislin (Basel)

Parents: Female red, male large

EG-Import documents: CH

Sex: male

Tortoise 2: since 19.06.2010

Studbook entry: **No. 24** (Documents from the Zoological Institute Basel

Hatching date:	14.02.2009, Viktor Mislin (Basel)
Parents:	Female blue, male small
EG-Import documents:	CH
Sex:	male

ACCLIMATION OF FRESHLY IMPORTED WILD CAUGHT TORTOISES

In December 2000, 1.1 *Pyxis planicauda* were acquired from the pet trade. In March 2001 an additional group of 1.3 tortoises could be acquired. These were freshly imported wild caught tortoises. After only a few days the tortoises were observed feeding. However, initial fecal examinations revealed a strong infestation with roundworms as well as hexamites which required deworming using Panacur and treatment with metronidazole and Flagyl.

Treatment against roundworms and protozoa:

13.12.2000	First treatment of roundworm infestation (Panacur)
02.01.2001	Second treatment of roundworm infestation (Panacur)
16.01.2001	Treatment of protozoa (Metronidazole & Flagyl)
26.01.2001	Third treatment of roundworm infestation (Panacur)
26.04.2001	Fourth treatment of roundworm infestation (Panacur)
29.04.2001	Treatment of protozoa (Metronidazole and Flagyl)

In May 2001 the treatment was completed by a follow-up examination.

On 7th of October 2006 three more *Pyxis planicauda* (1.2) were taken in from another keeper.

DETAILED DESCRIPTION OF HUSBANDRY CONDITIONS

PROJECT LEADER VIKTOR MISLIN (SWITZERLAND)

Project manager Viktor Mislin keeps his tortoises in open topped indoor enclosures measuring approximately 0.7 m x 0.8 m. The substrate consists of a three to 15 centimeter thick layer of de-dusted hemp litter and unfertilized sowing soil. The enclosures are planted with pothos (*Epipremnum pinnatum*), *Tradescantia* spec., flaming Katy (*Kalanchoe blossfeldiana*), and chandelier plant (*Kalanchoe manginii*). Some of these plants (*Tradescantia* spec. and *K. manginii*) are also eaten by *P. planicauda*. All enclosures are furnished with logs and roots (Fig. 6). Plastic flower pots cut in half, and a leaf litter covering the substrate to approximately 50 %, provide sufficient retreat and hiding places.

Fig. 6 Enclosures for *Pyxis planicauda* at the facilities of project manager Viktor Mislin.

Initially all enclosures were equipped with ultrasonic humidifiers, a fluorescent tubes for general lighting (Sylvania Reptistar 30 W also promoting vitamin D3 production) and HQI lights (70 W). The fluorescent tubes are installed at a close distance above the ground. Underneath, the HQI, temperatures reach up to 45 °C. In the past, additional Osram Ultravitalux bulbs were used to increase the UV supply (MISLIN et al. 2010). However, since the HQI lights were replaced with modern metal halide lamps equipped with a build it UV component (Lucky Reptile Bright Sun UV Desert 70 W) in 2009 additional UV supply is not necessary any longer. Since the UV-B emission of the light bulbs decreases rapidly, bulbs are checked regularly using a solar meter and have to be replaced at the latest once a year. The technical equipment of the enclosures is shown in Figure 7. Each of these enclosures holds a group of 1.2 *P. planicauda*.

Fig. 7 Technical equipment used for the *Pyxis planicauda* enclosures of Viktor Mislin.

The average ambient daytime temperature within the terrarium room ranges between 29° and 30 °C in summer. During the night temperatures drop to 17 °C in winter and 25 °C in summer. In order to best replicate the climatic conditions of the species' native range, the operating times of the lights and the ultrasonic humidifier vary according to seasons. Seasons are simulated in reverse to the area of origin. Therefore, the dry season starts between November and December and ends between March and April while the simulated rainy season starts between April and May and ends between September and November.

Table 1: Seasonal operating times of ultrasonic humidifiers and lights.

Dry season (December – April)	Rainy season (May – November)
Fogging system 5 x ¼ h, 1 x ½ h Turned down to 2/3	Fogging system 5 x ¼ h, 1 x ½ h Full power, taking up approx. 5L/week
Mean daytime temperature 24 - 26 °C	Mean daytime temperature 26 - 32 °C
Humidity 50 – 70 %	Humidity up to 80 %
HQI lights – by the hour	HQI lights – operated up to 9 h per day

A water dish for bathing and drinking was not used during the acclimatization period to avoid infection with protozoa (MISLIN 2006, MISLIN AND HERZOG 2011). However, later all enclosures were equipped with shallow water dishes. Even though no permanent water is available in the habitat during the dry season (GIBSON AND BULEY 2004), water is offered all year round.

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NUTRITION

In captivity, *Pyxis planicauda* readily accepts meadow greenery such as dandelion (*Taraxacum officinale*) clover (*Trifolium* spec.), ribwort plantain (*Plantago lanceolata*), or vetch (*Vicia* spec.). Hibiscus leaves, vegetables such as zucchini, carrot, pumpkin, all lettuces with bitter substances (radicchio lettuce, arugula, endive, and romaine lettuce), mushrooms and fruits are also accepted. Mango, blackberries, and currants are occasionally offered. All lettuces, fruit- and vegetable-parts are enriched with fiber (cut hay or hay flowers including seeds) and grated cuttlefish bone. Every 14 days the food is sprinkled with Korvimin or Vitakalk powder. The extent to which invertebrates such as insects, or millipedes, worms, and snails contribute to the diet in nature is unknown (GIBSON AND BULEY 2004). However, mealworm beetle larvae (*Tenebrio molitor*) which were offered on an experimental basis were ignored.

BEHAVIOR

Just as in nature the activity of *Pyxis planicauda* in captivity is strongly affected by seasonal climate fluctuations. During the rainy season the tortoises are substantially more active than during the dry season. However, even in the rainy season, the tortoises remain in their retreats except for brief excursions for food intake and thermoregulation. During the rainy season the tortoises are bathed once or twice a month. During the dry season the tortoises estivate and remain at the same place for several weeks. During estivation they do not feed. To maintain a certain fluid balance, the tortoises are also bathed once per month during the dry season. The basic lighting (fluorescent tube) is operated from 8:00 am to 6:00 pm. The HQI radiator is switched on between 9:00 am and 4:30 pm. After 3:30 pm the tortoises are rarely observed outside their hiding places. Although reports about the observation of a combat fight between two males suggest that the species is at least temporarily territorial (BLOXAM pers. obs. in GIBSON AND BULEY 2004) no dominance behavior or territorial conflicts could be observed so far. *Pyxis planicauda* does not seem to be particularly aggressive, but a strong structuring of the enclosure with many hiding places is recommended.

PROJECT LEADER TOBIAS FRIZ (GERMANY)

On 18.10.2010 two hatchlings (No. 25 and 28) were transferred from Viktor Mislin to Tobias Friz. In 2015 five additional tortoises of different ages were transferred (No. 35, 40, 7, 8, and 12). The animals 7, 8, and 12 as well as 25, 28, 35, and 40 are kept together in indoor enclosures (open-topped glass tanks) measuring 1.0 m x 0.5 m which are fitted with metal halide lamps (Lucky Reptile Bright Sun UV Desert 70 W) and 39 W T5 daylight fluorescent tubes (Fig. 8). The operation times of the lighting system follows the protocol by MISLIN AND HERZOG (2011). The light bulbs are replaced on an annual basis. The ambient temperature rises to 24-30 °C during the day, while underneath the metal halide lamp up to 45 °C can be reached. At night the ambient temperature drops to 20-22 °C. To increase the humidity, the enclosures are moistened four times a day for 30 minutes each with rainwater using an ultrasonic humidifier (Lucky Reptile Super Fog).

A mixture of cocos humus and hemp litter (free from dust) serves as substrate, while stones, logs, roots, pieces of cork oak bark, clay pots cut in half, beech tree leaves, bamboo leaves and moss provide a rich structuring of the enclosure (Fig. 8). It is furnished with various robust plants, among them several thick-leaved Euphorbiaceae, turtle vine (*Callisia repens*), *Kalanchoe* spec., golden pothos (*Epipremnum aureum*), spiderwort (*Tradescantia* spec.), spider plant (*Chlorophytum comosum*), and non-toxic ornamental grasses and sedges (e.g.: *Carex* spec.) which promote the microclimate and serve as hiding and retreat sites. Depending on availability, meadow herbs such as dandelion (*Taraxacum officinale*) and ribwort plantain (*Plantago lanceolata*) are planted into the enclosures. Water is permanently offered in two places in the enclosure. To avoid reinfection with protozoa, these are cleaned every day.

While the sexes of hatchlings 25 and 28, hatched in 2009 and 2010 respectively, as well as those of hatchlings 35 and 40, hatched in 2012, cannot yet be determined, the sexes of the hatchlings No. 7 and 8 hatched in 2006 and 2007 are already clearly distinguishable (7, 8 = male, 12 = female).

Fig. 8 Structuring and technical equipment of the enclosures at the facilities of Tobias Friz. Detailed photographs of plants used.

NUTRITION

Leaves and blossoms of dandelion (*Taraxacum officinale*) and ribwort (*Plantago lanceolata*) are regularly offered. A dry flower mix containing mallow (*Malva* spec.), chamomile (*Matricaria chamomilla*), rose, cornflower (*Cyanus segetum*), hibiscus (*Hibiscus* spec.) and a dry herb mix containing high-quality organic meadow herbs such as dandelion (*T. officinale*), ribwort and broad-leaved ribwort and greater plantain (*Plantago lanceolata* and *P. major*), and clover (*Trifolium* spec.) are offered alternately. These dried herbs serve as a raw fiber supplement throughout the winter months. During the estivation period (Nov - March) the tortoises do not feed. The composition of the dried herb mix was quickly extended to contain all available plant species, which according to MINCH (2013) are suitable for Mediterranean tortoises. Lettuce like endive, novita lettuce, radicchio, chicory, green and red coral lettuce, and dandelion greens as well as vegetables such as cucumber, zucchini, eggplant, carrot, opuntia, and brown mushrooms from organic cultivation are readily accepted and selectively picked out of mixtures. Vegetables and lettuces were fed more frequently in the beginning, but only rarely and in smaller quantities in later years. Pellets (Sera Raffy P), which are occasionally offered during bathing, are also accepted while the tortoises ignore the turtle vine (*Callisia repens*) planted into the enclosure. As a dietary supplement, a pinch of a vitamin mixture (Korvimin ZVT) or carob gum is sprinkled over the food every seven to 14 days. In addition, cuttlefish bone is always available in the terrarium. Since 2013, the tortoises were transferred to 1.0 m x 1.0 m outdoor enclosures from July to the beginning of September. The enclosure is made from Douglas fir wood and protected from predators and unwanted human access by a framed chicken wire cover. In order to keep radiant heat longer and provide protection against rain etc., the enclosure is partly covered with twin-wall polycarbonate panels. However, the tortoises seem to avoid the covered area. Garden soil and soil collected from molehills is used as substrate for the outdoor pens.

One enclosure facing south is partly shaded by wild wine. In the morning and evening hours the enclosure is exposed to plenty of sunlight, while around noon the vines provide sufficient shade.

The base of the enclosure is planted to about two thirds with mint (*Mentha* spec.), poppy (*Papaver* spec.), blackberry (*Rubus* spec.), stonecrop (*Sedum* spec.), etc., providing a cool and humid retreat. In one third of the area, the vegetation is kept short to maintain some rather open, sunlit spots. While kept outside the tortoises were extremely agile and mobile, which could not be observed to this extent in the terrarium. Most of the times the tortoises stay in the intensively overgrown part of the enclosure but constantly switch between shade and the small sunny spots. Although the tortoises were frequently observed feeding, a weight loss was observed. Nevertheless outdoor husbandry of *Pyxis planicauda* is considered positive and recommended. From the beginning of September the weather is too unstable for outdoor husbandry and the tortoises are returned to the indoor enclosures.

