Best Practice Guidelines for the Mountain Chicken (*Leptodactylus fallax*)

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EAZA 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.

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Cover photo: Gerardo Garcia

Introduction

The information in these Best Practice Guidelines have come from a variety of sources including an extensive literature review, the experience of the authors, and direct observations of *Leptodactylus fallax* in the field. Much of the non-husbandry related information was lifted directly from the Species Action Plan for *L. fallax* (Adams et al. 2014), to which a number of the authors also contributed.

Captive breeding of *Leptodactylus fallax* is essential for the long-term survival of the species, ensuring the viability and growth of the ex-situ population. This ex-situ population represents the potential founder stock for reintroductions and translocations of *L. fallax*, as well as a resource for research on the species. As such, these Best Practice Guidelines form a key component of the global conservation effort for *L. fallax* in maximising the effectiveness of the captive management of the species.

Captive management of *Leptodactylus fallax* has proven particularly difficult compared to other anurans, the breeding program limited by loss of fertility and the development of chronic health problems. These issues may relate to insufficient nutrition in the captive diet compared to the wild, though further work is required to confirm this. Furthermore, the reintroduction program progresses slowly due to the difficulties of identifying suitable release sites in light of the risk of amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) in the environment. These are active areas of research.

Important lessons can be learnt from the *Leptodactylus fallax* conservation program. The following guidelines not only apply to *L. fallax* but could also be used as a starting point for the management and breeding of other large frogs under quarantine conditions.

Key husbandry points

1. The supply of environmental UV-B and dietary supplementation with calcium are important in ensuring healthy growth and development of *Leptodactylus fallax* and in preventing metabolic bone disease (MBD).
2. Facilities should be designed to mimic wild conditions as closely as possible. Such designs should include a diverse range of resting areas and refugia, a diverse range of thermal environments, and opportunities for free range foraging for live prey. Such facilities should also facilitate easy maintenance by staff.
3. Much of the ex-situ *Leptodactylus fallax* population must be maintained in quarantine under biosecure conditions. This requires specially designed and maintained facilities.
4. Close replication of the nutritional profile of the wild diet in captivity is potentially of great importance for the health of captive *Leptodactylus fallax*. This is currently limited by the difficulty of maintaining sufficiently large captive colonies of prey species that match the nutritional profile of wild prey.
5. Biosecurity and barrier management are an important part of the management of the captive population of *Leptodactylus fallax*. In the European region there are two ex-situ metapopulations of *L. fallax*, the biosecure population (managed for future conservation translocation/ supplementation) and the non-biosecure population

managed for conservation education and conservation research. At the very basic level, all populations of *L. fallax* should be managed so that they do not pose a risk to native amphibian species both in the ex-situ locality and upon future reintroduction to the wild.

6. Bespoke techniques for handling and identification of individual *Leptodactylus fallax* have been developed and are outlined in these guidelines.
7. Bespoke veterinary techniques have been developed for *Leptodactylus fallax* for carrying out health checks and for treatment of specific conditions and are outlined in these guidelines.

Contents

Section 1: Biology and field data

Biology

1.1 Taxonomy

ORDER: Anura Fischer von Waldheim, 1813
FAMILY: Leptodactylidae Werner, 1896
GENUS: *Leptodactylus* Fitzinger, 1826
SPECIES: Leptodactylus fallax Müller, 1926

Common names:

Mountain Chicken (Hedges et al. 2019)
Dominican white-lipped frog (Frank & Ramus 1996)
Giant ditch frog (Hedges http://www.caribherp.org/)
Giant woodland frog (Groome 1970)
Crapaud (Kaiser et al. 1994, Breuil 2002, Adams et al. 2014)

Subspecies:

The Dominican and Montserrat mountain chickens (*Leptodactylus fallax*) represent the same species and the same evolutionarily significant unit (Hudson et al. 2016a). Both populations likely arose from a single founder stock or were moved between islands, presumably by Amerindian settlers.

Leptodactylus fallax was formerly more widespread than just Dominica and Montserrat, also occurring on the Eastern Caribbean islands of Martinique, St Lucia, Saint Kitts and Nevis, and possibly on Guadeloupe and Antigua (Schwartz & Henderson 1991; M. Breuil pers. comms.) prior to multiple local extinction events (reviewed in Adams et al. 2014). Of these extinct populations, museum specimens are only available from Saint Kitts (Hedges & Heinicke 2007). Specimens from St Kitts have not been subject to comparative taxonomic study.

1.2 Morphology

Leptodactylus fallax is the largest living *Leptodactylus* species, the largest native Caribbean amphibian, and one of the largest extant frog species (Kaiser 1994). With wide mouths and powerful hind limbs, *L. fallax* can reach a snout to vent length of over 20 cm and weigh over 1,000 g (Rosa et al. 2012); although most adults are more typically between 16 and 17 cm in length. The large body size observed in *L. fallax* could be considered in association with the island effect, which is a known cause of gigantism (Guarino et al. 2014).

The body colour and pattern of *Leptodactylus fallax* is variable, but they are usually a reddish brown on the dorsum, often orange-brown on the flanks and cream coloured on the ventral surface. The hind limbs, forelimbs and lateral surfaces of the head are commonly patterned with dark bars and blotches; the dorsum may be uniform in colour but is usually also broken into darker and lighter brown patches. This patterning is quite striking but effectively camouflages frogs against leaf litter on the forest floor (Adams et al. 2014).

Female *Leptodactylus fallax* tend to be larger than males although colouration and patterning are similar. Males alone have a 'spur' below the thumb. Males use this spur to grasp the female during mating and in combat with other males, stabbing and slashing each other whilst grappling (G. Garcia pers. obs.). This spur is keratinised and typically black and hardened during the mating season but white and fleshy outside of this season (Fig. 1A and B). The presence of the spur is the most reliable way of distinguishing between the sexes in sexually mature individuals (Adams et al. 2014). Mature males also have a number of more subtle distinguishing features that develop during the breeding season (G. Garcia pers. obs.): Noticeable hypertrophied forelimbs (Fig. 1C) and the development of darker colouration (grey to purple) in the area of the vocal sac (Fig. 1D).

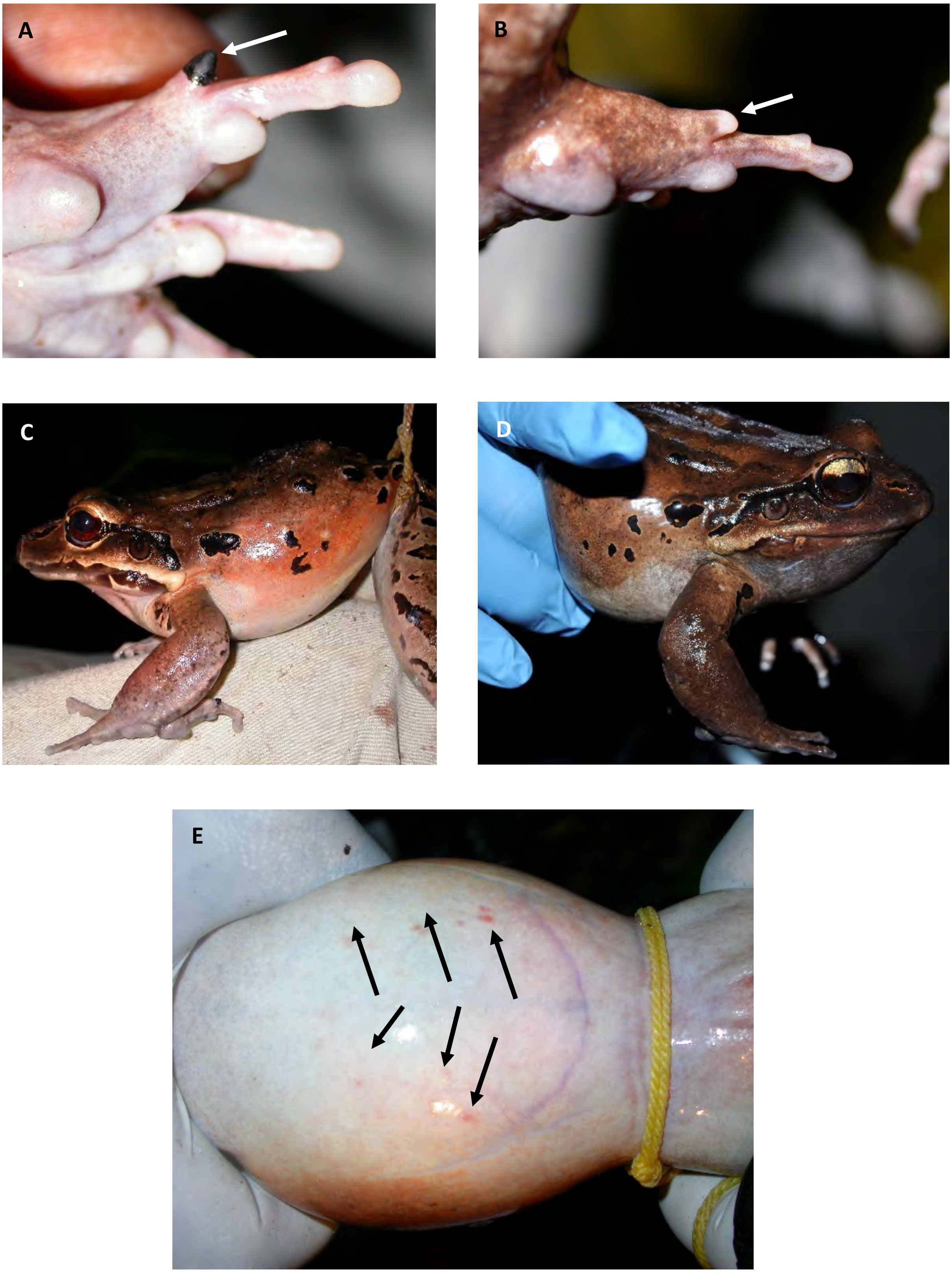

Figure 1. Secondary sexual characters in male *Leptodactylus* fallax: Spurs of male *L. fallax* (A) during the breeding season (arrow) and (B) outside the breeding season (arrow). (C) Male *L. fallax* with hypertrophied forearm and keratinised spur during the breeding season. (D) Male *L. fallax* with dark vocal sac. (E) Female with marks on venter from male spurs during amplexus (arrows). (G. Garcia).

1.3 Physiology

1.3.1 Blood physiology

The blood oxygen capacity (BOC), erythrocyte (RBC) density, and haemoglobin (Hb) concentration of *Leptodactylus fallax* is within the same range as other terrestrial and semi-terrestrial frogs that have been studied. The mean BOC of *L. fallax* is significantly different for males and females, 12.5 and 8.5 vol.% respectively. Higher BOC in males is potentially associated with high activity level involved in territoriality. This dimorphism is consistent with that seen in large ranid frogs. BOC/g of bodyweight decreases with increased weight in *L. fallax* as in other ranid frogs. The mean RBC counts in *L. fallax* are significantly different for males and females, 600,000/mm^3 and 744,000/mm^3 respectively. Mean Hb concentration is 10.9 g% with no significant sexual difference (Gatten & Brooks 1969).

1.3.2 Skin peptides

Two unique skin peptides have been discovered to be produced by *Leptodactylus fallax:* Fallaxin (Rollins-Smith et al. 2005), and *Leptodactylus* Aggression-Stimulating Peptide (LASP) (King et al. 2005).

Fallaxin has antimicrobial properties, inhibiting the growth of gram-negative bacteria. Fallaxin is structurally similar to members of the ranatuerin-2 family of peptides previously isolated from the skin of frogs of the genus *Rana* only distantly related to *Leptodactylus.* This supports the hypothesis that frog skin antimicrobial peptides evolved from a common ancestor early in the evolutionary history of the group (Rollins-Smith et al., 2005).

LASP is only found in males of *Leptodactylus fallax;* this peptide has no pheromone-like action on females but has a chemo-attractive effect on males and stimulates aggressive behaviours, such as rearing and leaping. It is hypothesised that LASP may play an important role in initiating the competitive male-male interactions that are associated with the onset of reproductive behaviour in *L. fallax* (King et al. 2005).

Leptodactylus fallax also produce significant amounts of skin secretions when handled (especially if never handled before) (Fig. 2) that have an irritant effect on mucous membranes (G. Garcia pers. obs.). This has implications for proper handling of the species both in the wild and in captivity (see section 2.6.5).

Figure 2. *Leptodactylus fallax* producing defensive skin secretions whilst being handled (G. Garcia).

1.3.3 UV-B and vitamin D_3 metabolism

Vitamin D_3 plays a critical role in calcium metabolism as well as important roles in organ development, muscle contraction, and the functioning of the immune and nervous systems in vertebrates. In most vertebrates vitamin D_3 is synthesized via exposure to ultraviolet B radiation (UV-B) (Wright & Whitaker 2001). Deficiencies in calcium have been show to result in Metabolic Bone Disease (MBD) in *Leptodactylus fallax* in captivity even in the presence of a calcium and vitamin D_3 supplemented diets (King et al. 2011). Tapley et al. (2015a) showed that both dietary supplementation and artificial provisioning of UV-B are required in captivity to stimulate healthy calcium metabolism and prevent MBD. See section 2.7.2 for further details.

1.4 Longevity

In captivity, most *Leptodactylus fallax* live for between 6 and 12 years. Data from the studbook have identified that the oldest recorded male and female in captivity were both about 15 years old. Both these individuals were wild caught; most captive bred individuals do not reach this age (G. Garcia pers. obs.).

Skeletochronology on wild *Leptodactylus fallax* (dead frogs collected following the chytrid-mediated decline in 2009 in Montserrat) provided a maximum estimated age of 7 years in wild frogs (Guarino et al. 2014). In 2016, there were two individuals in Montserrat that had been caught as adults in 2009 so are at least 9 years old. As the last two known remaining individuals on Montserrat, this may have represented an unusually long lifespan made possible by a lack of competition.

Field data

1.5 Conservation status/Zoogeography/Ecology

1.5.1 Distribution

Currently, *Leptodactylus fallax* is restricted to the islands of Dominica and Montserrat in the Eastern Caribbean; it was formerly far more widespread (Fig. 3), occurring on the Eastern Caribbean islands of Martinique, St Lucia, Saint Kitts and Nevis, and possibly on Guadeloupe and Antigua (Schwartz & Henderson 1991; M. Breuil pers. comms.). *L. fallax* were extirpated on many islands through a combination of habitat loss (forest and freshwater), introduced predators (especially rats and mongoose) and probably over-exploitation for food. *L. fallax* had disappeared from Martinique by the 19th century and from Guadeloupe, St. Kitts and St. Lucia in the early 20th century (see review in Adams et al. 2014). The introduction of the mongoose (*Herpestes javanicus*) was likely a major driver of *L. fallax* island extinctions. *H. javanicus* were never introduced to Montserrat and Dominica (Hays & Connant 2007).

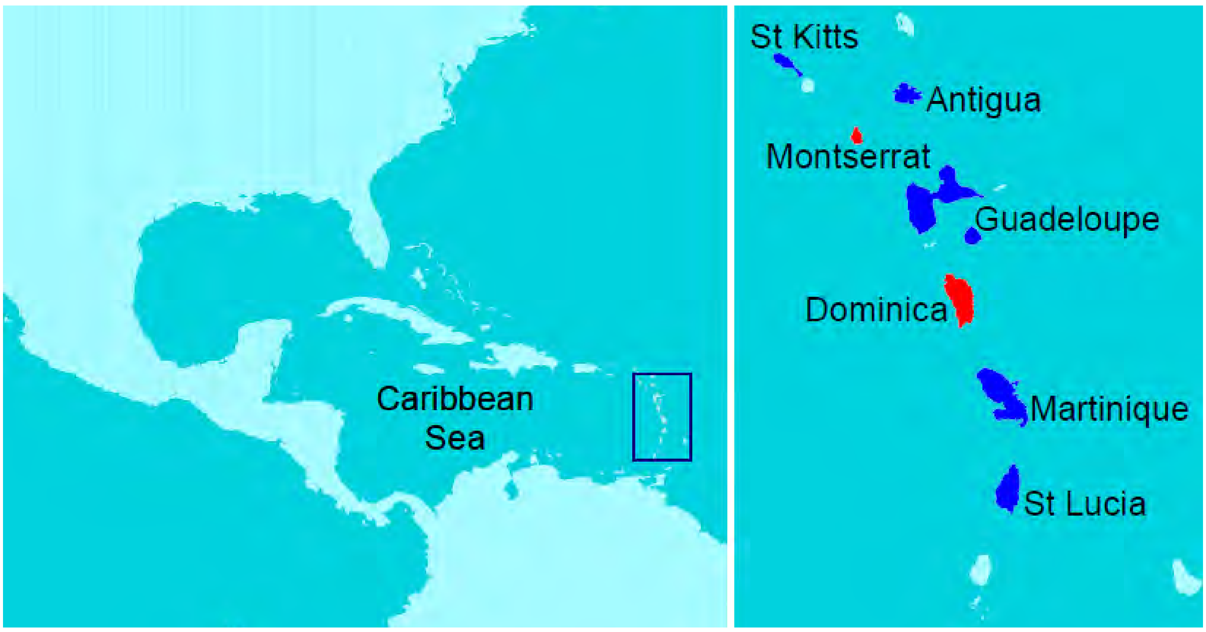

Figure 3. Current distribution of *Leptodactylus fallax* (red) and historic distribution of *Leptodactylus fallax* (blue). Taken from Adams et al. (2014).

Historically, *Leptodactylus fallax* was widely distributed on Dominica. The species was found on the west coast of the island at low elevation in natural and semi-natural habitats. There were also a number of small isolated populations introduced along the east coast of the island (see Fig. 4A). *Batrachochytrium dendrobatidis* (*Bd*), the causative agent of the disease chytridiomycosis, arrived on Dominica in the early 2000's (McIntyre 2003; Magin 2004) and in December 2002 *L. fallax* mortalities were reported (Magin, 2003). There was subsequent severe population decline (McIntyre 2003; Magin 2004). By 2007, the range of the area occupied by this species has declines by more than 60% with small populations restricted to the west coast of the island (Fig. 4A).

On Montserrat *Leptodactylus fallax* was historically widespread and occurred in the Centre Hills, Soufrière Hills, St Georges Hill, Molyneaux, and on Garibaldi Hill (Daltry 1998; see Fig. 4B). The range of *L. fallax* contracted due to habitat destruction after the Soufrière Hills volcanic eruption. Eruptions have been ongoing since 1995 and the Soufrière Hills and Garibaldi Hill populations were likely destroyed by pyroclastic flows and ash fall. Populations in the Silver Hills and St Georges were also likely extirpated due to ash fall. The remaining *L. fallax* population was restricted to approximately 1,500 ha of forest in the Centre Hills. *Bd* arrived on Montserrat in late 2008 or early 2009 (Garcia et al. 2009) causing further range contraction; currently the species is restricted to the east side of the Centre Hills on Montserrat (Fig. 4B) (Adams et al. 2014).

The current Extent of Occurrence (EOO) of *Leptodactylus fallax* is 1,568 km^2 and its area of occupancy (AOO) is approximately 10 km^2 (IUCN SSC Amphibian Specialist Group, 2017).

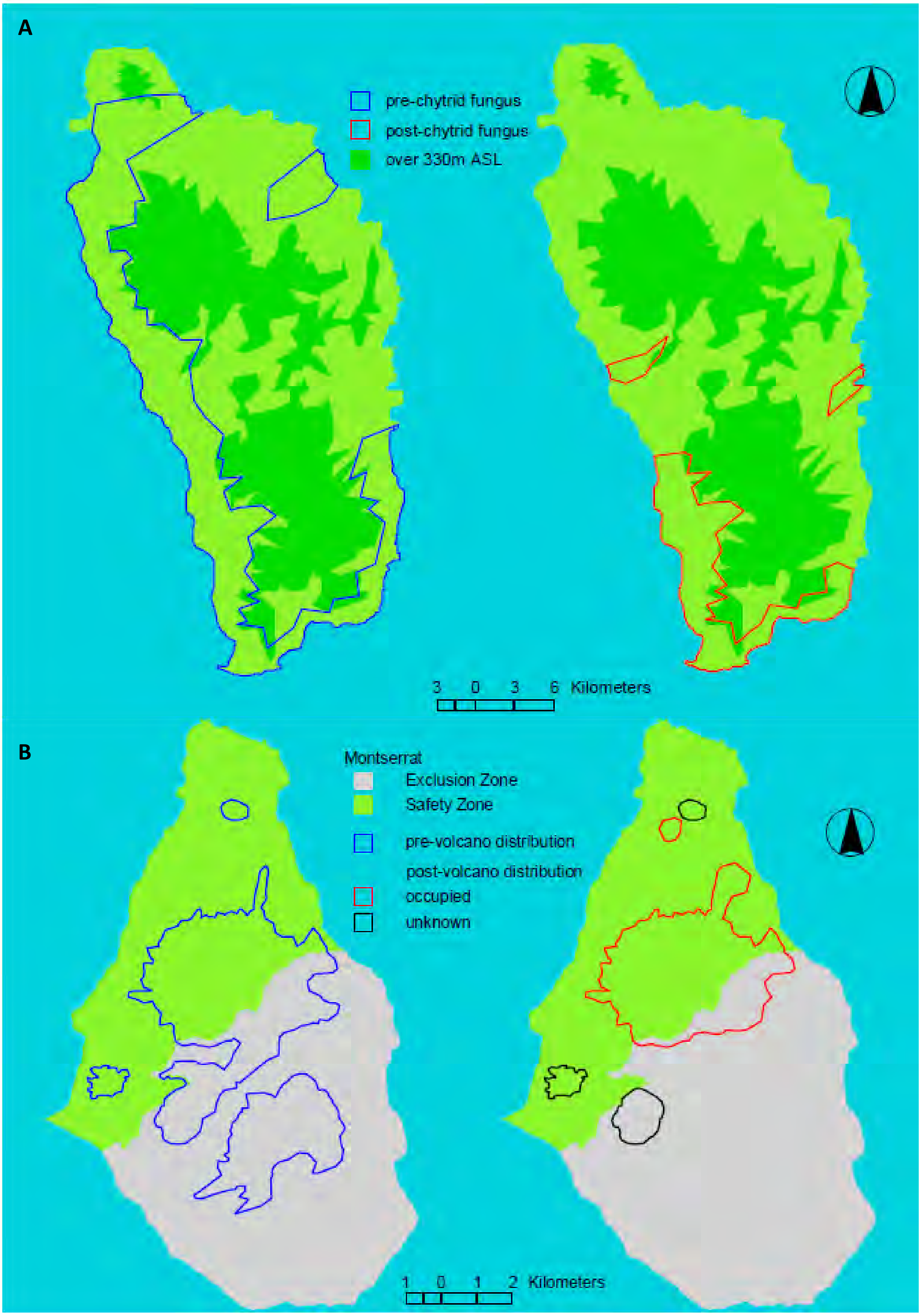

Figure 4. Distribution of *Leptodactylus fallax* pre and post (A) emergence of chytridomycosis on Dominica, and (B) volcanic eruption on Montserrat. Taken from Adams et al. (2014).

1.5.2 Habitat

On Montserrat, *Leptodactylus fallax* have been recorded at a range of elevations, from near sea level to as high as the once forested peaks of the Soufrière Hills at 1,000 m above sea level (ASL) (Gray & Martin pers. comms.; Daltry 1999) (Fig. 5). On Dominica *L. fallax* are believed to be restricted (both before and after the arrival of the *Bd* fungus) to areas below about 330 m ASL (A. James pers. comm.; McIntyre 2003), occurring down to almost sea level. It is not clear why this difference in elevational preference occurs. *L. fallax* on Montserrat tend to be found in forests, as well as highly disturbed areas such as ghauts running through cassava plantations, in contrast to Dominica where, as well as being associated with lower elevation mesic and wet forest below about 330 m ASL, they are also found in gardens and plantations. This difference between the two islands has persisted after the introduction of *Bd*. The species does not occur in arid habitats where natural vegetation is dominated by scrub and cactus, presumably because the frogs would desiccate.

A study on Montserrat in 1995 (Daltry 1999) provided information on finer scale habitat associations of *Leptodactylus fallax*. Within mesic and wet forests, *L. fallax* were associated with areas with permanent water and good canopy cover and appeared to avoid areas of thick undergrowth. However, the latter two habitat variables are often correlated as closed canopy retards the growth of understory vegetation. Forest with closed canopy may generate deeper leaf litter and maintain humidity, which likely promotes high invertebrate prey abundance. The optimal habitat for *L. fallax* therefore is likely to be undisturbed, mature forest with permanent watercourses (Fig. 5). During the dry season, DOE staff have only found *L. fallax* close to streams in ghauts, however, the frogs are more widely distributed during the wet season.

Figure 5. Examples of *Leptodactylus fallax* habitat. (G. Garcia).

1.5.3 Population

Abundance and population trends On Dominica

Leptodactylus fallax were historically abundant on Dominica. Annual legal harvests on Dominica were estimated at between 8,000–36,000 individuals per annum (Malhotra et al. 2007). In 2002, a programme to monitor the relative abundance of *L. fallax* on Dominica was initiated; *Bd* was first confirmed from specimens collected during this survey work (McIntyre

2003). *Bd* spread across the island and the population of *L. fallax* declined by >85% in 18 months of the first confirmed cases (Hudson et al. 2016a). Detections of *L. fallax* were very low at survey sites by 2007, and by 2008 no *L. fallax* were detected at the survey sites, although one frog was heard calling from one site outside the survey area (Cunningham et al. 2008). Regular survey efforts were not conducted on Dominica between 2008 and 2012. Small clusters and scattered individual *L. fallax,* including recently metamorphosed individuals and juveniles were detected during preliminary searches in 2011 (Tapley et al. 2014). Further ongoing fieldwork documented a number of *L. fallax* at a number of sites. Prior to Hurricane Maria in September 2017, the Dominican subpopulation was estimated to contain around 130 individuals (both adults and juveniles), captured since 2014, based on pit tag identification. These results could suggest nascent recovery of the *L. fallax* population on Dominica prior to Hurricane Maria that devastated much of the island, or at least some level of coexistence with *Bd* (Adams et al. 2014). Today, the remaining wild population on Dominica has been estimated to be less than two hundred individuals (IUCN SSC Amphibian Specialist Group, 2017) but the impact of Hurricane Maria on *L. fallax* has not yet been quantified.

Abundance and population trends on Montserrat

No historical or current estimates of population size exist for *Leptodactylus fallax* on Montserrat. Unpublished data from a study carried out in 1979-80 on Montserrat estimated the total frog harvest for 1979 at 1043 individuals and 1980 as 1680 individuals (J. Blankenship pers. comm.). Based on comparing hunting data alone this suggests that the abundance of *L. fallax* and/or hunting pressure on *L. fallax* prior to major population decline was far lower on Montserrat than Dominica.

The ongoing monitoring programme run by the Department of Environment (DOE) is designed to generate indices of abundance as a surrogate of population size and trend rather than produce absolute abundance estimates (Daltry & Gray 1999; Young 2007). However, in 2005, a number of repeat surveys of three monitoring sites were carried out to estimate the size of these local populations using mark-recapture. At that time (prior to the arrival of *Bd*), in the highest density site of Fairy Walk 252 ± 75.7 (s.e.) frogs were estimated to occur along a 200 m stretch of ghaut. This site was previously the area most exploited by hunters, being only half an hour walk from the nearest habited area (C. Fenton pers. comm.). Following the establishment of the exclusion zone following the 1995 volcanic eruption *L. fallax* at Fairy Walk ceased to be hunter, leading to rapid population recovery (C. Fenton pers. comms.). In Sweetwater Ghaut and Cat Ghaut, 39 ± 6.2 (s.e.) and 7.5± 3.0 (s.e.) frogs were estimated along 200 m stretches respectively. It is not possible to extrapolate these estimates to the wider population, but they did show that *L. fallax* density within the Centre Hills varied widely between sites. The reason for such variation is unclear, but likely factors were habitat quality, the effects of hunting, and presence of invasive vertebrates.

