EAZA Amphibian TAG
Best Practice Guidelines for the black-eyed leaf frog (*Agalychnis moreletii*)

1st edition

Coordinated and edited by Olivier Marquis

Collaborators: Andrew Gray, Christopher Michaels, Adam Bland, Alejandra Zamora, Rachael Antwis, Richard Preziosi, Ben Baker.

EAZA Amphibian TAG Chair: Dr Gerardo Garcia, Chester Zoo, United Kingdom, CH2 1LH.
EAZA Amphibian TAG Vice-chairs: Dr Benjamin Tapley Zoological Society of London, Regent's Park, London, United Kingdom and Dr Olivier Marquis, Parc Zoologique de Paris, 53 avenue de Saint-Maurice, 75012 Paris, France.

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Cover image. Amplexus of *Agalychnis moreletii.* Picture by A. Zamora.

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Preamble

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

Summary

The Black-eyed tree frog (*Agalychnis moreletii*) was assessed as Critically Endangered by the IUCN from 2004 to 2016. It was reclassified as Least Concern in 2016 due to the release of certain potential threats in the field (IUCN SSC Amphibian Specialist Group, 2017).
Since then, phylogeographic studies tend to show that the taxonomic status of this species is certainly more complex than it seemed in the past and within a very fragmented distribution area.
In the context of this confusing and contrasting situation and given the low number of captive animals in Europe, it seems essential to harmonise the knowledge and practices of captive care and breeding of this species. That is the role of the Best Practice Guidelines edited by the European Association of Zoos and Aquaria (EAZA).
This document has been written in collaboration with the best specialists of this species and aims to share the more up to date knowledge about *Agalychnis moreletii* in its natural environment as in captivity. The text is divided into two sections: 1) Biology and field data, and 2) Management in zoos and aquariums.

Acknowledgement

This work is the result of the close collaboration between zoo keepers and curators, veterinarians, researchers and private breeders. Our special thanks go to Tobias Eisenberg (private breeder), Calvin Allen (Sparsholt College Hampshire) and Ben Sutcliffe (Reaseheath College) for having shared their experience and pictures about captive care of this species.

(Photo credit T. Eisenberg)

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Section 1: BIOLOGY AND FIELD DATA

Biology

1.1. Taxonomy

The genus *Agalychnis* contains 14 recognised species (Frost 2020):

Agalychnis annae (Duellman, 1963)
Agalychnis buckleyi (Boulenger, 1882)
Agalychnis callidryas (Cope, 1862)
Agalychnis dacnicolor (Cope, 1864)
Agalychnis danieli (Ruiz-Carranza, Hernández-Camacho, and Rueda-Almonacid, 1988)
Agalychnis hulli (Duellman and Mendelson, 1995)
Agalychnis lemur (Boulenger, 1882)
Agalychnis medinae (Funkhouser, 1962)
Agalychnis moreletii (Duméril, 1853)
Agalychnis psilopygion (Cannatella, 1980)
Agalychnis saltator Taylor, 1955
Agalychnis spurrelli Boulenger, 1913
Agalychnis taylori Funkhouser, 1957
Agalychnis terranova Rivera-Correa, Duarte-Cubides, Rueda-Almonacid, and Daza-R., 2013

The current classification of *A. moreletii,* (following Frost et al., 2006) is:

- Class Amphibia
- Order Anura
- Family Hylidae
- Sub-Family Phyllomedusinae
- Genus *Agalychnis*
- Species *Agalychnis moreletii*
- Common name Black-eyed leaf frog

The taxonomical classification of this species is currently subject to review. According to the latest published papers, *A. moreletii* seems to be phylogenetically close to *A. annae* (Gomez-Mestre et al. 2008, Faivovich et al. 2010, Gray 2011) as well as concealing several regional variations that appear genetically distinct. Recent work across the natural range of the species show strong phenotypic (morphology and calls) and genetic (nuclear DNA and mtDNA) geographical structuring (A. Zamora and R. Preziozi, pers. communication). All together, analyses reveal three major clades (Figure 1) that remain to be taxonomically identified. Results could have major implications in terms of conservation needs. Current evidence, especially levels of genetic differentiation, suggest that individuals collected from the three main areas indicated in Figure 1 should be managed separately. Central Mexican populations are already limited in distribution and may represent additional subgroups when examined.

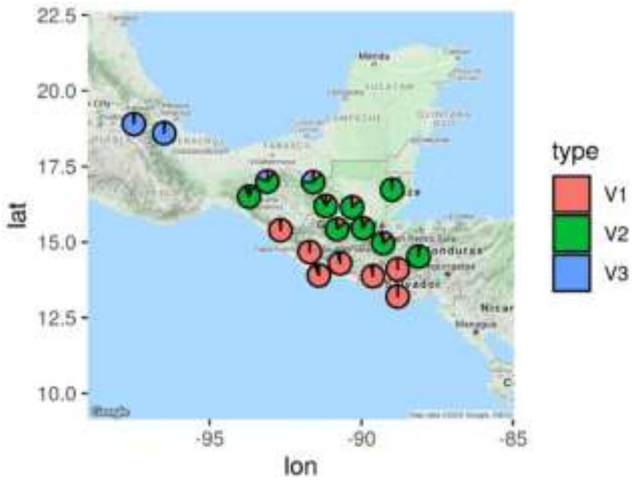


Figure 1 : Genetic structuration (nuclear DNA) of *Agalychnis moreletii* across the natural range of the species (unpublished data from Alejandra Zamora and Richard Preziosi).

1.2. Morphology

As illustrated in the Table 1, size and weight show some variations according to origin of animals. Maximum size can vary between populations (individuals from Belize and some parts of Guatemala being generally smaller (A. Zamora, pers. obs.).

Table 1 : Body size and weight variations in *Agalychnis moreletii*.

	Mean SVL (mm)		Mean weight (g)		Source	Comment
	Male	Female	Male	Female		
	60	75	*	*	Lee, 1996; Duellman, 2001	
	58,5	70,1	6,4	18,8	A. Zamora, pers. obs.	heaviest female probalby gravid
	62,7	79,6	8,25	19,05	Briggs (2008)	mean values for two consecutive field seasons (2004-2005)
	71,6	87,55	6,1	23,6	Briggs (2013)	Las Cuevas Research station, Belize
	56,1	72,5	10,9	25,1	B. Baker pers. obs.	Captive animals from Chester Zoo
Mean :	**61,8**	**77,0**	**7,9**	**21,6**		

Freshly metamorphosed frogs appear, as with most *Agalychnis sp.*, to be firstly uniformly grey/brown dorsally turning to light green in few days with a pale ventral surface. Note that the colour can turn from brown to green between day and night (
Figure 2).

Figure 2 : Colouration of *Agalychnis moreletii* metamorphs (Photo credit T. Eisenberg)

With increasing age, the animals exhibit striking orange sides (turning to soft pink in some populations) and thighs along with the jet-black eyes the species is known for (Figure 4). The eye itself has a bright blue rim around the black red iris (Figure 3). When disturbed, the colouration changes and the uniform green dorsum becomes blotchy, varying in colour from a pale green/yellow to a darker blue/green (Figure 4). This colouration is also exhibited at night where, presumably, the leaf green colouration is less important as camouflage. Some individuals exhibit white spotting/tubercles covering the back (Figure 5, right), and over time the number and density of these spots can increase.

Figure 3 : *Agalychnis moreletii* iris colour (Photo credit: Adam Bland).

Photo credit : B. Baker

Photo credit : T. Eisenberg

Figure 4 : *Agalychnis moreletii*, general aspect.

Figure 5 : Dorsal pattern variation in *Agalychnis moreletii* (Photo credit: B. Baker)

The skin is known to contain pterorhodin within its melanophores (Misuraca, Prota, Bagnara, & Frost, 1977) which allows reflection of near infra-red light. *Agalychnis moreletii* is one of several *Agalychnis* species that have extremely webbed feet to allow gliding/parachuting between/from trees (Stebbins & Cohen, 1997) together with the eye colouration this makes the species easy to identify throughout its range.
This species can be easily distinguished from other *Agalychnis* species having dark iris colouration by the absence of strips on white/yellow flanks and white spots on the back not outlined in black (
Figure 6,
Figure 7).

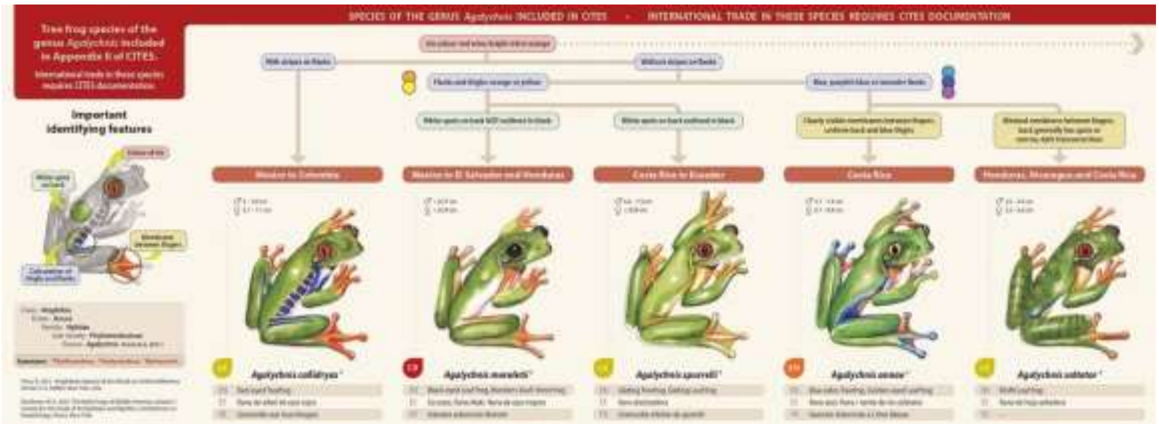


Figure 6 : Identification criteria for *Agalychnis moreletii*
(source : https://cites.org/sites/default/files/common/com/ac/26/26-10i-A2.pdf)

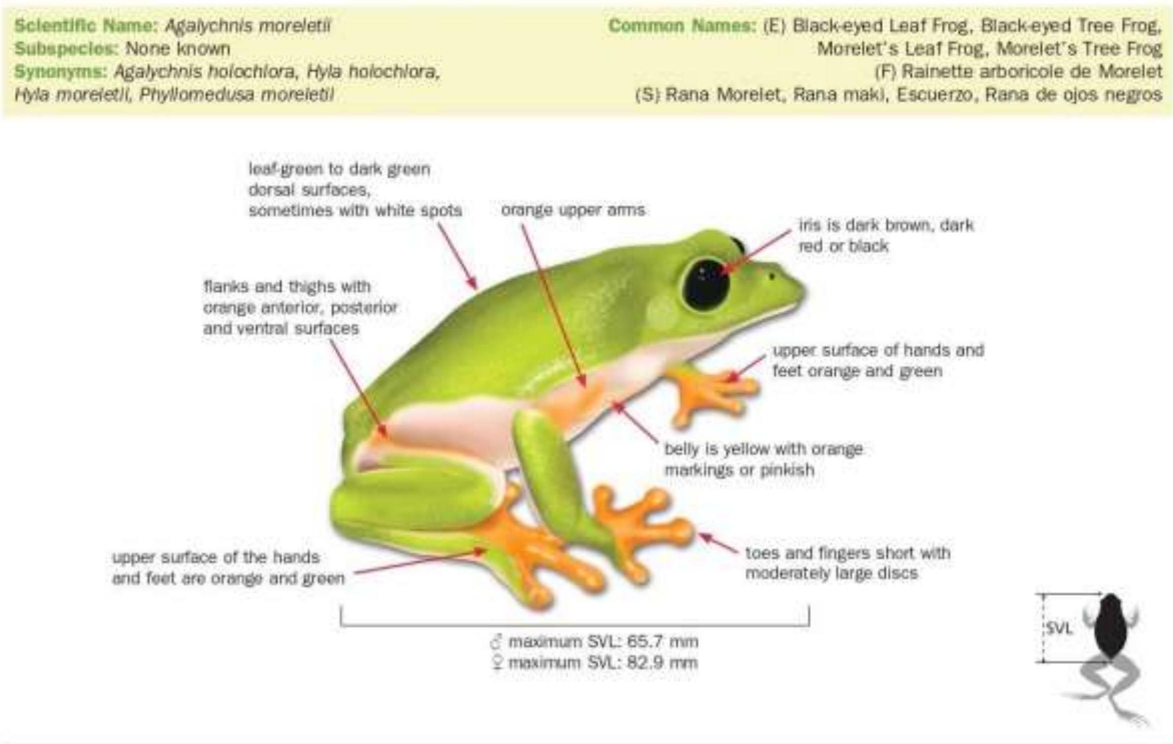


Figure 7 : Identification of *Agalychnis moreletii*
(from: CITES identification sheets Identification sheet 48 - publications.gc.ca/pub?id=9.579018&sl=0)

1.3. Longevity

Little is known about the longevity of this species in the wild but estimations around four to five years seem adequate when comparing the species to other similarly sized *Agalychnis* (Snider & Bowler, 1992). The founder animals within Chester Zoo population were all metamorphosed in August 2008 the last of which, a male, died in 2019 suggesting an upper lifespan of around 11 years for males and less for females.