BEHAVIOR

Initially, the younger hatchling only ate when there was no direct eye contact to the slightly older tortoise. Therefore food was offered in several places. When bathing both hatchlings together, the younger tortoise tried to escape indicating stress or intra-species aggressiveness. However, no such behavior was observed in subsequent years. From November to March, both tortoises estivate but hatchling 25 always retreats a little earlier than the about one year younger hatchling. During the estivation both turtles are bathed every two weeks.

PROJECT PARTICIPANT 1

On the 18th of October 2010 two juveniles (No. 26 and 30) were transferred from Viktor Mislin to project participant 1. On the 24th of November 2013 two more juveniles (No. 16 and 19) that had hatched in 2008 followed. Finally a fifth tortoise (No. 2, male, hatched in 2005) was handed over to project participant 1 on the 29th of November 2014. Initially, all tortoises were kept together in an enclosure measuring 1.5 m x 0.7 m. In 2015, however, the group was moved to a self-constructed wooden enclosure measuring 1.5 m x 1.0 m (Fig. 9). The technical equipment includes a T8 ReptiGlo fluorescent tube, two metal halide lamps (Lucky Reptile Bright Sun Desert 50 W and 70 W) as punctual heat sources as well as an ultrasonic humidifier (Lucky Reptile Super Fog). The enclosure is sprayed with a garden sprinkling system two to five times a day. Ambient temperatures vary between 22-25 °C during the day and decrease to 15-18 °C at night. Operation hours of the lamps and the humidifier vary according to the seasons. During the tortoises' main activity season the lighting system is switched on for up to 13 hours. To prepare for the dry rest, the lighting is gradually reduced and completely switched off in January. During the winter months, the humidifier is operated at 30-minute intervals between 09:00 am and 05:00 pm. Occasionally, lights and humidifiers are switched off to simulate bad weather. During the estivation, the enclosure is occasionally sprinkled with water. Even when inactive, the tortoises are bathed every 14 days for about 10 minutes and their weight is recorded.

Initially, a mixture of Floraton 3 and hemp litter was used as substrate. However, it started molding quickly at high humidity and was replaced by loamy substrate in 2012. The enclosure is equipped with a water dish for drinking and bathing and is structured with cork oak bark, willow wood bridges, grapevine wood, stones, and plants such as turtle vine (*Callisia repens*), Baobab tree (*Adansonia* spec.), *Kalanchoe* spec., Christ plant (*Euphorbia milii*), as well as specially acquired Malagasy plants for this purpose (including *Euphorbia geroldii*, *Euphorbia suzannae-marnierae*, *Pachypodium rosulatum*, and *Cyphostemma laza*).

Fig. 9 Self constructed enclosure made from wood at the facilities of project participant 1.

During the summer months in 2011, 2012, 2014, and 2015 the tortoises were moved into a cold frame enclosure located on the southwest facing terrace. The cold frame measures 1.5 m x 1.5 m and was placed in a wind-sheltered, semi-shaded spot to avoid overheating, and is sprinkled several times a day. The substrate used is a mixture of volcanic rock, gravel, and natural soil. The enclosure is planted with various kinds of stonecrops (*Sedum* spec.), aloe vera, cactus, *Kalanchoe* spec., Baobab trees (*Adansonia* spec.), and agaves and is structured with logs, stones, stone slabs, and roots. It is equipped with a shallow water dish and a food plate. While keeping *P. planicauda* seasonally outdoors has a positive effect on the tortoises' activity and is therefore generally recommended, the relocation also represents stress. In response, the tortoises are restless for several weeks and need a long time to get used to the new environment after the transfer. The same behavior could also be observed when returning them to the room indoor enclosures. The ideal solution is therefore a transportable enclosure that can be seasonally moved outdoors.

NUTRITION

Plants consumed by *P. planicauda* include turtle vine (*Callisia repens*), pumpkin, garden cress, iceberg lettuce, endive, lamb's lettuce, radicchio, carrot, mushrooms, chanterelles, herbs such as dandelion (*Taraxacum officinale*), and plantain (*Plantago* spec.) and, depending on availability, blossoms of hibiscus (*Hibiscus* spec.), rose, hollyhock (*Malva* spec.), common evening primrose (*Oenothera biennis*), and cucumber. Once a week the food is sprinkled with Korvimin ZVT.

BEHAVIOR

Only after the first estivation period, the tortoises began to establish preferences regarding their sleeping places and visited the feeding place as well as the shallow bathing dish independently. Especially in July and August the tortoises are very active, grow intensively and gain weight in the process. From mid-September, their activity decreases until they finally stop eating around October. After about three months of complete inactivity (January - March), the estivation period ends. Usually, the tortoises start feeding again in April. Project participant 1 observed one of the tortoises (No. 26) to feed on fresh feces of another tortoise (No. 30). Offspring no. 26 occasionally produces cracking sounds with its jaw. These may be warning sounds that are generated in the event of danger.

PROJECT PARTICIPANT 2

On the 26th of November 2011 two tortoises (No. 31 and 33) which had hatched in early 2011 were transferred from the project leader Viktor Mislin to project participant 2. On the 05th of October 2013 this group was supplemented with two additional juvenile tortoises (No. 41 and 42) that had hatched in 2012. Until 2015, all four tortoises were kept together in an open-topped enclosure measuring 1.2 m x 0.8 m. The enclosure was located in a greenhouse with a roof of twin-wall polycarbonate panels and was planted with stonecrop (*Sedum* spec.), turtle vine (*Callisia repens*), *Kalanchoe* spec., spiderwort (*Tradescantia* spec.), bromeliads, various aloe species, and some Malagasy plants (Fig. 10). A mixture of sand, loam, and hemp litter was used as substrate and stone slabs, cork oak bark, and rosewood were used for structuring. The enclosure was fitted with a Lucky Reptile Bright Sun Desert 70 W as heat source and a humidifier (EURO Zoo Fogmaster). A shallow water dish and a food plate were permanently available.

Fig. 10 Technical equipment and structuring of the old enclosures at the facility of project participant 2.

Since the tortoises no. 31, 33, and 41 died in 2014, the remaining tortoise (No. 42) has been kept in an enclosure measuring 1.0 m x 0.6 m which can be transported to the greenhouse in summer. Two additional tortoises (No. 6 and 22) which hatched in 2006 and 2008 respectively, were taken in on the 05th of October 2013. As those were considerable larger, they were kept separately in an indoor enclosure (winter) but were moved to a bigger enclosure (1.2 m x 0.8 m) with access to an outdoor pen in summer. Since 2016, all three tortoises are kept in an enclosure located in the green house. This enclosure provides a separate section for the smaller tortoise No. 41 (Fig. 11). The technical equipment and structuring correspond to the original enclosure. A mixture of sand, terrarium soil, cork litter, and volcanic rock is used as substrate.

Fig. 11 Technical equipment and structuring of the new enclosures at the facility of project participant 2.

Due to the south/southwest orientation of the green house and the exposure to direct sunlight, ambient temperatures rise up to 37 °C in summer (May - October). The metal halide lamp is operated from 08:00 am to 02:00 pm and locally provides temperatures of up to 45 °C. At night, temperatures do not drop below 20 °C. At first, the ultrasonic humidifier was switched on every three hours for 30 minutes between 09:00 am and 06:00 pm, but the frequency was increased to every two hours in subsequent years. In addition, the enclosure is sprayed manually in the morning and evening hours. During the activity period the tortoises were bathed in lukewarm water for ten minutes every 14 days. While bathing, they usually also drink. Bathing significantly increases the tortoises' activity and encourages them to feed. Switching on the humidifier after subjecting the tortoises to a bath further encourages food intake. During the estivation, the metal halide lamp is only switched on for two hours between 09:00 am and noon, reaching 40 °C right underneath. Ambient temperatures reach 22 °C during the day but drop to 15 °C at night. During the estivation the enclosure is moistened in the morning and in the evening to maintain a humidity of about 70 %. The Malagasy plants are deciduous, shedding their leaves during the dry season. Initially, the tortoises were also bathed weekly during the estivation but since they always returned to their resting sites quickly and food intake was only observed in very rare cases, bathing during the estivation period was omitted in recent years.

NUTRITION

Meadow herbs such as dandelion (*Taraxacum* spec.), ribword and greater plantain (*Plantago lanceolata* and *P. major*), chickweed (*Stellaria media*), clover (*Trifolium* spec.), white clover (*Trifolium repens*), leaves of strawberries (*Fragaria* spec.), daisies (*Bellis perennis*), turtle vine (*Callisia repens*), lamb's lettuce, iceberg lettuce, romana lettuce, carrot, courgette, raspberry, cucumber, oyster mushrooms (*Pleurotus ostreatus*), mushrooms and flowers of hibiscus (*Hibiscus* spec.), rose, apple, cherry, and hollyhock (*Malva* spec.) are regularly offered and eaten. Every two weeks the food is dusted with a mineral powder and carob bean gum. In addition, ground cuttlefish bone and MicroCalcit are offered.

BEHAVIOR

Increased humidity, for example by switching on the ultrasonic humidifier, increases the tortoises' activity and the willingness to eat considerably.

MISCELLANEOUS

After a weight loss was determined, hatchling no. 33 died on the 16th of August 2014. After consultation with the project management, the remaining tortoises (No. 31, 41 and 42) were handed over in on the 18th of August 2014 to an experienced veterinarian reptile specialist for examination and treatment. The deceased tortoise was sent to the laboratory of EXOMED (head Dr. Mutschmann, 10266 Berlin) for autopsy and microbiological diagnostics. Despite artificial nutrition and infusions tortoise no. 31 died on the 25th of August 2014 followed by tortoise 41 three days later. A postmortem examination was performed on both tortoises. The remaining tortoise (no. 42) was treated in Leipzig and released on the 09th of September 2014.

PROJECT PARTICIPANT 3

On the 03rd of May 2013 two juveniles (No. 37 and 39) were transferred to project participant 3. So far, both tortoises are kept together in an open-topped glass terrarium measuring 1.0 m x 0.5 m x 0.6 m, but will be moved to a larger enclosure in the near future. The substrate consists of sand and soil mixed with hemp litter. In one spot, more solid rock gravel from Mannheim was added. The terrarium is structured with roots, stones, and alluvial wood, and is equipped with a shallow water dish as well as a stone tile on which food is offered. The terrarium is planted with *Sedum* spec., *Kalanchoe* spec., *Tradescantia* spec., *Aloe* spec., and a ponytail palm (*Beaucarnea recurvata*) and fitted with an ultrasonic humidifier (Trixi), a metal halide lamp (Lucky Reptile Bright Sun Desert 50 W) as heat and UV source, as well as a T8 fluorescent tube (Arcadia Reptile 30 W) for basic lighting. In addition, a halogen spot (35 W), a thermometer and a hygrometer are mounted. During the estivation (December - April) the basic lighting is operated between 07:00 am and 04:00 pm, while the heat sources are only switched on from 08:00 am to 03:00 pm. Via the balcony door the enclosure is exposed to natural daylight. During the day, ambient temperatures reach 23 °C, while right underneath the metal halide lamp temperatures of up to 35 °C can be achieved. At night, temperatures drop to 20 °C. The humidifier is operated five times a day for 30 minutes between 07:00 am and 05:00 pm. During the dry season, the tortoises are bathed and weighed every 14 days. After bathing, they immediately return to the same retreat as before (Fig. 12). During estivation the tortoises do not feed.

Fig. 12 Resting places of *Pyxis planicauda* during the simulated dry rest at the facility of project participant 3.