The dry season monitoring data between 1999 and 2005 suggests little overall change in *Leptodactylus fallax* numbers during this period despite considerable fluctuation in the encounter rate index. Post- *Bd* in 2009, however, the population crashed very rapidly.

Since the first detection of *Bd* on Montserrat in 2009, the local population has experienced catastrophic decline. Three individuals were found at Killikrankie in 2011, but no *Leptodactylus fallax* have been detected at any of the annual population monitoring transects outside of those on the eastern slopes of the Centre Hills. No frogs were detected or known after 2016 and the population on Montserrat is considered functionally extinct.

1.5.4 Threats to wild population

Chytridiomycosis caused by *Bd*

Amphibians are in crisis around the world (Gascon et al. 2007). Since the 1970's, many amphibian populations have undergone precipitous declines, sometimes leading to the complete extinction of amphibian species. Most worryingly, many of these declines and extinctions have occurred in protected areas. The extent of these declines and extinctions is without precedent in any class of animals over the last few millennia, and has been the focus of much scientific research. At least 43% of all known amphibian species are declining and 42 %, (i.e. about 3,300 species), are now threatened with extinction. Over 120 species may have gone extinct since 1980 (Daszak et al. 2003).

Whilst habitat loss and degradation continues to be a serious problem for amphibians, the emergence of the amphibian disease, chytridiomycosis has had a great impact on amphibian species over a short period of time (Berger et al. 1998). Chytridiomycosis is caused by *Bd* or *Bsal* (*Batrachochytrium salamandrivorans*), species of chytrid fungi (Martel et al. 2013). *Bd* is known to infect all extant classes of Amphibia (Gower et al. 2013). Amphibian chytridiomycosis attributed to *Bd* was first discovered in 1998, when it was found to be the main cause of amphibian declines in the rain forests of Australia and Central America (Berger et al. 1998). Since then, this disease has been associated with amphibian declines and extinctions in many countries. Indeed, it has been found to be an emerging threat to >300 species (of frog, toad, newt and salamander) representing at least 14 amphibian families across six continents (see Daszak et al. 2007 for a review).

On Dominica, *Bd* first emerged in 2002 when large numbers of reports of dead and dying *Leptodactylus fallax* were received by the Forestry and Wildlife Division (McIntyre 2003). The disease quickly spread across the island and the population of *L. fallax* declined by >85 % within 18 months of the first confirmed cases (Hudson et al. 2016a). The population of *L. fallax* on Dominica is now relatively small and there is ongoing fieldwork to determine the state of the population. In 2007 A captive breeding program was established in Europe to help safeguard the future of the Dominican mountain chicken. Unfortunately, none of the animals bred in captivity and this population had died out a decade later (B. Tapley pers. comm.). In 2003, a chytrid and ranavirus surveillance survey was conducted on amphibians in Montserrat; the pathogens were not detected (Garcia et al. 2007).

Despite the actions outlined in the 2007 Mountain Chicken Species Action Plan (Martin et al. 2007), which identified the need for strict island wide biosecurity protocols and a chytrid screening program throughout the Eastern Caribbean, the first reports of the arrival of *Bd* on Montserrat were made by the Montserrat DOE to Durrell Wildlife Conservation Trust in February 2009. Forest rangers and a local hunter observed unusual behaviour when frogs were sighted during the day and were congregating in large numbers around water sources in Cedar ghaut. They also reported dead and sick looking frogs and described clinical signs of chytridiomycosis. In March 2009 the presence of *Bd* was confirmed at multiple sites on

Montserrat with losses expected (Garcia et al. 2009), and subsequently confirmed, to be similar to those seen in Dominica in 2002.

Bd has also been found in swabs from cane toads (*Rhinella marina*) and eleutherodactylid frogs on both Dominica and Montserrat. Since the cane toads appear clinically healthy and *Eleutherodactylus* sp. populations are very dense and therefore recover quickly from potential losses, they are acting as reservoir hosts and potential disease vectors (Hudson et al. 2016a). Vector amphibians appear to be the main way that the disease is spread from one country, or area, to another. In the Caribbean region, for example, eleutherodactylid frogs are frequently transported internationally with bananas and other produce. As much of Montserrat's fresh produce is imported from a known *Bd* -positive country (Dominica), this route of transmission is thought to be the most likely way the fungus could have arrived into Montserrat, though there are many other possible routes.

Volcanic activity on Montserrat

The Soufrière Hills Volcano, located in the south of Monserrat, began its first eruption for 350 years on 18th of July 1995 with a series of steam and gas explosions. Lava extrusion later commenced in November 1995. Following a series of short-term evacuations, a state of emergency was declared in April 1996. The capital, Plymouth, was abandoned along with all other communities located in the southern two-thirds of the island. In June 1997, a small dome collapse generated widespread pyroclastic flows to the north of the dome that killed 19 people in the village of Streatham. Pyroclastic flows from the same event also reached the W.H. Bramble airport to the NE of the dome, resulting in its permanent closure (Kokelaar 2002).

Between 1995 and 2010 there were five phases of volcanic activity lasting up to 3 years separated by periods of little or no activity of up to 2 years. Activity during these phases has included the repeated growth and collapse of a lava dome and associated pyroclastic flows, more than 100 large volcanic explosions and frequent ash falls, some of which affected areas in the Centre Hills and the north of the island. The last activity occurred in February 2010, ten months after *Bd* was first discovered on Montserrat, when a major dome collapse impacted 11 km^2 to the north and northeast of the volcano, including the Farm River Valley and Fairy Walk in the Centre Hills. Pyroclastic flow deposits also added 1 km^2 of new land to the coastline between Trant's Bay and Spanish Point (Cole et al. 2010; Stinton et al. 2014a, b). Since the collapse in 2010, there has been no evidence of further dome growth and volcanic activity appears to remain at a minimal level. An assessment issued in 2018 by the UK Scientific Advisory Committee on Montserrat Volcanic Activity (SAC) indicated that significant surface activity at the volcano is currently paused and has been for the past eight and a half years. Pyroclastic flows and major rock falls are absent, however, temperatures of volcanic gases that escape through fractures and fumaroles remain high (SAC, 2018). At present, nearly 60 % of Montserrat's land area is within the exclusion zone.

There is little data on habitat loss or habitat regeneration of the areas affected by the volcano since 1995. There was substantial defoliation after major ash falls (Fig. 6), but this was followed by a rapid recovery (within weeks to a few months), and there was also some acid rain and ash damage during periods of chronic ash fall (C. Fenton pers. comm.). Research indicates large but ephemeral effects of ash fall on canopy insects, with some suggestion that ground-dwelling insects were most seriously impacted but recovery again appeared to be rapid (Marske et al. 2007). There were consequent knock-on effects on some vertebrate consumers and not others. Pederson et al. (2012) documented dramatic decreases in bat populations and increases in several sub lethal pathologies associated with accumulation of ash. Alternatively, Dalsgaard et al. (2007) showed that most bird populations in the Centre Hills were not strongly impacted overall. It seems highly likely that the complete loss of habitat in the south of Montserrat resulted in the loss of *Leptodactylus fallax* in these areas. This led the IUCN to infer a severe population decline in their red listing of the species in the assessment of 2004. Data on the impacts of more ephemeral volcanic events (ash falls) do not exist. There are several anecdotal reports of *L. fallax* found covered in ash, or in water acidified by ash, and, to outward appearances, healthy; but follow-up data on the fates of these individuals does not exist along with the effect of the ash on the presence of the chytrid fungus.

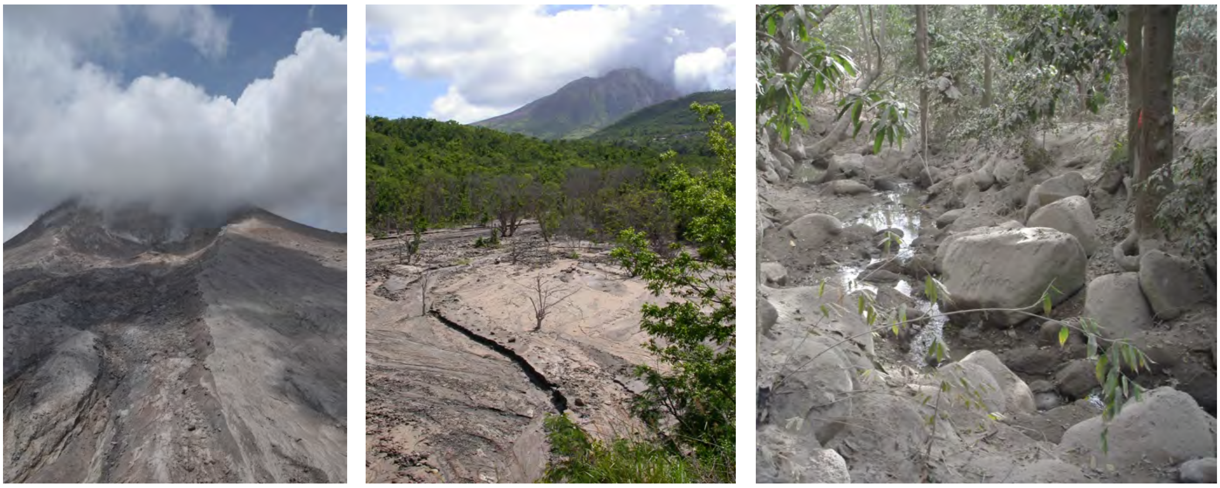

Figure 6. Impacts of volcanic ash fall on Monserrat. (G. Garcia).

Unsustainable hunting

Unsustainable hunting had been identified as one of the most important threats to the survival of *Leptodactylus fallax*; both on Dominica and Montserrat before the arrival of *Bd*. *L. fallax* were hunted for both domestic consumption as a traditional dish and also sold to restaurants where they were offered to tourists. Under the Forestry, Wildlife, National Parks and Protected Areas Ordinance, *L. fallax* are listed as a partially protected wildlife in Dominica, for which it is possible to have a closed and open season. Prior to the volcanic emergency, there were no enacted regulations in Montserrat on the hunting of *L. fallax*, for example there was no open and closed hunting season or licensing system. CEMA legislation in Montserrat giving full protection to *L. fallax* was passed in 2014 (Conservation and Environmental Management Act 2014).

Prior to the volcanic emergency, it is believed that roughly 60-70 hunters in Montserrat were hunting regularly and about 15-20 restaurants and hotels were serving mountain chicken dishes. Unpublished data from a study carried out in 1979-80 on Montserrat estimated the total frog harvest for 1979 at 1043 individuals and 1980 as 1680 individuals (J. Blankenship pers. comm.). Significantly more of Montserrat was at that time available to hunt in. Local knowledge and data that have been collected since 1997 (see section 2.2.1) indicate that (since the volcanic emergency) in areas where hunters have easy access, the population of *Leptodactylus fallax* has declined. This clearly suggests that hunting in these areas is unsustainable. Since the arrival of *Bd* on Montserrat, the population of *L. fallax* has declined to such drastically low numbers that hunting frogs is now an unreliable income source and no evidence of recent hunting activity has been reported.

Dominica provides an example of regulating the impacts of hunting on *Leptodactylus fallax.* Dominicans had traditionally hunted *L. fallax* with no restrictions or regulations until the mid-1970s when the Forestry and Wildlife Act was enacted in July 1976. In the earlier days, most of the frogs hunted were for domestic consumption, as the frog is considered a delicacy and was promoted as the island's national dish. However, over the years, with promotion and the development of eco-tourism, the demand for the frog by hotels and restaurants grew significantly, to the point where a local supermarket even sold local frogs' legs sometime in the late 1990's. In 1998, a survey of hunters and freshwater fishermen was undertaken by the Forestry and Wildlife Division. The survey results identified *L. fallax* as one of the three most hunted game species on the island (McIntyre 2003).

The Dominican Forestry and Wildlife Act provides for the issuing of licenses to hunt game wildlife (including *Leptodactylus fallax*) and the setting of open and close seasons for the taking of game wildlife. These measures – among others – first came into force in 1976. From this date until 1999, hunting of game was permitted for six months in any one year, from the first day of September of one year through the last day of February of the following year. The Act in its current form does not provide for the setting of bag limits or catch quotas, nor does it provide for regulating the sale of wild meat. New amendments to the legislation, expected to be enacted soon, are felt needed to address such issues.

Although *Bd* is believed to be the primary cause of the catastrophic decline *Leptodactylus fallax* in numbers on Dominica (IUCN SSC Amphibian Specialist Group, 2017), it also rendered the frogs more lethargic and easier to catch and hunting was feared to be compounding the decline. From 1 January 1999, the Government of Dominica had imposed a total ban on the hunting of *L. fallax* and all other forms of wildlife on the island. As of 2000, this ban was temporarily lifted annually but for only two to three months of the year. However, due to concerns for the *L. fallax* population because of the outbreak of chytridiomycosis among the frog's population in 2002/2003, the Government enacted regulations to impose an indefinite ban on the hunting of *L. fallax* as of April 2004.

The enforcement of the provisions of the Forestry and Wildlife Act is carried out mainly by the Director and officers of the Forestry, Wildlife & Parks Division, although Police Officers have similar enforcement powers under the Act. While regular forest offences may be "compounded" or dealt with by the Director of Forestry and Wildlife without the matter going to court, all wildlife cases, including those pertaining to *Leptodactylus fallax*, must be dealt with in the Magistrate's Court. In the period 1997-2004, 12 wildlife cases went through the courts, though none relating specifically to *L. fallax*. In 2013, there were two unofficial reports of continued illegal hunting although no evidence of this was ever received by the Forestry, Wildlife & Parks Division (A. Blackman and M. Sulton pers. comm.).

Invasive species

Several species of invasive alien mammal occur in Montserrat and Dominica (summarised in appendix 1), including rats (two species), domestic cats, dogs, pigs, goats, cows, and donkeys. Both islands have been fortunate enough to escape having small Indian mongoose (*Herpestes javanicus*) introduced, unlike many islands in the Caribbean. Rats (*Rattus norvegicus* and *R. rattus*) are among the most damaging invasive species and the multiple impacts of rats on island ecosystems have been described in many publications (Atkinson 1985; Atkinson & Atkinson 2000; Towns et al. 2006; Global Invasive Species Database 2007). As abundant, opportunist omnivores, they predate many native invertebrates and smaller vertebrates, and have driven declines and extinctions of numerous species through processes such as competition, predation and modifying habitats. Rats are thought to have played a dominant role in the declines and extinctions of numerous New Zealand amphibian species (Towns & Daugherty 1994). Impacts are generally thought to be greatest on nocturnal amphibian species (Global Invasive Species Database 2007), although it is also believed that species on tropical islands on which land crabs are native tend to suffer fewer impacts, because land crabs are ecologically rather similar predators (Atkinson 1985).

European boats brought brown rats (*Rattus norvegicus*) and black rats (*Rattus rattus*) to Montserrat and Dominica. Black rats reached the Caribbean as early as the beginning of the seventeenth century, with brown rats perhaps two centuries later (Varnham 2007). Both species of rats are currently very abundant in Montserrat's Central Hills forests. Snap-trapping data indicates that, in the forest, black rats are somewhat more abundant than brown rats, although both are present throughout. In general, in the Central Hills, black rats are more abundant at higher altitudes, and are more arboreal, than brown rats. Both species' population levels at this site appear to be linked to the local abundance of large fruit trees and clearings (Young 2007). Rats, probably mainly brown rats, are also abundant in the settled lowland areas of Montserrat and Dominica. Captures of *Leptodactylus fallax* on Montserrat during the dry season of 2005 showed a high percentage (5-27 % depending on capture sites) of frogs with old and, more commonly, fresh rat bites. Most of the bites were observed on the hind limbs.

Since the late nineties, feral pigs (*Sus scrofa*) have spread rapidly through Montserrat's Central Hills forest following the release of domestic pigs from farms evacuated in the wake of the volcanic crisis. The main source of invasion is thought to have been from the Harris area to the southeast of the Central Hills (J. Daley pers. comm.), and consequently the invasion has spread progressively from the southeast of the hills. Pigs were first noted as a substantial presence in the Central Hills forests during 2001 (Buley 2001). There is no evidence of a feral pig population in Montserrat prior to the volcanic crisis. In the following years they spread rapidly through most of the forest, but substantial control efforts by local forest rangers in 2004 and through a project led by the RSPB from 2009 to 2013, the population of feral pigs in the Central Hills has been greatly reduced. In Dominica, the presence of feral pigs in the forest has been identified as one of the biggest threats to farmers land, crops, and private gardens, and as a result, the Government declared them a pest in 1982, allowing them to be hunted without license.

Pigs can have a major impact as invasive species on some island tropical forests. Like rats, they are opportunistic omnivores, and can cause declines and extinctions in terrestrial animals that they prey on (Cruz et al. 2005 and references therein; Global Invasive Species Database 2007). However, there is little specific scientific information regarding their effects on amphibians. In some island forests, especially Hawaii, feral pigs have had profound impacts on the vegetation structure of the forest itself, through soil-rooting and consumption of seedlings, tree-ferns, and through spreading propagules of invasive plants such as guava (*Psidium guajava*) (Global Invasive Species Database 2007).

In Montserrat, goats (*Capra hircus*) and cattle (*Bos taurus*) are also encountered in the Central Hills forests. As with pigs, cattle were released to fend for themselves when people were evacuated from the south in 1995 and their population has grown significantly over time (S. Mendes pers. comms.). Goats meanwhile have had free roaming populations for years prior to 1995 (S. Mendes pers. comms.). When introduced to islands, these two species can affect forest structure and native plant communities through their grazing and browsing (Fig. 7), with knock-on effects for native animals (Atkinson & Atkinson 2000; Campbell & Donlan 2005; Global Invasive Species Database 2007). In Dominica, feral goats cause limited damage to farmer crops and private gardens but are not present in large enough numbers to be considered a major threat (M. Sulton pers. comms.).

Figure 7. Impact of feral goat grazing on vegetation on Monserrat. (G. Garcia).

There is almost certainly a feral cat (*Felis catus*) population in Montserrat, although this has not been confirmed by formal study, and anecdotal information rarely distinguishes between detections of wandering domestic cats and true feral animals. The distribution, population density and ecology of the feral population is not known. Feral cats are devastating invasive species on many islands, through predation on native vertebrate species. Mammals and birds are most commonly affected (Global Invasive Species Database 2007), and indeed, in some circumstances, feral cat predation on introduced rats may be beneficial to native island ecosystems by reducing rat impacts (Courchamp et al. 2003), but negative impacts on some island herpetofauna populations, such as various Caribbean island iguanas, have also been recorded (Varnham 2006).

The agouti (*Dasyprocta antillensis*) was probably introduced by Caribs as a food source at some time before the arrival of Europeans, and is now widespread through forested areas of Montserrat (Young 2007). Agoutis, like the more recently introduced red-footed tortoises (*Geochelone carbonia*) are not thought to impact native wildlife significantly and are not considered invasive.

The common green iguana (*Iguana iguana*) has been introduced to or has otherwise invaded almost all the Lesser Antillean islands in the last decade and is highly invasive (e.g. Falcon et al. 2013; Vuillaume et al. 2015; Haakonsson 2016). On Dominica, the first breeding animals were discovered in 2018. The situation on Montserrat is more complex because the island harbours a native but genetically distinct form, which is not readily distinguishable from the invasive, common green iguana of American mainland decent (Stephen et al. 2012). A stark green iguana population increase was reported from Montserrat in 2017 (S. Mendes, pers. comm.) suggesting an invasion of the non-native common green iguana, which possesses a much higher reproductive potential than any native island forms. While not directly affecting *Leptodactylus fallax* on either island, invasive green iguanas are known to be able to alter habitats drastically and catastrophically through overgrazing of seedlings, shrubs and trees, thereby able to affect overall vegetation structures and food sources of Mountain chicken prey species (Haakonsson 2016; Burton pers. comms; M. Goetz, pers. obs.).

On Dominica the common opossum (*Didelphis marsupialis*), known locally as the manicou, is widespread (A. Blackman and J. Spencer pers. comms.). Its impact on *Leptodactylus fallax* is currently unknown, but has a generalist diet, which includes small vertebrates such as frogs (Tyndale-Biscoe 2005).

Cane toads (*Rhinella marina*) have been introduced to Montserrat and are now widespread and common throughout the island (Lescure 1979). In the Central Hills forests, they appear to be highly clustered around watercourses, rather than dispersed throughout; this is in contrast to the (wet season) distribution of *Leptodactylus fallax* (DOE pers. comms.). The cane toad is a generalist and opportunist predator. It feeds nocturnally, primarily on terrestrial invertebrates and small vertebrates. It also produces toxins in its skin that can directly kill native predators (Global Invasive Species Database 2007). As such, the cane toad is potentially an important predator of juvenile *L. fallax*, and a competitor for food and water resources. Some cases of ticks (*Amblyomma rotundatum*) found on *L. fallax* in Montserrat could be a sign of the close contact with cane toads as this species shows a high prevalence of these parasites during all seasons (Kevin et al. in press). It is possible these ticks could be transmitting a wide variety of pathogens to *L. fallax* (Liu & Bonnet 2014). This close contact between cane toads and *L. fallax*, may also have assisted the spread of *Bd*, for example during the Bd outbreak of 2009 cane toads were observed in amplexus with weakened *Bd* infected *L. fallax* (G. Garcia pers. obs.).

Cane toads have not been successfully introduced to Dominica. In 2013, however, a container that had travelled directly from Montserrat arrived at the port in Dominica carrying a number of live cane toads. The arrival of the cane toads was reported to Forestry staff and intensive surveys were immediately undertaken at the port and surrounding areas. Twenty-six toads were caught and removed from the port and there was no evidence to suggest the toads had spread to other areas (M. Sulton pers. comms.).

More recently, in 2017, the highly invasive Cuban tree frog (*Osteopilis septentrionalis*) has been found on Dominica. The tree frogs likely arrived with the aid that reached the island after Hurricane Maria. Cuban tree frogs have the potential to spread amphibian parasites (Ortega et al. 2015) and the presence of an invasive amphibian could cause a shift in the patterns of infectious disease transmission and disease transmission pathways. Several water bodies have been found on Dominica with developing tree frog tadpoles. Attempts are currently underway to eradicate the species

Habitat degradation

Water availability - *Leptodactylus fallax* are associated with areas with permanent water (Daltry 1999), and it seems likely that moist conditions are essential for successful breeding. These conditions will depend upon, amongst other factors, the intensity and distribution of rainfall and the abstraction of water from capped springs for human consumption (Fig. 8). Water conservation depends on spring yield and length of ash fall as demand can easily increase during dry periods and ash fall. Increasing abstraction tracked increasing rainfall over the period 2003-2007. The average rainfall over the Central Hills decreased substantially in 2007 (to levels similar to those found during 1999-2001) but without a corresponding decrease in abstraction. The Montserrat Water Authority (MWA) notes that there is not currently enough data to indicate a minimum level of rainfall required to ensure suitably moist conditions for the survival of *L. fallax*. The dome collapse at the Soufrière Hills volcano would be expected to affect the availability of water, covering existing pools of water, and also increasing surface runoff that, by extension, will reduce water available for the persistence of suitable microhabitat.

Figure 8. Water abstraction at a ghaut in Monserrat. (G. Garcia).

Chemical contaminants

Boone et al. (2007) review the impacts of contaminants on amphibian populations and conclude that there are serious gaps in current knowledge on what contaminants amphibians are exposed to in nature, and in what combinations, as well as on how contaminants interact with other stressors. However, there is clear evidence that contaminants can impact amphibians at the individual, population, and community level. At present, there is no evidence of any impacts of chemical contaminants on *Leptodactylus fallax* on Montserrat. However, a number of the agrochemicals currently in use on Montserrat are known to have adverse impacts on other amphibian species (e.g. glyphosate found in the herbicide Roundup or carbaryl in SevinTM can both greatly increase tadpole mortality) or on their food source (insects and other invertebrates) (Boone et al. 2007). Furthermore, significant pollution of habitat with waste that may leach contaminants into the environment is present on both Dominica and Montserrat (Fig. 9). The extent to which these potential impacts are being realized on Montserrat remains unknown.

Figure 9. Pollution on Monserrat. (G. Garcia).

Observations from captivity show that at least some chemicals adversely affect *Leptodactylus fallax*. On one occasion, an individual held at Jersey Zoo escaped their enclosure and was found on a surface that had been heavily swabbed with disinfectant. Where the individual had been in contact with the disinfectant the skin sustained chemical burns (G. Garcia pers. obs.) (see section 2.7.2).

Built development

Development of land for both housing and agriculture can result in the loss and degradation of *Leptodactylus fallax* habitat (Fig. 10), the introduction of invasive alien predators (cats and dogs) and possibly chemical contaminants. Again, at present, there are no data to indicate whether such impacts are occurring on Montserrat although the boundary of the Central Hills Reserve seems likely to be an area of high sensitivity in this regard. Montserrat's national GIS (housed at the Physical Planning Unit, PPU) may be able to provide a mechanism for monitoring such pressures, both from the recent past and in the future.

Figure 10. Banana plantation on Monserrat built around a ghaut. (G. Garcia).

1.5.5 Conservation status

Leptodactylus fallax is currently listed as Critically Endangered by the IUCN (IUCN SSC Amphibian Specialist Group, 2017). *L. fallax* is one of the most threatened frogs in the Caribbean region and, indeed, one of the most critically endangered species in the world (Tapley et al. 2014).

Unsustainable hunting had been identified as one of the most important threats to *Leptodactylus fallax* throughout its range. Under the Forestry, Wildlife, National Parks and Protected Areas Ordinance in Dominica *L. fallax* were listed as partially protected wildlife, and hunting was only permitted at certain times of the year. The Government of Dominica placed an indefinite ban on the hunting of *L. fallax* as of April 2004. There were no enacted regulations in Montserrat on the hunting of *L. fallax* until 2014 when CEMA legislation, which gives full protection to *L. fallax,* was passed (Conservation and Environmental Management Act 2014). In 2013, there were two unofficial reports of illegal hunting on Dominica; however, no evidence of this was ever received by the Forestry, Wildlife & Parks Division (Adams et al. 2014).

Chytridiomycosis caused by *Bd* is currently the most severe threat to *Leptodactylus fallax.* Mitigative measures against this disease in nature have only succeeded under a unique set of circumstances (Bosch et al. 2015). Currently the immediate future of the species is uncertain with the most realistic hope being through captive breeding and release. In response to the population crashes resulting from the arrival of chytridiomycosis on Montserrat and Dominica, ex-situ safety-net populations were established in 2002, 2009, and 2011 at several institutions in Europe and the range state of Dominica.