Field data

1.4. Conservation status/Zoogeography/Ecology

1.4.1. Distribution

As shown in
Figure 8, the distribution is composed of disjunct populations from both Atlantic and Pacific slopes from Veracruz, adjacent Puebla, and Guerrero through Chiapas, Mexico, to the Maya Mountains of Belize, Guatemala, north-western Honduras, and El Salvador, from 200 to 2130 m elevation.

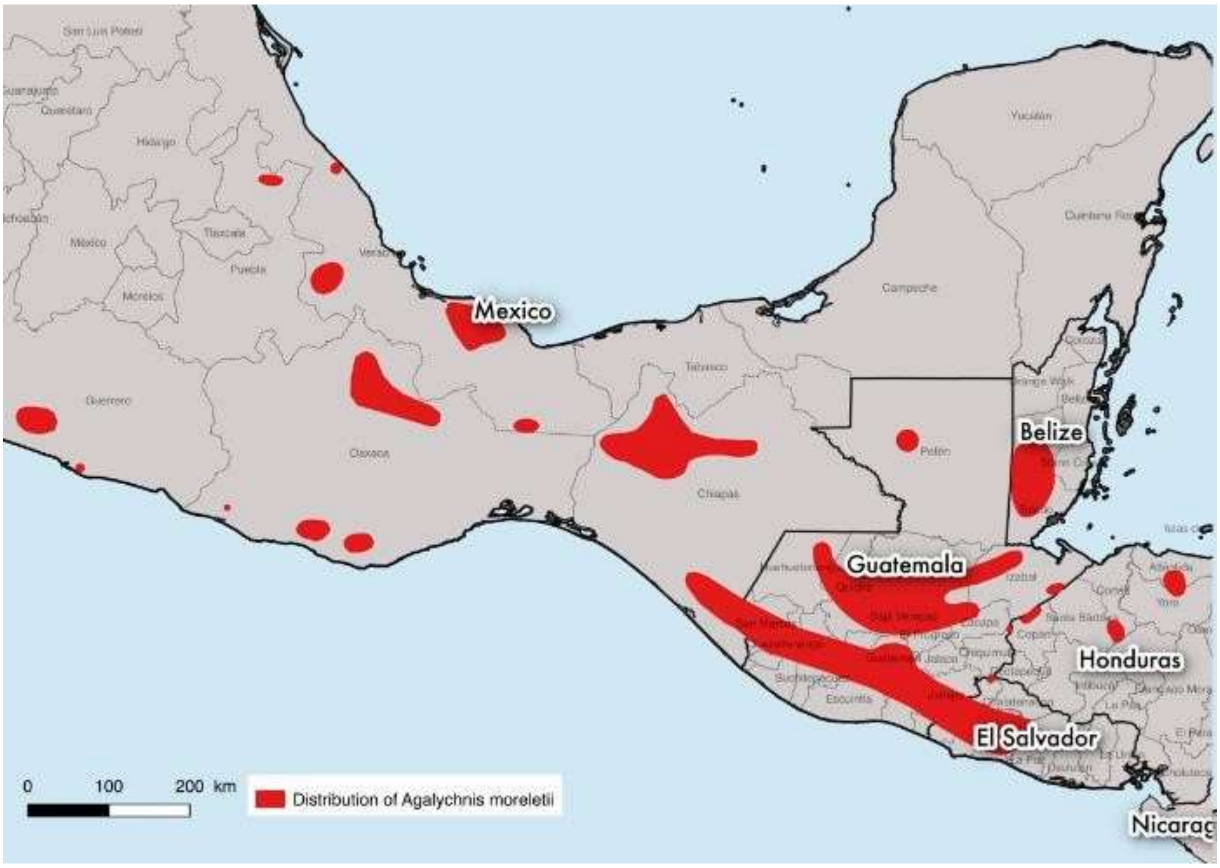


Figure 8 : Distribution of *Agalychnis moreletii* (data extracted from IUCN (International Union for Conservation of Nature), Conservation International, NatureServe & CONABIO (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad). 2016. *Agalychnis moreletii*. The IUCN Red List of Threatened Species. Version 2020)-3)

This was once a wide spread species and could be found throughout central America from Mexico south to Honduras and El Salvador (Duellman, 1970) however the last ten years has seen a major reduction in the population (Felger et al. 2007; Lips et al. 2004) with the species close to extinction in Guerrero, Oaxaca and Chiapas Mexico (Santos-Barrera, Lee, Acevedo, & Wilson, 2004). Genetically, the population appears to be split geographically into three clusters, Atlantic coast Pacific coast and the Veracruz Mexico populations in turn the Atlantic coast and Pacific coast clusters also each contain three distinguishable populations spread over several countries (Figure 1, R. Preziozi, unpublished data). In the future, these population clusters should be managed as separate conservation units.

1.4.2. Habitat

The species is found in primary and secondary forests usually in close proximity to water (mainly temporary pools) (**Error! Reference source not found.**), the species is commonly encountered between 6 and 10 m from the ground when not involved in any reproductive cycle but certainly lives much higher (R. Antwis, pers. comm.), with females often choosing higher resting sites than males (Greenhalgh, 2012).

As indicated in Figure 10, the distribution of *Agalychnis moreletii* mostly coincides with the mountainous environment. Looking at the Köppen climate classification map, the distribution of *A. moreletii* seems to be restricted to regions where tropical and mid temperate climates occur (blue and green color in Figure 11).

Figure 9 : Example of habitat with temporary pond during the rainy season in lower montane forest at Reserva Natural Privada Sac Wach Já, Alta Verapaz, Guatemala (from Serrano et al., 2018).

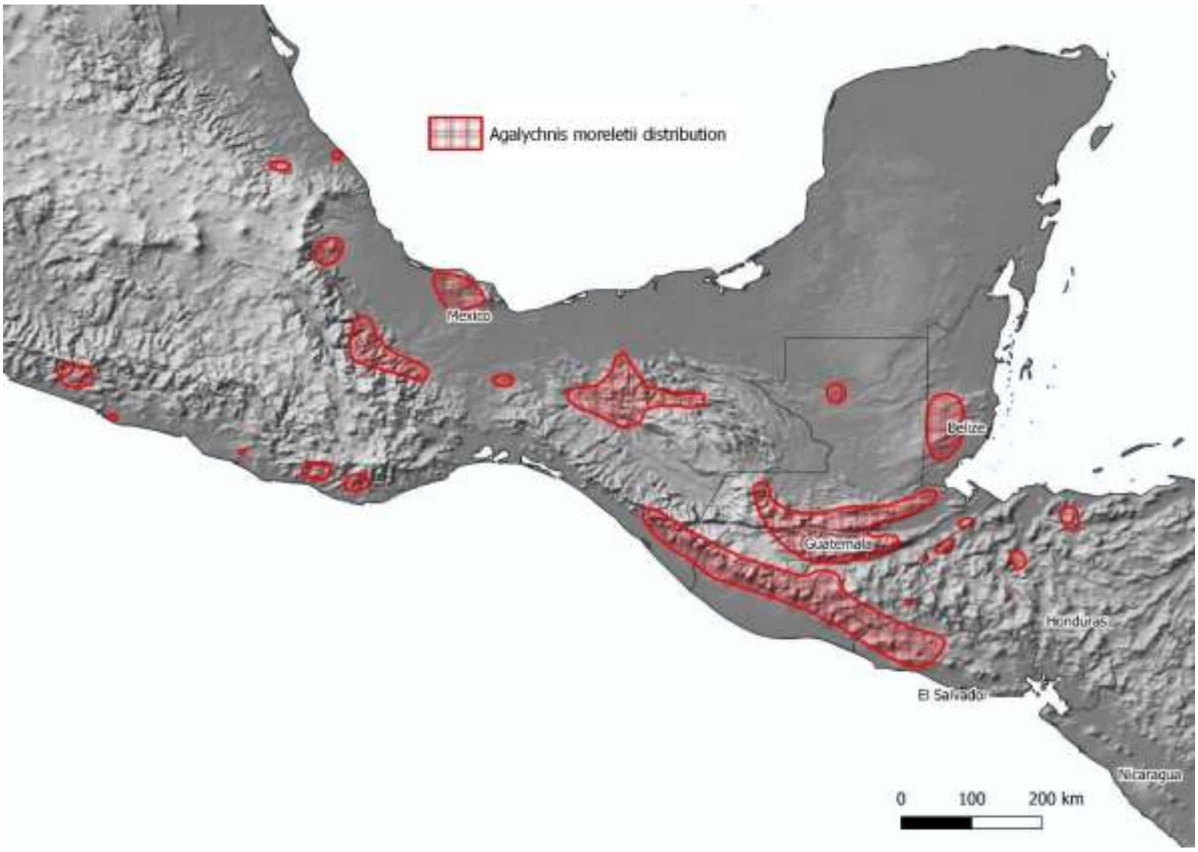


Figure 10 : Distribution of *Agalychnis moreletii* superimposed on the Central America relief map (data extracted from IUCN database (see Figure 8 for references))

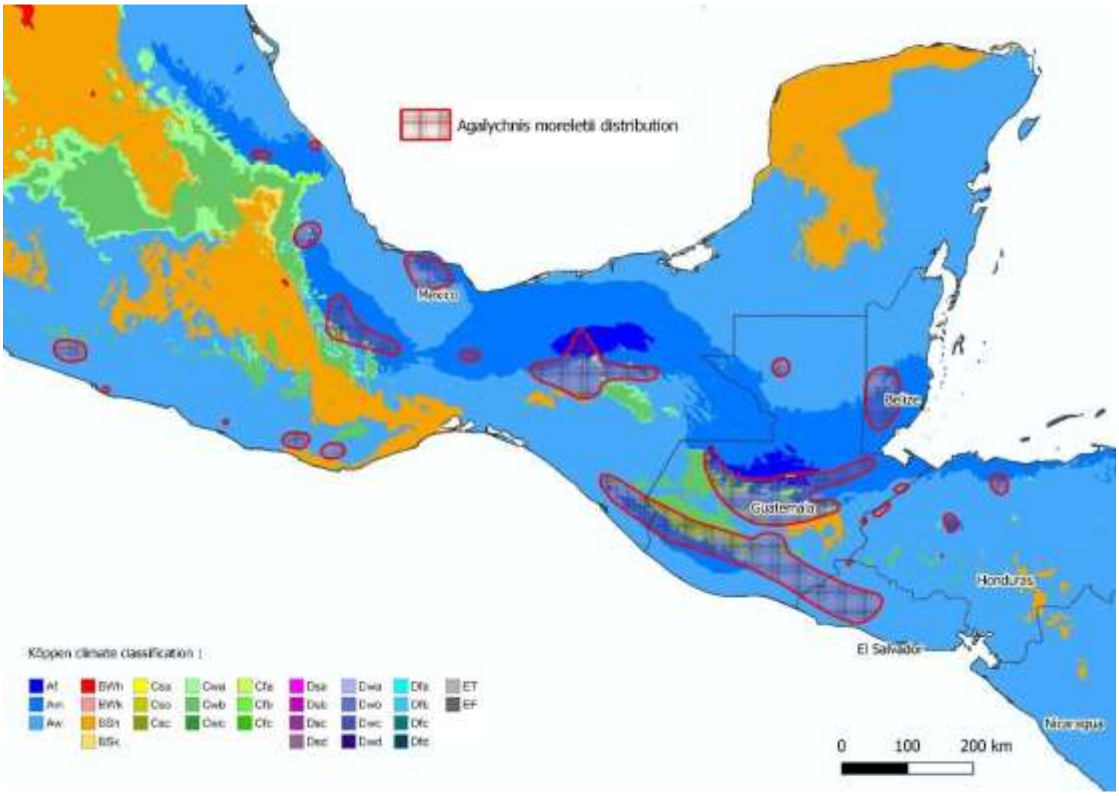


Figure 11 : Distribution of *Agalychnis moreletii* superimposed on the Köppen climate classification map(data extracted from IUCN database (see Figure 8 for references))

Figure 12 : Location of weather stations (see date in Figure 13).