During the rainy season (May - November) the basic lighting is operated from 06:00 am to 05:00 pm, while the heat sources are switched on from 07:00 am to 05:00 pm. Ambient temperatures range from 23 °C to 28 °C. Underneath the metal halide lamp up to 40 °C are reached. The ultrasonic humidifier operates six times a day for 30 minutes between 07:00 am and 05:00 pm. During this time, the tortoises show increased activity (Fig. 13) often drink water that collects on the ground, and are encouraged to feed. During the activity period, the tortoises are bathed and weighed every two weeks.

Fig. 13 An increased activity of *Pyxis planicauda* can be observed during operation of the humidifier.

NUTRITION

Meadow herbs such as ribword and greater plantain (*Plantago lanceolata* and *P. major*), clover (*Trifolium* spec.), dandelion (*Taraxacum officinale*), and chickweed (*Stellaria media*), as well as blossoms of bellflower (*Campanula* spec.), hibiscus (*Hibiscus* spec.), evening primrose (*Oenothera biennis*), mallow (*Malva* spec.), and roses are eaten. Especially at the beginning of the activity period, a preference for fungi such as oyster mushrooms (*Pleurotus ostreatus*), mushrooms, and porcini (*Boletus edulis*) can be observed. Zucchini, carrots and lettuce are rarely offered. In order to increase the fiber and crude fiber content, the food is regularly sprinkled with a dry herb mixture. Cuttlefish bone is permanently available for calcium supply. Occasionally dried freshwater shrimps (*Gammarus* spec.) are offered.

PROJECT PARTICIPANT 4

On the 1st of November 2014 the tortoises no. 38 and 43 were transferred to project participant 4. The tortoises were initially maintained in an open-topped enclosure measuring 1.0 m x 0.54 m, but were recently moved to a larger facility (1.35 m x 0.66 m) with deeper substrate (Fig. 15). This new enclosure is located in the turtle room where the ambient temperature ranges from 34 °C (day) to 20 °C (night). The room is equipped with a humidifier maintaining a constant humidity level of 60 to 90 %. Depending on the season, the enclosure is manually sprinkled between once a week and several times a day. A fluorescent tube (Lucky Reptile Daylight Sun T5 daylight 54 W) provides basic lighting. In addition, a metal halide lamp with a build in UV component (Lucky Reptile Bright Sun UV Desert 70 W) is installed as a heat source under which up to 45 °C can be achieved. Food is offered on a glazed stone tile and a shallow water dish, which is cleaned daily, is permanently available. The substrate is about eight centimeters thick and consists of a mixture of hemp litter, unfertilized sowing soil and river sand. The enclosure is planted with golden pothos (*Epipremnum aureum*), a Malagasy palm (*Pachypodium* spec.), snake plant (*Sansevieria spec*.), and devil's backbone (*Kalanchoe daigremontiana*) as well as *Crassula* spec. and structured with pieces of bark. The devil's backbone as well as its seedlings is also eaten by the tortoises.

Fig. 14 Enclosure to rear *Pyxis planicauda* hatchlings.

NUTRITION

Since the devil's backbone (*K. daigremontiana*) was planted into the enclosure, both tortoises feed largely on the leaves and seedlings of this plant (Fig. 15). In winter, organically grown dried herb mixtures containing mallow blossoms (*Malva* spec.), chamomile (*Matricaria chamomilla*), rose and hibiscus leaves (*Hibiscus* spec.), dandelion (*Taraxacum officinale*), clover (*Trifolium* spec.), and plantain (*Plantago* spec.), are also served. Occasionally radicchio, lamb's lettuce, mushrooms, and chicory are sprinkled with dried herbs. Once a week the offered greens are dusted with Korvimin ZVT and ground cuttlefish bone. In addition, cuttlefish bone is permanently available in the enclosure. During the dry season, grasses and wild herbs collected on the project participant's property are offered.

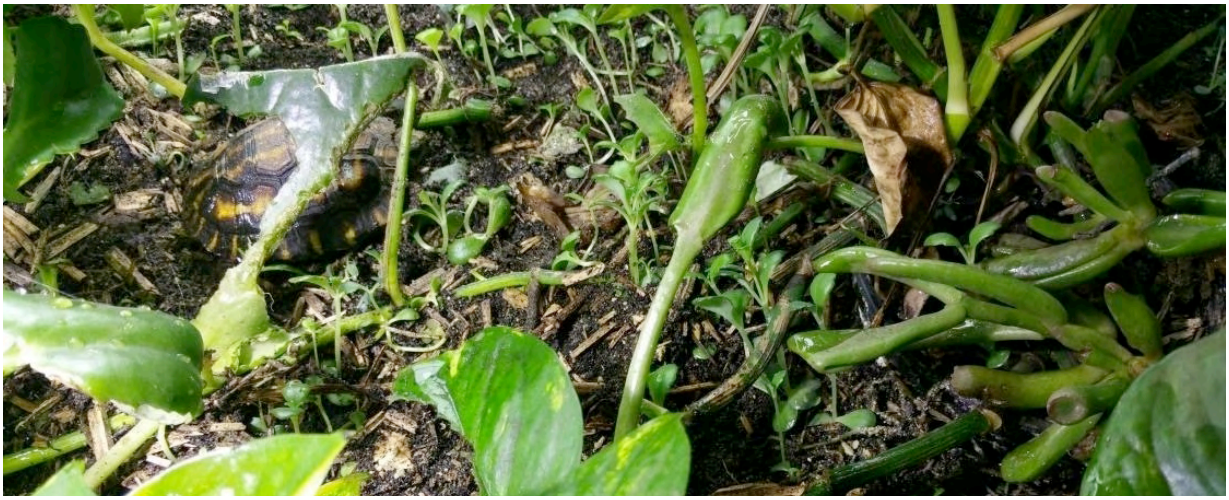

Fig. 15 *Pyxis planicauda* enclosure with seedlings of devil's backbone.

BEHAVIOR

Activity is highest in the morning hours. While reared together, the young tortoises appear to avoid each other which might indicate some sort of intraspecific stress. Therefore, it is considered to rear them individually in order to detect a possible change in behavior.

PROJECT PARTICIPANT 5

On the 30th of October 2014 two juveniles (No. 36 and 44) that had hatched in 2012 and 2013 respectively were transferred from Viktor Mislin to project participant 5. One month later, (30.11.2014) two additional tortoises, hatched in 2005 and 2006 were transferred from a former project participant. Until June/July 2016 all tortoises were kept together in an open-topped enclosure (1.55 m x 0.90 m) which was subdivided into two sections, one for the younger and much smaller tortoises and the other for the older ones. The enclosure was constructed with Forex panels and a glass front held together by an aluminum frame. In the meantime, the tortoises have been moved into a 1.8 m^2 green house (Beckmann) covered with twin-wall polycarbonate panels (Alltop). The substrate consists of loamy soil mixed with sand. The enclosure is planted with golden pothos (*Epipremnum aureum*), spiderwort (*Tradescantia* spec.), spider plant (*Chlorophytum comosum*), royal poinciana (*Delonyx* spec.), three Malagasy Euphorbia species, and various wild herbs. Stones, grapevine wood, and cork oak bark are used for structuring. The food is served on a stone tile. A shallow water dish is permanently available (Fig. 16). A metal halide lamp (Lucky Reptile Bright Sun UV Desert 70 W) is installed as a local heat source. Thanks to the twin-wall polycarbonate panels, the fluorescent tube is no longer necessary. Ambient temperature reaches up to 35 °C, in winter and 40 °C in summer, while a fan heater (Green-Phoenix) maintains a minimum temperature of 28 degrees all year round. While the old enclosure was equipped with a humidifier, the soil is now only moistened in the mornings and evenings of the rainy season.

Fig. 16 New enclosure to rear *Pyxis planicauda* at the facilities of project participant 5.

NUTRITION

While dried herbs, lettuces, and mushrooms were initially spurned, the tortoises did feed on *Kalanchoe*, *Plantago lanceolata* and *P. major*, vetch (*Vicia* spec.), clover (*Trifolium* spec.), and common chicory (*Cichorium intybus*) planted into the enclosure. Blossoms of hibiscus (*Hibiscus* spec.), hollyhock (*Malva* spec.) the evening primrose (*Oenothera biennis*), opuntia, lettuces such as endive, Romaine lettuce, arugula, lamb's lettuce, radicchio, and vegetables such as carrot, courgette, mushrooms, chanterelles (*Cantharellus cibarius*), and oyster mushrooms (*Pleurotus ostreatus*) are also eaten (Fig. 17). Lettuces are sprinkled with a dried herb mix (Testudo Baby, Agrobs) and occasionally (Raffy P) to increase the fiber content. Once a week, the food is dusted with a mineral supplement (Herpetal Mineral D3) while cuttlefish bone is permanently available.

Fig. 17 Juveniles of *Pyxis planicauda* feeding.

BEHAVIOR

On the 30th of January 2017 one of the juvenile tortoises (no. 44) died surprisingly. The autopsy report revealed pneumonia due to a mycoplasma infection. It can therefore be assumed that the remaining tortoises are also infected with mycoplasma and are therefore no longer available for exchange within the project.

PROJECT PARTICIPANT 6

On the 15th of August 2015 hatchling no. 10 was transferred from a former project participant to project participant 6. On the 26st of September 2015 four more tortoises (No. 17, 18, 21, and 32) were transferred from Viktor Mislin to project participant 6. After initial keeping in an indoor enclosure with a seasonal transfer to an outdoor pen in summer, the tortoises are now kept in an indoor enclosure made from phenolic resin coated plywood (2.55 m x 1.25 m x 0.65 m) that provides access to an adjacent outdoor enclosure (2.30 m x 1.35 m x 0.55 m). The outdoor enclosure is covered with chicken wire to protect the tortoises from predators and unwanted human access (Fig. 18). The indoor enclosure is equipped with an ultrasonic humidifier (Dragon Magic Fog) as well as two spotlights, locally providing heat and UV (Lucky Reptile Bright Sun Dessert 70 W, Power Sun 100 W). A fluorescent tube is used for basic lighting. Initially, a mixture of garden soil (25 %), hemp litter, and 25 % coco fiber was used as substrate. Meanwhile, a garden soil, pine bark mixture is used. The enclosure is planted with hibiscus (*Hibiscus* spec.), spiderwort (*Tradescantia* spec.), spider plant (*Chlorophytum comosum*), flaming Katy (*Kalanchoe blossfeldiana*), *Scindapsus* spec., and turtle vine (*Callisia repens*).

Fig. 18 Indoor enclosure with access to the outdoor pen.

Dandelion (*Taraxacum* spec.), lady's mantle (*Alchemilla mollis*), ribwort and greater plantain (*Plantago lanceolata* and *P. major*), chickweed (*Stellaria media*), clover (*Trifolium* spec.), white clover (*Trifolium repens*) as well as daisies (*Bellis perennis*), bamboo, and various succulents are planted into the outdoor enclosure. The indoor as well as the outdoor enclosures are equipped with large water dishes and structured with stones and cork oak bark. Food is offered on a slate plate. The outdoor enclosure is equipped with a small shelter that provides a heat source (Dragon Breeding Light 120 W).

NUTRITION

Mushrooms (white and brown mushrooms) and oyster mushrooms (*Pleurotus ostreatus*) are regularly offered. Wild herbs such as ribwort plantain (*Plantago lanceolata*), clover (*Trifolium* spec.), chickweed (*Stellaria media*), and ground elder (*Aegopodium podagraria*) are also eaten. In addition, blossoms of dandelion (*Taraxacum officinale*), hollyhock (*Malva* spec.), hibiscus (*Hibiscus* spec.), clover (*Trifolium* spec.), rose, poppy (*Papaver* spec.), common evening primrose (*Oenothera biennis*), common chicory (*Cichorium intybus*), and nasturtium (*Tropaeolum* spec.) are readily consumed. Rarely, vegetables such as courgettes and grated carrots, or lettuces such as romaine lettuce, radicchio, lamb's lettuce, arugula, and endive are offered. In addition, the species of *Kalanchoe*, *Tradescantia*, *Scindapsus*, *Hibiscus* as well as *Callisia repens* which are planted directly into the enclosure are also eaten. For calcium supply cuttlefish bone is permanently available. Occasionally commercial turtle food pellets (Sera Raffy P) dusted with grated cuttlefish bone is offered.