In 2002, a safety net population was removed from the wild following the arrival of *Bd* to Dominica. In total 12 wild *L. fallax* were captured in Dominica and relocated to specialised quarantine facilities at ZSL, these frogs never bred successfully, and the last animal died in 2015. In 2005, a specialised captive breeding facility was built in order to establish the capacity for a *L. fallax* captive breeding programme on Dominica (Cunningham 2008). Forestry staff were trained in the UK in the husbandry techniques required to keep *Leptodactylus fallax* in captivity (Cunningham 2008) and to develop live food colonies using native species (Nicholson et al. 2017). The aim of this captive breeding facility was to breed wild caught Dominican *L. fallax* in-situ that could be introduced back into the wild without the issues of biosecurity. The facility (Fig. 11) has housed *L. fallax* since mid-2011; the frogs have yet to breed in the facility although non-viable foam nests have been produced historically (Tapley et al. 2014). The facility sustained severe damage during Hurricane Maria in September 2017 and breeding efforts were all but abandoned afterwards. Efforts are now focused on monitoring the wild frogs on Dominica.

Figure 11. (Left) Mountain chicken conservation breeding facility in Dominica. (Right) Supporting mountain chicken conservation at Carnival, Dominica 2012. (M. Gaworek-Michalczenia).

In 2009, 50 (25 male and 25 female) *Leptodactylus fallax* were collected from Montserrat and exported to Europe where the frogs were held in biosecure facilities to minimise the risk of them picking up novel pathogens in captivity. The captive programme is managed through EAZA (European Association of Zoos and Aquariums) as an EEP (EAZA Ex-situ Programme) (Garcia & Schad 2015). The aim of these captive breeding populations is to maintain a viable population of *L. fallax* free from novel pathogens and removed from *Bd* infected sites and also to provide a captive-breeding stock to use for experimental releases and to potentially restock depleted wild populations at a later date. Although less than half of the original founders of 2009 have been successfully bred, the Montserratian biosecure captive population has produced viable clutches every year since the programme began in 2009.

A series of four experimental releases were conducted by reintroducing captive bred *Leptodactylus fallax* into the Central Hills in Montserrat. The aim of these releases was to determine factors affecting variation in *L. fallax* mortality when released into a *Bd* positive environment. Analyses were being conducted as part of a PhD investigating the ecology of *Bd* and interactions between the fungus and *L. fallax*. Factors that were tested included the age of the frogs and the season of release (Hudson 2016c).

Susceptibility to *Bd* varies between species and although individual frogs can be treated for *Bd* other species act as disease vectors. *Bd* positive apparently healthy eleutherodactylid frogs have been found on both Dominica and Montserrat. There is ongoing research into the emergence, epidemiology, and the impact of chytrid in Montserrat and Dominica with longitudinal monitoring of *Leptodactylus fallax* at key sites as well as the prevalence of *Bd* by using eleutherodactylid frog as indicators of *Bd* status of a given site (Hudson et al. 2019).

When *Bd* was first detected on Montserrat an in-situ treatment of *L. fallax* using the antifungal drug, itraconazole was trialled (Hudson et al. 2016b). Infection probability was lower in treated animals and whilst long-term post treatment survival was not observed, the antifungal treatment prolonged the estimated time to population extinction. In-situ itraconazole treatment may be a viable method to augment other conservation interventions for amphibian species threatened by chytridiomycosis.

In 2016 only two *Leptodactylus fallax* were thought to remain on Montserrat, these animals (a male and female) were wild and had not been part of any translocation program. Whilst occurring in roughly the same area, the home ranges of these frogs did not overlap. The mountain chicken recovery programme translocated the female frog into the male's territory and constructed a number of artificial nest chambers. Although both frogs were seen for several months after the translocation, neither have been seen for nearly two years as of 2018. Attempts to establish a population of *L. fallax* on Montserrat are ongoing. The next step is to evaluate the efficacy of a temperature refuge from chytridiomycosis on the disease's pathogenicity in *L. fallax*. Captive bred *L. fallax* from Europe are to be translocated to Montserrat in 2019 where they will be housed in semi wild conditions in large enclosures. The environment within these enclosures is manipulated with a variety of techniques, designed to raise the temperature of the environment out of the range suitable for the chytrid fungus. This plans to mitigate the impact of the disease on the population; these methods may be scalable to the island at large but also potentially to other amphibian populations.

The Dominican Forestry, Wildlife, and Parks Division and the Department of Environment in Montserrat run education and outreach in order to raise awareness of *Leptodactylus fallax* locally (Adams et al. 2014). In Dominica, initial outreach focused on reducing hunting activity (McIntyre 2003) but since the arrival of *Bd*, a greater emphasis has been placed on the conservation efforts in order to build and maintain a large protected area for *L. fallax* conservation. This has included disseminating information to the Dominican public using information boards, posters, leaflets, radio and television, and giving talks to schoolchildren. A campaign entitled "Have you seen me? Have you heard me?" was launched in 2011; people are encouraged to report *L. fallax* sightings and vocalisations. This campaign included a series of public engagement events (Fig. 11), such as a community group promoting the project in the 2012 carnival, a 2014 carnival queen contestant using her talent and costume round to promote the *L. fallax* story, an annual mountain chicken hike on the island, and an annual Mountain Chicken Day. A crucial component of the outreach campaign is the involvement of local contributors. This helps to foster a sense of local pride and ownership for saving the species. Recently a talented local poet published a poem in support of the project called the "Crapaud Story", and collaboration with local artists has taken place to help use local talent and business to develop interest in the project. The use of social media proved a very effective tool at raising local and international awareness

A communications strategy was produced for Montserrat in 2011 (Adams & Mendes 2011) to guide a comprehensive programme of education and outreach activities between 2011 and 2013. Activities included a series of radio and television programmes, production of educational posters and leaflets, community and school presentations, production of two calypsos, creation of a website and social media pages dedicated to *L. fallax* conservation and a forty minute wildlife documentary featuring *L. fallax* and the conservation efforts conducted by DOE and Durrell Wildlife Conservation Trust. Island wide surveys showed an increase in the knowledge, understanding, and empathy towards the species was achieved over the three-year project.

Objective 4.6.1 in the Mountain Chicken long term recover strategy 2015-35 is to "scope the feasibility of engaging historic range state countries in the captive breeding of mountain chickens" (Adams et al. 2014). Both Martinique and (maybe) Guadeloupe (see section 1.5.1) historically contained *Leptodactylus fallax* and their governments are keen to be involved in the mountain chicken project. Zoo de Guadeloupe/Parc des Mamelles and Zoo de Martinique have expressed their interest to participate in the mountain chicken project in conjunction with island governments. There is the potential to reintroduce *Leptodactylus fallax* to both these islands. *Bd* has so far been confirmed as present on Guadeloupe, with assessments on Martinique still ongoing as of 2019 (G. Garcia unpublished data).These institutions are happy to start the process to evaluate the possibility of holding this species in semi-natural conditions or totally isolated under the umbrella of the EEP Program (see section 2.7).

1.6 Diet and feeding behaviour

Like most frogs *Leptodactylus fallax* are carnivorous, with both sexes and all ontogenetic stages feeding on almost any animal that they can catch and swallow. This includes a wide range of insects, millipedes, spiders, tarantulas (Rosa et al. 2012), land snails, and slugs, as well as vertebrates. Tree frogs, anoles, and snakes have been reported as vertebrate food items (Brooks 1982; Rosa et al. 2012) as have geckos (G. Garcia pers. obs.). Cannibalism has never been recorded (except mothers feeding eggs to their tadpoles). The main food item is crickets, but beetles, harvestmen, millipedes, and snails are also important in wild populations (Fig. 12) (Brooks 1982; Jameson et al. in press). Some minor seasonal differences in diet exist between the wet and dry season as shown in Fig. 13.

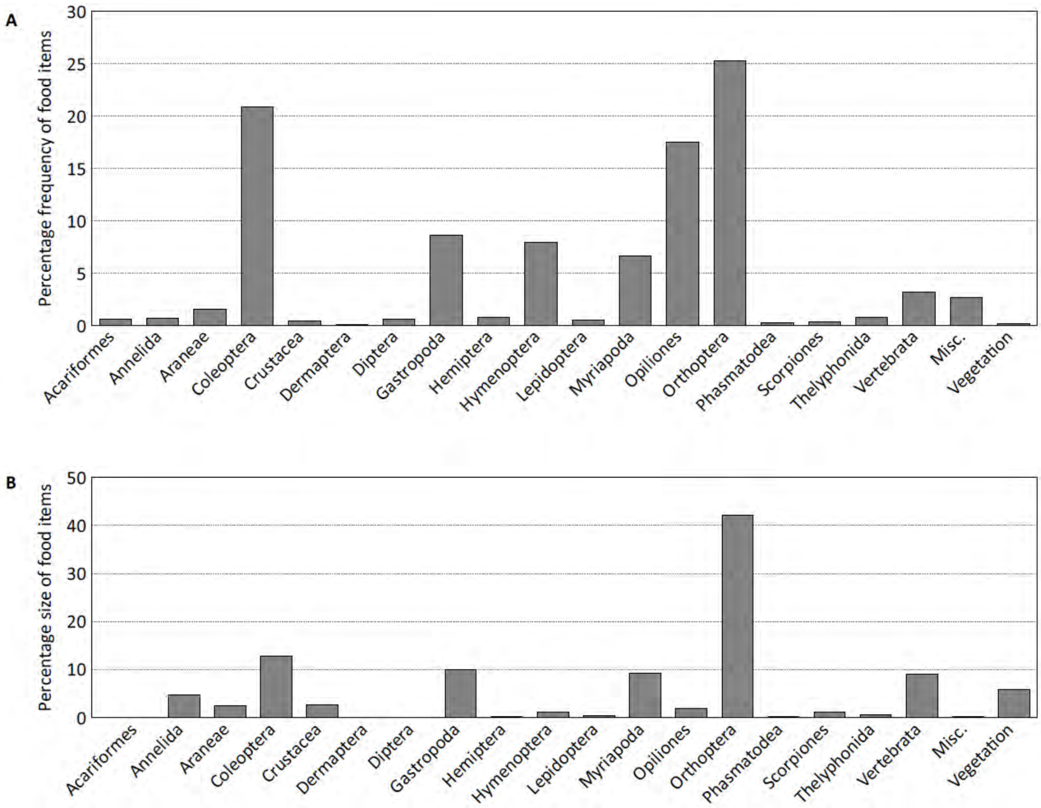

Figure 12. (A) Percentage of total frequency of food items of each prey category, and (B) Percentage of total size (proportional volume and proportional dry mass) of food items of each prey category for *Leptodactylus fallax* across datasets from Dominica (1965-66), and Montserrat (1979-80 and 2009). From Jameson et al. in press.

Despite being a native apex predator, little is known about the ecological role of *Leptodactylus fallax* or whether it functions as a keystone species. It is suspected that with such a wide diet and voracious appetite (*L. fallax* have been recorded to eat up to 100 crickets per individual per week in captivity; M. Goetz pers. comm.), it is highly likely that *L. fallax* plays an important role in ecosystem function. Following the precipitous decline of *L. fallax*, the effects of the reduction in pressure on local invertebrates are being seen in Dominica, with reported (but never quantified) cases of an increase in centipedes, millipedes, and crickets (C. Fenton & M. Sulton pers. comms.). The decline of *L. fallax* on Montserrat was followed by a reported (but never quantified) increase in sightings of the invasive cane toad (*Rhinella marina*) (C. Fenton & L. Martin pers. comms.). It is speculated this is due to a reduction in competition for food and is predicted to result in increased negative impacts on native species.

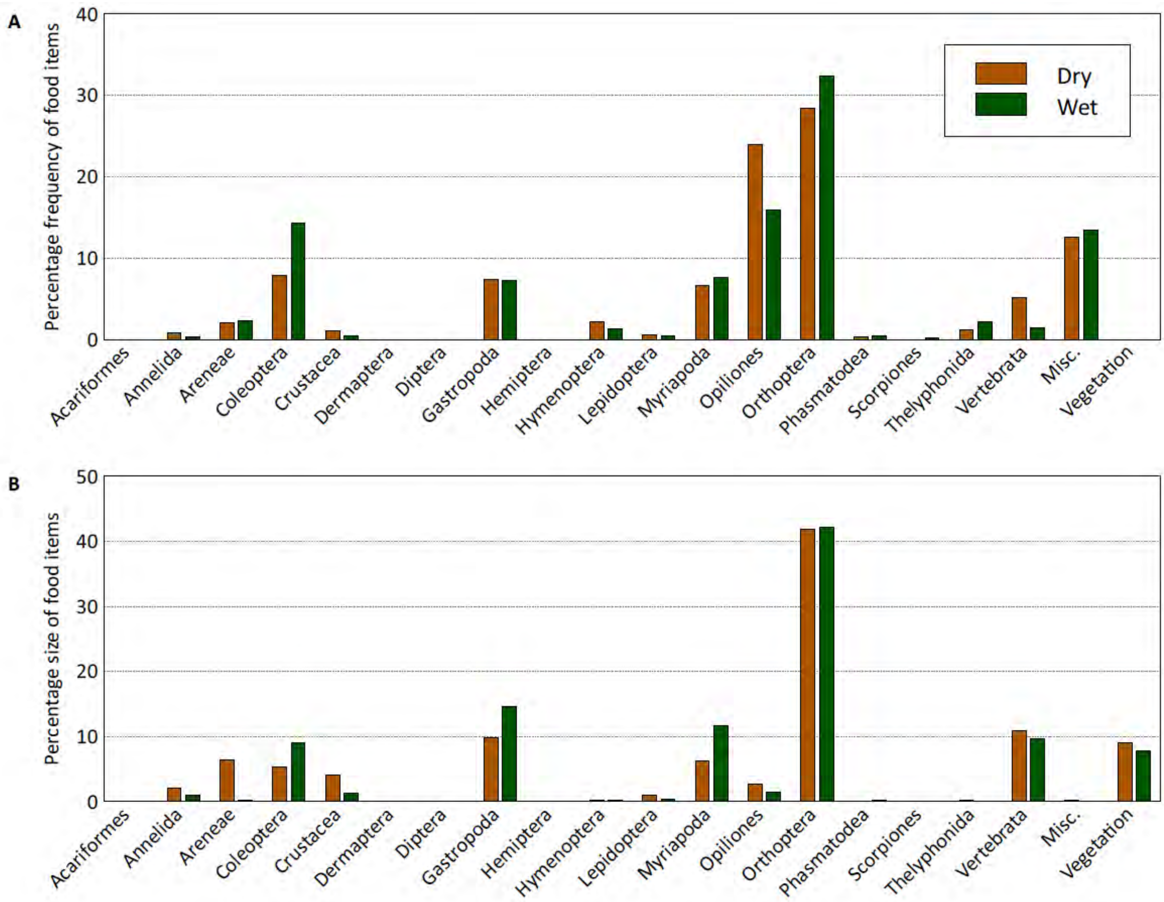

Figure 13. Proportional composition of diet by prey category of *Leptodactylus fallax* in the dry and wet season by (A) percentage of total frequency of food items for each prey category and (B) Percentage of total size of food items for each prey category. From Jameson et al., in press.

Leptodactylus fallax tadpoles feed exclusively on a diet of unfertilised eggs provided by the mother (see sections 1.7 and 1.8) (Fig. 14).

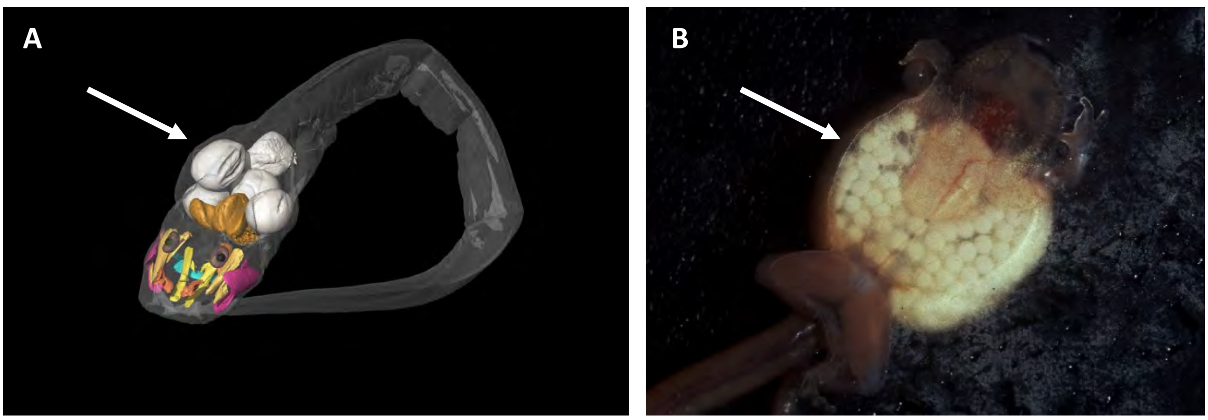

Figure 14. *Leptodactylus fallax* tadpoles eat unfertilised eggs: (A) False colour 3D visualisation of tadpole showing eggs in stomach (arrow) (R. Boistel). (B) Photograph of ventral surface of metamorph showing eggs in stomach (arrow) (R. Gibson).

1.7 Reproduction

Leptodactylus fallax have a unique reproductive strategy for an amphibian, with sophisticated maternal care, which includes obligatory oophagy in which mothers feeding tadpoles their own unfertilised eggs (Gibson 2001; Gibson & Buley 2004). Everything known about mating and parental care in *L. fallax* is based upon observations of captive animals.

1.7.1 Developmental stages to sexual maturity

Leptodactylus fallax eggs hatch 7-10 days after being laid. Larvae take approximately 6-8 weeks to metamorphose. Tadpoles attain a total length of 150mm. At metamorphosis frogs weigh between 1.6 and 3.6g (Gibson & Buley 2004) and measure 23-29mm long (SVL). It takes 2-7 days for all individuals to metamorphose and leave the nest, after which they receive no more direct parental care. However, juveniles often remain in close proximity to the nest and the mother animal for the next 1-2 weeks and seem to be recognised as conspecific kin as they are not predated upon by adult frogs which will feed happily on other frog species of similar size (M. Goetz pers. obs.). Young frogs grow rapidly but growth rates for wild animals have not been reported. In captivity, males develop secondary sexual characteristics (keratinized spurs) at an age of 8-10 months.

1.7.2 Age of sexual maturity

Sexual maturity is reached within two years. A captive female reproduced at 22 months at Jersey Zoo while males, depending on size, can be heard producing calls from as early as 6 months old (M. Goetz unpublished data), the youngest breeding male was 1 year old (G. Garcia pers. obs.).

1.7.3 Seasonality of cycling

Breeding is thought to be initiated by rainfall and the breeding season starts in February with the onset of rain (Fig. 15) and extends through to September (Davis et al. 2000).

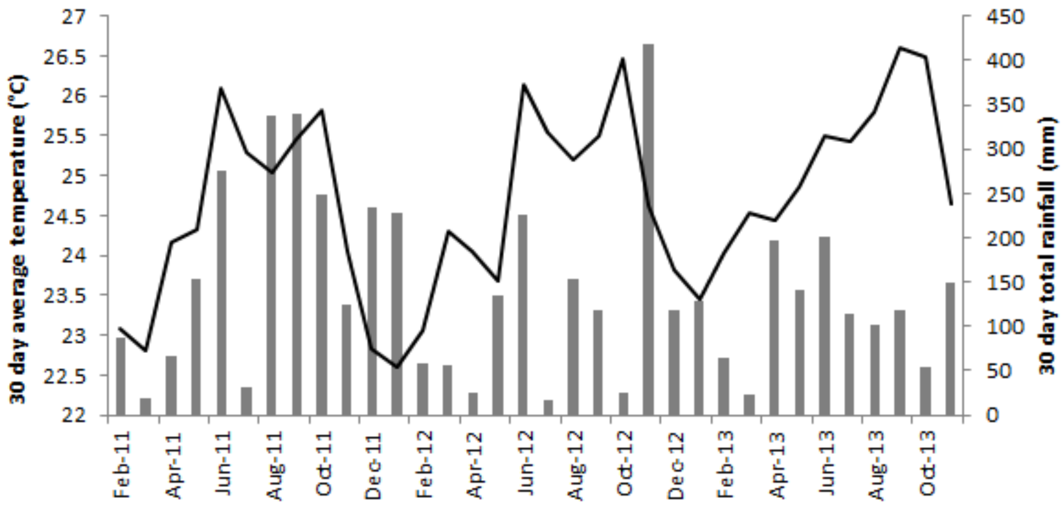

Figure 15. Monthly estimates of 30-day mean temperature and 30-day accumulated rainfall averages for Sweet Water Ghaut, Montserrat. Black line represented temperature, and grey bars represent rainfall. (From Hudson et al. 2019).

1.7.4 Clutch size

3-78 froglets have been recorded metamorphosing successfully per nest in captivity. Tadpoles are fed unfertilised eggs by the female attending them. Attending females may feed tadpoles in the nest 10-13 times during larval development, supplying an estimated 10,000-25,000 eggs (Gibson & Buley 2004). In captivity, females usually only lay one fertile, i.e. developing clutch per year although up to nine infertile nests can be produced per pair/year (M. Goetz unpublished data). One female from ZSL has been recorded laying two fertile clutches in a single year. The first clutch was small, producing six tadpoles, only two of which survived to maturity. The second clutch produced 33 tadpoles of which 31 survived (F. Servini pers. comms.)

1.8 Behaviour

1.8.1 Activity

Leptodactylus fallax are primarily nocturnal, spending the days hiding in burrows and rock crevices, or relying on their camouflage to hide in the leaf litter. They emerge at dusk to feed and, in the breeding season, to seek mates. In the wild, *L. fallax* are particularly active on cooler, humid nights. In captivity, frogs can be seen basking around spotlights during the day (M. Goetz & B. Tapley pers. obs.). This is a relatively infrequent behaviour but occurs often enough that mild basking lamps should be provided to account for this behaviour. During experimental releases of captive bred *L. fallax* conducted between 2011 and 2013, frogs that were tracked showed that during dry periods, individual *L. fallax* accumulated around available water sources such as ponds. In these ponds multiple frogs (including cane toads that are carriers of *Bd*) were often recorded in close proximity, sitting in the same water. This tendency for *L. fallax* to congregate in water during dry periods increases the risk of transmission of *Bd* from infected to healthy frogs and amplifies the prevalence of *Bd,* turning these ponds into ‘hot spot’ areas of infection. Similarly, congregations increases the risk of transmission of ticks (*Amblyomma rotundatum*) and associated blood-borne pathogens. During periods of heavy rainfall and/or elevated levels of water in the ghaut, *L. fallax* disperse more widely up the banks of the ghauts and spread out increasing the distance between them and neighbouring conspecifics and cane toads (Hudson 2016c).

1.8.2 Locomotion

Leptodactylus fallax have powerful hind limbs and are capable of jumping distances of over two meters.

1.8.3 Predation

Introduced rats have the potential to eat *Leptodactylus fallax*. During the dry season of 2005, 27% of frogs at one site on Montserrat were found with old and, more commonly, fresh rat bites. Most of the bites were observed on the hind limbs (Adams et al. 2014). On Montserrat, there are also populations of invasive pigs that could potentially feed on *L. fallax* and their nests opportunistically. Other invasive species such as the cane toad on Montserrat and the common opossum on Dominica could potentially consume juvenile *L. fallax* (Adams et al. 2014). Although unproven, the only native predator of adult *L. fallax* might be the Montserrat racer snake (*Alsophis manselli*); whilst the Montserrat tarantula (*Cyrtopholis femoralis*) and land crab (*Gecarcinus sp.*) may feed on small juvenile frogs.

1.8.4 Vocalisation

Both male and female *Leptodactylus fallax* will move into the open and call (Fig. 16), possibly to advertise their presence to potential mates. *L. fallax* primarily vocalise at night although they may also call during the day (Davis et al. 2000). Calling is most intense in March and April, coinciding with the onset of increased humidity (Adams et al. 2014). The male produces a distinctive whooping call, which can be heard up to a kilometre away. Advertisement calls of different males do not overlap (Davis et al. 2000). The call of Female *L. fallax* is quite different and much quieter (Davis et al. 2000). Gibson & Buley (2004) report that males entice females into their nests with a trilling bark call (100-120 calls per minute).

Animals of all sizes and sexes can emit a very loud and piercing distress call, e.g. when caught and handled. This call is often lost in captive individuals, likely as they get used to being handled.

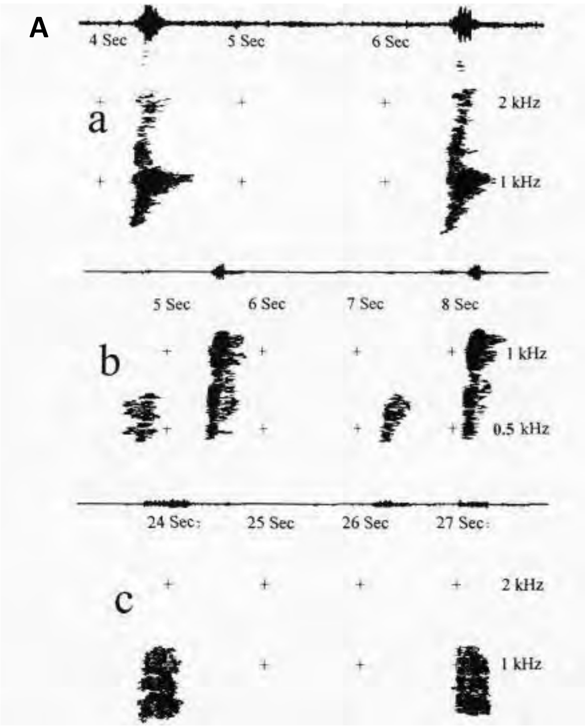

Figure 16. (A) Male advertisement call with harmonics, oscillography: (a) Sonograph bottom; (b) duo of type 1 female call and male call; (c) type 2 female call (taken from Davis et al. 2000). (B) Male *L. fallax* vocalizing. (ZSL).

1.8.5 Sexual behaviour

Male *Leptodactylus fallax* are territorial, with individual males seeking to seize and defend territories through combat. Males rear up on their hind legs; each contestant supported by the other's body weight and attempt to wrestle the other to the ground (Gibson & Buley 2004). Skin secretions seem to stimulate aggression in other males (King et al. 2005).

Males prepare nest chambers prior to breeding, using their forelimbs and head to shape the chamber (Fig. 17A). Males attract females by calling from burrows (Gibson & Buley 2001). The call is a distinctive "whooping" increasing in tempo to a "trilling" sound (at 100–120 calls/min) that can be made at day or, more commonly, night. Calling is most intense in March and April, coinciding with the onset of increased humidity in Montserrat. Calls appear to attract a female to enter a males burrow where mating occurs, with the male using his forelimbs to grasp the female in front of the forelimbs (a position called axillary amplexus – Fig. 17B). Males seem to use their hind legs during mating to stimulate females to produce a foam nest into which her eggs are laid (Davis et al. 2000).

A foam nest is produced at the bottom of the male's burrow (Fig. 17C) and the surface becomes thickened after approximately 24 hours, this may provide protection against desiccation, some potential predators, and also possible fungal / bacterial infection. The volume of the foam decreases after the first 7-10 days to produce a mucosal puddle. A sign of nest fertility is that the foam returns to its initial consistency after the first feeding visit by the female to the nest. The tadpoles develop in the foam nest and, uniquely, feed only on eggs that their mother deposits in the nest every 1-7 days (Fig. 17E). The female will also deposit more foam in the nest to prevent the eggs and larvae from becoming desiccated. The female, and commonly the male, guards the nest, and will actively defend it against intruders (Davis et al. 2000; Gibson & Buley 2004) including other female *Leptodactylus fallax,* until tadpoles metamorphose (Fig. 17G and H). Females have been observed wrestling and vigorously kicking other conspecifics that enter the nesting chamber (B. Tapley pers. obs.). *L. fallax* have not been observed eating metamorphosed frogs when they leave the nest or in the days after emergence when juveniles often stay very close to the nest burrow and the mother animal but have been observed eating similar sized invertebrates and frogs of different species. This suggests they are be able to distinguish their young from potential food items (B. Tapley & M. Goetz pers. obs.).