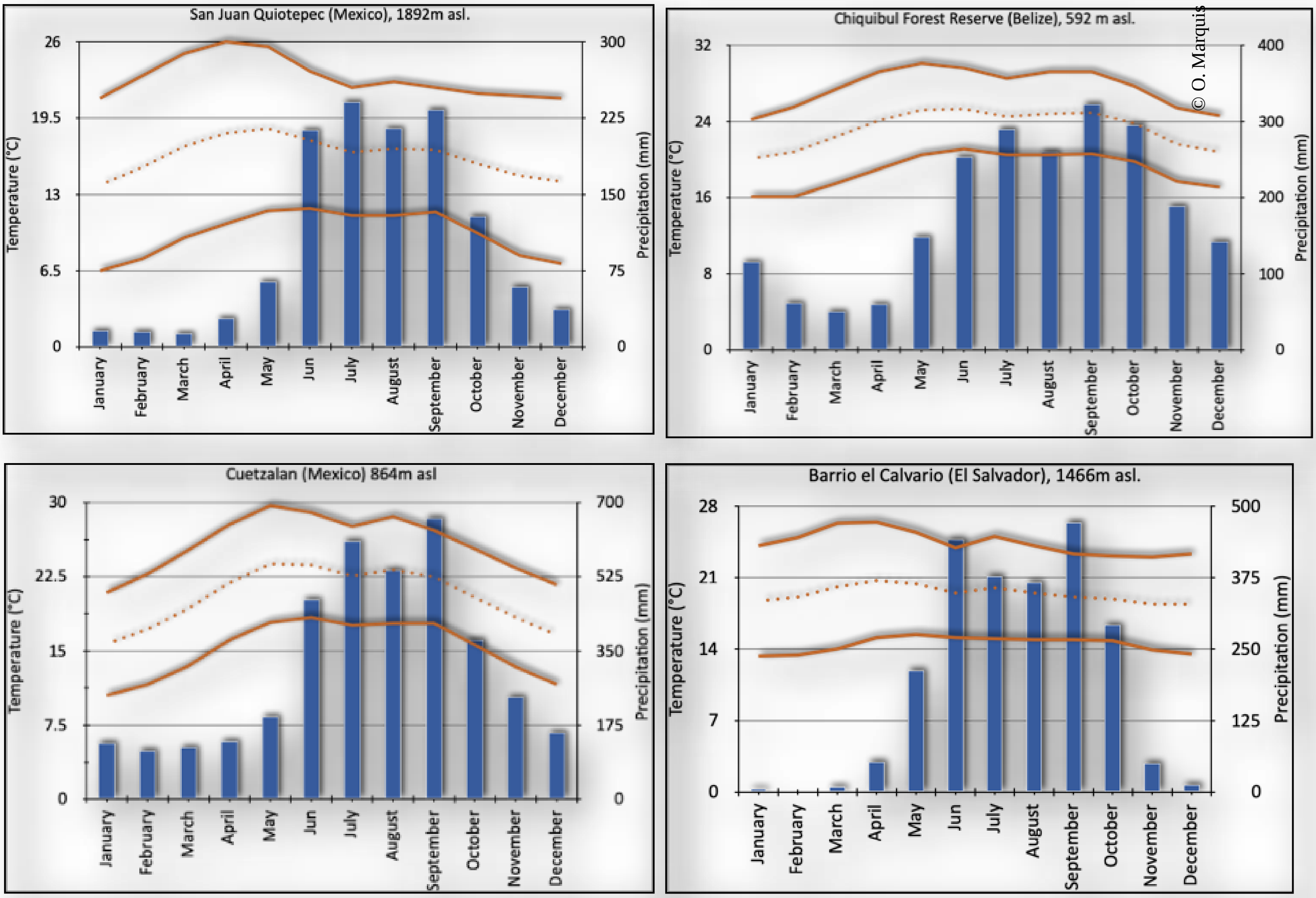


Figure 13 **: Monthly climatic data across the geographic range of *Agalychnis moreletii* (Source: https://en.climate-data.org)**

Analysis of climatic data in 5 locations (source : https://en.climate-data.org) at various altitudes across the distribution area (Figure 12 and Figure 13) show a strong seasonality in precipitation with the rainy season beginning in June and ending in October (mean amount of precipitation of 336.1 mm (206mm to 532.2mm, Table 2). Precipitation is lower for the rest of the year (mean amount of precipitation of 87 mm (33.1mm to 156.9mm, Table 2).

The rainy season seems to coincide with a slight increase in mean ambient temperature of 0 to 3.5°C according to the locations (Table 2). During the dry season the mean temperature seems to be around 18°C whereas it is about 20°C during the rainy season.

Night temperature seem to be quite low with a mean of 16.6°C during the rainy season and of 14.1°C during the dry season. There is an important variation across the altitudinal gradient.

Maximum daily temperatures remain quite low with a mean of 25.3°C during the rainy season and 24.6°C during the dry season.

There are strong variations in temperature fluctuations and precipitation between sites and, not surprisingly, along the altitudinal gradient. Living under large leaves during the day may prevent direct sunlight exposure but we can imagine that this kind of microhabitat exposes the animal to ambient climatic fluctuations. It is unclear if lowland and highland populations are adapted to the same amplitude of climatic variation, or if some local adaptation exists.

Table 2 : Summarised mean temperatures and precipitations between rainy (June to October) and dry season (November to May) (Source: https://en.climate-data.org).

		Rainy season		Dry season	
		mean	SD	mean	SD
Cuetzalan (Mexico), 864m asl	Mean T° (°C)	22.5	1.3	19.0	2.9
	Average min T°(°C)	17.4	1.1	13.5	2.7
	Average max T° (°C)	27.5	1.4	24.5	3.2
	Precipitation (mm)	532.2	113.5	156.9	45.8
San Cristobal Verapaz (Guatemala), 1389m asl.	Mean T° (°C)	19.3	0.5	17.9	1.4
	Average min T°(°C)	14.7	0.5	12.3	1.3
	Average max T° (°C)	24.0	0.5	23.6	1.6
	Precipitation (mm)	268.4	27.3	86.3	41.2
Barrio el Calvario (El Salvador), 1466m asl.	Mean T° (°C)	19.4	0.4	19.4	1.0
	Average min T°(°C)	14.9	0.1	14.1	0.8
	Average max T° (°C)	23.9	0.7	24.8	1.4
	Precipitation (mm)	389.6	69.8	49.1	74.8
Chiquibul Forest Reserve (Belize), 592 m asl.	Mean T° (°C)	20.5	0.5	17.7	1.6
	Average min T°(°C)	24.7	0.6	22.1	1.9
	Average max T° (°C)	28.8	0.8	26.6	2.3
	Precipitation (mm)	284.3	27.9	109.8	53.2
San Juan Quiotepec (Mexico), 1892m als.	Mean T° (°C)	16.7	0.7	16.0	2.0
	Average min T°(°C)	11.1	0.8	8.6	1.9
	Average max T° (°C)	22.4	0.7	23.4	2.2
	Precipitation (mm)	206.0	45.2	33.1	21.2

Interestingly, as some other leaf sitting neotropical frogs, the skin of *A. moreletii* has been show to reflect near infrared wavelength (700-900 nm) (Schwalm et al.,1977). Herrerías-Azcué, et al. (2016) demonstrated that reflectance in the near infrared spectrum allows frogs to absorb less heat than non-IR absorbing frogs and this reflectance may prevent overheating and also diminish water-loss.

1.4.3. IUCN conservation status

In general, the species' habitat is threatened due to a lot of human pressures. The populations in the south of Guatemala, Mexico and El Salvador are surviving on people's backyards and in some small private reserves (A. Zamora, pers. comm.). Populations like those in Las Cuevas Research Station in Belize are some of the few in a good state, which is why it is so important to preserve those genetic resources.
The species seems to be adapting to habitat degradation and alteration to some extent. However, the possibility of genetic reclassification leading to distinct separate populations or even subspecies means that this needs to be monitored closely as many of these populations seem to be barely adapting to the changed environment, and also show signs of low genetic diversity. Based on the study of 19 populations, the populations at Las Cuevas Research Station, Chiquibal Forest reserve in Belize, Sach Wa Já, Alta Verapaz in Guatemala and some sites in Chiapas in Mexico having good expected levels of diversity (A. Zamora, unpubl. data).
Among other threats, chytrid fungus (*Batracochytrium dendrobatidis*) gets a lot of attention. Lawson et al. (2011) recorded a prevalence up to more than 89% in the tadpoles of *A. moreletii* in several infected populations in El Salvador. Thanks to her PhD work, Alejandra Zamora demonstrated that Bd infected animals from the Guatemala populations exhibited a massive decline in the diversity of skin microbiota when compared to animals from non-infected populations (
Figure 14). Even though there are no documented cases of dramatic population declines in *A. moreletii* following Bd infection, the situation should be closely monitored.

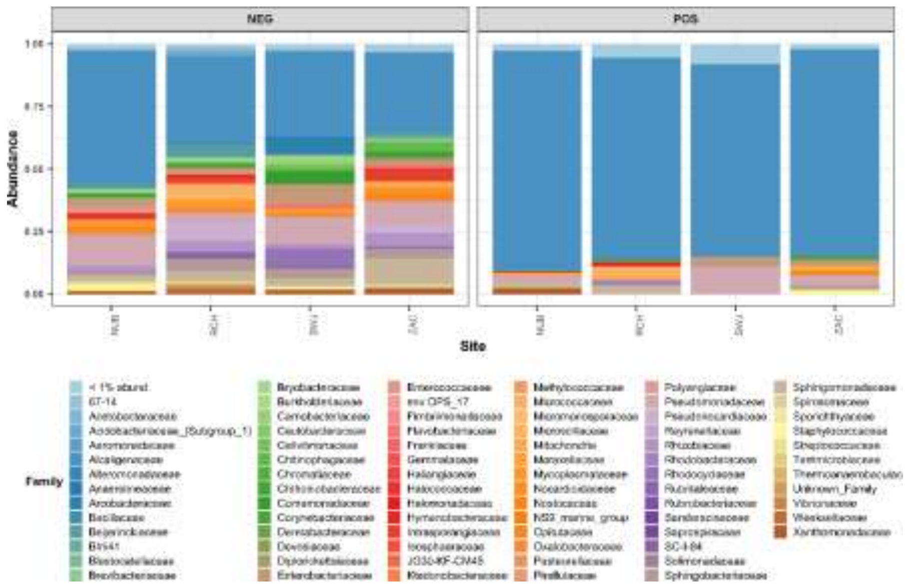


Figure 14 : Comparison of skin bacterial diversity between individuals from Bd non infected (NEG) and Bd infected (POS) population of *Agalychnis moreletii* in Guatelama (from A. Zamora, unpublished data).

The species is currently assessed as Least Concern on the IUCN Red List (IUCN SSC 2017). This species has undergone population declines across much of its range due to habitat degradation and the spread of Bd (Lips et al., 2004; Pounds, et al., 2006) and was originally assessed as Critically Endangered in 2004 due to the expected threat Bd posed to isolated populations. Fortunately, these populations have persisted, leading to the reassessment of the species in 2016.

The international trade of this species is controlled by the CITES regulation as the entire genus *Agalychnis* has been included on Appendix II since the 13th of March 2010. According to the CITES database at the time of writing (2021), since 2010, 495 individuals have been captured in the wild and exported (see Table 3 for details). Interestingly, only one international movement of captive bred animals (4 individuals) had been recorded by the CITES database (source C) exported from the UK to the USA in 2014 for scientific purposes.

Table 3 : Summary of exportations of wild *Agalychnis moreletii* between 2010 and 2021. (CITES trade statistics derived from the CITES Trade Database, UNEP World Conservation Monitoring Centre, Cambridge, UK.)

Year	Exporter	Importer	Quantity	Term	Purpose
2010	GUATEMALA	UNITED KINGDOM	6	bodies	Science
2010	GUATEMALA	UNITED KINGDOM	13	specimens	Science
2011	USA	ARGENTINA	1	specimens	Science
2016	EL SALVADOR	UNITED KINGDOM	69	specimens	Trade
2017	BELIZE	GUATEMALA	41	specimens	Science
2018	GUATEMALA	UNITED KINGDOM	365	specimens	Science

1.5. Diet and feeding behaviour (Wild)

Natural diet is not well known but nutritional analysis in Las Cuevas research center (Belize) show that the global diet was mainly composed of orthopterans. Surprisingly, diet analysis showed a very poor calcium content (R. Antwis, unpubl. data).

1.6. Reproduction

Reproduction is similar to other *Agalychnis* species. Males attract females by calling from a vantage point overlooking permanent or seasonal water bodies (Lee, 1996). The availability of water bodies dictates the reproductive periods of different populations. The breeding in some populations is controlled by the seasonal availability of pools for tadpoles to develop in with others using permanent water sources. In Las Cuevas Research Station (Belize), the breeding season usually starts in June and ends in September, depending of the extent of the rainy season (R. Antwis, pers. comm.). Amplexus (
Figure 15) can take place over several days before spawning. Successful spawning can lead to periods of axillary amplexus in excess of 6 hours (Briggs, 2008) during this time neither individual is able to feed, but males will continue to call (Briggs, 2008). Briggs (2008) reports a maximal density of 0.21 frogs/m^2 around one breeding pond. In the same study, Briggs reported a positive correlation between the SVL of amplectant males and females indicating that larger males amplect larger females. Ogilvy (2011) has put in light

variation of coloration between individuals partly due to carotenoids content especially in males. Nevertheless, she was been unable to show that carotenoid content would have been a sexual selection criterion for females as males calling for females did not have a higher carotenoid content than males already in amplexus with a female. Despite this result she was able to show that brighter males are more often associated with brighter females when comparing carotenoid content of pairs in amplexus.