PROJECT PARTICIPANT 7

On the 19th of June 2010 two juvenile tortoises (no. 23 and 24) were transferred to project participant 7 by Viktor Mislin. During the summer months (June - September) the tortoises are kept in an outdoor enclosure (cold frame) with a lid made from twin-wall polycarbonate panels (Alltop). In order to maintain adequate ambient temperatures even on cold and overcast days, a radiator and a heating mat are installed on the inside of the lid just above the sleeping area. From September on, the tortoises are kept in an indoor enclosure.

NUTRITION

Mushrooms, courgettes, and oyster mushrooms (*Pleurotus ostreatus*) are eaten with pleasure, porcini mushrooms (*Boletus* spec.) however were spurned so far.

GENERAL RECOMMENDATIONS FOR CAPTIVE HUSBANDRY

In the EHAP project, both open-topped and closed enclosures such as glass terrariums, wooden terrariums and enclosures made of Forex or phenolic resin coated plywood have proven to be suitable for keeping and rearing *Pyxis planicauda*. Depending on size of the tortoises and the size of the group, enclosures with an area of 0.7 m x 0.8 m are suitable to house 1.2 adults; 1.0 m x 0.5 m and 1.2 m x 0.5 m to care for two juvenile tortoises; 2.0 m x 1.0 m to keep a group of 1.4; 1.2 m x 0.8 m or 1.55 m x 0.90 m to rear four juveniles or two sub adults and an enclosure measuring 2.55 m x 1.25 m to rear five juvenile tortoises. Mixtures of hemp litter and unfertilized sowing soil, loamy soil, river sand soil mixtures, a sand-loam mixture with hemp litter, as well as a mixture of coco humus and dust free hemp litter or a garden soil and pine bark are used as substrate. A mixture of Floraton 3 and hemp litter is no longer recommended as it was found to mold quickly in humid environments. The substrate layer used by the project participants is between eight and 15 centimeters thick. The substrate is partly covered with a layer of leaves (e.g. beech or bamboo). The enclosures are structured with stones, roots, logs, cork oak bark, willow wood bridges, alluvial wood, branches, rosewood, grapevine wood, and moss. In addition, clay or plastic flower pots cut in half serve as hiding places. Food is offered on a natural stone slab, slate slab, or tile. Although, during the dry season permanent water is likely not available in the species habitat (GIBSON AND BULEY 2004), water is offered all year round in shallow dishes. To avoid reinfection with protozoa, these dishes are cleaned and refilled daily.

According to the conditions in the natural habitat, the enclosures and terrariums of all project members are furnished with a variety of plants. These include the following Malagasy plants specially obtained for this purpose; thick-leaved euphorbia species such as Christ plant (*Euphorbia milii*), *Euphorbia geroldii*, *Euphorbia suzannae-marnierae*, Elephant's Foot (*Pachypodium rosulatum*), and other *Pachypodium* species, *Cyphostemma laza*, stonecrop (*Sedum* spec.), Baobab tree species (*Adansonia* spec.), pony tail (*Beaucarnea recurvata*), snake plant (*Sansevieria* spec.), *Crassula* spec., royal poinciana (*Delonyx* spec.), and the chandelier

plant (*Kalanchoe manginii*). In addition, flaming Katy (*Kalanchoe blossfeldiana*), devil's backbone (*Kalanchoe daigremontiana*), turtle vine (*Callisia repens*), golden pothos (*Epipremnum aureum*), pothos (*Epipremnum pinnatum*), *Scindapsus* spec., spiderwort (*Tradescantia* spec.), spider plant (*Chlorophytum comosum*), various bromeliads and aloe species, cacti and non-toxic grasses and sedges (e.g.: *Carex* spec.) have been cultivated successfully within the enclosures. These plants significantly promote the microclimate and provide hiding places and retreats for *P. planicauda*. Some of these plants (*Tradescantia* spec. and *K. manginii*) and in some cases other species of *Kalanchoe*, *Scindapsus*, and *Hibiscus* as well as the turtle vine (*Callisia repens*) are eaten by *P. planicauda*. Depending on availability, herbs such as dandelion (*Taraxacum officinale*), and ribwort and greater plantain (*Plantago lanceolata*, *P. major*) are directly planted into the enclosures and readily eaten by the resident tortoises.

In order to replicate the climate experienced by *P. planicauda* in its native range, all enclosures are fitted with ultrasonic humidifiers (e.g.: Lucky Reptile SuperFog, EURO Zoo Fogmaster, Dragon Magic Fog, Fa. Trixi),daylight fluorescent tubes (e.g: T8 ReptiGlo, Sylvania Reptistar 30 W, Light Strip T 5 Power 39 W, Biovital tube from Narva 80 W or Arcadia Reptile 30 W) for general lighting, and metal halide lamps with a build in UV component providing local basking spots and promoting vitamin D3 production (Lucky Reptile Bright Sun Desert 50 or 70 W). Since the UV emission decreases during time, the light bulbs are replaced every year. Lamps and humidifiers are seasonally operated according to seasonal climate fluctuations. Because conditions such as enclosure size and location affect ambient temperature and humidity, operation times vary. For an example of seasonal operating times, see Table 1. A thermometer and a hygrometer are mounted to monitor the climatic conditions within the enclosures. During the simulated rainy season between May and November, a constant humidity of about 80 % is maintained by operating the ultrasonic humidifier at appropriate intervals. In addition, the enclosures are regularly sprinkled by hand. During this time, average ambient temperatures between 22-33 °C are maintained, but may rise up to 37 °C during the day. Underneath the metal halide lamp, up to 45 °C should be reached. At night, temperatures should not drop below 20 °C. During the rainy season, the basic lighting is operated for about twelve hours, while the metal halide lamps are switched on for about ten hours.

To simulate seasonal fluctuations and prepare the tortoises for estivation, the operation hours of the lighting is gradually reduced. During the dry season between December and April, a constant humidity of 50-80 % is maintained by adjusting the intervals of the ultrasonic humidifier. During this time, the average ambient temperatures vary between 24-26°C during the day, and drop to around 15-20°C at night while operation times of the metal halide lamps are reduced to five hours. Occasionally, lights and humidifiers are turned off to replicate overcast conditions and cold days.

In summer (July - September) some project members keep *P. planicauda* in wind-protected, south-facing outdoor enclosures. Both, cold frames made of twin-wall polycarbonate panels, and enclosures made from Douglas fir wood, which are partly covered twin-wall polycarbonate panels and secured with framed chicken wire to prevent predator access, have been used successfully. Some keepers offer a shelter fitted with a heating mat or a Dragon Breeding Light 120 W as a heat source. To avoid overheating, the enclosures should be partially shaded. Garden soil, molehill soil, and mixtures of volcanic rock, gravel, and natural soil are used as substrates. The outdoor enclosures are structured and planted similarly to the indoor enclosures, but with a larger variety of native herbs. Plants cultivated successfully include: lady's mantle (*Alchemilla mollis*), ribwort and greater plantain (*Plantago lanceolata* and *P. major*), chickweed (*Stellaria media*), clover (*Trifolium* spec.), white clover (*Trifolium repens*), and daisies (*Bellis perennis*), mint (*Mentha* spec.), poppy (*Papaver* spec.), blackberry (*Rubus* spec.), stonecrop (*Sedum* spec.), *aloe vera*, cacti, *Kalanchoe* spec., Baobab (*Adansonia* spec.), and agave species. These plants provide shelter and contribute to a cool and humid microclimate. The vegetation should be kept short in approximately one third of the area to create open, sunny spots for basking. Just like the indoor enclosures, the outdoor facilities are equipped with a shallow water dish. While keeping *P. planicauda* seasonally outdoors undeniably has a positive effect on the activity of the tortoises and is therefore generally recommended, relocating the tortoises is quite stressful. In response to translocation, tortoises were restless and needed a long time to get used to the new environment. The same behavior was observed when returning the tortoises to the indoor enclosures. Therefore, a transportable enclosure that can seasonally be moved outdoors is recommended.

Within the EHAP project the following herbs and blossoms were consumed by *P. planicauda*: dandelion (*T. officinale*), clover (*Trifolium* spec.), white clover (*T. repens*), ribwort and greater plantain (*P. lanceolata*, *P. major*), common chicory (*Cichorium intybus*), chickweed (*Stellaria media*), vetch (*Vicia* spec.), daisy (*Bellis perennis*), poppy (*Papaver* spec.), common evening primrose (*Oenothera biennis*), nasturtium (*Tropaeolum* spec.), bellflower (*Campanula* spec.), chamomile (*Matricaria chamomilla*), ground elder (*Aegopodium podagraria*), cornflowers (*Cyanus segetum*), leaves of strawberries (*Fragaria* spec.), hollyhock (*Malva* spec.), hibiscus (*Hibiscus* spec.) and fruit trees such as apple and cherry, as well as rose trees. Dried flowers and dried herb mixtures serve as raw fiber supplement especially during the winter months. Lettuces such as chicory, red and green batavia lettuce, endive, romaine lettuce, novita lettuce, radicchio lettuce, oak leaf lettuce, iceberg lettuce, lamb's lettuce, and arugula are readily consumed. Vegetables such as zucchini, garden cress, carrot, cucumber, eggplant, pumpkin, opuntia, white and brown mushrooms, chanterelles (*Cantharellus cibarius*), oyster mushrooms (*Pleurotus ostreatus*), and porcini (*Boletus edulis*) are also eaten. At the beginning of the activity period the tortoises show a preference for fungi. Vegetables and lettuces were fed more frequently in the beginning, but were reduced in later years. Now they are only offered rarely and in small quantities during the transitional period. Fruits such as mango, blackberries, currants, and raspberries are occasionally offered. To increase the fiber content, all lettuces, fruits and vegetables are supplemented with commercial mixed dry herbs (e.g. Testudo Baby, Agrobs) or mixed dry hay flowers with seeds, and grated cuttlefish bone. In addition, the food is supplemented with a pinch of a vitamin supplement (e.g. Korvimin ZVT, Vitakalk powder, MicroCalcit or Herpetal Mineral D3) every seven to 14 days. As a source of calcium, cuttlefish bone is permanently available in the enclosures. Some keepers have occasionally offered commercial turtle pellets (Sera Raffy P) in small quantities during bathing. The extent to which invertebrates such as insects, larvae, millipedes, worms, and snails may enrich the species diet in nature is not known (GIBSON AND BULEY 2004). However, freshly skinned mealworm beetle larvae (*Tenebrio molitor*), which were offered on an experimental basis, were not eaten. Also the woodlice inhabiting the enclosures are spurned.

However, dried freshwater shrimps (*Gammarus* spec.) occasionally offered by one keeper were eaten. In preparation for the estivation, the food is gradually supplemented with more grasses and wild herbs such as dandelion and plantain. During the estivation the tortoises do not feed.