Figure 17. Sexual and reproductive behaviour in *Leptodactylus fallax*: (A) Male covered in mud having prepared nest (G. Garcia). (B) Axillary amplexus (G.Garcia). (C) Production of the foam (B. Tapley). (D) Freshly hatched tadpoles visible in the foam (G. Garcia). (E) Female feeding tadpoles (I. Stephen); (F) Late stage tadpoles in the nest (G. Garcia); (G) Metamorphs in the nest (G. Garcia). (H) Metamorph in the process of reabsorbing the tail (G. Garcia).

Section 2: Management in Zoos and Aquaria

2.1 Enclosure

Due to their large size and ability of adult animals to jump up to two meters, it is important that *Leptodactylus fallax* have large enclosures. The size of the enclosure will vary according to the size of the animal being housed. Newly metamorphosed frogs can initially be reared in large plastic Hagens Faunariums (Fig. 18A) (Exo Terra, Rolf C. Hagen (UK) Ltd., Castleford, UK) but will soon need to be moved to larger enclosures (Fig. 19 and 20) a few weeks later as they grow rapidly (see also 2.4.5). *L. fallax* are good at climbing and are able to scale mesh or nets with ease.

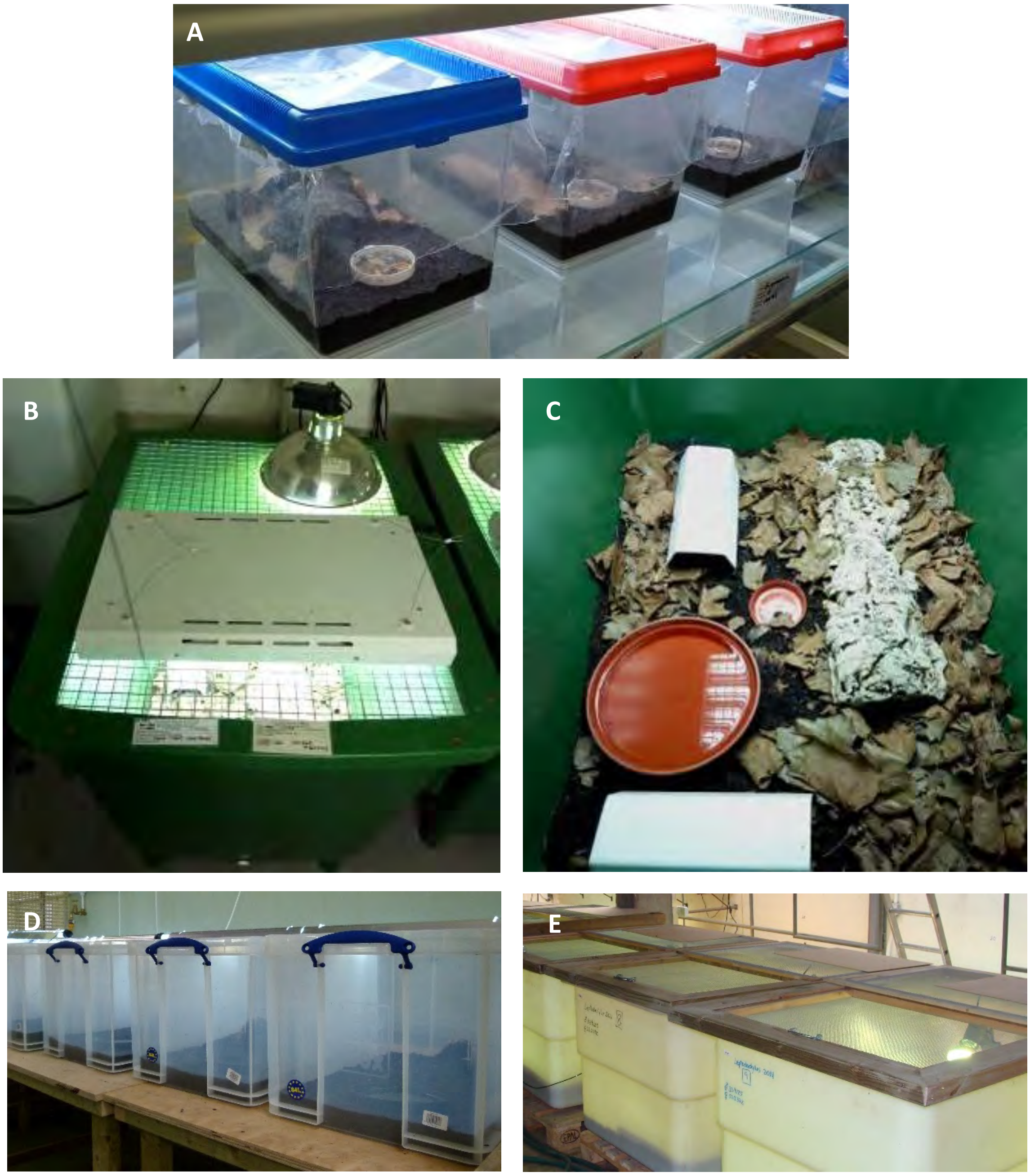

Figure 18. Enclosures for rearing juvenile *Leptodactylus fallax*: (A) Faunariums are appropriate for rearing metamorphs (B. Tapley). Various plastic boxes have been utilised effectively for rearing larger Juveniles at ZSL London Zoo (B and C) (L. Harding), Jersey Zoo (D) (G. Garcia), and Barcelona Zoo (E) (G. Garcia).

Figure 19. Various off show enclosures for adult *Leptodactylus fallax*: *L. fallax* were easily able to jump between enclosures (A), these had to be modified to prevent frogs from escaping (B) (I. Stephen and L. Harding). (C) A converted room is appropriate for housing large juveniles and adult frogs (D. Lay). (D) Specially designed container units (APODS) are used at Chester Zoo as large biosecure enclosures (G. Garcia). (E and F) It may be necessary to house animals as pairs for planned breeding events, converted paddling pools have served well at some institutions (M. Goetz and G. Garcia).

Figure 20. Various display enclosures for adult *Leptodactylus fallax*: (A) Example of large public display enclosure, suitable for multiple animals at Chester Zoo (G. Garcia). (B) Example of medium sized public display enclosure at Jersey Zoo (D. Lay). Examples of small public display enclosures, suitable for pairs of animals at Barcelona Zoo (C) and Norden's Ark (D) (G. Garcia).

2.1.1 Boundary

Leptodactylus fallax have powerful hind limbs, capable of jumping distances of over two meters in length and nearly two meters in height. They are also surprisingly adept climbers for a large terrestrial frog; as such, precautions need to be taken in establishing a sufficiently high and secure boundary to prevent escape (Fig. 19B). Fortunately, despite the force behind the powerful jump of *L. fallax,* this species does not seem prone to damaging themselves by jumping into boundaries at full force; even glass barriers are usually recognized as such after a very short period of time. Therefore, most types of materials commonly used are acceptable as boundaries. Regardless of boundary material injuries to the face from excess rubbing against walls may occur if stocking densities are too high, this can be alleviated my maintaining suitable stocking densities (see section 2.1.5). In all cases, boundaries must completely enclose the enclosure, ensuring a suitable roof boundary is present to prevent escape by jumping or climbing. Care needs to be taken where boundaries meet as *L. fallax* are able to squeeze through gaps much smaller than initially thought possible.

2.1.2 Substrate

A suitable substrate should be used such as peat moss, bark chip, and leaf litter. A 6 cm substrate layer should be placed at the bottom of the enclosure with a top layer of dried leaves (Fig. 21). It is important that leaves are kept in discrete areas within the enclosures to avoid frogs being stepped on when keepers are servicing the enclosure and to ensure excess leaf litter doesn't impede UV absorption by frogs. In addition, it is important not to have too many leaves on the floor as this may make it difficult for *Leptodactylus fallax* to find food and could mean that they do not encounter food whilst it is still dusted/ well fed with supplement, which will compromise the nutritional value of the diet offered. This mix of substrate for adults and juvenile specimens not only provides security for the frogs but also helps to maintain humidity levels within the enclosure (Gibson 2001). An important consideration in regards to substrate is drainage. Care should be taken to avoid over saturating the substrate that should be moist but not wet. Methods such as having a layer of gravel with a membrane over the top underneath the substrate layer help to improve the drainage within the enclosure.

Substrate used for juveniles and metamorphs should be compacted so that it does not stick to the skin and cause irritation or damage.

The introduction of planting provides visual barriers and humid microhabitats within the enclosure. Recommended plants are palms and plants native to Dominica and Monserrat such as members of the genus *Monstera*. Visual barriers are important and give the frogs a feeling of security.

Consideration should be given within the bio-secure population with regards to the sourcing of substrate, leaf litter, and live or artificial plants – trying to minimise the possibility of potential contaminants that the leaf litter/substrate/ plants may have been susceptible to. Leaf litter should be adequately dried before being introduced into enclosures within a bio-secure facility.

2.1.3 Furnishings and maintenance

Sufficient furnishings should be provided to provide a variety of refugia whilst leaving the majority of floor space as open ground to allow the animals to move around freely without too many obstacles. Leaving open areas is also important to ensure individuals encounter food whilst it is still dusted with supplement/ well fed to ensure the nutritional value of the diet offered is maximised.

As *Leptodactylus fallax* usually remain mostly hidden throughout the day, both humid and drier areas need to contain shelter. A variety of different types of shelters should be provided to create different microhabitats and suit different sized animals and different behaviours (Fig. 21). These should include furnishings creating large well-aerated refuges such as large plants, large upturned bowls, and upturned plastic dog beds. The furnishings should also create tight, humid spaces such as under nearly flat cork bark and upturned saucers. Other objects creating intermediate sized shelters and also dry places to hide are required, such as cork bark tubes, ceramic piping, and halved plastic flower pots.

In the wild, *Leptodactylus fallax* are found in association with permanent water bodies (Daltry 1998) and spend much of their time sitting in shallow water (Adams et al. 2014). As such, a permanent clean water source should be provided in enclosures, to be changed daily. Such a water source need not be large or deep; deep enough to cover an individual's legs when sitting and large enough to allow a couple of individuals to sit together. A large shallow dish is sufficient. Dishes should be fitted with a small length of cork at one side (Fig. 21) to prevent food insects from drowning. Frogs often defecate in water dishes, as such, daily water changes provide a good opportunity to collect faeces for health screening when required (G. Garcia pers. obs.).

Enclosures of breeding individuals should contain artificial nesting burrows (Fig. 20). These can be constructed from opaque plastic boxes (approx. 35 X 25 X 25 cm) or lidded plastic plant pots. The nest chambers can contain a shallow (1 cm) layer of moist bark-chippings or a lining of potting clay although nests are also laid onto normal soil or onto bare plastic. Boxes/ pots should have a length of plastic piping (30-100 cm) of 10 cm diameter attached to create a single tunnel entrance (Gibson & Buley 2004). Nest boxes should be built with a tightly fitting but easily removable lid to allow nests to be easily checked and serviced with minimal disturbance to animals.

Enclosure furnishings should be selected to allow all animals within the enclosure to be checked with ease, and to allow the enclosure to be spot-cleaned daily. Keepers should be able to move furnishings with ease and all furnishings must be firmly placed to avoid crushing the frogs. Furnishings should be cleaned if soiled. It is important that porous items in the enclosure are not cleaned with chemical disinfectants and reused as these may leach into the environment and be harmful to the frogs.

Consideration should be given within the bio-secure population with regards to the sourcing of furnishings – trying to minimize the possibility of potential contaminants which furnishings may be susceptible to.

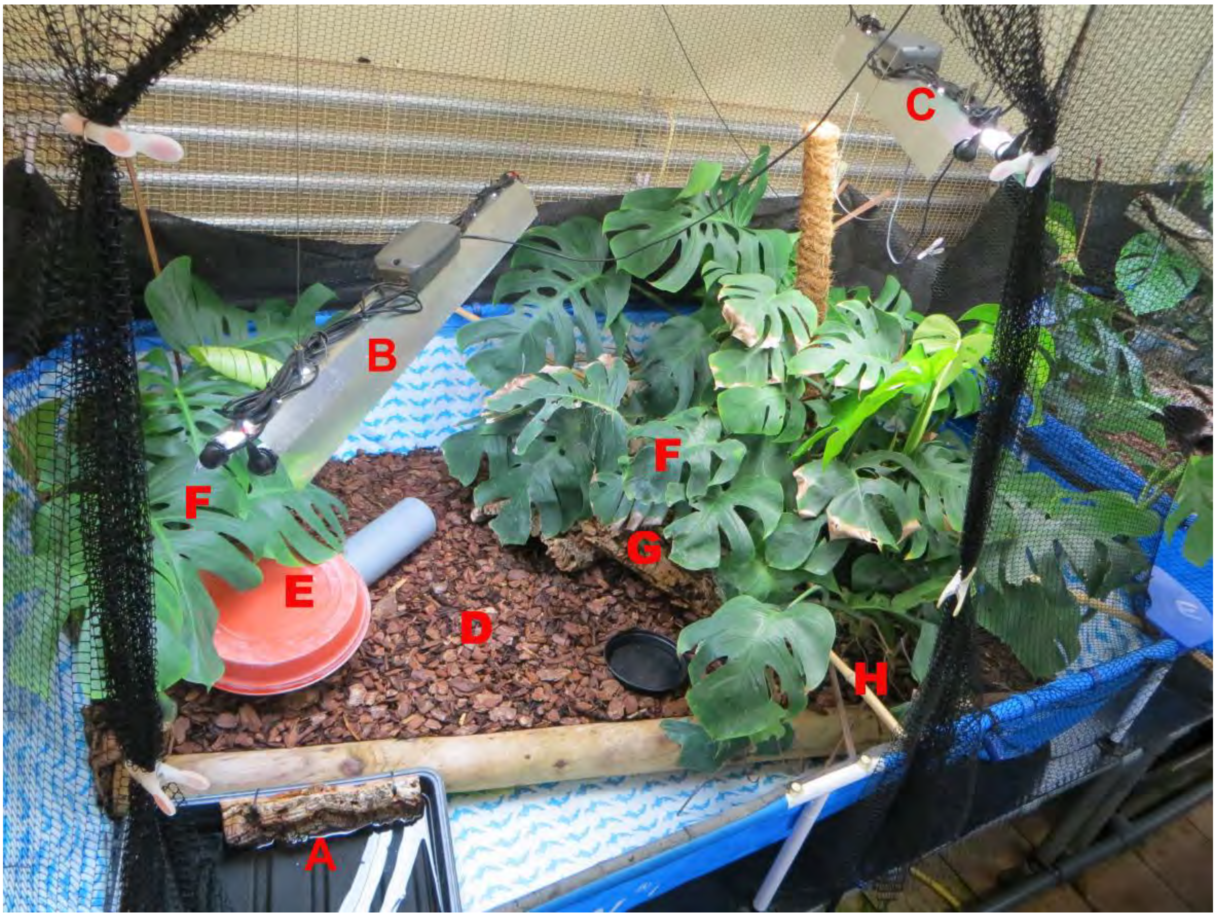

Figure 21. Example of an off-show enclosure (a baby swimming pool) measuring 1.5 x 2.2m used to hold a breeding pair of *Leptodactylus fallax* and up to 3 adults or a pair of adults and up to four juveniles. A) water bowl with cork side to prevent food insects from drowning; B) double T5 fluorescent tubes providing an UV-Index gradient of 1.5 - 3 across the open area of the enclosure; C) additional lighting for plant growth; D) bark-chip substrate humid under the plants, drier in the open area; E) nesting burrow; F) plants providing cover; roots throughout the substrate help with adjusting humidity levels and nitrogen waste removal; G) cork bark hides; H) horizontal bamboo sticks keep the plants off the ground providing an open area underneath with cover from above. (M. Goetz).

2.1.4 Environment

As with all amphibians, *Leptodactylus fallax* need to be provided with appropriate gradients of humidity, temperature, and light, including ultraviolet A and B wavelengths. Enclosures must be large enough that gradients of these parameters can be established which range from a humid (but not soaking wet) area on the darker, cooler side of the enclosure, to a drier or actually dry area on the warmer side which also receives much of the light.

If *Leptodactylus fallax* are kept on constantly too wet or waterlogged substrate without the opportunity to choose a dry spot to rest, ulcerations on the legs, feet, or the ventral body can on occasion occur (Fig. 22). This is usually due to over-acidification and rotting of waterlogged substrates combined with the relatively heavy weight of the species; it is therefore recommended, especially in the drier, non-breeding season, to keep this species drier rather than too wet. If enclosure floor space is restricted, a drier area to sit on can be provided e.g. by a layer of dry leaves that are placed in one corner of the enclosure.

Figure 22. Ulcerations on the fingertips of *Leptodactylus fallax* as a result of inappropriate substrate. (G. Garcia).

Over the years, different automated watering and/or spray systems have been employed in a variety of enclosure types and sizes to varying degrees of effectiveness. In some institutions such systems invariably overwatered substrates and the time saved on spray and water routines was less than the time spent on additional substrate changes when they became water-logged. In these institutions spraying only little to moderately (depending on substrate and ventilation) once per day or every two days in the winter months is usually sufficient to keep the substrate underneath a dry layer humid. In the breeding season spraying once or twice per day might be needed to provide a much higher overall enclosure humidity and humid substrate throughout.

In other institutions automated watering systems have been used to great effect, saving on keeper time and allowing heavy sprays to be provided without breaching biosecurity without issues of water logging. Such systems can be set to simulate seasonal cycles of rainfall and to create a gradient of rainfall and humidity throughout the enclosure, providing a more naturalistic environment with less human disturbance. Such changes in seasonal rainfall and humidity are a key breeding determinant, an increase in frequency of showers from spray systems coinciding with the initiation of calling behaviour at some institutions (C. Michaels pers. comms.). Automated watering systems may also be of value is in recreating the storms of the wet season a couple of times per year to mimic natural seasonal cycles. Here, after the initial water changes and checks of an enclosure the watering system can be left on at a high intensity for several hours provided that the enclosure is fitted with suitable drainage. The use of such systems must be approached on a case-by-case basis, depending on other features of the enclosure. In large well-drained enclosures automated systems may be preferable, whereas in smaller more poorly drained enclosures manual spraying may be optimal.

Ambient temperatures for each season are summaries in table 1. A spotlight should be provided that offers a very mild basking spot (Fig. 21), elevating local temperatures by about 4-6°C.

Table 1. Temperature parameters for *Leptodactylus fallax* enclosures matching day-night and seasonal (dry season – November-April; wet season – May-October) cycles.

Season	Time	
	Day	**Night**
Dry	26-28^oC	20-24^oC
Wet	28-30^oC	23-28^oC

Leptodactylus fallax seem to respond to seasonal changes less through changes in temperature and more through changes in day length, humidity and rainfall. Thus, lights should be timed to illuminate enclosures 10-11 hours in winter months and then gradually increase to up to 13-14 hours in the middle of summer. This is slightly more variation than the light levels in the Caribbean but might enhance the effect.

As for all amphibians, naturalistic lighting should be provided including all wavelengths and of course also including UV-A and UV-B radiation. Especially UV-B radiation has proven to be essential (Baines et al. 2016) and is particularly important for growing frogs (Tapley et al. 2015a). UV radiation has beneficial effects on calcium metabolism and (mainly through Vitamin-D_3 production and regulation) on reproduction, immune system, gene expression and general wellbeing (Baines et al. 2016). A gradient of UV-B radiation with a maximum UV-Index of 3 (measured with a Solarmeter 6.5 UV Index meter [Solartech Inc., Harrison Township, Michigan, USA]) at one open area or around the basking spot and then fading to zero at the other end of the enclosure is considered suitable for *Leptodactylus fallax* (Fig. 21); in small enclosures where no such gradient can be provided, e.g. in rearing boxes, a maximum UV Index of 1 - 1.5 throughout the enclosure is recommended (see also Baines et al. 2016). UV gradients can be achieved by placing a suitable UV light at one end of an enclosure with more open ground cover, with vegetation and cover increasing to the other end of the enclosure (Fig. 21B).

Leptodactylus fallax will benefit from furbishing which provides some cover from above. If such areas are provided throughout the enclosure, the frogs are much more likely to be active and use more of the enclosures during the day, which also aids in feeding since the frogs will better distributed throughout the enclosure. Although open hides such as larger cork bark tubes are often accepted by adult frogs, many individuals and especially younger animals prefer tighter hiding spots they can wedge under and should hence be provided with e.g. upturned plant saucers or nearly flat cork bark.

Leptodactylus fallax do not seem to like air movement due to the desiccating effect on their permeable skin and can be seen in the wild pressing themselves flat to the ground if wind is present (G. Garcia pers. obs.). The same behaviour can be observed if stronger ventilators or air conditioning systems are used in captivity and it is advised to direct the airflow in a way that no draft is experienced in the enclosures.

In the wild, males often sit on elevated positions, e.g. rock boulders, to emit territorial calls (Fig. 23). Such elevated calling sites might be worth replicating in larger enclosures. Where multiple males are present a large number of such sites should be provided.

Figure 23. Male *Leptodactylus fallax* calling from elevated positions (G. Garcia).

2.1.5 Dimensions

The recommended minimum enclosure dimensions for permanently housing adult *Leptodactylus fallax* are about two meters squared but it is beneficial to offer more space as *L. fallax* is an active species often suffering from muscle atrophy when space is too limited. Due to the large size of *L. fallax*, offering suitable and necessary microclimatic and microphotoic niches and gradients demands considerable space. Juveniles must be kept in progressively larger enclosures as they grow and age (see section 2.4.5). Enclosure sizes roughly equivalent to ten times snout-vent length squared are recommended.

2.1.6 Biosecurity and barrier management

In Europe there are two ex-situ metapopulations of *Leptodactylus fallax*, the biosecure population (managed for future translocation / supplementation) and the non-biosecure population managed for conservation education and conservation research. A non-biosecure population managed in an integrated fashion with the European population is present in the USA. The goals of these programs differ and therefore the management differs too. At the very basic level, all populations of *L. fallax* should be managed so that they do not pose a risk to native amphibian species. It is recommended that all wastewater is filtered to remove organic waste, prior to it being disinfected with a suitable disinfectant (e.g. Virkon S, Anigene, F10 or Safe4) following the manufacturers guidelines prior to being discharged into municipal waste. To avoid spreading disease within a collection, equipment should be disinfected between enclosures or ideally, dedicated equipment should be in place for each enclosure. It is recommended to use non-powdered latex or nitrile gloves with amphibians in captivity in order to protect the handler and the frog's skin and to prevent the transfer of pathogens between individuals and/or species.

Representatives from the biosecure population must be housed in dedicated and isolated facilities with dedicated equipment to minimise the chances that they will come into contact with novel pathogens that could then be translocated with the frogs and introduced to wild habitats. The institutions housing the biosecure population must make sure all of the above minimum requirements are in place. Furthermore, some more stringent management protocols should be adopted with the agreement of all biosecure holders. These include:

- Wearing freshly laundered uniform under overalls or changing clothing completely to overalls.
- Ensuring that staff working in the dedicated facility have not come into contact with any other amphibian or reptile prior to commencing their work with *Leptodactylus fallax.*
- The maintenance of a consistent / directional flow of routine.
- Undertake regular health screening of the frogs (analysis of blood samples, ultrasound and radiographs).
- Restricting access to the facility (essential personnel only).
- Communicating any breach in biosecurity honestly and transparently to the EEP species committee.

2.2 Feeding

Adult *Leptodactylus fallax* should be fed 4-5 times a week depending on the size of the frogs, their body condition, and the season. For the first 4-6 months, juvenile animals should be offered food daily. Adults should be fed more in the lead up to the breeding season and in the aftermath of the breeding season to aid recovery of lost body mass. The amount of food offered should be adjusted in accordance with the body condition of the frog. All food that is offered to the frogs should be well fed itself. Uneaten food items should be removed from the enclosure the day after being offered as the nutritional value of the food item would likely have decreased due to a lack of food for the prey items inside the enclosure. Removing the food the day after feeding is good practice as the frogs should be slightly hungry prior to the next feeding event so that the introduction of new food triggers a good feeding response; this is important as the frogs will then consume food that is well fed and dusted with dietary supplements when it is offered.

2.2.1 Basic diet

Leptodactylus fallax will accept a variety of large invertebrates and small vertebrates. The size of the food offered should not be larger than the width of the frog's head. Field crickets (*Urogryllus rufipes*), Cave crickets (*Amphiacusta* cf *annulipes*) and Cockroaches (*Blaberus* sp.), slugs (*Veronicella* and *Sarasinula* species), snails (*Austroselenites* sp.) and millipedes (Unknown species) were offered to the frogs in the facility at Roseau (Dominica) (Dale 2009; Nicholson et al. 2017). In European collections, a variety of invertebrates can be offered to *L. fallax* including black crickets (*Gryllus bimaculatus*) and brown crickets (*Gryllus assimilis*), locusts (S*chistocerca gregaria*), cockroaches (*Blaptica* sp. and *Blaberus* sp.), sun beetle grubs (*Pachnoda* sp.), snails (*Helix aspera*), stick insects (*Extatosoma tiaratum*) (and other phasmids), and earthworms (*Lumbricus terrestris).*

At Chester Zoo adult *Leptodactylus fallax* are fed eight crickets per individual per feed on Mondays and Wednesdays, and five crickets per individual per feed on Wednesdays and Fridays. Other prey items (see above) are not regularly available in large numbers and are therefore only provided alongside crickets and locusts when available as enrichment feeds.

2.2.2 Special dietary requirements

Sub-optimal nutrition and nutritional disease is a known issue in the captive husbandry of amphibians (Antwis & Browne 2009; Dugas et al. 2013; Gagliardo et al. 2008; King et al. 2005; Verschooren et al. 2011; Ogilvy et al. 2012; Tapley et al. 2015ab) as the nutritional requirements of most amphibians are unknown. Even when the diet is known, it is often impossible to replicate in captivity (Jayson et al. 2018a). Diets for captive amphibians are often limited by the commercial availability of food species and the ability to establish breeding colonies of appropriate species, as well as difficulties in providing prey species themselves with suitable diets (Tapley et al. 2015b).

A recent study analysed the nutritional content of food items that comprise 91% of the wild diet of *Leptodactylus fallax*, by dry weight of food items, and all food items offered to captive *L. fallax* at two European collections (Jayson et al. 2018a). The captive diet at one institution, without dusting of nutritional supplements, was higher in gross energy and crude fat and lower in ash, calcium, and calcium: phosphorus ratio than the wild diet. Most of the food items in the captive diets had a high omega-6: omega-3 fatty acid ratio and in the wild diet had a low omega-6: omega-3 fatty acid ratio. The authors recommended a combination of modifications to the captive diets to better reflect the nutritional content of the wild diet (Jayson et al. 2018a).