Photo credit : V. Briggs

Figure 15 : ***Agalychnis moreletii*** **amplexus (top) and clutch (bottom).**

Gestation period/incubation

Clutch/litter/brood/offspring size/number

Sánchez-Ochoa et al. (2020) observed a strong preference for the plants of genus *Inga* as oviposition sites (48%). Clutch size may vary depending on the health and fitness of the parents. Briggs (2013) observed that number of eggs per clutches (N=39) ranges from 93 to 334 (mean 200.4 ±9.30) in animals from Belize. Gomez-Mestre (2008) noted an average clutch size of 71.5 ± 4.8 eggs (N= 20 clutches) deposited by animals studied in Guatemala. Gomes-Mestre et al. (2008) observed 15 *A. moreletii* clutches and noted that hatching occurred mainly at night, the peak of hatching being approximately 11 days after oviposition. The time taken for all the embryos to hatch after the first one has hatched is approximately 32h (temperature range from 20.58°C to 24.58°C). Hatching success observed by Briggs (2007) from wild animals that laid eggs in captivity was, on average, 38.05%. In the Neotropical Natural Protected Area of Nahá (Mexico), Sánchez-Ochoa et al. (2020) observed that 40.6% of the eggs developed to the tadpole stage. Hernández-Herrera et al. (2019) reported water temperatures from 16.5°c to 34.9°C in ponds where clutches were found in Chiapas region of Mexico.
The captive population held at Chester Zoo between 2010 and 2014 had an average clutch size of 145 eggs (N=14), this clutch size is corroborated by records from other holding facilities in the last few years 2017-2018 (B. Baker, pers. comm).
Briggs (2007), recorded in captive conditions that the average hatchling size (SVL) ranged from 5.6 to 9.9 mm (mean = 7.2 ± 0.03 mm), and tadpole total length ranged from 12.2 to 25.1 mm, (mean = 19.1 ± 0.08 mm). Briggs (2013), showed that bigger males produced bigger froglets.

1.7. Behaviour

Like most *Agalychnis*, the species is predominantly nocturnal. It is occasionally encountered awake during the day but this is usually in response to some form of disturbance and normally returns to sleep as soon as possible.
As with many *Agalychnis*, locomotion is generally a slow and *A. moreletii* is a hand over hand climber, occasionally reaching a bit of a jog, however they are capable of explosive leaps to escape danger or whilst hunting or searching for a mate. The individuals can, given the space and the inclination, glide from tree tops down in a controlled parachuting fall utilizing the extensive webbing between the toes.
Predominantly a solitary species, they are really only encountered together during the breeding season or when metamorphosing. Generally accepted as a non-explosive breeder, regional populations, reliant on temporary water bodies, do form small aggregations coordinated with the flooding of the breeding sites.
Vocal behavior of this species is well studied (Lee, 1996; Duellman, 2001; Briggs, 2010). Calls can vary within populations as well as between individuals (Figure 16); males with the most intense and varied calls appearing more attractive to females (Briggs, 2010).

A

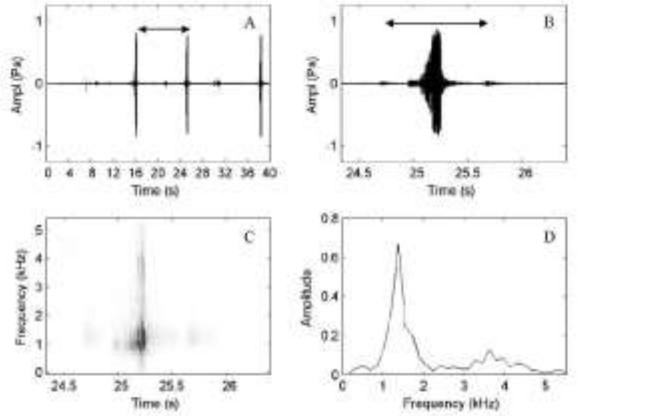


FIG. 1.—Advertisement call of *Agalychnis moreletii* as (A) an oscillogram of a sequence of calls separated by intercall intervals (denoted), (B) an expanded view of a single call illustrating the pulsed structure per call and the duration of the call (denoted), (C) spectrogram and (D) a power spectrum that shows the energy distribution across the call.

B

TABLE 1.—Variation of call traits of male advertisement calls of *Agalychnis moreletii*. Mean, standard deviation (SD range and between-male and within-male variability (CV = coefficient of variation) of call traits. *N* = total n recorded males followed by total number of calls analyzed, duration = mean length of call, DF = frequency and call intensity as MinAmp = minimum call amplitude per call, and MaxAmp = ma per call.

Call trait	Grand mean ± SD (*N* = [30] [575])	Range	Mean within-male CV, %	P
Total calls	19.2 ± 14.4	4–68		
Call rate (no./s)	0.03 ± 0.02	0.004–0.1		
Duration (ms)	39.3 ± 18.8	21.5–8		
MinAmp (dB)	54.2 ± 3.4*			
MaxAmp (dB)	60.6 ± 5.0*			
Intercall interval (s)	44.6			
Total pulses				
Pulses/call				
DF (Hz)				

Figure 16 : (A) Acoustic description of advertisement call of *Agalychnis moreletii* and (B) variation of calls traits among and between males (Extract from Brigss 2010)

Males become sexually active with the onset of the rainy season and descend from the tree tops to form aggregations around viable breeding sites and from here they begin to call to advertise their physical fitness and their location to the females and other males. The females usually respond after a few days if the rains continue and they then join the males around the spawning site (Briggs, 2010).

Multiple factors have been identified as possible determinants of mate choice by females. These include male body size (Briggs 2008), call trait/variation (Briggs 2010) and coloration (Ogilvy 2012). All probably have a role in the decision but ultimately the female will select the most attractive/suitable male. On occasion, multiple males will attempt amplexus with the same female at which point the individual's fitness and strength will almost certainly come in to play. Males defending a calling site will sometimes threaten conspecifics by shaking leaves and twigs with their forearms whilst balancing on them (Figure 17).

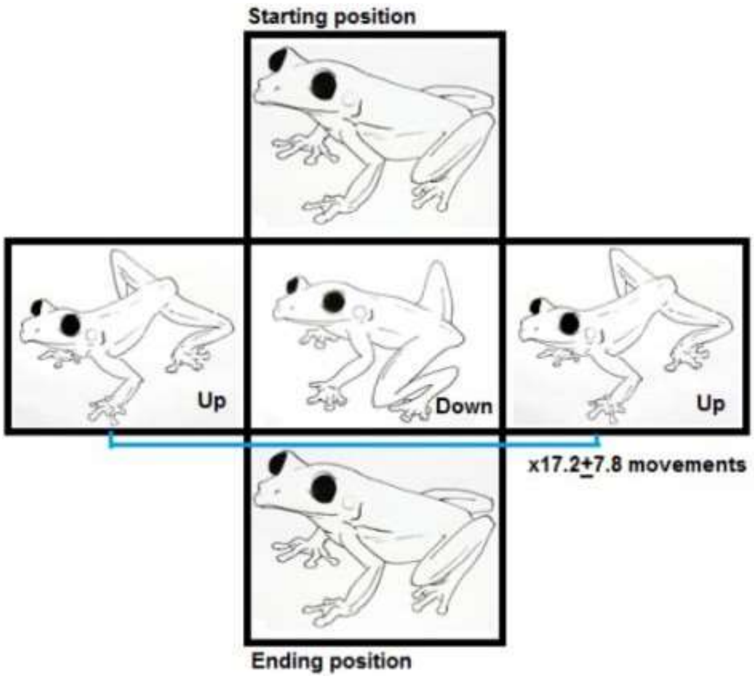


Figure 17 : Agonistic behaviour in *Agalychnis moreletii* (from Serrano et al. 2018).

2.1. Enclosure

2.1.1. Substrate

Being an arboreal species, there is no strict necessity to have substrate in *A. moreletii* enclosures. Any classical substrate like soil or coir will inevitably stick to the frogs when they come down to walk around, causing stress and skin issues. Therefore, if any "natural" substrate is used for growing plants, it should be covered in leaflitter or heavily compacted to prevent it sticking to the frogs. However, as this species naturally absorbs water through its permeable undersides, such as rainwater from leaves when in the wild, a method used by several collections is to add wet paper towel (to prevent any potential intoxication avoid chlorine bleached paper and printed paper) or matting on all or a section of the floor space of enclosure. These towels should be regularly changed, preferably daily. This, together with a daily water change, helps the frogs to hydrate in clean water. The cleanliness of the housing and water provided daily is key to maintaining the health of these frogs. During the changing of paper towel or matting, surfaces of the enclosure should be wiped clean with water and waste removed, as the frogs utilize the glass walls of an enclosure when active. The walls of the enclosure act as a substrate for the animals and therefore must also remain clean. Waste build-ups on glass surfaces may lead to irritation to the sensitive ventral surfaces of this species. Ideally, the substrate should be changed late in the day so that it is at its freshest when frogs become active. Being nocturnal, and best kept in the way highlighted above, it should be noted that these frogs do not make a good public display animal. However, where they are displayed, any substrate used should ideally be inert and easily cleaned, otherwise it will soon become a breeding ground for parasites and harmful bacteria, which can quickly affect the health of the frogs.

2.1.2. Furnishings and maintenance

In terms of physical structures within enclosures, in *Agalychnis callidryas* it has been shown that live plants are essential as they create many different microclimates amongst the leaves allowing the frogs to select their preferred resting place (Michaels et al, 2014). *A. moreletii* are leaf frogs and have adapted to naturally adhere and sleep on leaves, so the leaves need to be large enough and strong enough to support the frog across its whole body (
Figure 18). Leaves should also have a smooth, waxy surface – delicate or hirsute leaves are not suitable as resting sites. Plants that have been used with success with this species include *Ficus elastica*, *Anthurium andreanum*, *Aglaonema commutatum, Schefflera amate* and *Spathiphyllum wallisi*. When attempting to breed this species, leaf selection for egg deposition is an incredibly important factor to consider and plants used with success within rain chambers include *Philodendron* sp, *Monstera* sp, *Scindapsus aureum*, *Neoregelia spectabilis* and *Microsorium steerei*. Plants such as *Microsorium* sp. add a differing leaf shape but are rarely used by the frogs whilst the *Monstera* sp*., Scindapsus* sp*.* and *Spathyphyllum* sp*.* are used regularly, *Microsorum* offer a foliage that can provide a natural appearance and additional cover. *Neoregelia*, a bromeliad, is also used as a daytime resting area by sleeping frogs, whilst also providing a water above the ground. In

order to maintain good water quality inside the bromeliads, we advise that the water in leaf axels is replaced on a daily basis (manually or via the automatic misting system). Depending on the plant supplier it is important to verify if some chemicals (fertilizers, pesticides etc.) have been used on the plants. It is important to rinse the plants and change their substrate to prevent any chemical contamination which could be harmful to the frogs.

Alongside live plants every vivarium should have at least one piece of branch or liana to provide solid resting places. In addition, these act as raised vantage points and allow the food invertebrates to get off of the floor and up into the tree frog's preferred hunting zone. It is important that at least some of the branching is connected to the ground level of the enclosure to provide the frogs with easy access to the paper towel and water bowls to support hydration. Frogs can become very active at night and so climbing branches, while not used much during the day, can become an important means of moving around the terrarium at night.

Figure 18 : *Agalychnis moreletii* off-show terrarium at Chester Zoo. Note that adapted branches were not in place in the terrariums at the time of the picture. (Photo credit: A. Bland)

2.1.3. Environment

In terms of temperature, it is important to consider that *A. moreletii* is a mid- to high elevation species in comparison to the more commonly encountered relative, the red-eyed leaf frog, *Agalychnis callidryas*. *A. moreletii* requires considerably cooler temperatures. In the wild it lives in an elevation range of 600 m to approximately 1,300 m asl in montane tropical forest (Santos-Barrera, et al. 2004). It fills the same niche as

Agalychnis annae from Costa Rica, and is known to be the highest altitude frequenting leaf frog of all. As such, a general maintenance temperature of around 20-24°C should be maintained. Any temperatures above 24°C may affect this high-elevation frog's metabolic rate and overall health. Temperatures should not rise over 25°C for even short periods of time without affecting the frogs' health long-term and inducing periods of stress. Night temperature can drop to 15°C with no problem. Some captive specimens have been observed feeding at 10°C (C. Allen, pers. comm.). BIAZA UVTool (version 013, updated 15th of November 2021, http://www.uvguide.co.uk/BIAZA-RAWG-UV-Tool.htm) recommend a temperature range from 13-15°C (at night) to 18-20°C (during day) during summer with a drop to 10-12°C (at night) to 15-17°C (during day) during winter. A basking zone at 25°C should still be accessible.

A humidity level of around 50 - 70% relative air humidity is required during the day. Maintaining the enclosure in dry conditions during the daytime appears to be critical for this species. A misting system should raise the relative humidity to 80-100% in the evening before the frog's period of activity. A bowl of clean water is also required at all times. This allows the frogs to hydrate, slough and defecate at night when awake and this prevents dehydration during the day whilst they sleep. In the wild, these frogs only go down to permanent water bodies to reproduce and gain the moisture they need from absorbing rainwater or from condensation from the leaves on which they sit. Therefore, some collections effectively use damp paper towel or capillary matting to help the frogs sit on and hydrate more naturally. However, if used, this should be replaced or cleaned daily and fresh clean water used to remoisten. When misted during the afternoon it may be observed that this species uses its permeable ventral surface to absorb moisture from surfaces within the enclosure.