When moved into a new environment *P. planicauda* needs a long time to establish fixed sleeping places and to independently approach the shallow water dish or the feeding place. This corresponds to observations from nature suggesting a much localized lifestyle. As in nature, the seasonal activity of *P. planicauda* is strongly influenced by seasonal climate fluctuations. During the simulated rainy season (especially in July and August) the tortoises' activity significantly increases and individuals grow and gain weight. Nevertheless, *P. planicauda* exhibits an extremely secluded lifestyle and even during the main activity season, leaves its shelter only to search for food and to bask. Activity is highest during the early morning hours. During the dry season *P. planicauda* estivates for two to three months (December to January). To regulate the fluid balance, the tortoises are bathed once a month, even during estivation. Afterwards, they quickly retreat to their preferred hiding place. During the rainy season, bathing as well as increased humidity e.g. by operating the humidifier, significantly increases the tortoises' activity and encourages them to eat. Although reports on combat between two male tortoises suggests that the species is at least seasonally territorial (GIBSON AND BULEY 2004), no dominance behavior or territorial conflicts between juvenile or adult tortoises was observed within the EHAP project so far.

This chapter was recently published with Sacalia, the professional journal of the International Tortoise Association (ISV).

Mislin, V., Engl, K., Friz, T., Lessing, J., Pawlitzki, M., Petersen, T., Sandmeier, P., Scheffel, R., Trentmann, C., Ihlow, F. (2018). Empfehlungen zur Haltung der Madagassischen Flachrücken-Spinnenschildkröte *Pyxis planicauda* – Erfahrungen aus dem EHAP Projekt. Sacalia, 16(4): 12-21.

MATING

All adult *P. planicauda* kept by the project leader Viktor Mislin are permanently kept in mixed groups of either 1.1 or 1.2. Therefore, mating is possible all year round without purposefully introducing individuals. Mating takes place throughout the activity phase but is more frequent at the beginning of the rainy season. Moistening the enclosures encourages the tortoises to mate. Although mating can take place at any time of day, most mating attempts were observed in the early morning hours.

During the rainy season, males actively search for the females and try to mount. Receptive females remain motionless while unreceptive females retreat into a hiding place. No aggressive behavior such as ramming or biting was observed preceding or during mating. Mating (Fig. 19) lasts about 15 minutes and often there are several matings per day.

Fig. 19 Breeding pair of *Pyxis planicauda* mating.

EGG DEPOSITION

In captivity, a single egg is laid two to four times a year, with the first oviposition taking place around the end of August. Immediately before egg deposition, a females' readiness for nesting can be detected by an increased mobility of the plastron. Usually the females select a spot inside of one of the retreat sites for nesting. The nest cavity measures approximately five centimeters in diameter and is six to eight centimeters deep. Regular moistening during digging prevents the substrate from slipping back into the nest cavity. The egg deposition takes about 30 minutes. Usually, the females remain at the nesting site for a few days and do not take up any food.

INCUBATION

A total of 148 eggs have been deposited since 2003. Out of these, 55 have hatched to date (37 %). The egg development lasted between 187 and 239 days.

In 2001 and 2002, eight eggs were incubated in a laboratory incubator at a constant daytime temperature of 31.5 °C and a night set-back to 25.5 °C at night. A humidity of 85-90 % was maintained throughout the incubation period. Although the eggs were fertilized and initially developed, they died during incubation.

While cleaning an enclosure in June 2003, a fertile egg was encountered which had probably been laid the previous September or October. Since other keepers had already incubated eggs in the enclosure successfully (KÖNIG pers. comm.), the egg was marked and subsequently returned to the same spot within the enclosure. The substrate was moistened regularly until a hatchling (No. 1) with a bodyweight of 14.1 g finally emerged on the 18th of September 2003.

Due to this positive experience, ten eggs laid in 2003 were left within the enclosure. Unfortunately, these did not develop.

In 2004 a total of eleven eggs were laid by different females. All eggs were placed into moist vermiculite (coarse, 3-6mm). Mixing 400 ml vermiculite with 300 ml of water provides sufficient moisture that also raises the humidity within the incubator to 80-90 %. If necessary, the substrate is moistened repeatedly during the incubation. All eggs were initially incubated for four weeks at 21 °C, then cooled down to 15.5 °C for four weeks and again incubated at 21 °C for four weeks. Then temperature was gradually increased to 26.6 °C and maintained until hatching. Temperatures were always adjusted gradually over the course of several days. Between April and August 2005 three hatchlings emerged. Subjecting the eggs to a diapause appears to be indispensable for successful egg development. Using incubation conditions that replicate the natural environment as closely as possible, incubation takes between seven months and one year (MISLIN AND EBERLING 2009). Since 2008, incubation temperatures have been monitored and recorded at ten-minute intervals using temperature dataloggers (HOBO pendant) (see App. 5).

SUCCESSFUL INCUBATION METHODS (2004 – 2017)

Between 2004 and 2017, nine different incubation methods were tested. Across all methods a night set-back and a constant humidity of 85-90 % was used. The incubation consists of four phases, the first three of which were identical across all tested methods while the final phase was variable. Across all methods the eggs were first incubated for one month at 21.0 °C, then gradually cooled down to 15.5 °C for another month before slowly raising temperatures again to 21.0 °C. Then the incubation temperature was slowly raised and maintained until hatching. The maximum temperature over the last phase differs between the tested methods (see Table 2). The most successful incubation methods were M07 with a maximum daytime temperature of 27.7 °C and a night-time set-back to 24.7 °C (incubation period 112-125 days), M13 with 30 °C during the day and a set-back to 25 °C (incubation period 190-225 days), and M14 with a maximum day-time temperature of 31 °C and a night-time set-back to 26.5 °C (incubation period 196-206 days; Fig. 20). Using these methods a total of 41 of 73 eggs hatched (hatching rate: 56%; M07: 15 of 20 eggs = 75 %; M13: 19 of 39 eggs = 49 %; M14: 7 of 14 eggs = 50 %; Fig. 21). The remaining six methods tested were less successful. Combining all six, only 14 out of 36 eggs (39 %) hatched.

Incubation method	T [°C] max. last phase	T [°C] until hatching 12hrs.
M01	26.6	23.6
M04	29	26.0
M07	**27.7**	**24.7**
M09	30	27.0
M11	29	26.0
M12	30.5	25.0
M13	**30**	**26.5**
M14	**31**	**26.5**
M14/A	33	27.0

Table 2. Differences in the last phase of the tested incubation methods. The incubation methods with the highest hatching rate (M07, M13, and M14) are displayed in bold.

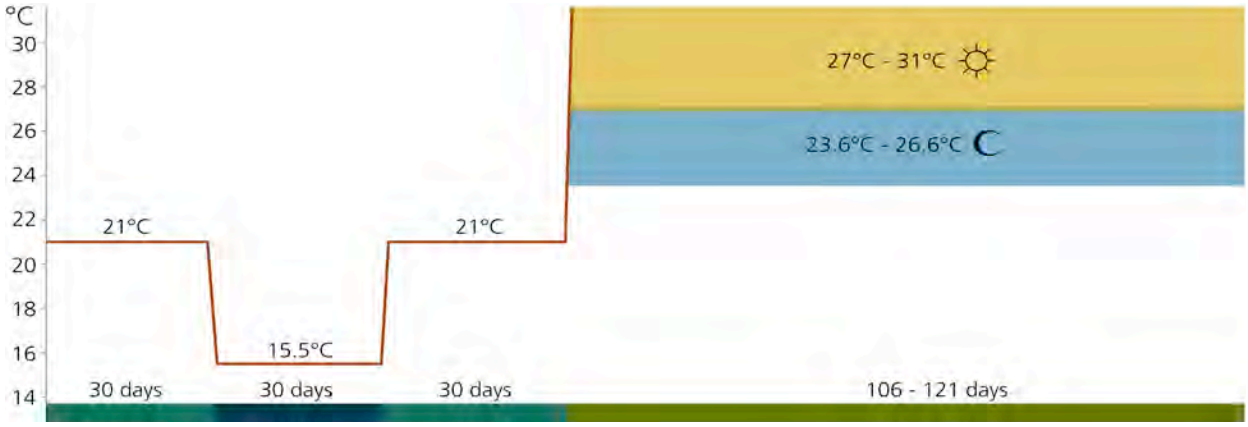

Fig. 20 Temperature curve of the most successful incubation methods (M07, M13, and M14).

Due to the small clutch size in combination with late sexual maturity and a relatively small breeding group it is extremely time-consuming and tedious to collect data on sex determination of *P. planicauda*.

Fig. 21 left) Hatching *Pyxis planicauda.* **right)** Incubators used for *Pyxis planicauda* egg incubation.

By now, the sexes of the first hatchlings can be determined. Although the number of successfully incubated tortoises is not yet sufficient for a statistical analysis, the collected data allow first conclusions. In the years 2003 (No. 1), 2005 (No. 2 and 3) and 2006 (No. 5 to 11) only male tortoises hatched using incubation within the enclosure or the incubation methods M01, M04 and M07. The eggs laid in 2007 were incubated using the methods M09 and M11. While M11 delivered hatchlings of both sexes, the only tortoise that had hatched using method M09 died without determining its sex. Therefore no statement can be made about this method. In 2008 method M07 was used again and this time tortoises of both sexes hatched (3. 3. 1). Interestingly, hatchlings produced by the same parents (female green and male large, and female blue and male small) that had produced male offspring exclusively using method M07 in 2005 and 2006 produced 100 % female hatchlings this time. However, another breeding pair (female white and male small) produced male hatchlings again. In 2009, the eggs were incubated using method M12. The two hatchlings whose sexes can already be determined are female. In the years 2010 to 2013 the eggs were incubated using method M13. Although not all sexes of the 19 hatchlings can be determined without any doubt yet, this method appears to produce a mixed gender ratio (4, 5, and 9). For the methods M14 and M14/A no results are available yet.

REARING OF HATCHLINGS

After hatching, the baby tortoises are placed into small, closed terrariums fitted with a spotlight (40 W) and an ultrasonic humidifier. After about ten days they are transferred to a larger rearing enclosure equipped with a metal halide lamp, a fluorescent tube (Sylvania Reptistar 30W) and an ultrasonic humidifier. The rearing enclosures are structured similar to those for adult tortoises using roots, logs, a leave litter and many hiding places. The hatchlings are immediately active and leave their hiding place in the morning for thermoregulation and feeding. Temperatures vary between 25-45 °C during the day and drop to 17 °C at night. Hatchlings that were handed over for rearing to other project participants in Europe from 2008 onwards often showed acclimatization phases of up to one year during which neither growth nor weight gain could be documented. In summer, the *P. planicauda* hatchlings can be transferred to protected outdoor enclosures.

DEVELOPMENT OF HATCHLINGS

The initial hatching weight of *P. planicauda* varies between 12.2 g and 18.3 g (average = 15.2 g). In the course of the first year, the hatchlings (n = 48) gained 21.0 g on average (Figs. 22, 23). The smallest increase documented across the first year was 5.3 g, while the largest increase was 38.2 g (Figs. 23). Weight gain is highest within the first six months.

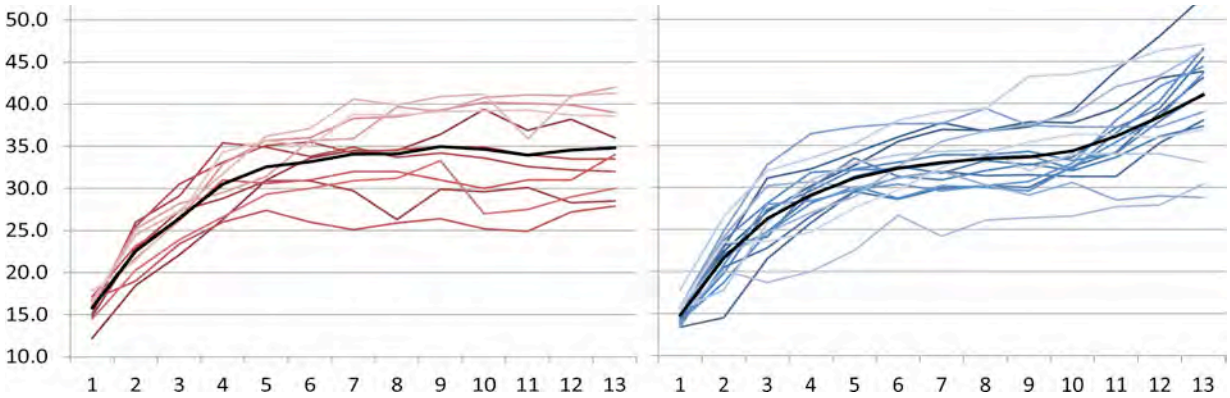

Fig. 22 Weight development of *Pyxis planicauda* hatchlings within the first year. **Left)** Females; **Right)** Males. The average monthly weight gain is displayed as a black line.