All arthropods should be dusted with an appropriate dietary supplement (e.g. Nutrobal) at least once per week. Dietary supplements are sensitive to high temperatures, high humidity and light and therefore should be stored in a refrigerator. Preliminary studies conducted at ZSL London Zoo have tested the effect of supplementation regimes on the development of gall bladder stones (choleliths). It was hypothesised that over-supplementation with calcium may be the cause of cholelith development. Although the results of this study were inconclusive it was found that animals raised with every feed supplemented had high levels of excess calcium stored in their dorsal lymph sacs (ZSL unpublished data). It may therefore be beneficial to supplement *Leptodactylus fallax* feeding with calcium more infrequently than every feed. However, further work is required to investigate this issue before any action should be taken (see section 2.9).

Vertebrates are a potentially important part of the diet of *Leptodactylus fallax* that haven't typically been accounted for in captive diets. In the wild, vertebrate prey makes up around 10% of diet of *L. fallax* by size (see section 1.6; Jameson et al. in press) and is potentially a very important component of diet, having a very different nutrient content to typical arthropod prey. Defrosted neonatal "pinkie" mice have been trialled as a feed item for *L. fallax,* however, *L. fallax* appear to be unable to recognise immobile dead prey as food and therefore rarely consume these food items (Chester Zoo staff pers. comms.). Further research should be carried out in the future to investigate suitable provisioning of vertebrate prey (see section 2.6).

2.2.3 Method of feeding

Leptodactylus fallax will only accept moving prey items. Food should be offered as late in the day as possible as *L. fallax* is nocturnal. Feeding events should coincide with the frogs' activity period so that food is consumed whilst it is still coated in dietary supplement and is itself well fed. This can be problematic for biosecure populations, which are managed in a way that they are serviced before keepers work with any other species, i.e. very early in the day. At ZSL London Zoo and in the facility on Dominica keepers return to the facilities at night after showering in order to feed the frogs. For food that is active on the surface broadcast feeding is a viable option. For food that may bury into the substrate (e.g. *Pachnoda* sp., *Blaberus* sp., and *Lumbricus terrestris*) feeding dishes are important and several can be used in one enclosure to prevent a single frog monopolising the food resource. Snails can be offered to adult frogs with the shell intact. Their slow movement may fail to illicit a feeding response, spraying the snails with water can encourage the snail to move.

2.2.4 Water

Water quality is an important consideration in keeping any amphibians, as all rely on some form of moisture, be it in a terrestrial or aquatic form (Odum & Zippel 2008). Monitoring water quality is vital to successfully rearing healthy captive amphibians (Odum & Zippel 2008); fluctuating water parameters create stress for the individuals, therefore, it is better to maintain constant conditions, even if these are slightly sub-optimal.

Leptodactylus fallax are known to be territorial and frogs housed in groups should either be provided with a large water body or several small water bodies (King et al. 2005). Water dishes should be deep enough to allow the frogs to fully immerse their drink patch. Frogs often excrete urine into the water and this can cause a build-up of toxic nitrogenous waste in the water body. Just because a dish looks clean, it does not mean that it is, water in dishes should be changed and scrubbed daily.

2.3 Social structure

In a breeding scenario, frogs should be housed as pairs. During the breeding season, males may become aggressive toward one another and behaviour should be closely monitored. Some who have bred *L. fallax* recommend a single female with two male frogs per enclosure (2.1) as they believe that the competition between the male may trigger reproductive behaviour. Other breeders have had success in breeding individuals that are kept permanently as a pair (1.1) and as larger mix sexed groups (G. Garcia, M. Goetz, and B. Tapley pers. obs.). Simply hearing males calling while not living with them may be enough to stimulate breeding behaviour in both males and females. As such, keeping multiple separate enclosures close enough together to allow calls to be heard may be a preferable set-up. The exact reproductive triggers for *Leptodactylus fallax* are unknown; if a response is not elicited from one set up consider swapping males around, always keep a close eye on all individuals when making changes to social structure.

Juvenile *Leptodactylus fallax* can be kept together but frogs will need to be size sorted into enclosures as they get older to minimise competition between individuals (see also 2.4.4). Underweight frogs should be separated for feeding to give them ample opportunity to feed. This said, on several occasions juveniles were left in adult enclosures, having avoided capture when others were moved to juvenile-only enclosures. On these occasions these left behind individuals grew faster than the separated members of the clutch (G. Garcia pers. obs.). As such, the separation protocol may need to be reconsidered, further research is required (see sections 2.4.5 and 2.9).

2.3.1 Basic social structure

Leptodactylus fallax is a territorial species and both males and females will defend territories or particular resources within those territories. It is therefore important that the appropriate stocking density is maintained, especially in smaller enclosures, and that animals are closely monitored to ensure that resources are not being monopolised by more dominant individuals. Male wrestling bouts can be particularly aggressive and there are cases where smaller male frogs have been killed by large dominant males (B. Tapley pers. obs.). Making sure that there are adequate resources in an enclosure (water dishes, refugia and potential nesting sites) and visual barriers may go some way to minimise aggressive interactions between frogs. In the laboratory aggression was triggered by males being exposed to skin secretions from other males (King et al. 2005; see also section 2.5.5). The optimal social structure for *L. fallax* is unknown but male biased sex ratios should be avoided if seeking to minimise aggression, though may prove beneficial in encouraging mating. Frogs housed together should be of a similar size to minimise competition for resources.

2.3.2 Changing group structure

Generally, *Leptodactylus fallax* can be kept in pairs or in groups as long as minimum space requirements are observed, enough appropriate hiding areas are provided and it can be assured that enough food is available and taken by all animals. At Jersey Zoo exhibit animals were kept in groups of up to 14 frogs of mixed sex (enclosure size 4.5 x 3 x 3m); in this situation great care needs to be taken to monitor every animal regularly to ensure food is adjusted and weights are maintained. In a similar setup at Chester Zoo, 13 frogs were kept together. Here smaller weaker frogs regularly became underweight due to high levels of competition for food, ultimately leading to separation of the group (G. Garcia pers. obs.). It is therefore recommended to keep group sizes below 10 animals for ease of management, ensuring only the largest and healthiest frogs are kept in large groups with high potential for competition for food.

Changing group structure can trigger breeding. In general, smaller setups are recommended for breeding this species. It is noticeable that breeding females with foam nests are prone to disturbances and many are likely to abandon their nests if disturbed repeatedly. In a group situation, although initially conducive to breeding due to male-male competition and calling, it seems that frogs trying to enter burrows occupied by nesting females can prevent successful nest care, possibly due to continued disturbance of the guarding female

It is therefore recommended to try to keep breeders in pairs and experiment by introducing a second male for a few days to promote male-male combat and stimulation for both sexes. One downside of the dual male approach is that it may be difficult to determine the sire of any potential nests. One male (the sub-ordinate animal if this can be determined, or, after spawning the male not guarding the nest) should then be removed as prolonged domination of sub-ordinate males in smaller breeding enclosures can lead to exhaustion. Any changes in group structure should therefore be carefully monitored for an effect on body condition of the animals involved. Male *Leptodactylus fallax* produce a skin peptide that triggers aggression in other males linked to breeding behaviour (King et al. 2005). As such, introduction of this peptide from another individual to a male may be enough to stimulate breeding behaviour. This may be one way to stimulate breeding whilst avoiding interspecific aggression.

2.3.3 Sharing enclosure with other species

If any other species are added to the enclosure, they should be naturally sympatric with the *Leptodactylus fallax* in their wild state. At Jersey Zoo *L. fallax* have been housed with *Iguana delicatissima*, a sympatric species from the Lesser Antilles, for many years without any problems and both species bred repeatedly in this setup. The introduction of Martinique anoles (*Anolis roquet summus*) was initially successful until plant growth enticed the anoles to spend more time in the lower areas of the enclosure resulting in successful predation by the frogs.

A large colony of *Eleutherodactylus johnstonei* that was established in one large exhibit enclosure was predated to extinction within a few months after the introduction of a group of *L. fallax* even though plenty of suitable leaf litter, logs, and crevices were available.

At Chester Zoo *Leptodactylus fallax* have been kept in a public exhibit also containing Caribbean hermit crabs (*Coenobita clypeatus*) and Haitian galliwasps (*Celestus warreni*) without any conflict. London Zoo has successfully housed *L. fallax* and Montserrat orioles (*Icterus oberi*) together. Thoiry Zoo has also successfully maintained *L. fallax* with Utila iguanas (*Ctenosaura bakeri*) and red-footed tortoises (*Chelonoidis carbonarius*).

2.4 Breeding

Precise reproductive triggers are unknown for *Leptodactylus fallax*. All specimens should be subject to the natural temperature and humidity regime described in section 1.7.3 to encourage natural physiological cycles. Humidity can be increased during the wet season using spray systems and heavy misting. For information on social structure for breeding see section 2.3. Adults should be fed more in the lead up to the breeding season and the amount of food offered should be adjusted in accordance with the body condition of the frog.

2.4.1 Mating

Mating occurs in a burrow or under refugia. Both sexes can be left together after mating and egg laying.

2.4.2 Egg laying

A foam nest is produced at the bottom of the male's burrow and the surface becomes thickened after approximately 24 hours, this may provide protection against desiccation, some potential predators and also possible fungal / bacterial infection (see 1.7 and 1.8.5 for more details).

2.4.3 Assisted reproductive techniques

Assisted reproductive techniques (ART) have not yet been attempted with *Leptodactylus fallax*. The following points must be considered before attempting an ART program:

Reproductive dysfunctions are common in conservation breeding programs; they are usually the result of poor nutrition, stress, or the absence of environmental stimuli. Poor nutrition can usually be corrected, providing the appropriate environmental stimuli can be more challenging (Kouba et al. 2012). ART may ameliorate common problems by ensuring that founder animals are not lost before they have reproduced, so that the maximum amount of genetic diversity is maintained in the captive population. ART can also facilitate multiple paternity of clutches as well as the transfer of sperm between facilities rather than live animals (Kouba et al. 2009). There is also the potential to select for disease resistance (Clulow et al. 2012). Several conservation breeding programs already use ART with success (Browne et al. 2006).

Hormone efficacy may be predicted by phylogeny (Silla & Roberts 2012) but protocols tend to be species-specific and the development of protocols can be hampered by the differing needs of each sex (Browne et al. 2008; Mann et al. 2010). Incorrect hormone dosages can have adverse effects and may result in death (Michael et al. 2004). Moreover, the use of ART can make it seem as if programs are achieving their goals whilst veiling underlying husbandry issues that are likely the reason why animals are failing to breed naturally. If individuals bred in captivity have reduced fitness or are of compromised health status, their post-release survival may be reduced and the chances of program success decreased. ART also remove sexual selection, this could adversely affect program success as sexual selection may in fact increase offspring health and survival (Wedekind 2002). ART should therefore not be viewed as a remedy to overcome the limitations of captive husbandry (Maruska 1986). Additional research into the effects of ART on program success, as well as into the husbandry practices required to stimulate natural breeding, is required.

ART may be particularly problematic for *Leptodactylus fallax* as any artificial hormonal intervention is likely to stimulate spawn of all the eggs contained within a female. This scenario would leave no follicles to develop to produce the infertile eggs required by the female to feed tadpoles. As such, any ART procedure on one female would have to be combined with fostering of the tadpoles produced by other females that were producing infertile eggs (see section 2.4.4).

2.4.4 Hatching

Actual timing of tadpoles hatching from eggs laid into foam nests is as yet unknown as the consistency of foam obscures any activity within. Tadpoles are usually noticed once they are seen swimming through the foam or when disturbed which can be quite late in development (usually 7-10 days). Especially if few tadpoles are present in a well-maintained foam nest and human disturbance is kept to a minimum it may be that the first tadpoles when first seen, are already several centimetres long. If a nest is infertile after 10 days it will degrade to a flat sheet of mucus (Fig. 24). Since the female is caring for the tadpoles until they metamorphose, keeper intervention is not necessary; regular disturbance can in fact often be detrimental as many guarding females are likely to abandon nests if disturbed too often. It is recommended that disturbance of nests is kept to a minimum, especially during the early stages of development.

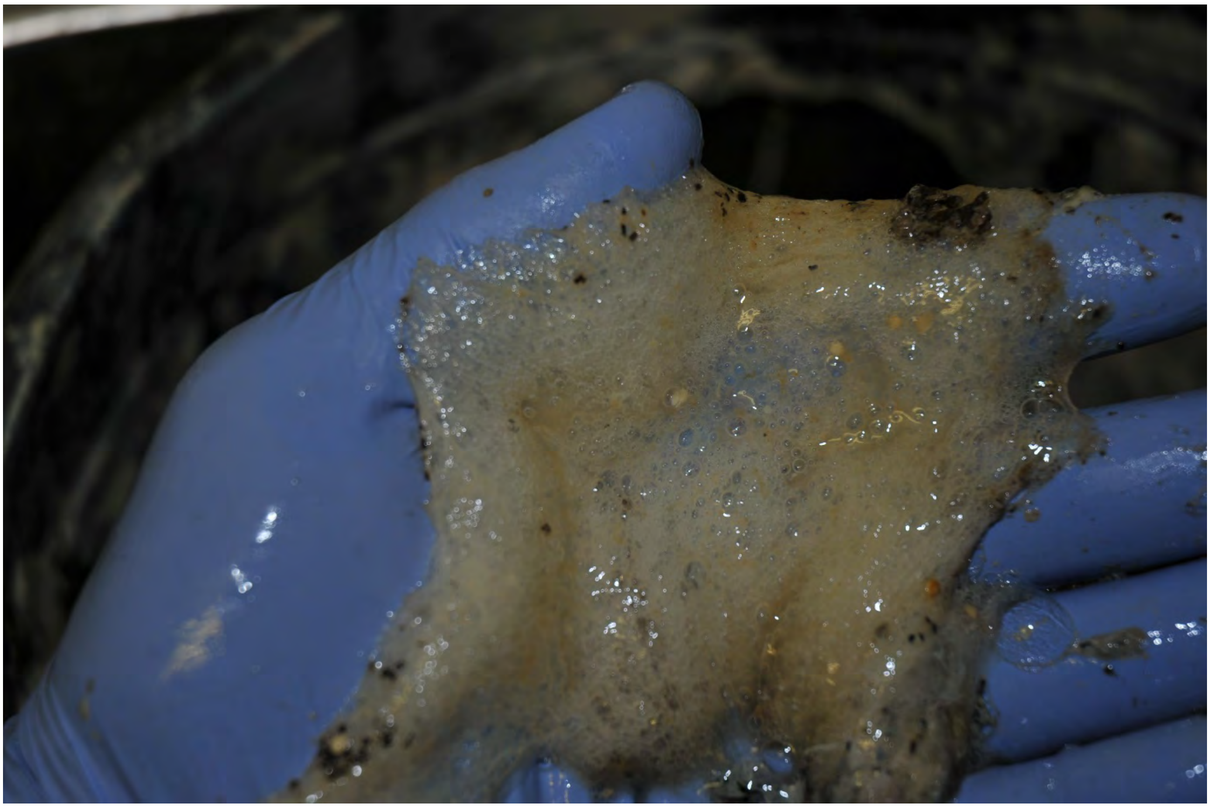

Figure 24. Mucosal remains of an infertile *Leptodactylus fallax* nest after 10 days (G. Garcia).

Females sometimes abandon nests for reasons that are not obvious. As this usually happens to nests which contain only very few tadpoles, a possible explanation might be that females use tactile stimuli from the tadpoles to gauge their presence. This possibility is further corroborated by the fact that females can adjust the number of nutritious eggs they release during each feeding event (Gibson & Buley 2004) and a possible trigger might be the intensity of tactile stimulation by the tadpoles.

Abandoned tadpoles would obviously starve to death but might be rescued by keeper intervention. Several attempts have been made to artificially feed abandoned tadpoles but without any success (G. Garcia & M. Goetz pers. obs.). One solution can be to have those tadpoles fostered by another female. This will only work if the other female is guarding her own nest, with tadpoles or without. In such a case, abandoned tadpoles can be released into the foster nest and might be fed by the fostering female. Durrell Wildlife Conservation Trust and Chester Zoo have carried out a number of foster trials successfully. In early attempts, abandoned tadpoles were transferred to a new nest, containing other tadpoles at a later stage of development. Though this was initially successful, the foster females ceased feeding the nest after the last of her own tadpoles had metamorphosed. As such, the foster tadpoles were unable to complete metamorphosis. In a later attempt, foster tadpoles were placed in a nest containing other tadpoles at similar stages of development. In this case, all foster tadpoles survived and metamorphosed (G. Garcia & M. Goetz pers. obs.).

2.4.5 Development and care of young

Metamorphosing juveniles will start leaving the nest when their tail is largely resorbed. Juveniles will stay in the vicinity of the nest and the females for many days and use both nest chamber and female as refuge when feeling threatened.

The entrance or tunnel to the nest chamber should be closed or covered as soon as the first metamorphed froglets are noticed to leave the foam. Juveniles with only the last millimetres of tail left should then be removed and housed in small groups in rearing enclosures. In these enclosures, the size of the groups and the group structure undergo quite rapid changes in the first months as the frogs grow: Care has to be taken that groups are very regularly size-sorted and only animals of similar size housed together as smaller frogs will continue to lack in growth and wellbeing if housed in groups with food-monopolising, bigger animals.

Occasionally less well-developed metamorphs may need to be removed from the nest (e.g. abandoned by mother). As long as they have stopped feeding from the mother (e.g. tadpole mouthparts changed to frog mouthparts) this is not apparently deleterious. Such froglets should be housed with a disk of wet clay covered in cork bark to provide nest-like conditions. Once the tail has been reabsorbed the dish can be removed.

A good size for a first rearing enclosure holding up to eight metamorphed is roughly 60 x 40 x 40cm. It is not advised to house these juveniles at high densities due to their great need for food. Over the following months, the group of juveniles will need to be distributed over more and larger enclosures; after 4-6 months, the juveniles will be big enough to be housed in the same setups as for adults.

Environmental parameters and enclosure furbishing for juvenile enclosures is as for adult enclosures as detailed in section 2.1.4. It should again be emphasized that even if mineral and/or vitamin supplements are used, rearing juvenile *Leptodactylus fallax* does require a UV-B radiation source to avoid problems with bone mineralization (Tapley et al. 2015a). As outlined above (see 2.1.4), juvenile rearing enclosures will typically have a UV fluorescent tube of appropriate strength illuminating the entire enclosure in which case an UV Index range of 0.5 to 1.5 is appropriate.

Most of the captive bred *Leptodactylus fallax* remain smaller as adults than their wild counterparts (M. Goetz pers. obs.; Guarino et al. 2014). It is assumed that the large body size of *L. fallax* is due to a more rapid and continuous growth than in most other amphibians (Guarino et al. 2014), a special trait it seems has not been properly supported in the past when rearing this species in captivity. It might be that growing juveniles need an even more constant and continuous supply of food than juveniles of other amphibian species. This is supported by observations on juveniles that left the nest without being noticed and "vanished" in the adult's large and complex structured enclosure at Jersey Zoo. These enclosures are well established and harbour a variety of invertebrates that live and breed there, be it as non-eaten food (e.g. cockroaches) or abundant substrate organisms like woodlice that are encouraged as custodians. At some point these juveniles were noticed amongst the adults and were invariably larger and in better body condition than all the separately reared clutch-mates (M. Goetz pers. obs.). Nathan (2013) found higher mortality and higher differences in growth rate when juveniles were reared in parent enclosures; however, no details of enclosure size, complexity of and possible "bio-activity" (i.e. established populations of various invertebrates) in these enclosures were given and a possible lack of such continuous food supply and/or the large number of juveniles kept with the parents exhausted food quickly.

It seems overall beneficial that metamorphs are initially reared in separate enclosures for 2-4 months after which small numbers of juveniles might be transferred back to parental enclosures if these enclosures are established, well-structured, and harbouring a large variety of invertebrates. In the absence of such enclosures and/or if juvenile numbers are too large, it is advisable to feed juveniles on a large variety of food species of appropriate size daily or every other day for the first 4-6 months.

Early trials at ZSL London Zoo have found that feeding juvenile's relatively large sized prey (e.g. medium brown crickets) resulted in faster growth rates and larger frogs than normally seen in captivity at any given age. The frogs seem to have a certain number of prey items that they will ingest, rather than a volume, and so feeding smaller items results in substantially less food being actually eaten (C. Michaels pers. comms.).

2.4.6 Hand-rearing

This species cannot be hand reared. The tadpoles will only survive on a diet of unfertilised eggs produced by female *Leptodactylus fallax*. However, these do not necessarily have to be the original parent of the tadpoles (see also 2.4.4).

2.4.7 Population management

The European Studbook (ESB) for *Leptodactylus fallax* was upgraded to a European Endangered Species Program (EEP) in 2016; in early 2018, EEPs were renamed as EAZA Ex-situ Programmes. The EEP also manages the small number of individuals in other institution outside of Europe. Individuals from both Montserrat and Dominica are managed together as each population has been shown to represent the same species and a single evolutionarily significant unit (Hudson et al. 2016a). The *L. fallax* EEP population is managed as two metapopulations: the biosecure population (managed for future translocation/ supplementation) and the non-biosecure population (managed for conservation education and conservation research).

The EEP population was founded from individuals caught to establish safety net populations. For the non-biosecure population the first 13 frogs were brought into an ESB institution in 1998 from Montserrat in response to volcanic activity on the island. The biosecure population was established in response to the *Bd* epidemics of each island of the early 2000s. The first seven frogs were brought into an ESB institution in 2007 from Dominica, with an additional 50 brought from Monserrat in 2009. Initially these two populations were managed separately, but have since been integrated following the recommendations of Hudson et al. (2016a).

The goal of the EEP is to maintain a demographically and genetically stable and behaviourally competent assurance population with the highest veterinary and health standards for potential future reintroduction in accordance with the 2014-2034 Long-term Recovery Strategy (Adams et al. 2014; Garcia & Schad 2016). To meet these demographic and genetic goals the 2016 ESB (Garcia & Schad 2016) recommended a target biosecure population size of 70 individuals and a non-biosecure population size of 200 individuals.

2.5 Behavioural enrichment

Enrichment of captive species is an important aspect of welfare and husbandry practice, but has been very little studied or put into practice with amphibians, including *Leptodactylus fallax.* This is probably due to a range of factors including a lack of understanding of amphibian behavioural motivations (Michaels et al. 2014b).

There are a number of areas that provide potential for enrichment of *L. fallax* in captivity. Enclosure design such as including more refugia can help to provide a more enriching environment for captive *Leptodactylus fallax.* Other opportunities such as increasing foraging and widening the diet offered can also influence the activity levels of the frogs. High standards of husbandry especially with maintaining heating and lighting gradients can help to increase activity levels and movement within the exhibit.

Mixed species enclosures may also provide a level of enrichment, whereby the activity of other species alters the environment providing novel feeding opportunities. For example, the activity of passerines feeding in leaf litter and understory vegetation may disturb resident invertebrates in well-established enclosures, making them accessible to *L. fallax* where they otherwise would not be.

2.6 Handling

2.6.1 Individual identification and sexing

Individual *Leptodactylus fallax* can be distinguished from one another by their unique pattern of blotches or by marking them with Passive Integrated Transponders.

Much of the body patterning of individual *Leptodactylus fallax* changes subtly as an individual matures (e.g. bars on the lips fading as an animal ages (Fig. 25)) however distinct tympanic/ post-tympanic marks remain relatively consistent throughout an individual's life. Visual identification can be achieved in *L. fallax* by noting the unique pattern of dark spots/ blotches that mark the body from the tympanum to the inguinal region (Fig. 26). Photographs of individuals to be identified should be taken in the lateral view and incorporated into an individual's records to aid identification.

Figure 25. Fading of lip bars with age in *Leptodactylus fallax*. Youngest to oldest, A-D. (G. Garcia).

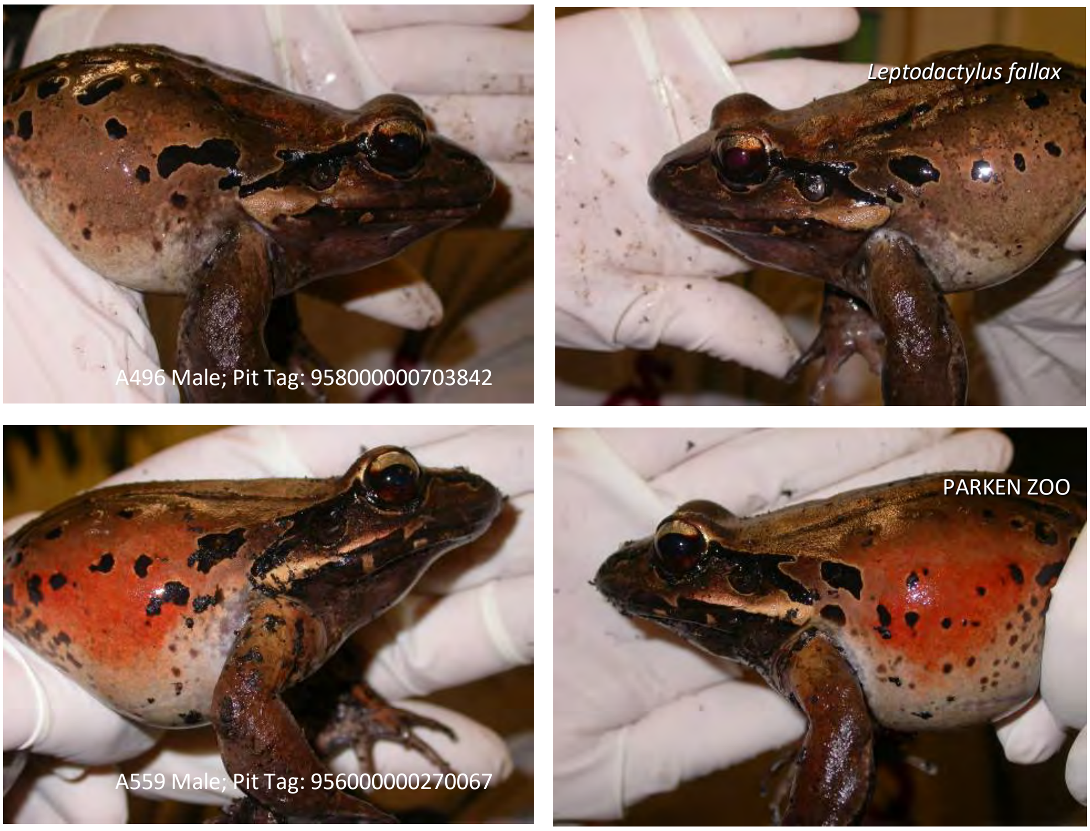

Figure 26. Example of visual ID sheet for two specimens of *L. fallax* based on unique pattern of distinct tympanic/ post-tympanic blotches. (Top) specimen A469; (Bottom) specimen A559; (left) lateral view of right-hand side of body; (right) lateral view of left-hand side of body. (G. Garcia).

Leptodactylus fallax can also be marked with passive integrated transponders ("microchips"), these can be injected, percutaneously, into the dorsal lymphatic sacs of large frogs (SVL >70.0mm) and scanned with a compatible scanner to recover the individual identification number associated with the microchip. The microchipping protocol for *L. fallax* requires two people and is as follows (Fig. 27):

1. Frog is restrained by person one: One hand should grip the frog firmly around the lower abdomen and the other behind the forelimbs. The hind limbs should be angled between 45 and 90^{o} to the axis of the body that will facilitate microchip implantation. Held thus the frog should be braced against a firm surface so that the lower dorsum is exposed (Fig. 27A).
2. Person two first cleans the implantation area using tissue of paper towel; if very dirty the body should be washed with water and then dried by dabbing with tissue/ paper towel. Once cleaned the microchip can be inserted: With the frog restrained the microchip insertion needle should be inserted percutaneously into the dorsum above the cloaca, the needle pointing anteriorly, the tip of the needle should be held in a position so that the visible bore of needle is orientated upwards (Fig. 27B). The microchip should then be injected, do not retract the needle until it is certain that the chip has been ejected from the needle; this should place the microchip into the dorsal lymphatic cavity of the frog. Be aware that the frog may struggle and emit distress calls during this process.