Vitamin D_3 is essential for calcium metabolism. Being mostly absent in insects, insectivorous reptile and amphibian dietary sources of vitamin D_3 are not sufficient to fulfil the needs of the organism. In most vertebrates, vitamin D_3 can be synthesized by exposing their skin to solar UVB radiation of 280-320 nm. The provision of the good amount of artificial UVB in captivity is now well documented in reptiles (see Baines et al. 2016 for a review) and more and more data are available in amphibians with varying results (Antwis et al. 2009, Antwis et al. 2014a, Michaels et al. 2015, Tapley et al. 2014, Verschooren et al. 2011, Whatley et al. 2020). If frogs are provided with insufficient levels of vitamin D_3 from the diet and via exposure to UVB, they may develop nutritional secondary hyperparathyroidism (NSHP) commonly known as nutritional metabolic bone disease (NMBD) as shown in *A. spurrelli* (Galante-Mulki et al. 2019). There are no published cases of this disease in *A. moreletii*, but it is certainly a possible condition in the species. Looking at the closely related species *A. callidryas*, Antwis et al. (2014a) demonstrated that a UV Index (UVI) of 1 as a background exposure (see Ferguson et al. 2004) with a sufficient calcium supplementation in the food (see "feeding" paragraph for more details) is sufficient to prevent any clinical signs of NMBD. Moreover, Michaels et al. (2014) show that in a planted terrarium allowing the creation of multiple microclimates *A. callidryas* can choose basking areas corresponding to an UVI around 6 and up to more than 9 (corresponding to a skin temperature increasing to nearly 30°C) without any noticeable negative health consequences. Nevertheless, *A. callidryas* living in a substantially different climate we cannot ensure that these parameters can be transposed to *A. moreletii*. The best way is, as usual, to provide UVB and temperature gradients within safe parameters in the enclosure and let the animals choose. Being a mid-altitude species subject in nature to significant variations in temperature and solar exposure during the day (see Figure 10 and Figure 11), we can hypothesise that this species is capable of withstanding significant environmental variations.

Too low UVB exposure can lead to metabolic bones diseases; however, too much UVB can equally be deleterious and can damage the skin and/or the eyes. Consequently, it is crucial that the output of UVB emitting lamps is checked and adjusted on a routine basis using a Solarmeter 6.5 (Solartech Inc, USA). Gradients have to be created using the branches and plant's leaves to create microhabitats and associated microclimates. The combination of more powerful lamps over lower-lying plants/branches and less powerful lamps over the higher resting sites can be an option to avoid frogs being over- or under-exposed to UVB. The BIAZA UVTool indicates that *A. moreletii* is a Ferguson zone 3 species, corresponding to an UVI mean exposure between 1 and 2.6 with a maximum range between 2.9 and 7.4 (Ferguson et al. 2010). Some exhibits use a combination of different lights including small 70-Watt spot lights to provide localized raised temperature (up to 30°C) over plants. These hot spots are frequently used as diurnal resting sites by the frogs. Other keepers use specialist LED plant growth lights and UVB emitting T5 fluorescent tubes. This combination allows for good plant growth as well as providing UVA UVB radiation and localized heat. However, this lighting array must be combined with good ventilation so that the temperature remains within the optimal parameters required for this mid-high elevation species (15-22°C). Heat spots may also cause more rapid dehydration and care must be taken to avoid this harming the frogs.

2.1.4. Dimensions

A minimum enclosure size of 45 x 45 x 60 cm is suitable for maintaining a small group, although for inducing a successful captive reproduction a larger vivarium will be required. Considering that this is an arboreal species, the height of the terrarium is an important factor. For this purpose, 60 x 45 x 90 cm glass vivaria have been used to maintain 3.1 – 4.2 reproductive individuals with success. Within these vivaria rain chambers can be created for breeding attempts by simulating a body of water and a period of rain. A breeding group of 4.4 animals was successfully maintained in a 90 x 90 x 60 cm at Berkshire College of Agriculture (BCA) (C. Allen, pers. comm.). Nevertheless, given the distance these frogs can jump and depending on the origin of the animals (wild caught *vs*. captive bred) we can recommend that they should be given as big enclosures as possible.

2.2. Feeding

2.2.1. Basic diet

This species is entirely insectivorous and as such should be offered good quality live invertebrates. Using crickets as a standard diet seems the best way of maintaining large populations due to their availability. Black field crickets (*Gryllus bimaculatus*) and locusts (*Locusta migratoria* or *Schistocerca gregaria*) are sometimes associated with rectal prolapses in the frogs and appear more difficult for them to digest (A. Gray, pers. obs.). Well gut-loaded brown crickets (*Acheta domesticus*) have proved to ensure a good frog health by some collections, such as at Manchester Museum, where they gut-load with bee pollen (A. Gray, pers. Comm.). Nymphs of brown crickets are also useful for smaller individuals, which may find them more palatable and easier to digest. It should also be noted that apart from black crickets being more difficult to digest they can also bite the frogs and injure them whilst they sleep. The use of black crickets is only recommended in moderation with other feeder invertebrates. A food source for crickets can be included in the vivarium to reduce the chance of insects injuring frogs.

The nutritional value of any feeder insect mainly comes from its diet. Consequently, all live food prior to being offered to the frogs should be appropriately "gut loaded" to ensure high nutritional value (Finke, 2003; Ogilvy et al., 2012; Bah-Nelson et al., 2021). As a general rule, insects are poor sources of calcium, vitamin D_3 and vitamin A. Consequently, the two more frequent nutritional diseases observe on amphibians in captivity are associated with improper Calcium:Phosphorus ratios in prey, and vitamin A deficiency (Ferrie et al., 2014).

Fresh fruits and vegetables are ideal for this, grated carrot provides high levels of beneficial carotenoids. Pure Beta carotene used as gutloading nutrient for crickets has been shown to have health benefits at least for *A. callidryas,* including improved fecundity and green colouration more similar to wild frogs (Ogilvy, Preziosi, & Fidgett, 2012). Carotenoids are important in antioxidant and immune defenses (Krinsky, 21994), and play a relevant role in vitamin A synthesis due to provitaminic activity (Blount, 2004). Other commercial insect diets may also be used, such as Repashy Super Load (REPASHY VENTURES Inc.). Live foods should also be dusted with a high quality vitamin and mineral supplement at least once per week.

As a general rule a 48h period of gut loading before feeding the frogs is recommended. It is important to note that the nutritional value of a gut loaded cricket decreases very quickly over time. Bah Nelson et al. (2021) have shown that adult black crickets lose all accumulated calcium content in less than 48h after gut loading period of 48h. As a result of this and the increasing chance of crickets attacking frogs as they become hungry, feeder insects should be added to vivaria late in the day and uneaten insects removed first thing the following morning so that they are only in the vivarium while frogs are actively hunting.

2.2.2. Method of feeding

Feeding regimes, sizes, and amounts will vary, but as a basic rule each adult frog can be fed the equivalent of no more than 3-4 crickets (approx. 20mm in size) 2 or 3 times per week. Food must be offered at the end of the day after the enclosure has had its daily spray, as this wakes the frogs as the lighting goes out and this species is only active at night. The food can be dusted with a quality calcium/vitamin powder (several commercial calcium complement can be easily found as, for example, Repashy calcium plus) and then scattered around the enclosure. Care must be taken not to drop live food into the water or release the dusting powder to where the frogs could come into direct contact with it whilst awake. As a general rule, prey should be consumed by the frogs as quickly as possible as it has been shown that approximately 50% of the powder dusted on the insects is lost in the first 2.5 minutes (Li et al. 2009). Moreover, Michaels et al. (2014) showed that the speed of calcium powder loss significantly differed between cricket species and between instars. Arboreal feed dishes containing dietary supplements powder can be used to increase the time that insects stay in the vitamin/calcium powder by preventing insects to spread in the terrarium and maintain insect's supplementation.

2.2.3. Water

A. moreletii often urinate in the water whilst hydrating. Although the water may look clear and clean, it requires daily changing.
Dechlorinated and filtered water to remove potential traces of heavy metals and other pollutants (nitrates, pesticides, etc.) should be provided in a shallow bowl (for example 20cm of diameter and 3cm of water depth). This can be Heavy Metal Axe (HMA) filtered tap water (heavy metal reduction) or re-mineralized reverse osmosis (RO) water but not pure RO water. Some commercial products are available for RO water remineralization but see Odum & Zippel (2008) for a DIY solution. The water should be changed daily and water dishes scrubbed to remove and bacterial build up/biofilms.

2.3. Social structure

2.3.1. Sex ratios

Captive social groups can vary greatly with all male, all female, and mixed sexed groups all cohabitating as long as the actual population density is not too high for the size of the space available (see 2.1.4). A terrarium of 45 x 45 x 60 cm is adequate for a group of four animals of mixed sex. When maintained outside of environmental breeding conditions multiple males do not cause each other stress, or undue stress on females. When attempting to breed these frogs, multiple males should be mixed with one or two females; male competition spurs the females into condition; however, males often suffer from stress if kept in breeding conditions for long periods of time: the increased physical outlay and intra-specific competition taking its toll physically and this may be by a rapid loss of body condition. For this reason, it is important to follow the humidity level guidelines herein when not wishing to induce breeding. In non-breeding setups, the humidity should be kept relatively low (around 60% RH) during the day and the enclosure should only be sprayed once per night as the lights go out. Frogs should only be put into rain/breeding chambers for short periods (1 week maximum) and should have a constant high humidity, otherwise these frogs will quickly succumb to health issues due to stress. The same stresses could also be triggered in collections where large numbers of calling males, housed within separate enclosures but within the same room, begin calling against each other (A. Gray, pers. obs.). This increased social stress can lead to secondary diseases/infections. It is also worth noting that, as with other *Agalychnis*, the females of these species may require breeding simply to ensure they do not become egg-bound, which can also lead to serious health problems in these frogs (A. Gray, pers. obs.).

2.3.2. Sharing enclosure with other species

Mixed species exhibits can make very interesting and educational displays and as long as the environmental parameters of the species are met and there is no risk of aggression or predation this can be successful. *Agalychnis moreletii* has been successfully displayed alongside dendrobatid frogs as well as with cone-headed lizards (*Laemanctus* sp.) (B. Baker, pers. obs.).
It is important to remember that these frogs sleep during the day and are indistinguishable from other leaf frogs of the genus *Agalychnis* when sleeping. Also, these frogs eat medium-large sized crickets so it is important to take this into account if thinking of mixing them with smaller species (such as *Dendrobates* sp.) which may be stressed by

having large crickets in their enclosure. If mixed with other diurnal species, it is important to provide adequate food regimes for this nocturnal species and feeding best takes place in the early evening, just before the animals become active.
There is no risk of hybridization between *A. moreletii* with other *Agalychnis* species despite with *A. annae* with which it can hybridize in captivity (Gray, 2011).

2.4. Breeding conditions/Triggers

As previously mentioned, this species requires a waterbody to lay eggs above and, in most cases, will also require simulated rainfall and increased humidity to trigger reproductive activity. To achieve this, the frogs will need to undergo appropriate seasonal cycles of temperature and humidity in preparation for breeding. According to the seasonal climatic variations experimented in the field by this species (see 1.4.2) the frogs should be maintained in the dry daily environment before being moved into a rain chamber with higher relative humidity and artificial rainfall.
By increasing food and a period of slightly lower humidity the frogs will become conditioned ready for spawning. In Berkshire College of Agriculture, amplexus have been induced by lowering the temperature to 21°C during the day and 12-13°C at night for several weeks (C. Allen, pers. comm.). Animals are ready for mixing in rain chambers when the eggs can be seen through the transparent skin of the flanks of gravid females and when males to have developed nuptial pads on their 1st finger. Here the frogs are cooled for up to three days and no rainfall is provided. The water in the enclosure base should be cold (15 - 18°C). Following this period, an aquarium water heater is used and to increase the water temperature to 22 -24°C and the ventilation restricted causing a rapid increase in humidity. The rainfall is then switched on and this is controlled by an aquarium pump with connected pipework. Note that this cycling method has been shown to work with other *Agalychnis* species (Bland 2013; Bland et al. 2021). The artificial rain is to remain on during the daytime and turned off at night, coinciding with the activity period of the frogs. The frogs do not like being under the rain when awake, and in the wild they spawn after a rain shower, not during it. The rain provides the stimulus required to inform the frogs that it is the correct time to spawn, but continual rainfall causes stress, spawning during rainfall decreases success as eggs may be damaged and displaced from the spawning site. Males may often begin to call as soon as the humidity increases at night and amplexus and spawning usually takes place within three days (
Figure 19).

Figure 19 : ***Agalychnis moreletii*** **in amplexus (Photo credit C. Allen).**

The frogs usually spawn in the evening or early morning. If no spawning takes place, leave the rainfall regime as above but replace the water to prevent waste accumulation. If there is no spawning or signs of reproductive behavior after five days, the pump should be turned off and the standing water removed or the frogs should be removed from the rain chamber and returned to their dry-season vivarium if available. Longer periods of rain conditions will cause health issues such as eye infections, high stress levels, and the frogs will need to feed (as they should not be fed whilst in high reproductive mode in the rain chamber).