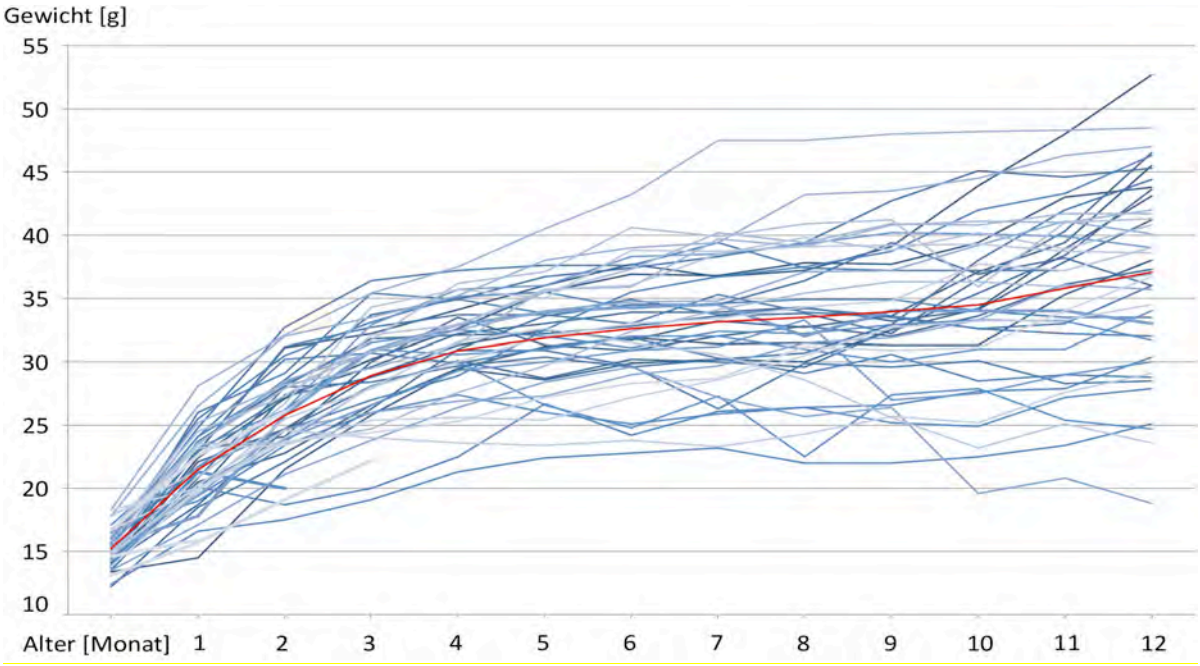

Fig. 23 Weight development of *Pyxis planicauda* hatchlings (n = 49) within the first year. The average monthly weight gain is displayed as a red line.

Monitoring the hatchlings' development across the entire project duration reveals phases of increased weight gain as well as phases during which only little weight is gained (Fig. 24).

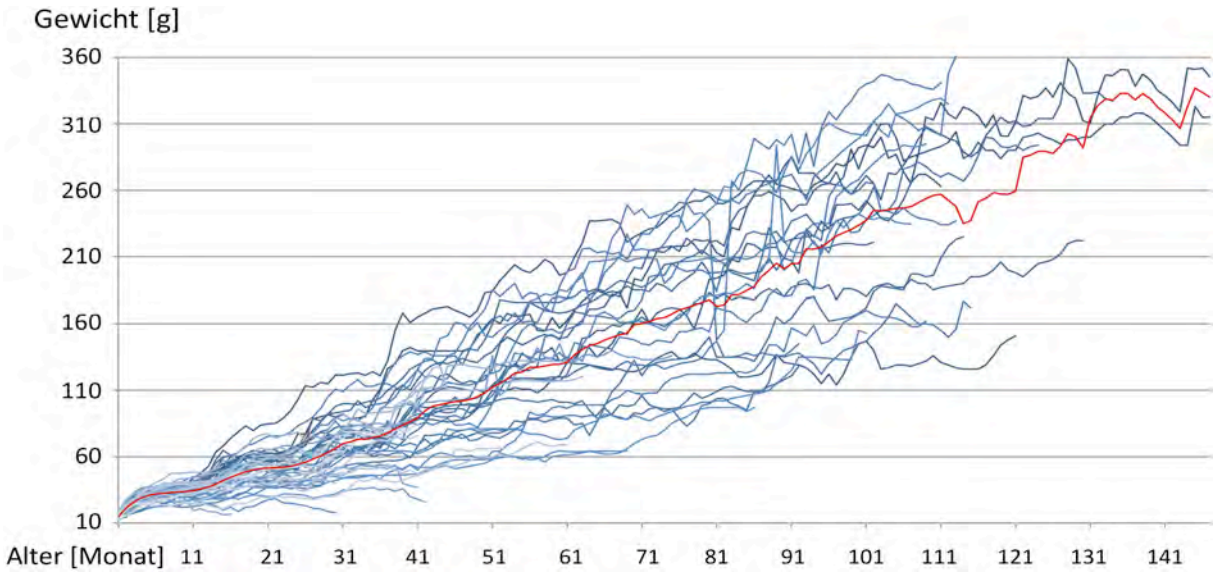

Abb. 24 Weight development of *Pyxis planicauda* hatchlings (n = 49). The average monthly weight gain is displayed as a red line.

RESULTS AND FUTURE PERSPECTIVE OF THE EHAP PROJECT

Over the past years the husbandry conditions within the EHAP project were optimized (project objective 1). Based on the collected experiences of all project members, recommendations for keeping *Pyxis planicauda* have now been formulated (see chapter: GENERAL RECOMMENDATIONS FOR CAPTIVE HUSBANDRY) and recently published with Sacalia, the professional journal of the International Tortoise Association (ISV).

Research on the optimal incubation method (project objective 3) has not yet been completed, but the incubation success achieved so far as well as the gender ratio of those hatchlings that can already be sexed with certainty indicate that the gender fixation is indeed temperature sensitive and that incubation experiments are close to reveal insights on the pivotal temperature. Moving of the project leader Viktor Mislin and his breeding group in May 2014, and the infection of wild caught tortoises with parasites of a so far undescribed species of the genus *Falcaustra* as well as the related treatment have led to a poor fertilization and hatching rate over two years. In the meantime the tortoises have recovered and recently more hatchlings have emerged. We hope to be able to answer the question of the ideal incubation method in the coming years.

Since so far only few individuals bred by Viktor Mislin have already reached sexual maturity, project objective 4 has not yet been achieved either, which delays the original project schedule. However, first growth curves could be established on the basis of the collected data (see Chapter: DEVELOPMENT OF HATCHLINGS, Figs. 23-24). Only one of the hatchlings transferred in 2007 has reached maturity, was grouped with a female and is reproductively active. In this group the first baby tortoises have recently hatched. However, this means that we are only at the beginning of conservation breeding with captive bred individuals. After 55 hatchlings have been produced from a small breeding group, an exchange of animals with other keepers is desired for within the coming years to increase genetic diversity. At this point a possible exchange is discussed with Matthias Goetz, curator for herpetology at the Durrell Wildlife Conservation Trust. As more offspring will reach maturity in the coming years, additional breeding groups will be put together. This will increase the number of offspring. In order to cope with the growing

number of individuals and the associated amount of data (pictures as well as the weights and measurements to be recorded on a monthly basis) and to facilitate the statistical analyses, an improvement of data recording and archiving is currently being worked on in cooperation with Dr. Flora Ihlow. The new tables will be made available to the project members shortly.

In February 2018 Viktor Mislin, Flora Ihlow, and Matthias Goetz visited the turtle conservation center in Ampijoroa, Ankarafantsika National Park, Madagascar to get a personal impression of the husbandry conditions and to exchange information with the station manager Ernest Bekarany. Details on the husbandry of *P. planicauda* at the turtle conservation center, was presented to other project members during the annual EHAP meeting 13. 7 - 15.7.2018, held in Basel.

Viktor Mislin and Flora Ihlow also visited the habitat of *Pyxis planicauda* during a seven-day expedition to the Kirindi National Park on the west coast of Madagascar. A total of three animals, two adult females and one male were discovered. A detailed description of the habitat was prepared, the daily activity and movement patterns of one female tortoise were documented, and forage plants were documented for later identification. In addition, two dataloggers (HOBO pendant) could be installed within the habitat to measure and record temperatures for the duration of an entire year. Observations from the biotic and abiotic conditions within the natural habitat will be used to further optimize the keeping conditions. These results were also presented at the annual EHAP meeting.

VETERINARY OBSERVATIONS

COCCIDIOSIS

PRASCHAG et al. (2010) reports on a case of protozoa infection (intranuclear coccidiosis) in *P. planicauda*. In chelonians, coccidiosis causes severe diseases with high mortality. According to PRASCHAG et al. (2010), there was an outbreak at the Behler Conservation Center, California, USA, which began in January 2008 and caused 29 deaths (71 % of the stock) over the course of two years. The same parasites were also confirmed from other populations in the USA and Europe by laboratory tests (ALVAREZ et al. 2012). Symptoms of a coccidiosis infestation include lethargy, rapid weight loss, weakness, gasping respiration, and swollen erythematous vents with severe epidermal necrosis (ALVAREZ et al. 2012).

DYSTOCIA

A case of dystocia was reported from the Jersey Zoo, UK. Treatment with calcium and oxytocin failed, but this was attributed to late treatment (GIBSON AND BULEY 2004).

CLOACAL INJURIES

In one female (female blue) the cloaca was injured during egg deposition in 2011 (Fig. 25). After consultation with a specialist veterinarian, the wound was not stitched, as a longitudinal tear usually heals well while a seam could tear again when eggs are laid. In order to avoid infections, the tortoise was bathed in nettle tea on a daily basis. By mid-January 2012 the wound had healed completely. In August another egg was laid without any problems.

Fig. 25 Injured cloaca of a female *Pyxis planicauda*. **Left)** 27.10.2011; **Center)** 28.10.2011; **Right)** 28.11.2011.

In 2013 the cloaca of the same female was infected and an abscess had developed. A veterinary examination including an x-ray revealed no abnormalities. Again the tortoise was bathed in nettle tea on a daily basis. In addition, an antibiotic ointment was applied. The healing process was documented using photographs (Fig. 26). According to specialized veterinarians, the healing of such an abscess can take months. In January 2014 it had still not healed completely.

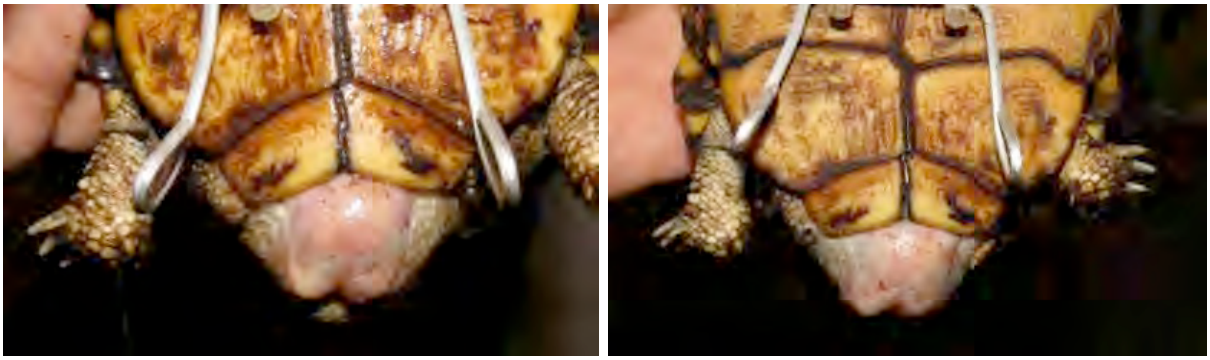

Fig. 26 Injured cloaca of a female *Pyxis planicauda*. **Left)** 30.11.2013; **Right)** 21.01.2014.