3. Person two seals the entry hole with tissue glue (superglue has been used without any negative effects, but is not recommended): With the frog continuing to be restrained, the area around the entry hole should be wiped with a tissue to remove any soil of blood. The entry hole should then be sealed with a drop of tissue glue (Fig. 27C). Excess glue should then be dabbed away with a tissue.

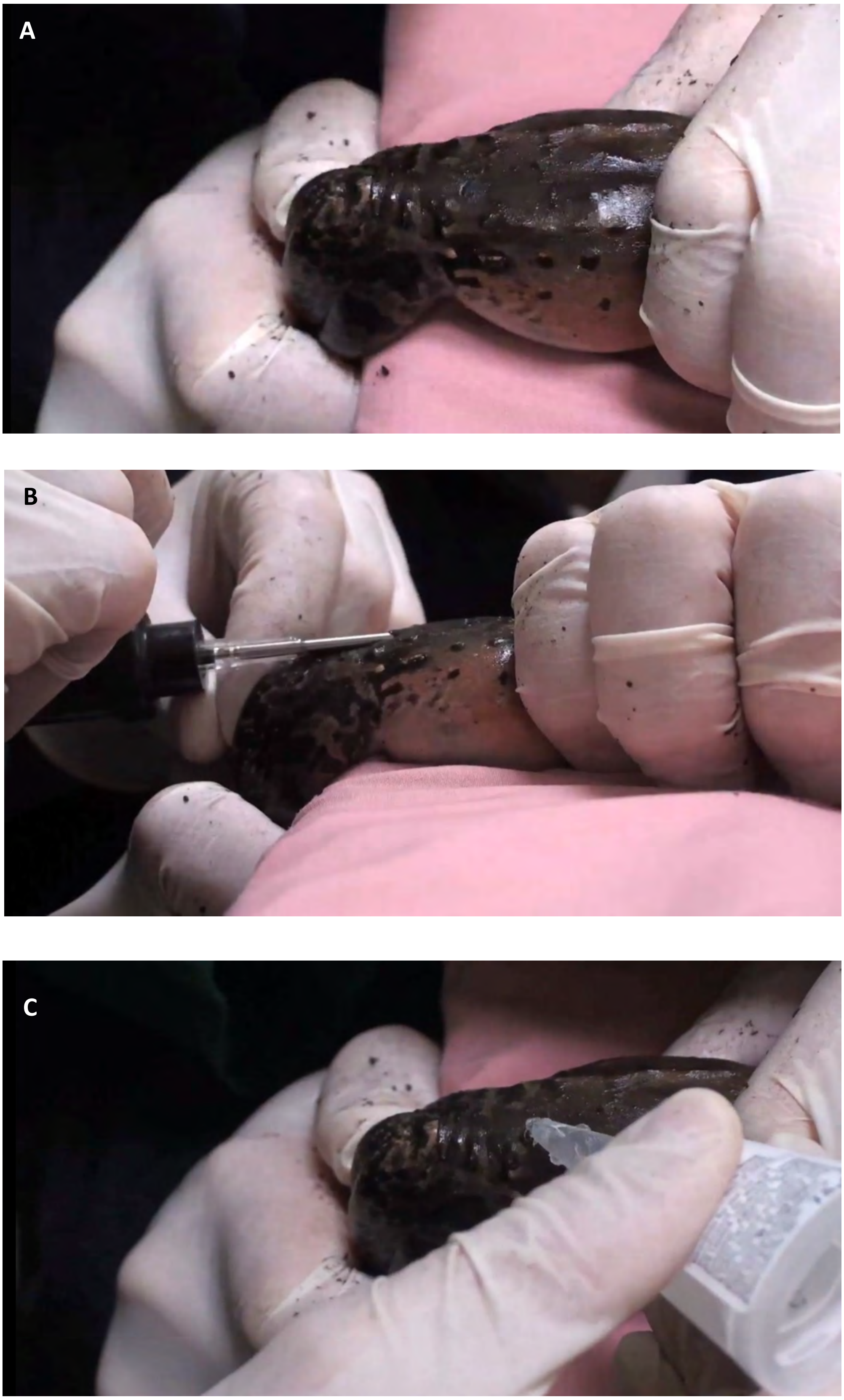

Figure 27. Protocol for passive integrated transponder (microchip) insertion into *L. fallax*: (A) Restraining position for *L. fallax* during procedure; (B) injection of microchip into *L. fallax;* (C) sealing of entry hole with tissue glue. (G. Garcia).

Animals that are too small to microchip may be marked with visible implant elastomer (VIE) (https://www.nmt.us/visible-implant-elastomer/). Tags consist of a biocompatible elastomer that can be injected subcutaneously, remaining visible through the skin. The tagging material consists of two components, a liquid elastomer material and a curing agent, which are mixed prior to injection. After injection, the mixture cures into a pliable solid within a few hours at room temperature. Once mixed, the elastomer can be stored as a liquid in the freezer for two to three weeks prior to injection. This technique has been successfully used in a large number of studies for marking amphibian eggs (Regester & Woosley 1998), larvae (Anholt et al. 1998; Belden 2006; Heemeyer et al. 2007), and adults (Antwis et al. 2014; Bailey 2004; Belden 2006; Davis & Ovaska 2001; Kendell 2001; Lampert & Linsenmair 2002; Marold 2001). In some species, VIE tagging has been shown to have good long-term retention, minimal marking effects, and meet the assumptions required in capture-recapture studies (Antwis et al. 2014; Davis & Ovaska 2001), making it an effective tagging tool, although marks have been seen migrate over time in *L. fallax* (B. Tapley pers. obs.). Details of how to select colours and code tags can be found at https://www.nmt.us/visible-implant-elastomer/. The VIE components should be kept refrigerated and mixed prior to use following the manufacturers guidelines. The VIE implantation protocol for *L. fallax* requires two people and is as follows:

1. Frog is restrained by person one: One hand should grip the frog firmly around the lower abdomen whilst the other should hold the limb in which the tag is being implanted. The frog should be held upside-down to expose the ventral side of the animal with hands and forearms of person one resting on a solid surface.
2. Person two first cleans the implantation area with water and inserts the tag: With the frog restrained the VIE insertion needle should be injected subcutaneously into the ventral skin or a limb as far from any joints as possible, needle pointing towards the posterior of the frog. The tip of the needle should be held in a position so that the visible bore of needle is orientated upwards. The VIE should then be injected, the needle being gradually retracted as pressure is applied to the plunger of the syringe. The needle should not be retracted fully until the VIE has ceased to be emitted from the bore of the needed as any external VIE may cause the mark to be lost. The mark site should be wiped clean of any excess VIE. Be aware that the frog may struggle and emit distress calls during this process.
3. Entry hole should then be treated following the same protocol as lain out above for microchip injection.

Male *Leptodactylus fallax* attain sexual maturity much earlier than females. *L. fallax* can be generally sexed from a size of 10cm SVL (see section 1.2 for details).

2.6.2 General handling

Handling should be kept to a minimum. Nitrile gloves must be used when handling amphibians to avoid disease transmission, damage and injury to the animals, and to protect the skin of the animals from any chemicals that may be present on the hand of the handler. Gloves must be powder free as such powder may be transferred to frogs, damaging their skin. Care must be taken when handling and restraining *Leptodactylus fallax* as if not done correctly it may cause damage to the specimen and cause an increase in stress. Some specimens display a loss of appetite after being handled or restrained and can take 2 weeks to a month to resume prior normal feeding patterns.

Preliminary research carried out at Chester Zoo suggests that handling of *Leptodactylus fallax* for veterinary procedures may raise body temperatures outside of a natural range (T. Jameson & G. Garcia pers. obs.). It is speculated that this may cause health problems and/or increase stress. Further data will be available as this research project progresses (see section 2.9).

When restrained, morphometric data can be gathered. Body mass is best measured by placing an individual restrained in a bag (see section 2.6.3) on a set of scales (Fig. 28). Body measures are best taken by two people, one holding the frog (see section 2.6.3) and the other measuring with a standard 30cm ruler (Fig. 29). The current recommendation is to weigh the specimens every 3 months, but specimens should not be weighed during the breeding season, when minimal disturbance is believed to be a contributing factor in successful breeding (ZSL Staff pers. comms.).

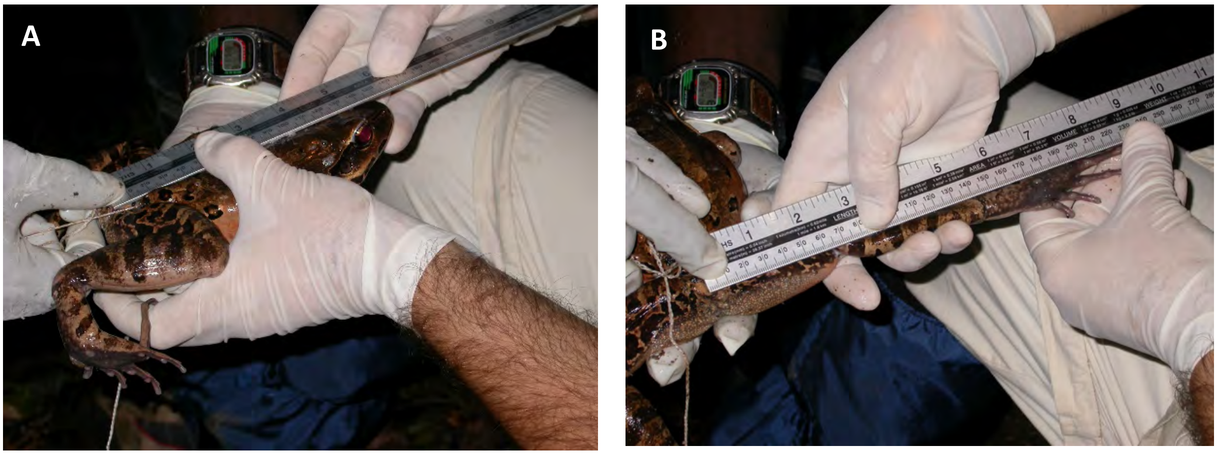

Figure 29. Morphometric measures of *Leptodactylus fallax*: (A) Measure of Snout-to-vent length; (B) measure of leg length. (G. Garcia).

2.6.3 Catching/restraining

When being handled, *Leptodactylus fallax* should be grasped firmly around the waist. They may emit an alarm call when restrained, if this happens, check that the frog is not being held too tightly.

When handling *Leptodactylus fallax* particular care should be taken not to cause damage to the legs when the specimen is being restrained. A firm but controlled grip should be maintained at all times to reduce the risk of harm to the specimen and avoid the risk of losing grip and dropping the specimen (Fig. 30).

Figure 30. Correct handling of adult *Leptodactylus fallax*: (left) Position of hand firmly gripping around the lower abdomen; (right) position of thumb and forefinger around the underside of the abdomen. (G. Garcia).

When handling juveniles, it is recommended to hold them round the waist between forefinger and thumb or enclosed in two hands this allows the specimen to be held safely whilst minimising the risk of harm to the animal if it were held too tightly at this young age.

It is recommended to use gloves when handling amphibians in captivity in order to protect the handler and to prevent the transfer of pathogens between animals. However, studies show that the use of latex gloves can be lethal to tadpoles (Cashins & Alford 2008) and therefore, handling tadpoles should be avoided where possible. Where it is necessary to handle tadpoles it is preferable to scoop them up into a dish or net rather than using hands. Work by Mendez et al. (2008) suggests that nitrile gloves kill *Batrachochytrium dendrobatidis* (*Bd*) on contact, with the affect reduced by washing. Similarly, bare human skin has a fungicidal effect on *Bd*, killing 100% of cells in 6 minutes, this affect reduced upon repeated washing with water/ ethanol. Alternatively, latex and polyethylene gloves have no effect on *Bd*. These results support the use of an unused pair of nitrile gloves for each new amphibian handled in either the field or the laboratory, and if this is not possible, bare hands are a preferable (although imperfect) alternative to continual use of the same pair of gloves.

Care must be taken to avoid restraining individuals in small solid containers (e.g. for short distance transport). When startled by handling *Leptodactylus fallax* will often jump when released. If such release occurs in a small solid container (such as a plastic box), individuals may damage their faces (Fig. 31).

Figure 31. *Leptodactylus fallax* with damaged faced from jumping into solid surfaces. (G. Garcia).

One alternative method when handling/restraining *Leptodactylus fallax* in order to carry out routine procedures or close body condition checks is to put them in clear food grade plastic bags. This technique is useful for procedures such as weighing or transporting *L. fallax* short distances. The use of bags is also helpful when *L. fallax* need to be removed out of the bio-secure environment, for example to be taken for veterinary treatment. The frogs are double bagged so there is a clean bag inside the outer bag to allow for better bio security. The second bag also acts as a second barrier as frogs have been known to break out of single bags. It is important that frogs are not left in the bags for prolonged periods and checked regularly and that new bags are used for each individual. At ZSL frogs have been bagged in this way for up to 40 minutes. Urine can be collected from these bags for veterinary analysis. Alternatively, cloth bags designed for bird ringing and pillow cases have been successfully used for the same purpose at Chester Zoo. The bags are strong and do not split, whilst also being soft enough to prevent abrasions to a specimen's skin. It is fairly common that the animals will pass urine while being restrained or held in the bags; therefore, cloth bags are recommended over plastic bags to prevent the animals sitting on their urine for a prolonged period of time. Additionally, cloth bags can be kept moist using electrolyte solutions such as amphibian ringer's solution (see appendix 2 for composition). These bags have the added advantage of being reusable, able to be washed in a standard washing machine.

2.6.4 Transportation

Small juveniles *Leptodactylus fallax* <8cm SVL should be shipped individually in appropriately sized ventilated plastic containers containing a layer of humid moss and a couple of dry leaves so the animal can choose whether to bury into the humid moss, hide underneath the leaves or sit on top of it. Sphagnum moss should be avoided, as it is extremely acidic.

Due to their large size and related volume-to-surface area, *Leptodactylus fallax* >8cm SVL can be transported "dry", i.e. without the need of including moisture-retaining substrates like moss or foam. Indeed, using humid or wet materials during longer transports can cause skin problems, can make cloth bags impermeable for air and can exacerbate problems caused by possible low temperatures.

Transportation in dry cloth bags, as is commonly done for snakes or lizards, is the only method by which sub-adults and adults of this species should be transported; enclosing individuals above ~8cm SVL in solid-walled containers like plastic boxes will result in skin abrasions and the animals injuring themselves (Fig. 32).

As largely nocturnal animals and being a relatively nervous but powerful species, *Leptodactylus fallax* will jump within the bags during transport and are able to move with the bags throughout the transportation box, often ending up on top of each other. This should ideally be prevented. Below is a guide on how to best prepare *L. fallax* for transport (Fig. 32):

1. Select an appropriate transportation box in line with IATA Live Animal regulations (IATA 2015).
2. Line the transport box with polystyrene (unless a polystyrene box is used alone, in cases when frogs are transported only a relatively short distance by private car).
3. Drill an appropriate amount of aeration holes through side of box and insulating lining.
4. Place a layer of shredded paper into the transport box.
5. On the shredded paper place plastic containers big enough to comfortably hold one frog. The containers should be relatively high to prevent the frog + bag from jumping out. Plastic containers of the approx. 16 x 16 x 12 cm (~ 3.0 litre) have proven to be ideal (Fig. 32A).
6. Place only one frog into a cloth bag, tie the top and either make a knot or secure with a cable tie (Fig. 32B). Cloth bags used for *Leptodactylus fallax* transports should be of a size to hold comfortably one frog but should not be too big. The bigger the bag, the more likely it is that the frogs are able to jump and move around with the bag. Place the bags in the plastic containers (Fig. 32C).
7. Cut a sturdy piece of plastic mesh and fit inside the transport box on top of the plastic boxes (Fig. 32D).
8. Fill the remaining space of the transport box loosely with more shredded paper (Fig. 32E). This should be loose enough to allow good ventilation but prevent the plastic containers from moving around. Make sure there is some light pressure applied from the lid of the transport box through the shredded paper onto the plastic mesh so that jumping frogs won't be able to lift the mesh and jump from the plastic boxes.
9. Seal the outer transport boxes appropriately (Fig. 32F).

Figure 32. Transport protocol for *Leptodactylus fallax*: (A) Transport box lined with shredded paper with individual containers for *L. fallax* specimens inside; (B) tied cloth bag containing *L. fallax* specimen; (C) cloth bags placed in individual containers in transport box; (D) plastic mesh secured over top of individual containers with bagged specimens inside; (E) Extra shredded paper packed around individual containers and mesh; (F) sealed transport box. (M. Goetz & G. Garcia).

Transport temperatures should ideally be maintained between 22°C and 25°C. For short periods of time temperature minima and maxima of 12°C and 30°C respectively will be tolerated but any further deviation up or down or an extended time at those temperatures can easily result in the death of frogs (G. Garcia, M. Goetz, and B. Tapley pers. obs.). When shipping frogs commercially by plane, extra precautions need to be considered. Airlines will ship live animals in a heated cargo hold; the pilots will be advised on the appropriate temperature the hold will need to be kept at and will engage the hold heating during pre-flight checks. However, the cockpit usually has no thermometer or any continuous influence over the hold heating that means that the cockpit might not be aware of a possible malfunction of the hold heating; in any case, there will not be anything that could be done during the flight. In addition, there may be delays in loading or unloading cargo onto or from the plane. Therefore, to make sure the animals survive a possible heating failure in the hold, longer commercial shipments by plane should only be undertaken when outside ambient temperatures on the ground are >24°C. Monitoring commercial shipments by plane through temperature data loggers enclosed in shipping crates indicate that hold temperatures drop by about 1°C/h at cruising altitude if the hold heating is not functioning as intended (Durrell Wildlife Conservation Trust unpublished data). Therefore, if the animal crate is loaded at 25°C air temperature, a critical minimum temperature in the transport boxes might be reached after ~10h flight time.

2.6.5 Safety

There are no reports of major skin reactions when coming into contact with the secretions of *Leptodactylus fallax*, however, secretions have been found to cause minor irritation to the eyes and nose if contact is made (G Garcia pers. obs.).

Leptodactylus fallax also produces volatile components (King et al. 2005) that give a characteristic smell to a frogs' skin when they are captured. Some people react with sneezing and a runny nose when exposure to the frogs' skin secretions is prolonged, e.g. when catching-up, handling, and temporarily housing a larger number of stressed frogs in a confined and poorly ventilated area (M. Goetz pers. obs.).

It has also been noted that *Leptodactylus fallax* can be negatively affected when coming into contact with secretions of other conspecifics. This usually happens when a number of frogs are caught and then placed in a plastic container for either weighing or temporary holding while another frog is caught. The stressed frog in the container releases mucus and when another frog is placed into the same container it is quickly evident that this frog becomes lethargic in its movements (M. Goetz pers. obs.). It is therefore important that frogs are only held in individual containers, even if only for a short while. If this is not feasible then any container must be rinsed and wiped carefully between housing different frogs.

2.7 Veterinary: Considerations for health and welfare

2.7.1 Biosecurity and health requirements for animal transfers

2.7.1.1 Biosecurity

As described elsewhere in these guidelines, there are biosecure and non-biosecure populations of *Leptodactylus fallax*. Biosecure populations are destined for potential release and exposure to alien pathogens should be minimised. Non-biosecure populations are likely to have been exposed to "non-mountain chicken pathogens".

Biosecure populations can only receive new specimens from other biosecure populations and the specimens must be free from pathogens and parasites (other than the accepted normal parasite fauna for the species). The specific management measures for these populations are covered elsewhere in these guidelines.

Non-biosecure populations can receive specimens from biosecure and non-biosecure populations. Specimens carrying non-mountain chicken parasites or pathogens can be accepted in the population based on risk assessment for the collection.

2.7.1.2 Pre-export health screening

Before a *Leptodactylus fallax* transfer, the receiver should request and the exporter should provide the following:

- Isolation from new contacts or animals of different health status for at least 6 weeks.
- Faecal parasitology exam: examination by direct preparation and flotation of one three-day pooled faecal sample.
- PCR test for Chytrid within two months prior to transfer or evidence of testing negative in the last 2 years for the group.
- Declaration of any cases of ranavirus infection in the collection for the last 5 years.
- Declare any other health problems in the amphibian collection that could be of relevance to the amphibians exported, especially infectious diseases.
- Routine treatments given or required.
- Copy of the clinical history and relevant veterinary inspection/investigations/results or share ZIMS medical records at least 1 month before the transport date.

Animal moves should not proceed until the importer has received all the required information and agreed that it is cleared.

2.7.1.3 Post import quarantine

All new *Leptodactylus fallax* arrivals should be isolated from the rest of the collection for at least three months before introduction to resident population. If deaths or clinical signs of disease are observed, the quarantine period will be extended until all diagnostic and post-mortem investigations have been completed, and an infectious cause has been ruled out or resolved.

Quarantine requirements:

Location: isolation from any other amphibians in the collection in a dedicated enclosure.

Duration: At least 3 months.

Staff: enclosure to be accessed by experienced staff trained in biosecurity.

Equipment: maintain all equipment within quarantine enclosure. If equipment needs to be removed it should be disinfected.

Disinfectants: Disinfectants that have been proven to work against *Bd*, *Bsal* (Van Rooij 2017), and ranavirus (Bryan et al. 2009) are:

- Clorhexidine at 0.75% for 1 minute.
- Virkon S® at 1% for 2 minutes.
- Bleach 4% for 1 minute.
- Note: F10® at 1:1000 for 1 minute and Safe 4® undiluted for 30 seconds have proven to be effective against *Bd* and *Bsal* but not tested against ranavirus.

Protective personal equipment: nitrile gloves (see section 2.6.3), boots, and overalls to be worn at all times while accessing the enclosure.

Waste: Solid waste to be placed in double bags and disposed of by incineration. Wastewater to be treated with sodium hypochlorite 4% before releasing into the environment.

Testing requirements during quarantine:

- Screen for *Bd* and *Bsal* once during quarantine (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 chytrid 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* status.
- Faecal parasite status (free from novel parasites, report to EEP any novel parasites observed).

2.7.2 Specific health problems

1. <u>Gastrointestinal adenocarcinoma:</u> Intestinal adenocarcinoma is a common cause of death in captive *Leptodactylus fallax* - up to 30% of all the deaths reported to the EEP. A cause has not been identified although in other species this type of carcinoma has been linked to viral infection, diet (excess or lack of some dietary components), exposure to irritant/carcinogenic substances, and chronic intestinal inflammation. The condition has been observed in both sexes, from three years of age, in all generations including F1 and in multiple institutions. Typically, one or multiple tumoral lesions develop in the distal intestinal region. These grow in size overtime, eventually leading to blockage and distension of the intestine proximal to the lesion. Adhesions with other organs are common but mainly with the urinary bladder where a fistula often forms leading to leakage of intestinal contents into the bladder. The most common clinical sign is weight and body condition loss despite eating (weight loss may be masked by severe fluid accumulation in the obstructed intestine - Fig. 33). Other typical signs are reduced activity, foul smelling diarrhoea or urine, and a palpable mass in the abdomen (best felt under anaesthesia after deflating the lungs). Large carcinomas might be detected by palpation, radiographically, or by ultrasound examination, but confirmation or detection of smaller masses requires exploratory coelioscopy or coeliotomy. Although surgical resection might be possible early on, the course of the disease is insidious and often, the condition is too advanced for treatment by the time it is detected.

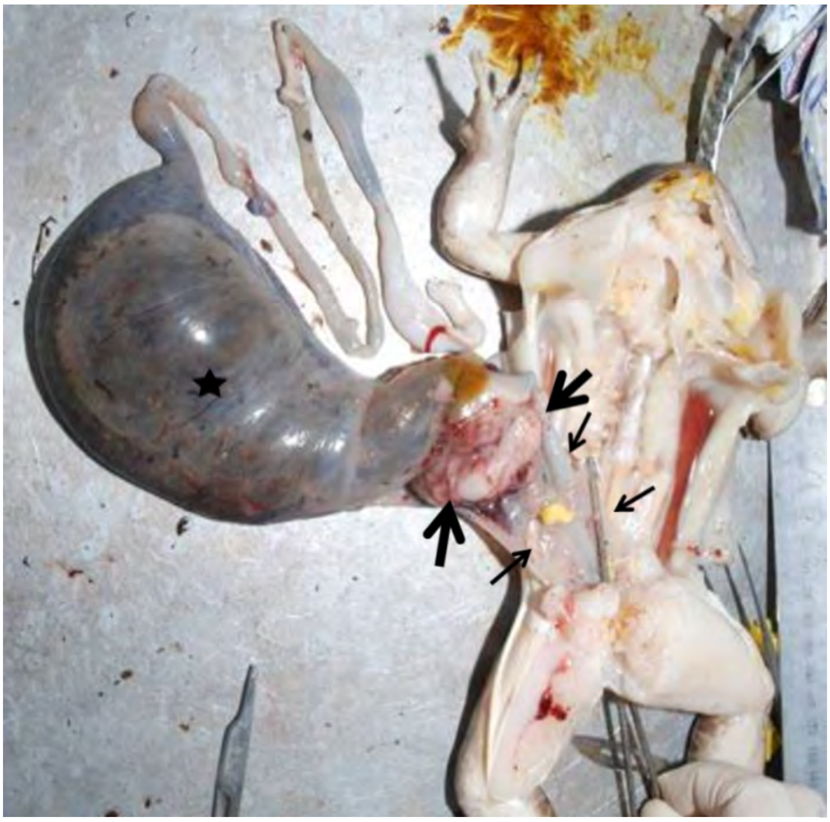

Figure 33. *Leptodactylus fallax* necropsy showing a large adenocarcinomatous mass (thick arrows), a severely distended large intestine (star) containing foul smelling faecal contents with the urinary bladder (thin arrows) adherent to the mass. (A. Barbon/ J.Lopez).

2. Urinary bladder pathology: This occurs in the form of foreign bodies (FB) or accumulation of faecal matter in a distended bladder of *Leptodactylus fallax*. FBs such as pieces of substrate, bark, etc. can be found within the urinary bladder. Most likely, FBs have been incorporated into the urinary bladder after an intestinal perforation as part of a physiological mechanism to seal intestinal perforation and remove FBs from the coelom. Alternatively, FBs may have been incorporated into the bladder in a retrograde direction through the sphincter if an object is too large to go through the cloacal opening. Most often, there is no associated pathology in the bladder tissue so it is not clear if the bladder FBs are causing pathology or are just an incidental finding. Diagnosis is made by radiography, palpation, or cystoscopy. If pathology is confirmed the bladder FBs have been successfully removed surgically through standard cystotomy.

   Presence of faecal material within the urinary bladder is always pathological and normally accompanied by bladder wall pathology. It is normally a consequence of a cysto-colic fistula (Fig. 33). However, it has been occasionally reported in frogs with no obvious fistula in which case, a mechanical or physiological problem at the bladders opening has been suspected. The condition is normally diagnosed at post mortem. In vivo it is suspected radiographically or when foul smelling urine is produced. Diagnosis is confirmed by laparoscopy or cystoscopy. No treatment has been reported.