2.4.1. Pregnancy/Egg laying and incubation

The spawn is usually deposited on smooth clean leaves or on the vivarium glass (
Figure 20).

Photo credit : B. Baker

Photo credit : T. Eisenberg

Figure 20 **: Various egg-laying substrates for** ***Agalychnis moreletii.***

The spawn develops quickly and fertility can usually be confirmed by day two. In the literature clutch sizes for *A. moreletii* are given as 25-75 (Campbell, 1998) and 23-77 (Duellman, 2001; average 49) eggs. Duellman (2001) attributes a proven clutch of 94 eggs to presumably two and a clutch of 103 eggs to presumably three egg layers, because the eggs in the clutches contained different stages of development. Gomez-Mestre et al. (2008) observed a mean clutch size of 71.5 ± 4.8 eggs (N = 20 clutches).
In 2012 the captive population of Chester Zoo produced eight clutches over a four weeks period with an average clutch size of 145 eggs and a fertility rate of nearly 97% (B. Baker, pers. obs.).
Tobias Eisenberg, a German private keeper, obtained a first clutch composed of 205 eggs, of which 179 were fertilized and a second clutch several weeks later composed of 628 eggs (414 fertilized, 214 unfertilized) from a single female.
We recommend leaving the clutch in situ until tadpoles hatch. Tadpoles can be collected as they hatch by placing a water cup below the leaves / glass where the eggs have been deposited (

Figure 21). Nevertheless, the spawn can also be moved to continue its development in a separate vivarium. The spawn on the glass can be easily removed using a small laminated piece of card and then transferred on to a replica leaf over shallow water (40-50m). Spawn attached to leaves are best left attached and the leaf removed and similarly rested over water (
Figure 21).

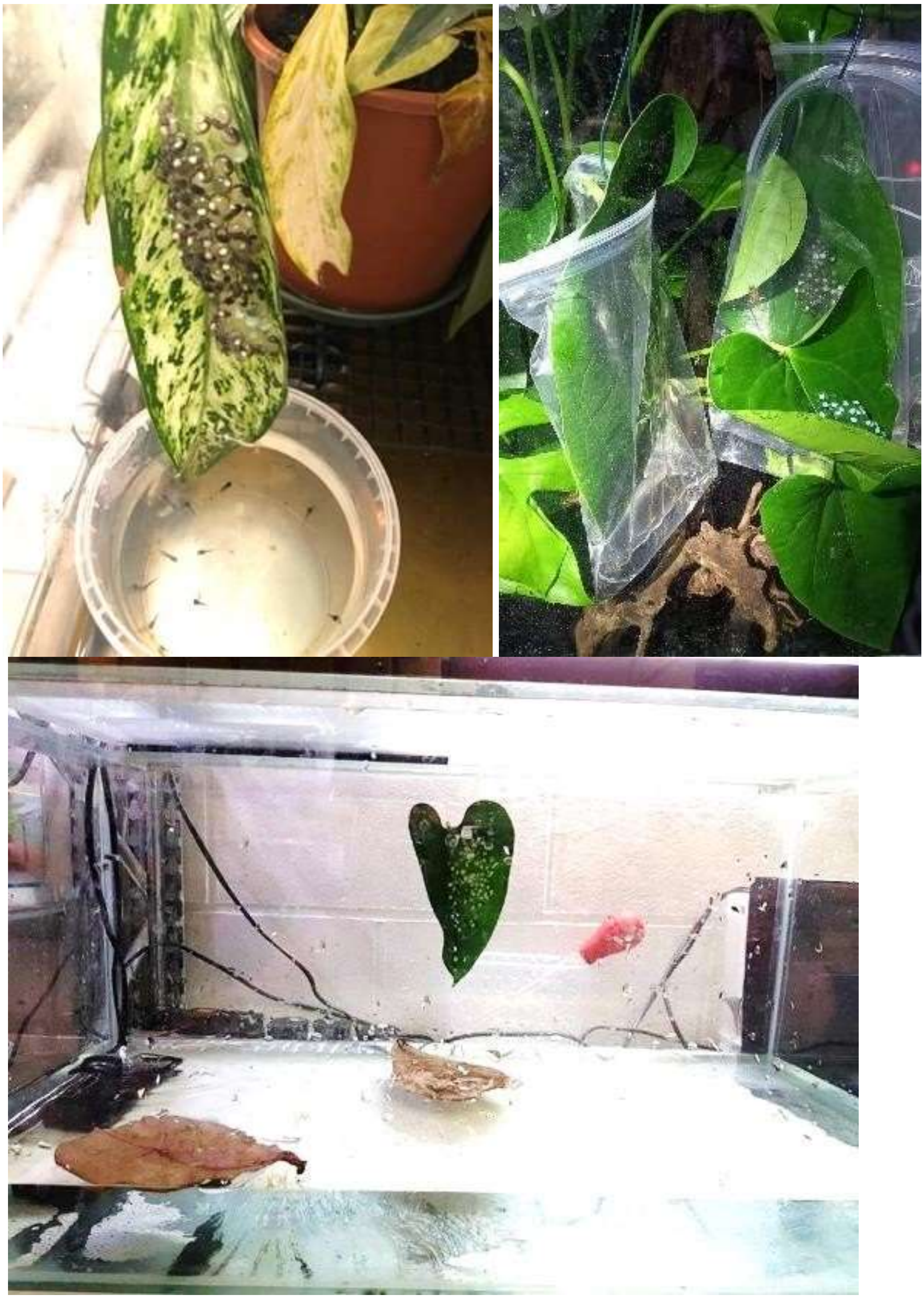


Figure 21 : Left: water cup collecting hatching tadpoles of *Agalychnis callidryas*. Center: example of "incubation tank". The leave where eggs mass was glued by the female is fixed over the water during the embryonic development. Right: plastic bags plastic bag wrapped around the leaf where the eggs are placed to collect the tadpoles when they hatch. The same methods can be employed for *Agalychnis moreletii*.

2.4.2. Birth/Hatching

Hatching can take place at any point between 7 - 10 days at 20-24°C although too much disturbance can cause earlier hatching times and lead to higher tadpole mortality (A. Gray, pers. comm.). In *A. callidryas* it has been shown that embryos react to certain vibrational stimuli that may indicate the presence of a predator by anticipating their hatching (Warkentin et al. 2006). Hatching plasticity in reaction to environmental biotic (i.e. snake predation) and abiotic (i.e. flooding) risk (up to nearly 30% of time earlier than spontaneous hatching) had been shown to be ancestral and shared by many phyllomedusine species (genera *Agalychnis* and *Pachymedusa*) and *A. moreletii* is able to hatch 36% earlier than the first spontaneous hatching following a disturbance (Gomez-Mestre et al. 2008) corresponding to 168h (7 days) after oviposition while the modal peak of normal hatching time is near 265h (11 days). Nevertheless, premature hatching has a cost while Gomez-Mestre et al. (2008) have shown that premature hatchlings of *A. moreletii* are 18% smaller than tadpole hatching spontaneously.
The tadpoles jump and wriggle their way down the leaf and drop into the water below. When hatching begins, it may also be beneficial to lightly spray the eggs at this stage as it may aid the process by stimulating tadpoles to hatch, the water run off also helps the tadpoles wriggle from the clutch and leaf to a point where there are able to drop into the water (A. Bland, pers. obs.).

2.4.3. Development and care of young

The tadpoles of this species are generally unproblematic to rear to metamorphosis. A 60-litre aquarium is suitable for rearing each clutch, filled to a depth of around 150-200 mm with treated tap water (see 2.2.3) at a temperature of 18-22°C, dead oak leaves or floating plants may be used for cover. The tadpoles can be fed high-quality fish flakes ground into a fine powder and sprinkled on to the water surface. Sera micron fish fry food (Sera GmbH) is an example of highly recommended food source as this contains a quality, varied powder diet, and is particularly useful when tadpoles are newly hatched and unable to graze on larger food items and more prone to filter small floating particles. Feed as much as can be consumed by the tadpoles within a few minutes, twice per day. Any uneaten food must be siphoned out and the water replaced. Partial water changes of 25% water volume should be undertaken one to two times per week and biological filtration may be provided by the use of air line sponge filters. Care must be taken not to cause a high flow rate as this species occupies still water and an increased flow rate causes stress and decreased body condition (A. Bland, pers. obs.). Tadpoles of this species seem to be quite resistant to variation in water pH and to nitrates but not tolerant to nitrite contamination (A. Bland, pers. obs.). Rachael Antwis has been able to analyse water quality in four breeding ponds of *A. moreletii* in Belize (R. Antwis, unpubl. data). Results show substantial variability of water quality between breeding ponds (

Figure 22) suggesting that tadpoles of this species might be tolerant to an important range of water quality. Nevertheless, in captivity water quality should be monitored on a weekly bases with the following reference values:

- Ammonia: <0.2 mg/L
- Nitrates: <50 mg/L
- Nitrites: 0 mg/L
- pH: 7

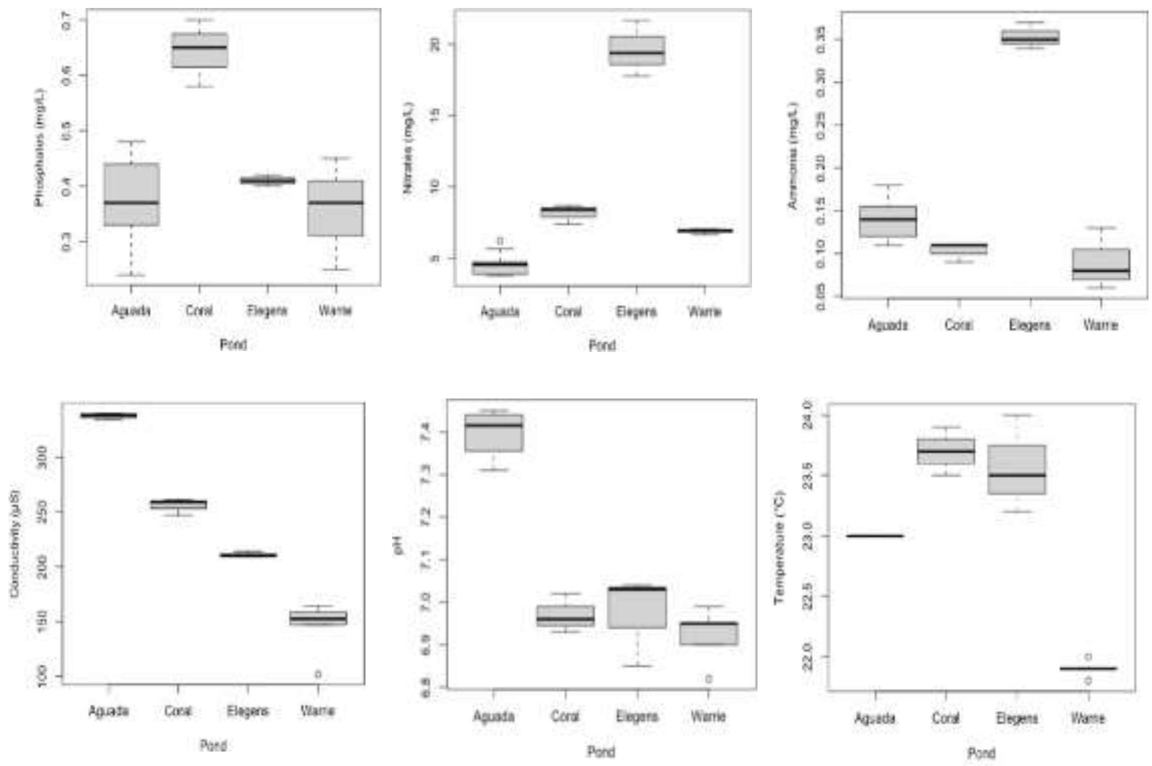


Figure 22 : Water quality analysis in four breeding ponds of *Agalychnis moreletii* in Las Cuevas, Belize (data collected by R. Antwis).

The tadpoles grow quickly and metamorphosis can occur as quick as 60 days; however, 80-100 days is more common, with some tadpoles within each clutch taking considerably longer. After metamorphosis frogs will try to leave the water with a nearly full tail (Figure 23) but absorb the tail over the first three to four days during which they will not feed; feeding takes place only once the tail is fully absorbed.