The therapy was continued with daily injections of an antibiotic (Baytril 2.5 %, 0.1 ml) for a duration of 14 days (28.11.2013 - 05.12.2013), resulting in a significant improvement (Fig. 27). The antibiotic was administered alternating to the right and left. Between the 23rd and the 31st of January 2014 injections were again administered on a daily basis. On the 2nd of January 2014 the therapy was finally completed. Although the abscess had not disappeared, the veterinarian considered it to be encapsulated and harmless.

Fig. 27 Injured cloaca of a female *Pyxis planicauda*. Pictures taken on the 5th of Feburary 2014.

Throughout the treatment the tortoise fed, was active and did not lose weight. On the 10th of April 2014 the condition suddenly worsened and the abscess was to be surgically removed. On the 14th of April 2014, one day before the planned surgery, the tortoise was transported to the clinic of a specialist veterinarian. In the morning of the 14th of April 2014 the tortoises' condition was so poor that the surgery had to be canceled. A few hours later the tortoise had died. A postmortem examination clarified the cause of death.

MICROFILARIA (BLOOD WORMS)

A minor injury to the cloaca was discovered in an adult female (female blue). During a microscopic examination of the scab roundworms of the Filarioidea type were discovered. Subsequently, blood was drawn from all wild caught animals by a veterinarian and examined in the laboratory. A blood smear analysis revealed three individuals (green, white, and red) to suffer from microfilariosis. However, the hatchlings appeared not to be affected. The infected tortoises were treated with 0.029 g Milbemax as follows. Green: 7.10.2014, 19.10.2014, 13.11.2014, 04.10.2014; White: 13.11.2014, 04.12.2014; Red: 09.10.2014, 19.10.2014.

On the 02nd of March 2015 one female (female green) was brought to the clinic to remove a small abscess (Fig. 28) which had developed at the base of its neck in response to blood being drawn (Fig. 29). After the surgery the wound has healed well and is hardly visible anymore.

Fig. 28 Female *Pyxis planicauda* before and after the surgery.

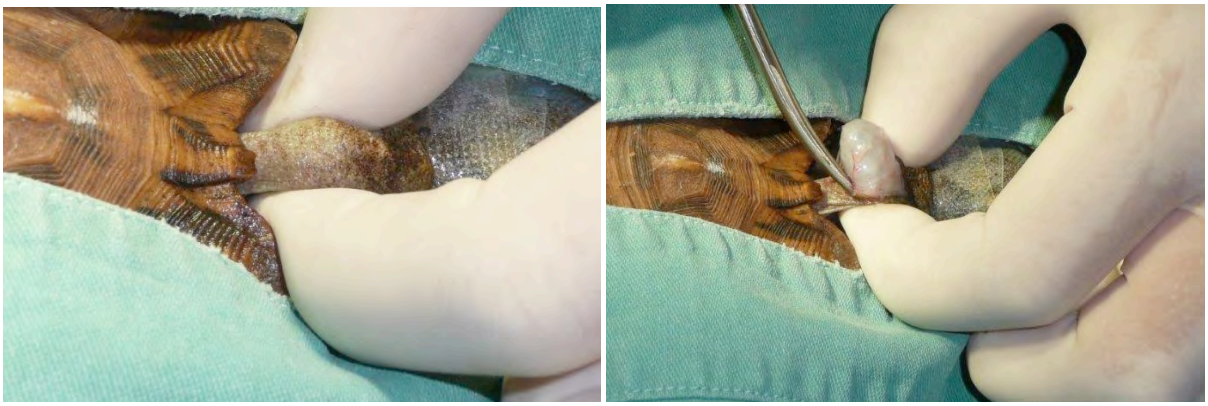

Fig. 29 Removal of an abscess at the base of the neck of a *Pyxis planicauda* female.

WORM INFESTATION (NEMATODES)

GIBSON AND BULEY (2004) report that *P. planicauda* are susceptible to severe nematode infestation which can be detected in the feces. Although no negative effect was observed in adult animals, infection with nematodes of the genus *Atractis* and the subfamily *Kathlamiinae* are suspected to be the cause of death of juvenile tortoises in the turtle conservation center in Ampijoroa, Madagascar (RAZANDRIMAMILAFINIARIVO et al. 2000). The latter migrates into the parenchyma, which was probably also the cause of death of *P. planicauda* female blue. The autopsy revealed that all organs have been interspersed with nematodes (Fig. 30). At first it could not be clarified which parasites were involved to decide whether the entire group had to be treated.

Fig. 30 Pictures of the autopsy of *Pyxis planicauda* female blue. The photographs show large numbers of nematodes.

On the 13th of April 2016 another tortoise (wild caught adult male) suddenly died. The tortoise was observed feeding and basking the previous day. The autopsy again revealed a massive infestation with nematodes (Fig. 31).

Fig. 31 Pictures taken during the autopsy of a male *Pyxis planicauda* infected with nematodes.

These parasites appear to be the same ones that caused death of female blue in 2014. Since the species could not yet be identified, the question of a suitable treatment arises. By killing of the nematodes, the treatment of a severe nematode infection can cause a sepsis. In addition, it is unclear whether all wild caught tortoises of the breeding stock carry these parasites.

Within the enclosure in which the deceased male had been kept, a small piece of excrement containing two worms was discovered on the 23rd of April 2016 (Fig. 32). The parasites were handed over for examination to the parasitologist Nicola Pantchev by Tobias Friz (project leader Germany) during the conference of the AG Amphibian and Reptile Diseases. In cooperation with parasitologists from Berlin a molecular genetic analysis was initiated. Preliminary results indicate that these nematodes likely represent a so far undiscovered species of the genus *Falcaustra* (Friz pers. comm.). Since the genus *Falcaustra* belongs to the same family, it appears likely that the same parasite caused the deadly outbreak in Ampijoroa. Profender was recommended for deworming.

ACKNOWLEDGEMENTS

I would like to thank my wife Silvia for her tireless help regarding feeding and maintenance of all our tortoises. I would like to thank Henk Zwarteporte and Hans-Dieter Philippen who supported this project until their unexpected deaths in May and October 2016 as members of the scientific advisory board. Further I thank all cooperation partners, in particular Professor Dr. Dieter Ebert from the Zoological Institute of the University of Basel, and all project participants. I would like to express my gratitude to the Schildkröten-Interessen-Gemeinschaft Schweiz (SIGS) and to various people who have financially supported the EHAP project, as well as to the company Lucky Reptile for providing light bulbs. I would like to thank Dr. Flora Ihlow for the help in preparing the project report and subsequently translating it into English, Falk Sebastian Eckhardt and Wojciech Doganowski for providing pictures and Dominik Ott for the maintainance of the project website.

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REGULAR DATA RECORDING

The tortoises' body weight is recorded at least once a month before and after bathing using a digital scale. In addition, the straight carapace length, width and height are measured using a caliper specifically designed for this purpose (Fig. 32). The collected data has to be entered regularly into the internal data collection system on the project homepage.

Fig. 32 Calipers used within the EHAP project to measure *Pyxis planicauda*.

In the future, images of the plastron and the tail region are to be taken and archived on a monthly basis in order to be able to pinpoint when precisely sexes become determinable. In order to facilitate later evaluation, a size reference, the individuals respective identification number, its body weight, as well as the straight carapace length in mm are to be added to each picture.

LIST OF PREVIOUS PUBLICATIONS, POSTERS, AND PRESENTATIONS

MISLIN V. (2004) Problematik bei der Haltung und Zucht von *Pyxis planicauda*. Annual meeting of the DGHT AG Schildkröten, Hofheim, Germany. (talk)

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MISLIN, V. (2012) Erfahrungsaustausch zur Haltung und Aufzucht von *Pyxis planicauda*. Schildkrötengruppe Region Basel SGRB, Basel, Switzerland. (talk)

MISLIN, V. (2012) Bebrütung der Eier von *Pyxis planicauda*. EHAP & EMI annual project meeting, Frieding near Andechs, Germany. (talk)

MISLIN, V. (2014) Kloakenverletzung bei *Pyxis planicauda*. EHAP & EMI Projekttagung, Herrsching, Ammersee, Germany. (talk)

MISLIN, V. (2014) Erfahrungsaustausch zur Haltung und Aufzucht von *Pyxis planicauda*. Annual meeting of the Vive, Neuss, Germany. (talk)

MISLIN, V. & IHLOW, F. (2016) 9 years of keeping and breeding the Madagascar Flat-tailed tortoise (*Pyxis planicauda*). 13th Annual Symposium on the conservation and biology of tortoises and freshwater turtles, New Orleans, USA. (poster)

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EHAP

THIRTEEN YEARS OF KEEPING AND BREEDING THE MADAGASCAR FLAT-TAILED TORTOISE (*Pyxis planicauda*)

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INTRODUCTION

The critically endangered Madagascar Flat-tailed Tortoise *Pyxis planicauda* is endemic to the Menabe region on the central western coast of Madagascar[1-3].

Although research has increased in recent years, knowledge on the species' life history remains poor. To date, no information regarding reproduction in the wild exist and despite several decades of considerable efforts by renowned zoos, knowledge on the species' breeding biology is still very limited[4-6]. Consequently, breeding attempts widely remain unsuccessful and the number of captive bred individuals is low.

The EHAP project is a captive breeding project established in 2007 that aims to enhance the exchange of experiences in captive care and breeding of *P. planicauda*. To date, the projects' participants produced more than 50 hatchlings using different incubation conditions of which nine proofed to be successful. Here we summarize our experiences.

CAPTIVE CARE AND INCUBATION

The EHAP project's breeding stock consists of nine adult specimens (3,6), which are kept in mixed groups of 1,1 or 1,2 in naturally vegetated enclosures measuring 70 x 80 cm. The enclosures are equipped with a UV fluorescent tube, a Bright Sun Desert UV spot, and a fogging system to simulate a seasonal climate resembling the conditions within the species' natural habitat.

Mating takes place throughout the rainy season (October - April), but is most frequent in the beginning (Fig.1)[4]. In captivity, females produce two to four clutches with a single egg each (Fig.2)[3,6]. Eggs are collected from the enclosures, half covered with vermiculite, and transferred into an incubator (Fig.3).

Fig.1. Breeding pair of *Pyxis planicauda* mating.

Fig.2. Female *Pyxis planicauda* after egg deposition.

Fig.3. Egg half covered with vermiculite.

Fig.4. Incubators used for *Pyxis planicauda* eggs.

To match seasonal conditions of the natural environment in Madagascar, egg incubation consists of four phases (Fig. 5) including a diapause that corresponds to the dry season. While the first three phases remain stable across all incubation methods, temperature during the final phase is variable. Throughout all phases of the incubation, humidity was 85-90%.

RESULTS & DISCUSSION

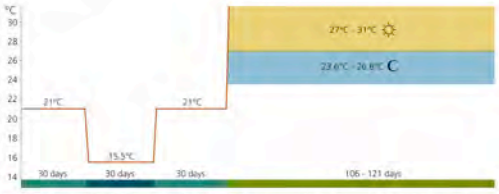

Fig.5. Range of successful incubation conditions. Yellow and blue area correspond to day and night time temperature ranges.