3. Cholelithiasis and cholecystitis: There has been an increase in reports of gall bladder stones (choleliths) and gall bladder inflammation (cholecystitis) in the past few years in captive *Leptodactylus fallax* populations. Cholelithiasis and cholecystitis cause severe pain in humans, so it is suspected that these are also painful in *L. fallax*. It is not clear whether gall bladder calculi or biliary sludge can cause blockage of the biliary duct and the consequent colic, anorexia, and other clinical signs that occurs in other species. In some cases, the cholecystitis observed in *L. fallax* was severe and likely linked to the cause of death but this has not been confirmed.

   Three gall bladder stones have been analysed and the main component in all three was calcium carbonate (95-100%), with one stone containing 5% aragonite and another 5% bile pigments. The aetiology of the calculi is yet to be determined. It is possible that a primary cholecystitis (possibly from ascending bacterial infection through the bile duct) lead to secondary formation of calculi in the bladder. Alternatively, choleliths might form spontaneously (possibly through dietary imbalance) leading to an eventual cholecystitis through chronic bladder trauma. Histopathological analysis of two surgically remove bladders showed lymphohistiocytic cholecystitis (erosive and fibrosing) reflecting chronic inflammation and consistent with the presence of choleliths. No yeasts or fungi were seen and Ziehl-Nielsen stains for acid-fast organisms were negative.

   Diagnosis is through ultrasound scanning of the gall bladder for presence of abnormal, heterogeneous or dense contents (Fig. 34C). Mineralised choleliths can be diagnosed on a dorsoventral radiographic view as an irregular, 1-5 mm diameter structure of mineral density, often just right of the vertebral column and caudal to the heart.

If a cholelith is observed, in the absence of clinical signs, is advised to monitor by ultrasonography and haematology-biochemistry although reference intervals for this species has yet to be established. Cholecystectomy has been carried out in two *Leptodactylus fallax* (see technique below). Although there were no surgical complications none of the patients survived in the mid-term. However, both patients were already chronically ill and in very poor condition and earlier intervention might result in better outcome.

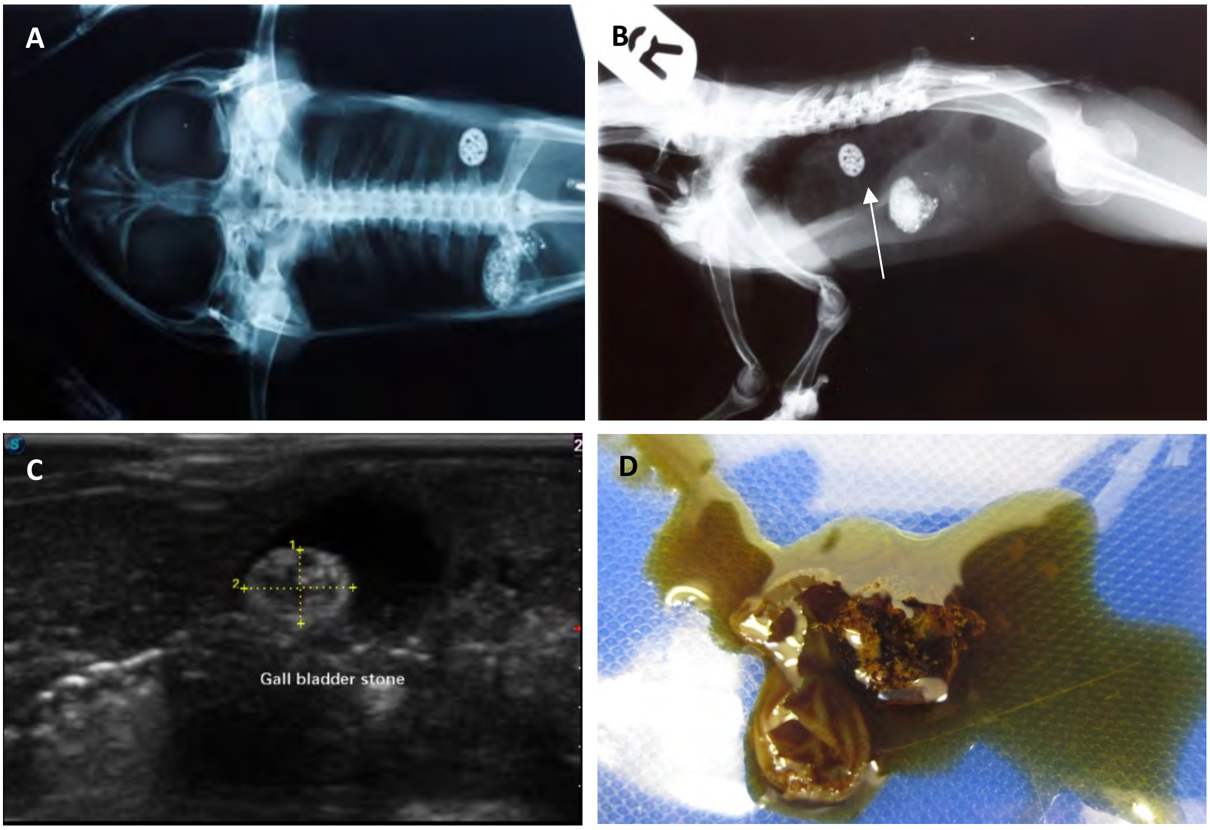

Figure 34. Presentation of Cholelithiasis and cholecystitis in *Leptodactylus fallax*. (A) Dorsoventral and (B) Lateral radiographs of *L. fallax* with cholelith (arrows). (C) Ultrasound of *L. fallax* showing longitudinal view of cholelith. (D) Post mortem appearance of *L. fallax* gall bladder with cholecystitis and sandy contents. (A. Barbon/ J.Lopez).

4. <u>Trauma:</u> Skin and soft tissue lesion from contact with irritant substances like disinfectants (Fig. 35) and rodent/predator bite wounds have been reported in *Leptodactylus fallax*. Skin suturing is recommend if wound is large. Absorbable monofilament materials such as polydioxanone (PDS) and polyglycaprone 25 (monocryl) have been used. A meshing technique has been used successfully when there is not sufficient skin available. Antibiotic cover (according to antibiotic sensitivity) and adequate hydration (amphibian ringer baths – see appendix 2 for composition) is required. Prognosis is usually very good and healing is quick if adequate husbandry conditions are provided.

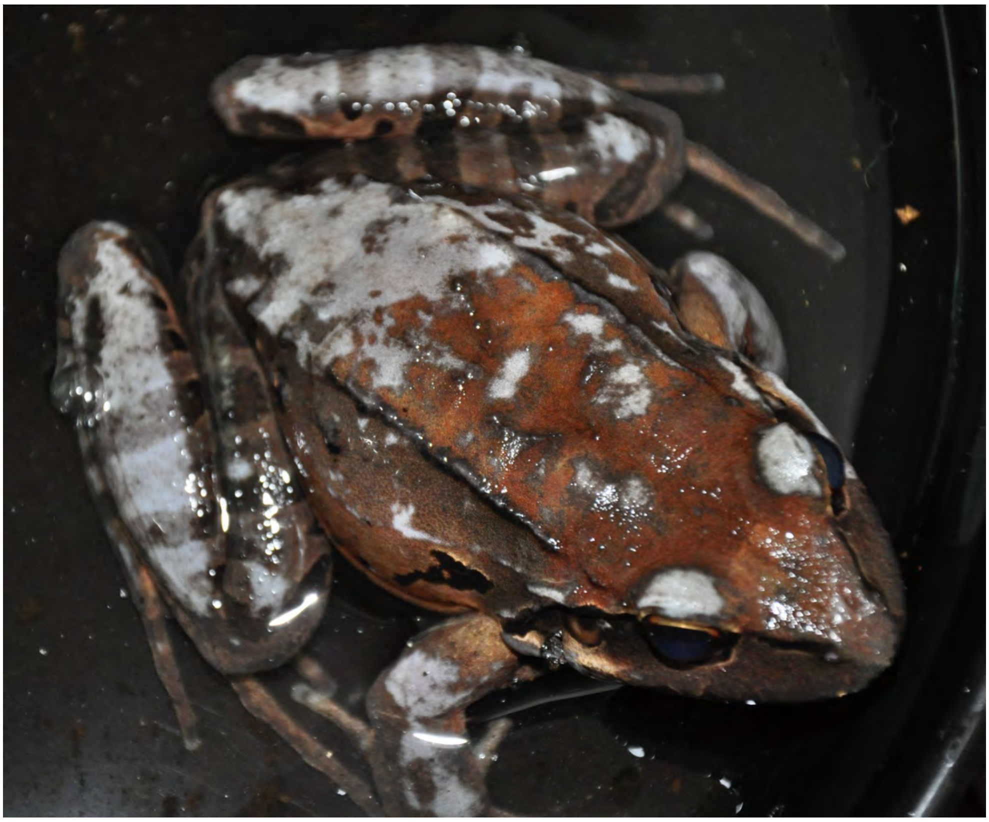

Figure 35. *Leptodactylus fallax* held at Jersey Zoo that escaped its enclosure and was later found on a surface that had been heavily swabbed with disinfectant. Where the individual had been in contact with the disinfectant the skin sustained chemical burns. (G. Garcia).

Bone fractures of long bones (femur and tibia) and ileum have been observed (Fig. 36). These have been successfully managed conservatively with cage rest. This is done by maintaining the frog in a small cage with a hide in order to minimise activity, offering food in front of the hide so animal does not need to move much, and minimising handling. If specimen is visually doing well it should be left for 4-6 weeks before catching for a follow up radiograph to minimize risk of reproducing the fracture. Femoral and tibia fractures have been successfully managed surgically by fitting of an intramedullary pin (see surgery section).

Figure 36. External signs of broken leg in *Leptodactylus fallax* displaying swelling. (G. Garcia).

5. Cachexia of unknown origin: Chronic cachexia in the form of a progressive loss of body condition, unresponsive to medical treatment and nutritional supplementation, is often reported. It has been observed on its own or associated to other underlying conditions such as cholecystitis, adenocarcinoma, and urinary bladder FB. Treating the underlying condition as well as antibiotics, antiparasitic drugs, and force-feeding have been attempted but the condition invariably resulted in the death or euthanasia of the frog. See decision-making tree (Fig. 37) when dealing with body condition loss.

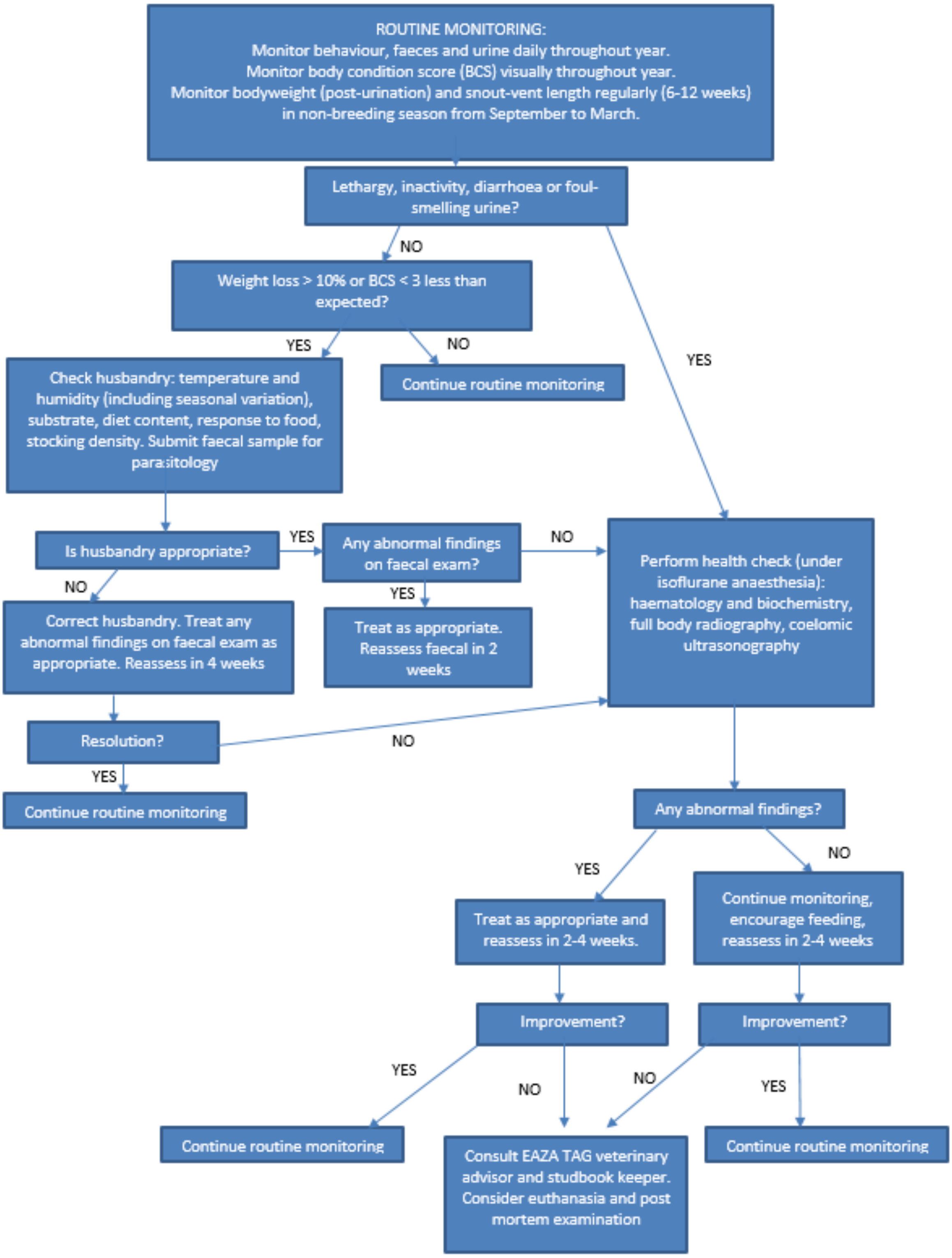

Figure 37. Decision making tree for management of body condition loss in *Leptodactylus fallax*.

6. Other nutrition-related health problems: Strict adherence to recommended calcium and ultraviolet light provision is required for *Leptodactylus fallax*; otherwise, Metabolic Bone Disease (MBD) occurs. This is diagnosed in radiographic examination as long bone deformities in juveniles and reduced bone calcification in adults (Fig. 38). Severe cases can appear with incoordination and muscle twitching. Bone deformities cannot be treated and, if severe, euthanasia must be considered. Reduced bone density or neurological signs can be reversed by provision of calcium and supplementation of UV-B radiation.

Figure 38. Metablic bone disease in *Leptodactylus fallax*: (A) wild-caught frog with normal cortical density and no fractures, (B) captive-born frog with cortical thinning and multiple fold fractures (arrows) of the hindlimbs. (A. Barbon/ J.Lopez).

7. Ocular pathologies: Cataracts have been observed in the captive population of *Leptodactylus fallax* (Fig. 39). Aetiology has not been established. It is important to ascertain that animals are still able to capture live prey.

Figure 39. *Leptodactylus fallax* specimen with cataracts. (A. Barbon/ J.Lopez).

8. Chytrid fungus: *Leptodactylus fallax* are highly susceptible to Chytridiomycosis caused by *Bd*. Affected frog's skin appears dull and sticky to the touch (Fig. 40C), losing its typical "cold beer glass" appearance (Fig. 40A and B). As the condition progresses, frogs can develop reddening of the ventral areas (Fig. 40D), ulcers on the toe tips, abnormal sloughing of the skin (small bits disintegrating, rather than a whole skin slough) and eventually uncoordination, muscular tremors when stimulated, and death.

   Recommended diagnosis is by qPCR on skin swabs. Using a dry swab, swab the ventral portion of the body, including the following areas: Drink patches, limbs, digits, body wall; swabbing 4-5 times each anatomical area.

   Treatment is with 0.01% itraconazole, 5-minute baths once a day for eleven days followed by ten days rest and repetition of the treatment for two or three cycles has been successful, with no toxicity observed. If treating a clinical case fluid therapy is also required, with 20-30 minutes baths in amphibian ringer's (See appendix 2 for composition) once or twice a day, depending on response. A specimen should be considered cleared after two consecutive negative chytrid tests at least 2 months apart.

Figure 40. Comparison of healthy and *Bd* infected skin in *Leptodactylus fallax*: Healthy *L. fallax* on Monserrat displaying typical "cold beer glass" appearance on dorsal (A) and ventral (B) skin. *Bd* infected *L. fallax* displaying lesions around the eyes and on the ventral skin and limbs (C) and inflamed ventral skin (D). (G. Garcia).

2.7.3 Health monitoring

Recommendations for routine health screening protocol: At least once a year full physical examination, radiographs, and ultrasonography under anaesthesia with a focus on the detection of intestinal neoplasia, cholelithiasis, cataracts, and assessment of body condition. It is highly advisable to do full haematology and biochemistry.

1. Haematology and biochemistry: Full haematology and the following biochemistry panel: Total protein, albumin, globulin, calcium, phosphorus, creatinine kinase (CK), lactate deshydrogenase (LDH), aspartate transaminase (AST), urea, and uric acid.

   At the moment there are not established reference values for haematology and biochemistry for this species. It is also important to point out that the diagnostic value of some of the biochemistry results has not been evaluated in this species and the panel may need to be adjusted in the future.

2. <u>Body condition scoring</u>: A non-invasive body condition score system has been published (Fig. 41) which can be used if the animals are not regularly handled (Jayson 2018b).

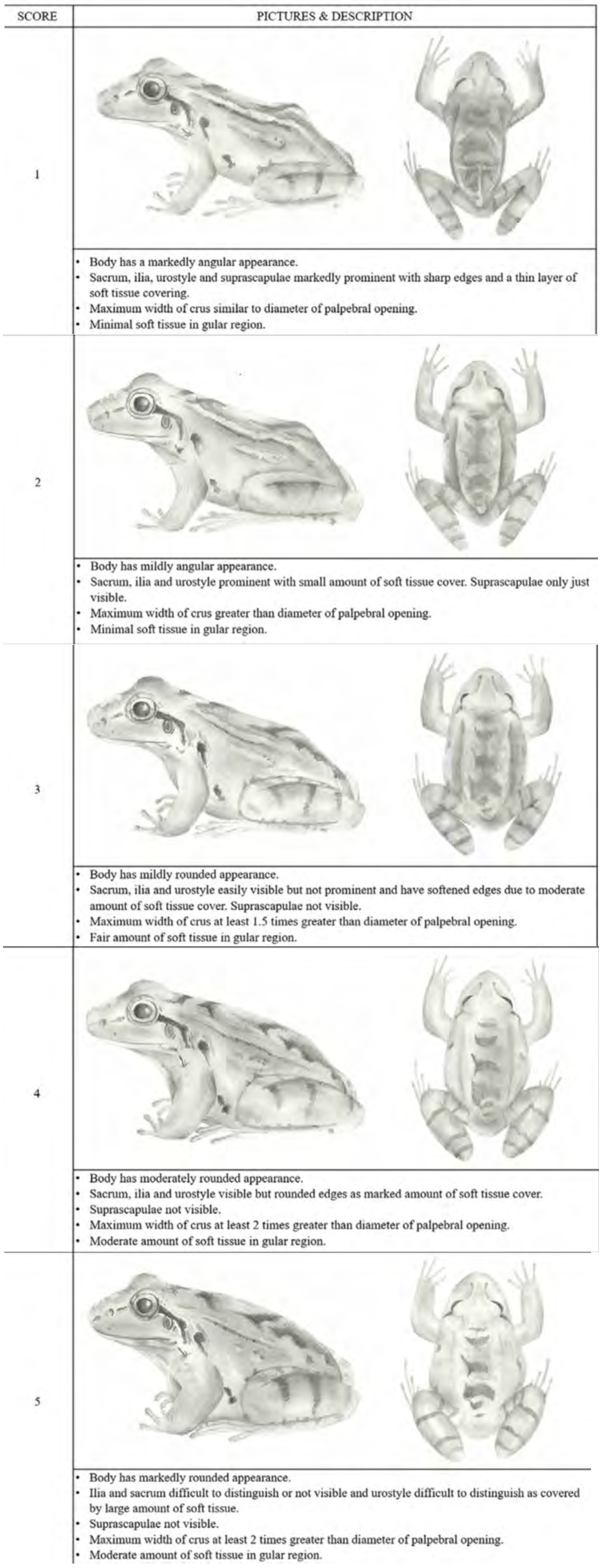

Figure 41. *Leptodactylus fallax* body condition scoring. Taken from Jayson et al. 2018b.

3. Physical examination:
Restraint:
Leptodactylus fallax can be restrained with one hand around the hips and hind legs (see section 2.6.3). This allows a limited physical examination, including integument examination and ophthalmological examination. Care must be taken when catching and handling frogs to avoid hip and long bone fractures.

Skin congestion in the ventral aspect of the hind limbs is commonly observed during physical restraint; this is a normal response and must not be confused with ventral petechial haemorrhaging associated with systemic infection. Physiologic congestion initially increases over time and is restricted to ventral and ventrocaudal thighs.

Leptodactylus fallax are likely to pass urine during handling or capture. Urine may have a strong rotting smell. This is associated with serious urinary bladder pathology and often seen with intestinal adenocarcinomas due to formation of fistulas connecting the intestine and the urinary bladder.

Physical examination:
Prior to capture or following release posture should be examined, limbs should be in a symmetrical position, with fore limbs partially extended keeping cranial portion of the body above the ground and hind limbs flexed.

Biometrics:
- Obtain body weight (record if has urinated or not) and snout to vent length. Weight can be obtained easily while keeping the frog in a cloth bag or pillowcase secured with a rubber band (see section 2.6.2). Body measures require two people, one restraining the specimen and one measuring as outlined in section 2.6.2.
- The integument should be examined for skin abnormalities, especially the ventral aspect of the body and palmar and plantar aspect of the digits.
- Coelomic cavity palpation is best carried out under anaesthesia as defensive lung over inflation prevents deep palpation of organs. Location of any abnormalities should be noted, dividing the coelomic cavity in four equal quadrants; cranial left and right, caudal left and right. This system is suggested to be used for the description of abnormalities also in radiographs and ultrasound. A full, firm stomach is often felt longitudinally on the left anterior and posterior quadrants. Fluid or a “gurgling” feeling on caudal quadrants suggest cystitis or large intestine dilatation.
- The oral cavity can be opened for examination using a finger or fine blunt instrument as a wedge at the cranial tip of the mouth and sliding the finger towards lateral to allow adequate oral cavity visualization. Mucous membranes are normally pale pink. Extreme pallor can be noted in specimens with low haematocrit.
- Ophthalmological examination can be carried easily under physical restraint, no detailed information is available regarding the ocular anatomy in this species but cases of unilateral and bilateral cataracts have been observed (Fig. 39).

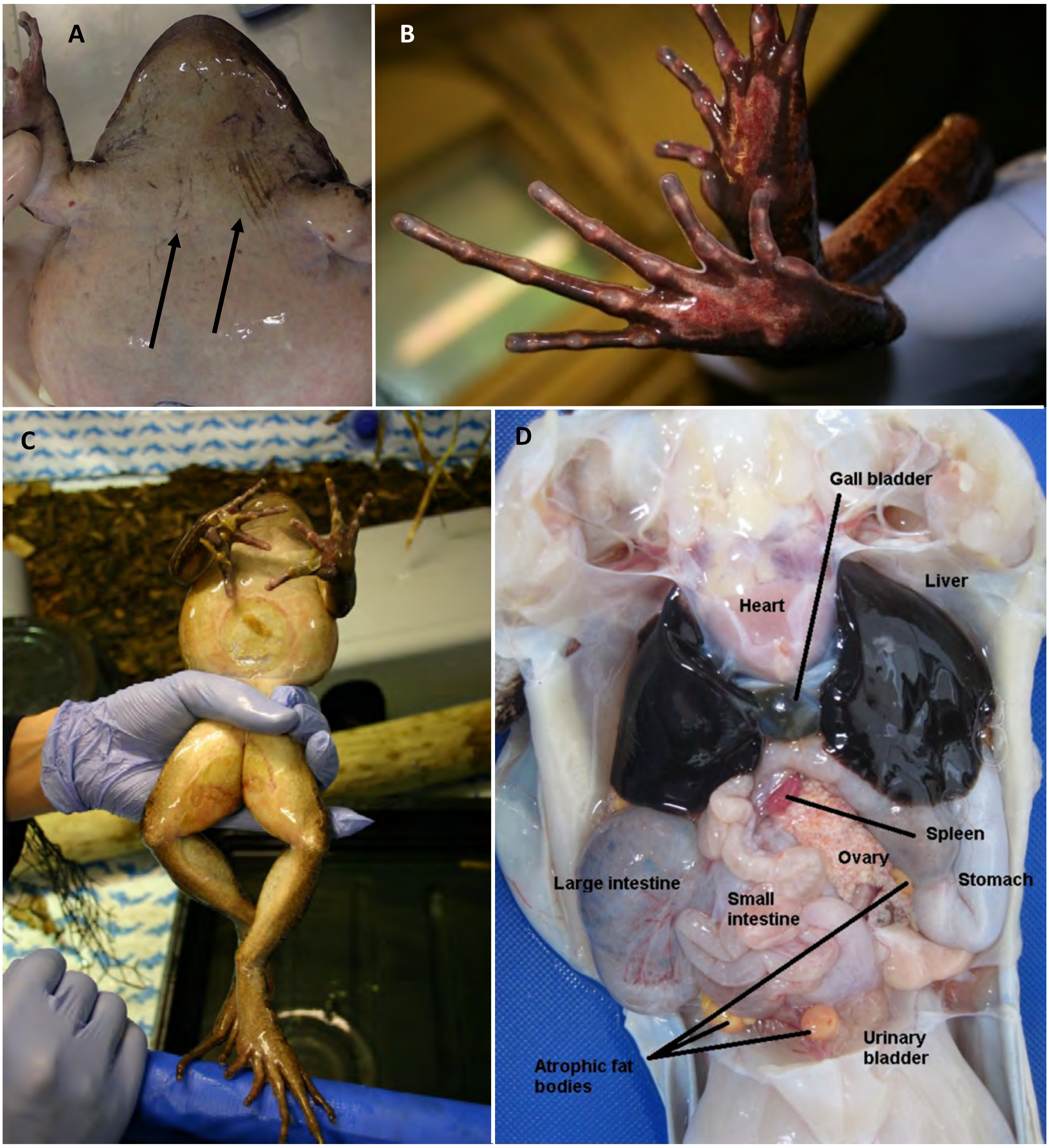

Figure 42. Reference images for physical examination of *Leptodactylus fallax*: (A) Scar tissue (arrows) secondary to superficial wounds in ventral aspect of adult female; (B) ulceration of feet; (C) contact dermatitis following exposure to disinfectant; (D) coelomic cavity organ distribution for reference for palpation, radiography, and echography. (A. Barbon/ J.Lopez).

4. Diagnostic techniques:

Blood sampling

Blood samples can be obtained from *Leptodactylus fallax* with the animal conscious or under anaesthesia, authors prefer to do it with the animal conscious but other veterinarians working with this species have found that sampling under anaesthesia is easier.

The preferable anatomical site to obtain the blood sample is in the midline abdominal vein, this is a relatively large vein located within the linea alba of the ventral musculature, along the ventral mid line. The vein can be visualised through the skin in most specimens.