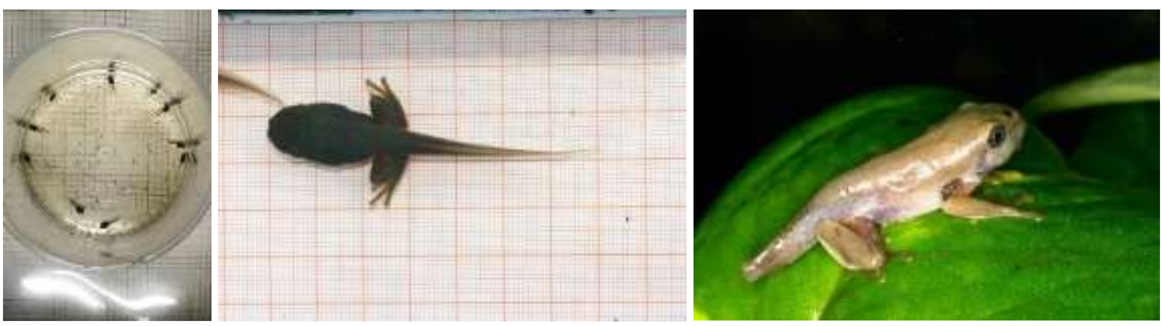


Figure 23 : Illustration of various stages of development in *Agalychnis moreletii* (Photo credit T. Eisenberg)

2.4.4. Population management

Animals may occasionally spawn without any planned cycling (B. Baker and T. Eisenberg, pers. obs.), although this is uncommon and potentially an effect of incorrect environmental conditions. Generally, using the above outlined methods breeding can be controlled which reduces stress on animals during non-breeding conditions by reducing stress between competing males and undue stress to females from males.

Considering the potential high fecundity of the species, in order to control the captive population in accordance with EEP recommendation eggs or tadpole's destruction could be needed. In this case, all or part of the eggs can be frozen in the freezer. If tadpole numbers have to be reduced, an overdose of anesthetic (MS222 is usually used) administered by a vet can be used to humanely euthanize the tadpoles.

2.5. Behavioural enrichment

Enclosure design can play a large part in enrichment, at least poor design and lack of live plants can have a negative effect on the animal's wellbeing (Michaels et al, 2014), whilst novel feeding devices that slow the emergence of the live foods could be used as a form of enrichment but have not been tested. Further research is required in this field for amphibians in captivity. Nevertheless, Michaels and Prezioi (2015) have shown that aquarium enrichment with live or artificial plants decreases *A. callidryas* tadpole's activity and leads tadpoles to metamorphose significantly later and at a larger size compared to no shelter conditions. Consequently, we can hypothesis that environmental enrichment could have a positive impact of the fitness of the tadpoles.

2.6. Handling

2.6.1. Individual identification and sexing

As mentioned previously males and females can be identified as adults primarily by their size, and in some case by the presence of skin distortion around the gular fold caused by the excessive calling of the males. Males in breeding condition can easily be identified by their dark nuptial thumb pads (
Figure 24), males also possess a more truncate snout when compared to the broad snout of a female (
Figure 25).

Figure 24 **: Nuptial thumb pad in *Agalychnis moreletii* (Photo credit: A. Bland)**

Photo credit: Morgan White - Josh' s

Figure 25: *Agalychnis moreletii* sexual dimorphism (bloodline from Guatemala).

Individual identification is usually done by the dorsal pattern of white spots. These do change over time as additional spots form; however, most animals can be identified by distinct clusters of dorsal spotting. This identification method has proven to be efficient in *Agalychnis lemur* for which dorsal pattern do not change drastically after 18 months of age (T. Skelton, pers. obs.). In addition to this, if more detailed identification is required the species can be microchipped using micro-RFID chips in the upper thigh, allowing the 7mm ISO chips to sit parallel to the limb between the skin and the muscle (
Figure 26). This method has been experimented at Chester Zoo with no apparent visible health issues and a good stability of the transponder (very few microchips lost). Nevertheless Antwis et al. (2014b) have shown that injection of a microchip drastically modifies the skin microbiota of *A. moreletii* within 24h after injection and during approximately two weeks. Consequently, microchipping can be a source of short-term weakening of the frog. For this reason, we recommend a limited use of this technique in *A. moreletii* and microchipping should only occur when other individual identification techniques are not possible. Moreover, we do not recommend the microchipping of individuals in the field and, if done on captive animals, they should be held in very strict hygienic conditions during at least two weeks after marking.

If microchipping has to be done, skin glue has been tried for re-sealing the entry incision. It is import to make sure that the glue is fully dried before releasing the frog otherwise or the legs may become stuck to the body of the frog. The microchipping process should be controlled at all times by the frogs' restrainer as even a slight movement by the frog could lead to a tear in the frog's skin. We have found it useful to use a slightly damp paper towel as a base whilst microchipping, the frog can be held firmly against the paper towel by one

hand and leaving the second hand free to manipulate the leg to allow free access to the upper thigh (
Figure **26**).

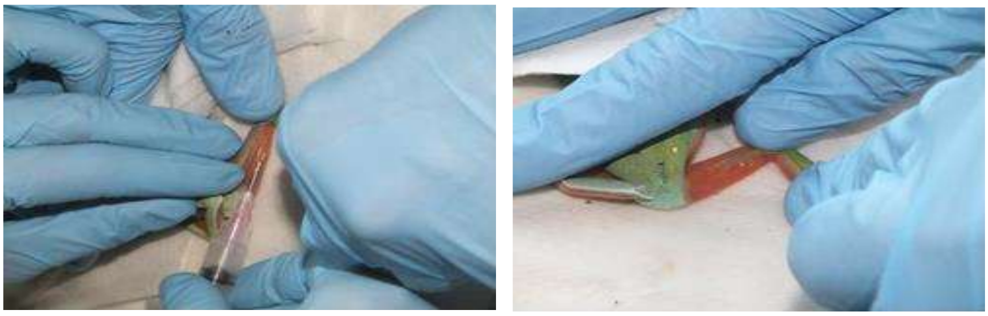
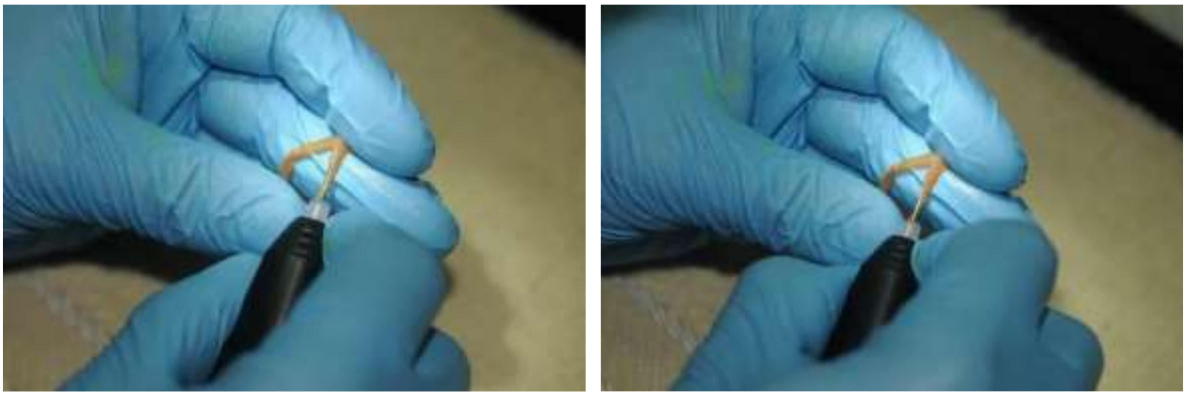


Figure 26 **: Injection of microchip for individual marking *Agalychnis moreletii* (Photo credit: B. Baker).**

The use of Visible Implant Elastomer (VIE) has been tested in Chester Zoo to mark froglets (
Figure 27). The harmlessness of the method and its stability will depend on the appropriate use of the equipment and the experience of the operator. The VIE can move under the skin and can be rejected through the skin at the injection point. It can also cause secondary infections in adults (B. Baker, pers. comm.). Moreover, in cane toads (*Rhinella marina*), VIE has been shown to be able to migrate into the kidneys and cause histologically apparent damage (Cabot et al. 2021). Nevertheless, Antwis et al. (2014c) have shown in *A. callidryas* that VIE injection in adults has no effect on physiological stress and a moderate impact on the epidermal microbiota anyway drastically lower than the effect shown in *A. moreletii* by microchipping in Antwis et al. (2014b).



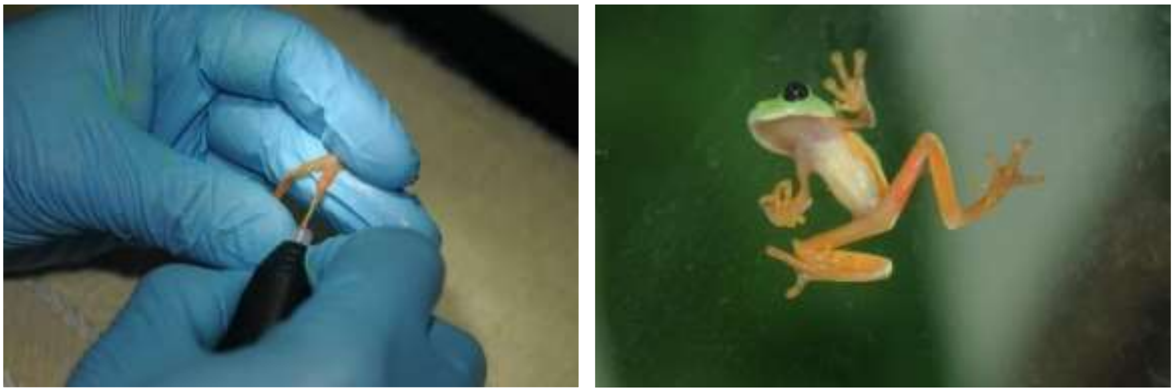


Figure 27: Injection of VIE to mark *Agalychnis moreletii* froglets (Photo credit: G. Garcia).

Chester Zoo also experimented VIE marking on tadpoles with no apparent detrimental effects and tadpoles (Figure 28).

Figure 28: Injection of VIE to mark *Agalychnis moreletii* tadpoles (Photo credit: G. Garcia).

More recently, Fouilloux et al. (2020) have tested VIE marking on small tadpoles of *Dendrobates tinctorius* (mean weight of 0.12g) with no effect on survival nor growth and a tag retention of 81% after 1 month and 50% after 3 month which correspond to metamorphosis time. Finally, tags were still visible in 36% of post-metamorphic individuals.

2.6.2. General handling

Handling of *A. morletii* is fairly straight forward as the frogs remain motionless even when peeled off of leaves or vivarium glass. However, there are a few basic techniques that can

help when handling frogs for veterinary examinations/procedures etc. when their activity will be increased and restraint necessary. As with all amphibians it is important to wear protective, powder free, nitrile gloves to prevent disease transfer as well as the negative effects of contact with human skin, for general examination a “thigh hold” allows easy access to the majority of the body and fore limbs, by holding both thighs, above the knee joint, the frog can be safely immobilised with one hand allowing the second hand to support the frog’s forelimbs and body (
Figure **29**). For examination of the rear limbs and underside a “cradle” technique can be employed, this can be undertaken belly up or belly down depending on the focus area, to do this the frog is grasped with its head facing the wrist and the legs between the thumb forefinger and index finger.

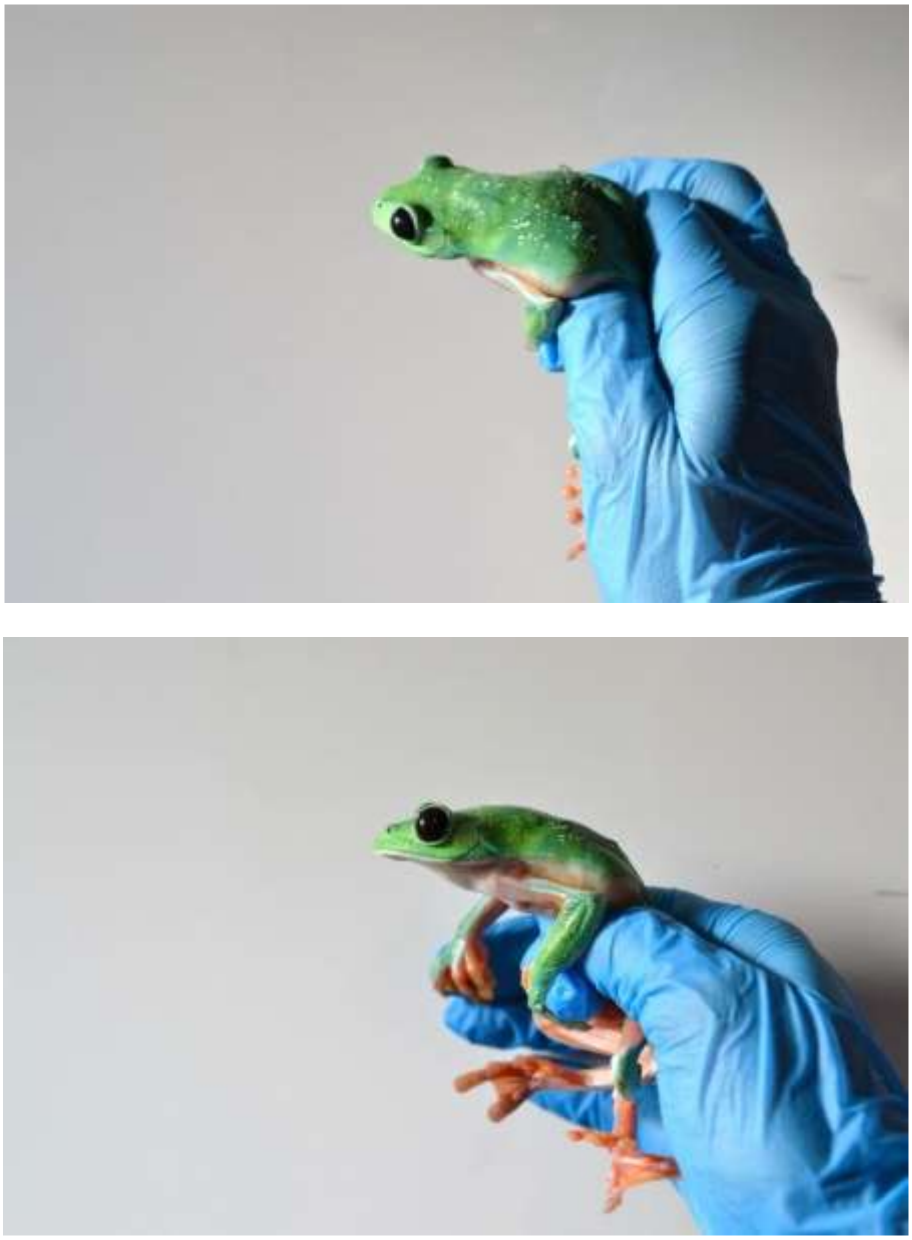


Figure 29**: Handling method for *Agalychnis moreletii* (Photo credit: A. Bland)**

Transportation of the frogs is much the same as other amphibian species and should only

be executed if the prevailing weather conditions rule out extremes of high (above 28°C) or low (below 15°C) temperature. A humid, clean substrate as paper towel can be used to prevent desiccation during transport (
Figure 30).