Table 1. The following three incubation conditions were the most successful.

METHOD	TEMPERATURE [°C]	DURATION [DAYS]	# EGGS	# HATCHLINGS	%
1	21.0 15.5 21.0 24.7 (night), 27.7 (day)	30 30 30 112 - 125	20	15	75%
2	21.0 15.5 21.0 25 (night), 30.5 (day)	30 30 30 190 - 225	39	19	48%
3	21.0 15.5 21.0 26.5 (night), 31 (day)	30 30 30 196 - 206	13	6	46%

To date, 50 *P. planicauda* hatched from a total of 102 eggs (49% hatching rate). Using the three methods listed above, 40 hatchlings emerged from 72 eggs (55%), while the remaining six incubation conditions produced only 10 hatchlings from 30 eggs (33%). However, it is uncertain whether eggs that did not hatch failed due to infertility.

Since 2008, incubation temperatures were recorded using a HOBO pendant temperature data logger set to record temperatures at intervals of 10 minutes.

Fig.6. Left: *Pyxis planicauda* hatchling emerging from its egg. Right: *Pyxis planicauda* hatchlings.

ACKNOWLEDGEMENTS

We are grateful to Silvia Mislin, Hans-Dieter Philippen who recently passed away, and Prof. Dr. Dieter Ebert (University Basel) for their kind support. Further, we thank all participants of the EHAP project as well as all sponsors who kindly provided financial support for this project.

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EHAP

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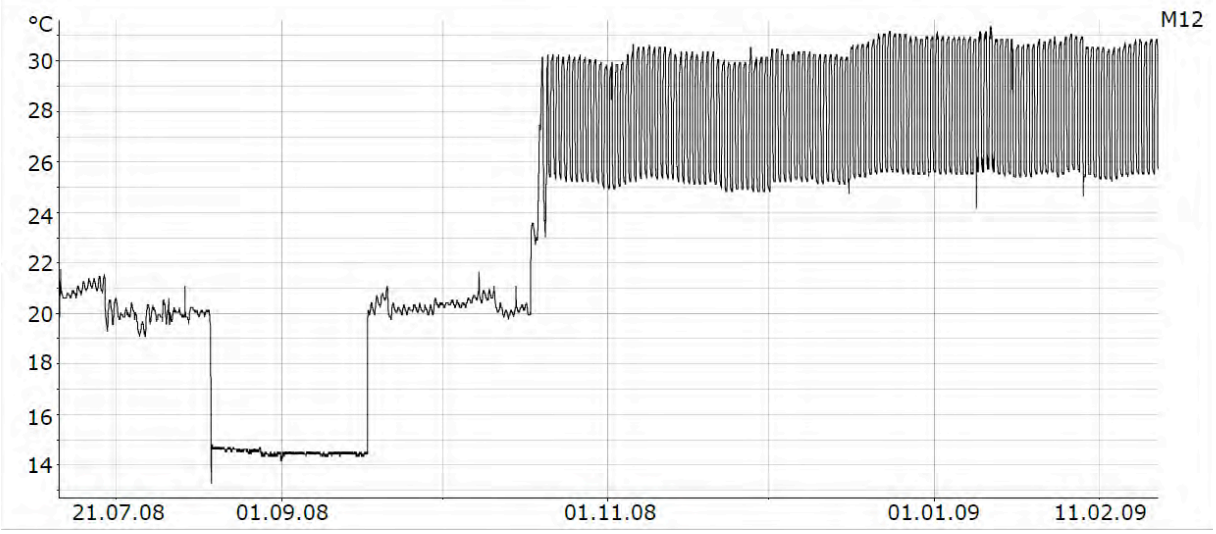
Fig. 4 Poster presented during the 13th Annual Symposium on the Conservation and Biology of Tortoises and Freshwater Turtles, New Orleans, USA.

APPENDIX 4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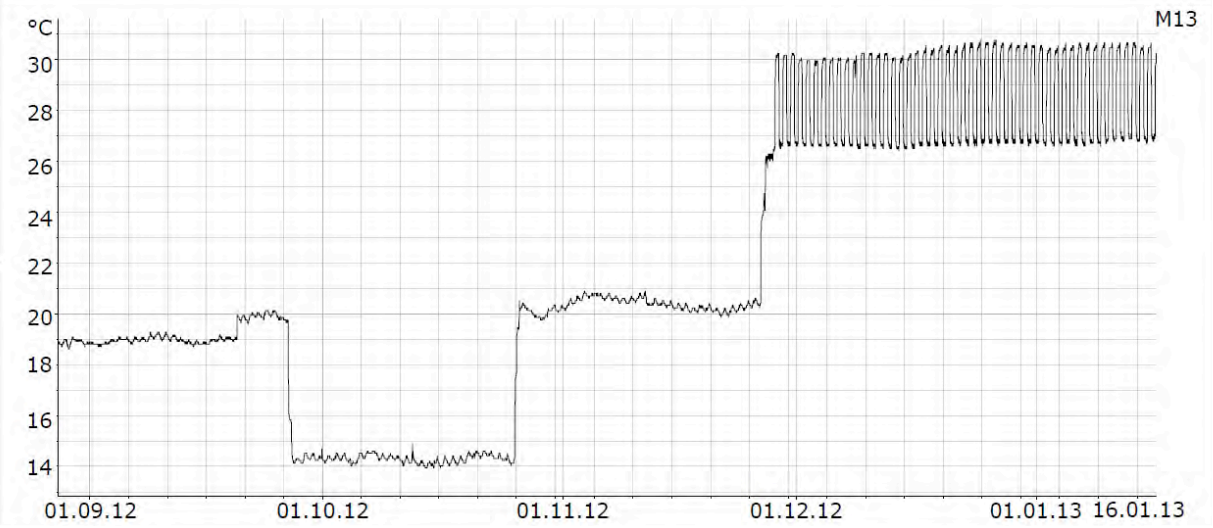
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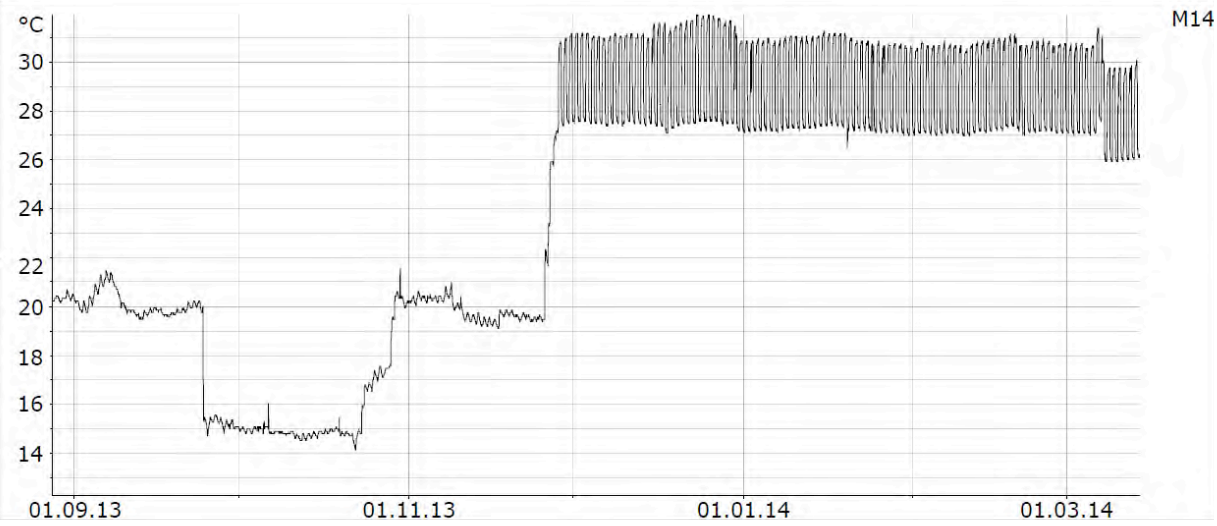
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APPENDIX 4

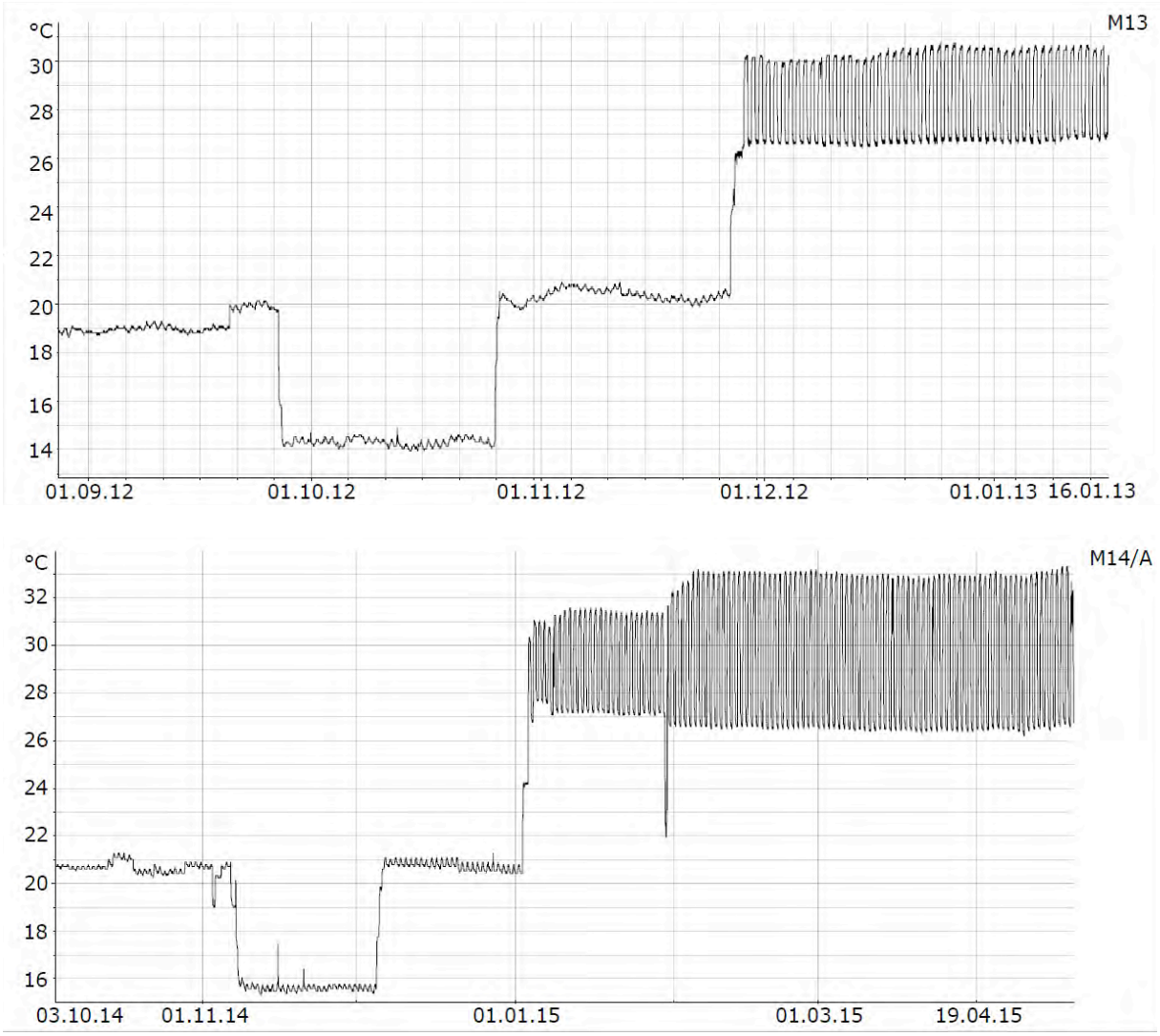

Fig. 5 Diagrams of incubation methods M12, M13, M14, and M14/A.