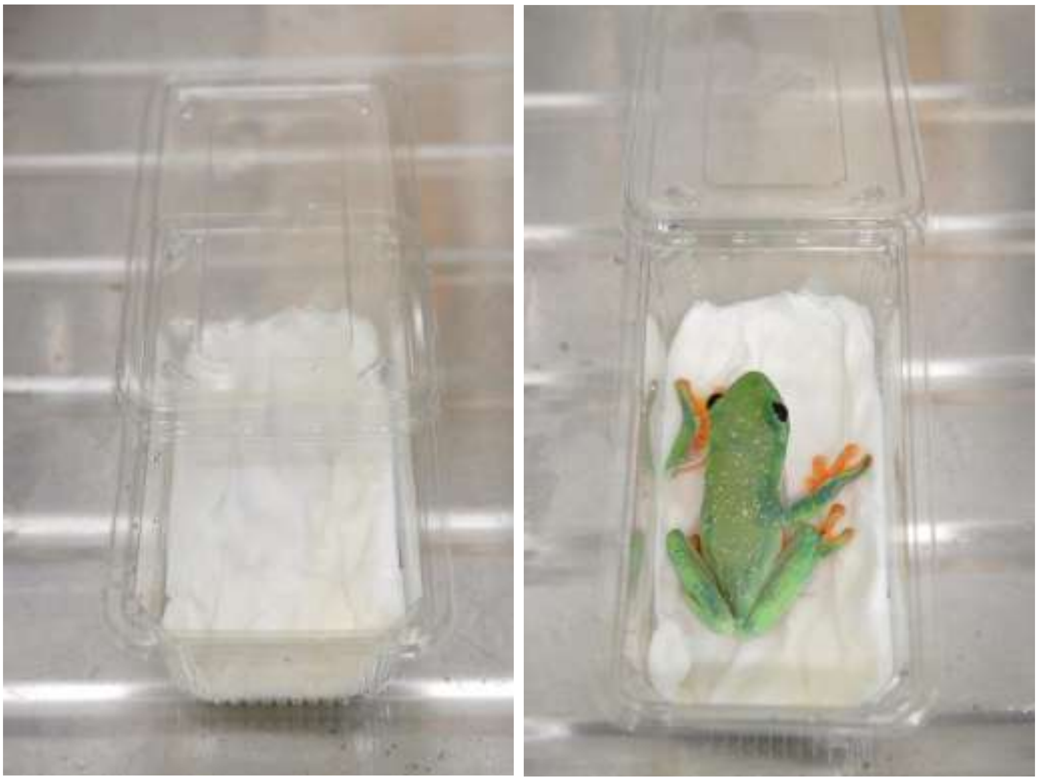


Figure 30: Example of transportation box (Photo credit: A. Bland).

2.6.3. Safety

As with all amphibians, disposable powder free vinyl gloves should always be worn to protect the frogs and the handler. The systematic use of gloves protects the skin of the frog from abrasion, prevent contamination between the animals and the handler and the spread of pathogens between animals (CCAC, 2003). Nevertheless, the glove type is important as it has been shown that latex and more marginally vinyl gloves can be toxic for at least some tadpoles (Gutleb et al., 2001; Cashins, Alford, and Skerratt, 2008).

2.7. Veterinary: Considerations for health and welfare

As for the majority of other anuran species *Agalychnis moreletii* are susceptible to be infected by the pathogenic fungus *Batracochytrium dendrobatidis* (*Bd*) and/or carry *Batracochytrium salamandrivorans (Bsal).* Considering its high pathogenicity Ranavirus is also currently feared. Consequently, a quarantine period is absolutely required for every new individual entering the captive population. This quarantine must take place in a dedicated room with dedicated equipment. The duration of the quarantine will depend on the result of health screening. For example, in Paris Zoo individuals are considered *Bd* free only after three consecutive negative PCR tests at two months of interval.

Disinfectants that have been proven to work against *Bd* and *Bsal* (Van Rooij 2017), and *Ranavirus* (Bryan et al. 2009) are:
- Clorhexidine at 0.75% for 1minute.
- VirkonS® at 1% for 2minutes.
- Bleach 4% for1 minute.
- Note: F10® at 1:1000 for 1 minute and Safe4® undiluted for30 seconds have proven to be effective against *Bd* and *Bsal* but not tested against *Ranavirus*.

During the quarantine, solid wastes have to be placed in double bags and disposed of by incineration. Waste water has to be treated with sodium hypochlorite 4% before releasing into the environment.

Testing requirements during quarantine are not specific to *Agalychnis moreletii* should include:
- Screen for Bd and Bsal during quarantine (minimum twice, two months apart if the status of the origin collection is positive or not known).
- Full post-mortem examination of any frogs that die during quarantine (including histology and testing for *Ranavirus* and chytrids as well as faecal parasitology).
- Faecal parasitology exam: examination by direct preparation and flotation of one three-day pooled faecal sample.

Before releasing from quarantine should have:
- Established cause of any death or illness.
- Confirmation of negative Bd/Bsal status.
- Faecal parasite status (free from novel parasites, report to EEP any novel parasites observed).

Several recurring issues have been identified with the most commonly occurring being the cyclic fluctuations of *Strongyloides* and *Rhabdias* sp. parasite burdens. These can rise to reach seriously life-threatening levels leading to rapid weight loss and sometimes even death. However fecal testing often gives negative or results indicating low levels of infections due to the fluctuating levels of infection, taking this into consideration as long as the enclosures are kept as clean as possible with regular paper changing and surface cleaning the frogs are able to cope with what is most likely a naturally occurring problem.

For more general considerations about amphibians' diseases in the context of ex-situ conservation programs you could refer to Pessier and Mendelson (2017).

2.8. Specific problems

As a general rule, any health issues with this species occur when it is maintained in temperatures that are too high or in overly humid conditions for extended periods.

Agalychnis are very prone to nematodiasis, which amplifies rapidly in soil or filthy water dishes and is very hard to resolve even when treated. Ivermectin and it's close relative Moxidectin have been used with good results against nematodes at the dose of 0.2mg/Kg (J. Lopez, pers. com.).

Cloacal prolapse is also quite common, especially in females and especially in animals with poor calcium status and/or high worm burdens. The causes of cloacal prolapse are multiple. The two most common are metabolic bone disease (MBD) and parasitosis. Other causes are possible such as egg retention, presence of a coelomic mass (organomegaly or tumor), or presence of an obstructing foreign body.
The first point before any attempt at treatment is therefore the identification of the cause of the prolapse. In the case of parasitosis, an antiparasitic treatment must be put in place. In the case of MBD, supplementation with calcium and vitamin D_3 as well as verification of the UVB dose accessible to animals is necessary. In the case of egg retention, hormonal triggering of egg laying can be considered. In the case of a tumor or the presence of a foreign body then surgery will certainly be necessary.
Management of the prolapse itself requires assessment of the viability of the prolapsed mucosa. If it is pink, it must be moistened with a solution of physiological liquid and put back in place manually. If it is necrotic, surgical trimming is necessary.
The prognosis is generally quite poor in the case where the cause is an MBD but is generally rather good in the case of a parasitic cause. For other causes the prognosis is quite variable.

Nose abrasion (
Figure 31) is extremely common especially in wild caught animals, but also in animals housed in poor conditions, too small tanks or where they have access to mesh wall of a terrarium. It can usually be resolved if the animals are kept dry and prevented from doing further damage. This can be done by avoiding any source of environmental stress: keeping animals at low density, furnishing enough and suitable resting places, avoiding use of abrasive materials in the terrarium (rocks for example). Opacifying the wall of glass terrarium can also prevent animals to jump against glass. Secondary bacterial or fungal infection may appear after nose abrasion. Increased monitoring is therefore necessary and cleaning and disinfection of the injured area is necessary.

Figure 31 : Nose abrasion in *Agalychnis moreletii.* © Chester Zoo

Irregular bacterial infections of the parotid region have also been observed (
Figure 32). These were initially suspected to be the results of secondary infections affecting abrasions caused by amplexus. However, they have been occasionally reported in male frogs. Initial treatments included combinations of antibiotics and barrier creams; however, it has been found that the regular handling and restraint for treating these infections caused increased stress and effectively negated the benefits gained from the use of the drugs. As a substitute, a 1:1000 solution of F10 was used as a spray to treat the

affected areas and the results were excellent and all subsequently infected animals have recovered within a few weeks.

(Picture credit: B. Baker)

Figure 32 : Bacterial infections of the parotoid glands of *Agalychnis moreletii* (different stages of healing).

More generally in all *Agalychnis* bacterial infection can be visible with small punctate lesions that start as small dots of skin that can't change colour and remain livid green even when the animal becomes darker. These are associated with wet/stale air conditions and if caught early resolve with correction of conditions – dry surfaces, good ventilation and slight increase in temperature (C. Michaels, pers. comm.,
Figure 33).

(Picture credit: Chester Zoo)

Figure 33 : Example of colouration anomaly that could indicate possible bacterial infection in *Agalychnis moreletii.* (arrows)

As previously mentioned, females may become egg bound and if not cleared of the eggs they will eventually toxify the frogs rather than be reabsorbed. This is especially the case in females that begin to pass eggs but then stop due to disturbance or rain stimuli stopping. The belly becomes 'heavy - looking' and the egg masses hang down within the abdomen in a mass when the animal is handled or sits vertically (C. Michaels, pers. comm.).

Self-toxification (or other) can be identified when the normally even coloured dorsal skin colouration in these frogs becomes patchy with dark and light skin colour variations on the animal during the day.

Eye cloudiness has been observed (see Figure 34). In that case it was bilateral and seen in multiple specimens so lipid keratopathy is the primary differential diagnosis. The cause may be age or hypercholesterolemia. However, other causes can lead to opacification of the eye: cataracts or lens sclerosis can be one of them.

Figure 34 : Eye cloudiness in *Agalychnis moreletii.* (Picture credit: Chester Zoo.)

Tadpoles may develop spindly leg syndrome (SLS), with reduced or absent front limbs at metamorphosis. In *A. callidryas* this has been associated with reproductive females in a suboptimal nutritional state, but may also be associated with water chemistry, diet or other parameters in this poorly understood condition (Lassiter et al. 2020; Claunch & Augustine 2015; Camperio Ciani et al. 2018).

2.9. Recommended research

Phylogeography and systematic :
This is certainly the major point that has to be clarified in this species in terms of its impact on a conservation strategy. The genetic structure observed in the field has to be translated in a systematic point of view and maybe help to define some Evolutionary Significant Units (ESU) as priority for conservation action.

Ecology:
This species from mid-altitude environments, it is therefore exposed to contrasting thermal environments. It would be useful to know more about the habitat selection, thermal ecology, body temperature variations, mean preferred temperature at various stages of development (i.e. from the embryos to the adult) or thermoregulation strategy (i.e. thermoregulator vs. thermoconformer).
In a context of global warming, one important point concerning *Agalychnis moreletii* would be to build habitat suitability models that could show how the habitat might evolve and how population distributions could change accordingly.

Global worldwide captive conservation approach:
European registered individuals of *Agalychnis moreletii* seem to all come from the same location in Belize. In the United States of America it seems that Mexican and Guatemalan bloodlines are represented in captive collections (A. Rea, pers. comm.). In the context of a complex phylogeographic situation, the different bloodlines from different locations/countries have to be recorded and a global strategy has to be decided.

***In situ* conservation:**
Natural habitat degradation being an important threat, habitat quality should be assessed in degraded areas. Restoration campaigns could be conducted in the most degraded parts of the range of the species by planting the preferred plants for breeding and managing the waterbodies use by this species to breed.

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