Conscious blood sampling requires two people. The handler restrains the frog with thumb and index or middle finger around the hips (Fig. 43A). The handler places the frog in dorsal recumbency over the edge of a table allowing the hind limbs to hang and controlling the anterior portion of body towards the table to prevent sudden jerky movements from the frog that could result in stabbing internal organs. Alternatively, the person taking the sample holds the frog and a helper controls the cranial part. Once in position, if the mid ventral vein is not readily visible bulging through the skin, letting the frog relax and then placing back on dorsal recumbency often helps. Once visualised a 25 to 30G needle (with a 1ml syringe) is inserted through the skin and then through the vein at a 20-30 degree angle (Fig. 43B). Larger syringes are not recommended as they tend to collapse the vein if excessive suction is applied. If large volumes are required multiple venepunctures may be needed. Minor haemorrhage or subcutaneous haemorrhage may occur following venepuncture but are not significant and can be controlled by gentle pressure.

Alternative blood collection sites that the authors have used are the brachial vein for small volumes (Fig. 43C) and the popliteal space where the sciatic and femoral vein form an anastomosis (Fig. 43D), although the possibilities for lymph contamination are greater, especially in the popliteal space.

Cardiocentesis has been used for administration of euthanasia agents but, given the alternative sites and the risks associated with cardiac access, the authors do not recommend this site for blood sampling in this species.

Lithium heparin is the anticoagulant of choice in amphibians as erythrocytes lysis has been reported in some species of amphibians (Wright 2001). A preliminary comparison of EDTA and lithium did not show haemolysis in EDTA samples after 24 hours so this anticoagulant may be also safe to use in *Leptodactylus fallax*.

Figure 43. Reference images for blood sampling of *Leptodactylus fallax*: (A) Correct handling of *L. fallax* during blood sampling; (B) blood sampling from ventral vein; (C) blood sampling from brachial vein; (D) blood sampling from the popliteal space. (A. Barbon/ J.Lopez).

Radiography

Radiographs can be easily obtained in conscious specimens while being restrained in a cloth or plastic bag (Fig. 44A); however, this may only be useful to diagnose certain pathologies. Due to the poor positioning and overlapping of anatomical structures is not recommended for health checks.

Obtaining adequate dorsoventral and lateral views requires anaesthesia to allow correct positioning. Position the fore and hind limbs away from body, elbows and stifles should be placed at approximately 90-degree angle in order to obtain comparable radiographs (Fig. 44B). Whole body and limbs should be included in the radiograph (Fig. 44C).

Lateral body radiographs should be obtained using a horizontal beam set up, positioning the animals in lateral can be challenging due to the shape of the body (Fig. 44D). Using a horizontal beam allows an easier to replicate positioning obtaining more comparable radiographs (Fig. 44E).

Figure 44. Radiography of *Leptodactylus fallax*: (A) Dorsovenral radiograph of *L. fallax* conscious in a bag; (B) anaesthetized *L. fallax* positioned for dorsoventral radiograph; (C) Dorsoventral radiograph of anaesthetized *L. fallax*; (D) anaesthetized *L. fallax* positioned for lateral radiograph; (E) lateral radiograph of *L. fallax.* (A. Barbon/ J.Lopez).

Ultrasonography

Ultrasound can be carried out with the specimen conscious, which allows visualisation of heart, liver, gall bladder and sections of the gastrointestinal tract and urinary bladder (Fig. 45A). Lateral placement of the ultrasound probe allows examination of the kidneys, adrenal glands, and gonads (Fig. 45B). However, due to the over-inflation of the coelomic cavity during conscious restraint image quality is reduced and it becomes difficult to do a systematic and thorough examination. Current recommendation is to carry out the echography under anaesthesia.

A 12-16 MHz or higher linear probe is recommended to obtain an adequate organ detail, ultrasound gel is not required, keeping the skin moist with amphibian ringer's (see appendix 2 for composition) is enough to obtain an adequate ultrasound transmission. Coelomic cavity should be scanned systematically in both transversal and sagittal sections, describing any abnormalities within the coelomic cavity. If possible, still images or video clips from the different organs should be kept for future analysis.

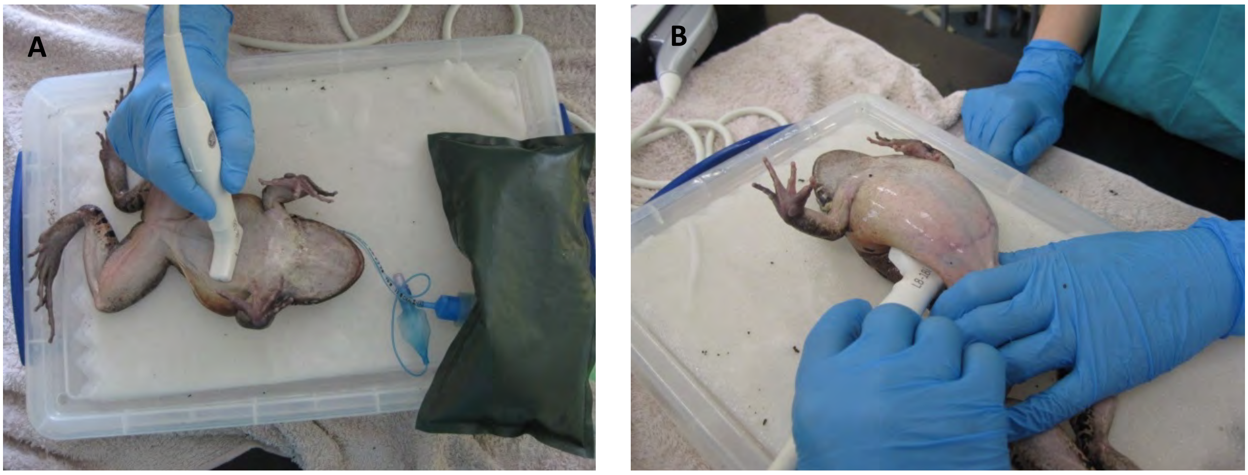

Figure 45. Echography of *Leptodactylus fallax*: (A) Dorsal recumbency placement of the ultrasound probe allows examination of the heart, liver, gall bladder, intestines, and urinary bladder; (B) lateral placement of the ultrasound probe allows examination of the kidneys, adrenal glands, and gonads. (A. Barbon/ J.Lopez).

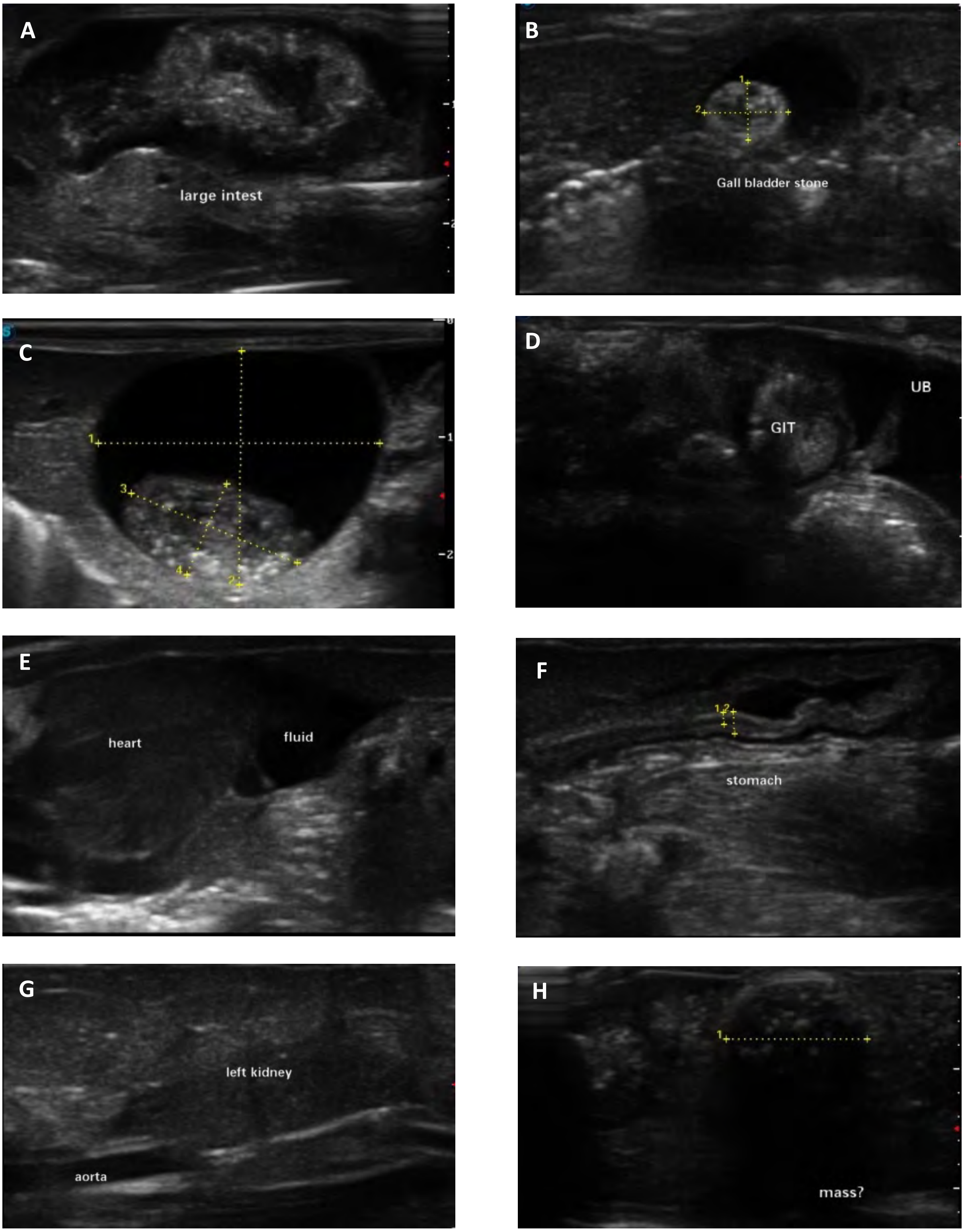

Figure 46. Reference ultrasounds for *Leptodactylus fallax* health checks: (A) Large intestine containing fluid and faecal material; (B) gall bladder containing a cholelith; (C) distended gall bladder with choelolith; (D) intestine and urinary bladder; (E) heart ventricle and gall bladder (marked as fluid); (F) stomach; (G) left kidney examination by probe placed on left flank; (H) foreign body in the right lobe of the urinary bladder. (A. Barbon/ J.Lopez).

2.7.4 Therapeutics

Table 2. Drugs used by the authors for the treatment of *Leptodactylus fallax*. The doses, route of administration, and frequency are completely anecdotal, no pharmacokinetic or phamacodynamic data is available to the authors' knowledge.

Drug	**Dose**	**Route of administration**	**Frequency**	**Comments**
Ceftazidime 90 mg/ml	20 mg/kg	IM	Q72 hours	
Enrofloxacin 25mg/ml	5 mg/kg	IM	SID	
Toltrazuril 25mg/ml	7 mg/kg	PO	SID	
Ivermectin 10mg/ml	0.2 mg/kg	IM	SID	
Silver suphadiazine		Topical	SID	
Meloxicam 5 mg/ml	0.2 mg/kg	IM, SC	SID	
Calcium gluconate	50m/kg	IM	BID	Metabolic bone disease
Vitamin D3	400 IU/kg	PO	SID	Metabolic bone disease
Amikacin 50 mg/ml	5 mg/kg	IM	Q48 hours	Can be combined with ceftazidime or enrofloxacin
Flubiprofen check	1 drop	Topical-eyes	SID	
Ofloxacin check	1 drop	Topical-eyes	SID	
Gentamicin 0.3%	1 drop	Topical-eyes	BID	

2.7.4.1 Anaesthesia

Anaesthesia is required for routine health checks in order to perform an adequate palpation of the coelomic cavity, positioning for radiographs and ultrasound scan, and for treatment and surgical procedures.

Injectable anaesthetics including alphaxolone, ketamine, medetomidine, and propofol alone or in combination have been used occasionally but did not provide consistent, repeatable anaesthetics with adequate depth. Similar results were observed with isofluorane or MS222® in a bath. In the particular case of MS222® very little effect was observed at standard doses of 1-2g/L and death was observed in two specimens at higher doses. The most extensively and successfully anaesthetic agent used by the authors for diagnostic and surgical procedures has been isoflurane by inhalation.

1. Induction: Specimen is placed in a clear plastic bag with a small volume (enough to provide about 1 cm depth at the bottom of the bag) of amphibian ringer's solution (see appendix 2 for composition). The bag is filled with Isoflurane at 5% and is sealed with a rubber band (Fig. 47A). Initially the animals may try to jump forward during the induction that may cause the rupture of the plastic bag, placing the hand in front of the specimen as a visual barrier may reduce the chances of this happening. Righting reflex is assessed at one-minute intervals by placing the specimen on its back within the bag, usually after 3-4 minutes most specimens lose the righting reflex (Fig. 47C). At this stage heart rate can be monitored by observing cardiac movement against body wall in the caudal section of the sternum. A basal heart rate can be established prior to the anaesthesia as reference. The specimen is kept in the bag for an additional 2 minutes after losing righting reflex.

   At this point, the specimen can be taken outside of the bag and position for radiographs, ultrasounds, and coelomic palpation. This method provides a short anaesthesia (approximately 5 to 15 minutes) after removal from induction bag, so it may be necessary to repeat the process if the examination is prolonged. For longer procedures, the frog needs to be intubated and anaesthesia maintained with positive pressure ventilation using isoflurane.

Figure 47. Induction of *Leptodactylus fallax:* (A) Induction in plastic bag; (B) Foam in plastic bag during induction; (C) Specimen after losing righting reflex. (A. Barbon/ J.Lopez).

2. Intubation: Low pressure, endotracheal cuffed tubes between 2.5 and 4.5 mm have been used for *Leptodactylus fallax* intubation and maintenance of prolonged anaesthesia. However, *L. fallax* do not have a well-defined trachea, the laryngeal cartilages connect directly with the lungs. A very fine, easily damaged membrane is present in the caudoventral portion of the larynx (Fig. 48). To prevent pulmonary intubation or damage to the laryngeal membrane the endotracheal tubes need to be modified to shorten the tip as explained below (Fig. 49).

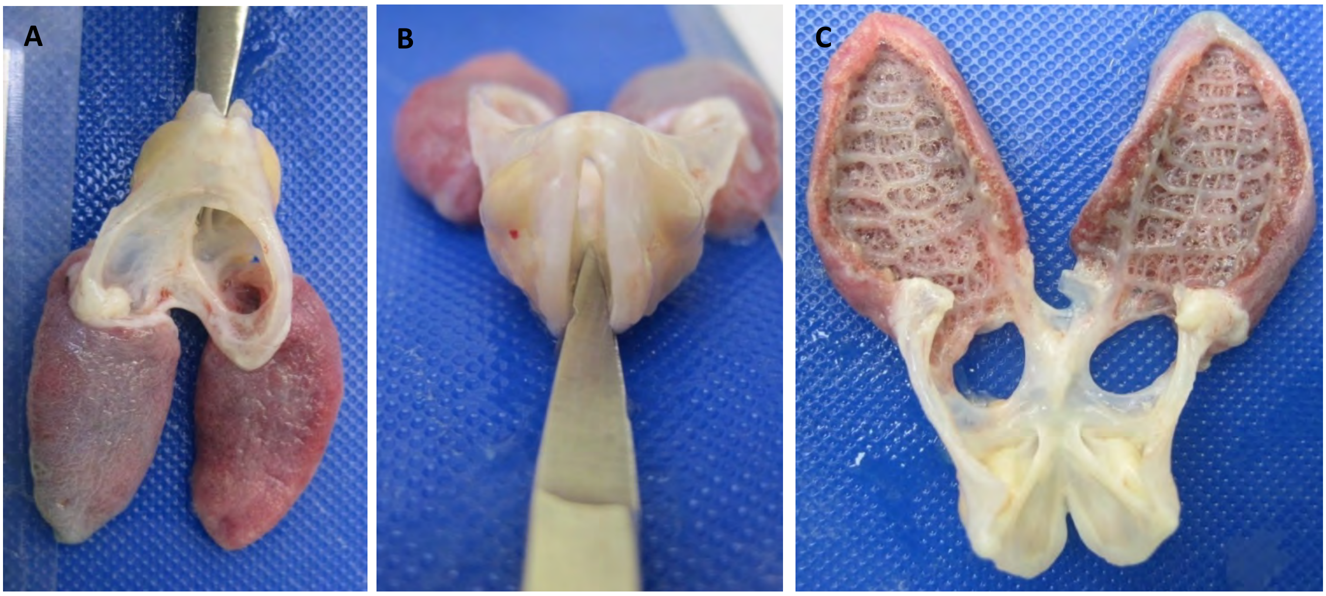

Figure 48. Larynx and lungs of *Leptodactylus fallax:* (A) Ventral view, the laryngeal membrane on the left side of the larynx has been removed for comparison; (B) cranial view; (C) cranial view following incision along the dorsal aspect of the respiratory tract, connective tissue in the ventral portion of the larynx has been removed. (A. Barbon/ J.Lopez).

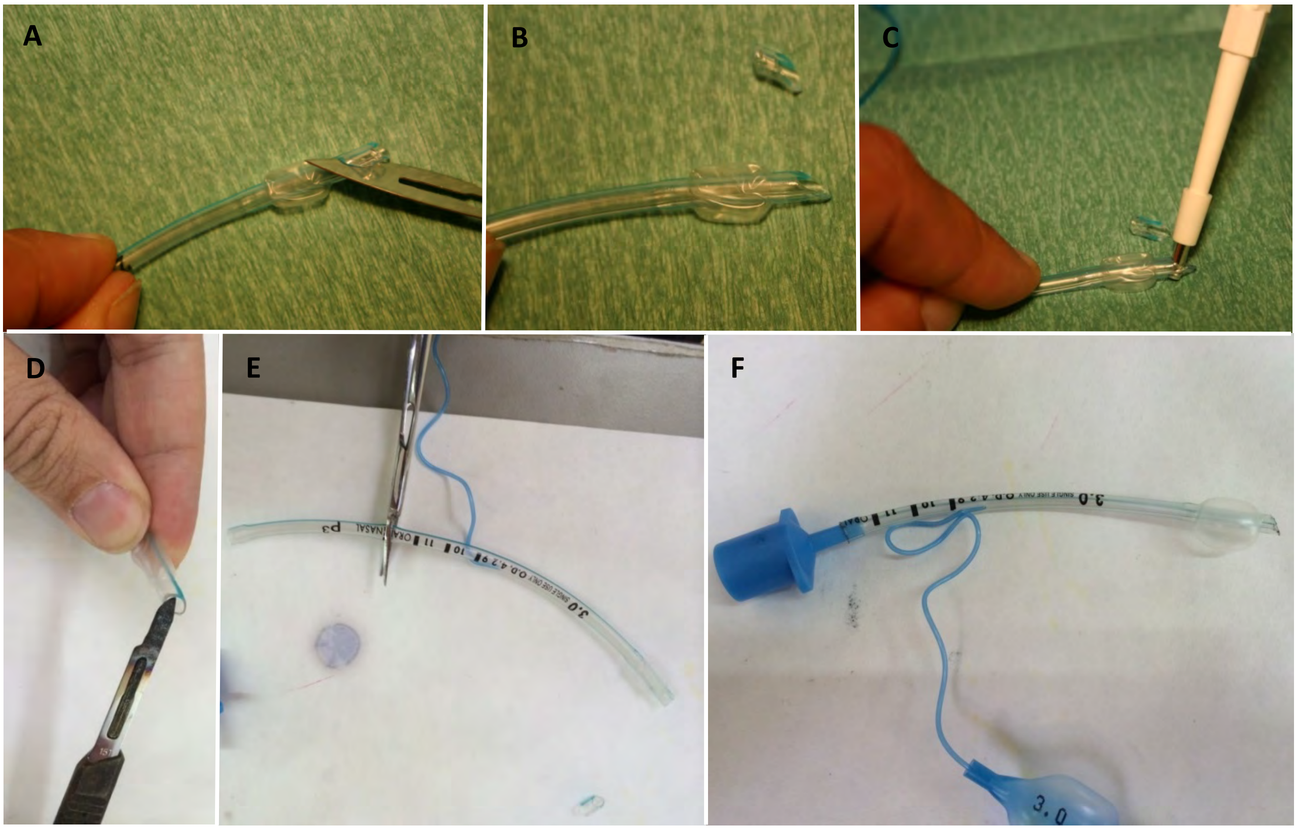

Figure 49. Modification of endotracheal tube for *Leptodactylus fallax*: (A +B) The tip of the tube is cut at a sharp angle using scissors or a scalpel blade, distal to the cuff leaving approximately a 0.5cm tube tip at the shortest point; (C) an additional hole in the side of the tip can be made with a punch biopsy to minimise the chance of the tube becoming blocked by mucus; (D) the small tube to inflate the cuff is open, so needs to be sealed with a heated blade or a small amount of glue; (E) the proximate end of the tube away from the cuff may need to be shortened to facilitate manipulation of the tube during intubation; (F) the final product with the inflated cuff. (A. Barbon/ J.Lopez).

2.1 Intubation technique: To prevent advancing the tube too far and damaging the laryngeal membrane or the lungs, the tube must be inserted with the cuff inflated. This will stop further advance and will produce a seal allowing positive pressure ventilation.

The intubated frog is restrained by an assistant keeping the back legs off the table to prevent kicking. Manipulation of the larynx elicits strong stimulation so the front legs need to be firmly restrained to prevent reaction and pulling the tube out (Fig. 50A).

The mouth is opened from the cranial edge and the finger is moved towards the side acting as a wedge to keep the mouth open (Fig. 50B); strong jaw tone is still present at this point.

While introducing the tube in the mouth care needs to be taken to avoid puncturing the cuff with the serrated borders of the frog's mouth and palatine teeth.

The larynx must be gently wedged open with the sharp tip of the tube, gently rotating the tube helps to achieve this.

The frog still may be reactive at this point so, once the tube is in place it must be held against the mandible between the index and the thumb (Fig. 50C) to keep it in place while positioning the animal. While still holding the tube in this position, the assistant should start to ventilate the animal to deepen the anaesthetic and ensure that the tube is placed correctly. Once there is no further reaction from the frog, the tube can be released. The tube is not taped or tied to the animal, two sandbags are used to keep the tube in position by placing the connector and end of the anaesthetic circuit between them and ensuring that it is positioned in such a way that there is always pressure from the cuff onto the larynx (as this is what will maintain the seal for ventilation) (Fig. 50D).

Figure 50. Intubation of *Leptodactylus fallax*: (A) Restraining of specimen and opening of mouth; (B) mouth wedged open whilst tube inserted; (C) tube held in place against mandible with forefinger and thumb until reaction stops; (D) placement of tubes with sandbags to hold tube in place. (A. Barbon/ J.Lopez).

3. <u>Maintenance:</u> Intermittent pressure ventilation is required throughout the procedure. This can be done manually or using a mechanical ventilator. Pressure is judged subjectively by observing the body wall displacement.

   Frogs' very thick mucus often produces blockages in one of the ET holes, resulting in unilateral lung inflation. If one ventilation under higher pressure does not clear this, the tube can be removed, cleared and replaced.

   Isoflurane minimal alveolar concentration has not been established in *Leptodactylus fallax*. In general, the frog is initially maintained at 3-4% and as the anaesthesia prolongs the concentration is reduced and adjusted based on stimuli response. As a rule of thumb, isofluorane is discontinued 10 minutes before the end of the procedure.

   Small specimens that cannot be intubated can be placed in a sealed mask, simulating a partial induction chamber, but anaesthesia may not be reliable for long procedures (Fig. 51).

Figure 51. Simulated partial induction chamber for small *Leptodactylus fallax* made using latex glove. (A. Barbon/ J.Lopez).

4. Recovery: Once that the procedure is finalised, intermittent positive pressure ventilation must be continued until the specimen recovers righting reflex. *Leptodactylus fallax* often appear to recover initially and try to remove the tube only go into apnoea again. To prevent this the authors advise to hold the intubation tube in place during the first attempt to recover. Once the frog attempts to reject the tube again, the frog is often breathing and has recovered the righting reflex. At this point the animal is extubated and placed in a box with amphibian ringer's solution (see appendix 2 for composition) (Fig. 52).

Figure 52. *Leptodactylus fallax* in recovery box following anaesthesia. (A. Barbon/ J.Lopez).

As part of the Mountain Chicken Frog Conservation Programme, 114 frogs were anaesthetised for intracoelomic radiotracer placement prior to release in Montserrat. Each frog was anaesthetized as described in these guidelines. Reflexes were monitored every 60 seconds and heart rate was monitored through the procedure at five-minute intervals (table 3). Righting reflex was lost at 3.4±2.3 minutes, 2-3 minutes later the frogs were removed from the bag for intubation.

Intubation was carried out by two persons at 7.6±2.2 minutes from the beginning of the anaesthesia. Manual intermittent positive pressure ventilation every 15 seconds was initiated and maintained through the anaesthesia; isoflurane concentration was reduced and maintained at 2%. Gular movement was lost at 7.6±2.7 minutes, followed by withdrawal reflex (10.6±4.8 minutes), and response to painful stimuli (11.1±2.9 minutes).

Surgical procedure from incision to last suture took 8.2±2.3 minutes. Isoflurane was discontinued as the last skin suture was placed. Total anaesthesia time since the animal was placed in the bag until the isoflurane was discontinued was 21±6.4 minutes. Intermittent positive pressure ventilation was continued until the animals recovered the righting reflex, which occurred at 40.4±10.1 minutes.

Table 3. Basal heart rate prior to anaesthesia obtained under physical restraint and during anaesthesia obtained at five-minute intervals. Number in brackets indicate sample size [n].

Time	Basal	5	10	15	20	25	30	35	40
HR	64±10	61±9	59±7	56±7	53±8	51±8	52±7	51±8	51±9
	[97]	[87]	[100]	[111]	[110]	[101]	[85]	[65]	[53]

HR: heart rate, beats per minute.

2.7.5 Surgical techniques

1. <u>Coeliotomy:</u> In *Leptodactylus fallax* coelotomies have been carried out to place intracoelomic radio trackers, for exploratory surgeries to establish the presence of intestinal adenocarcinomas, to perform cholecystectomies, for the removal of FBs, and to obtain intestinal biopsies (Fig. 53).

A paramedial incision is carried out in caudal half of the body, avoiding the mid abdominal vein. Forceps with teeth are recommended to grasp and elevate the skin, grasping the skin may elicit a painful response, the skin is thin and poorly vascularised making haemorrhage due to this incision rare (Fig. 53A).

The muscle layer should be visible under the skin allowing an incision that facilitates visualisation of the coelomic cavity. If intermittent positive pressure ventilation is being done for anaesthesia, is important to discontinue while practicing the incision in the muscle layer to avoid puncturing the lungs.

Closure is carried out in two layers, muscle and skin, using monofilament resorbable material such as poliglecaprone 25 (Monocryl). Muscle is closed using simple interrupted or continuous pattern, again care should be taken during this stage to avoid puncturing the lungs. Skin can be closed using an interrupted or continuous everting pattern using the same material. Suture material thinner than 4-0 (1.5 metric) has been observed to tear through the skin, so it is not recommended. Round body needles are recommended for muscle layer and reverse cutting needles for the skin.

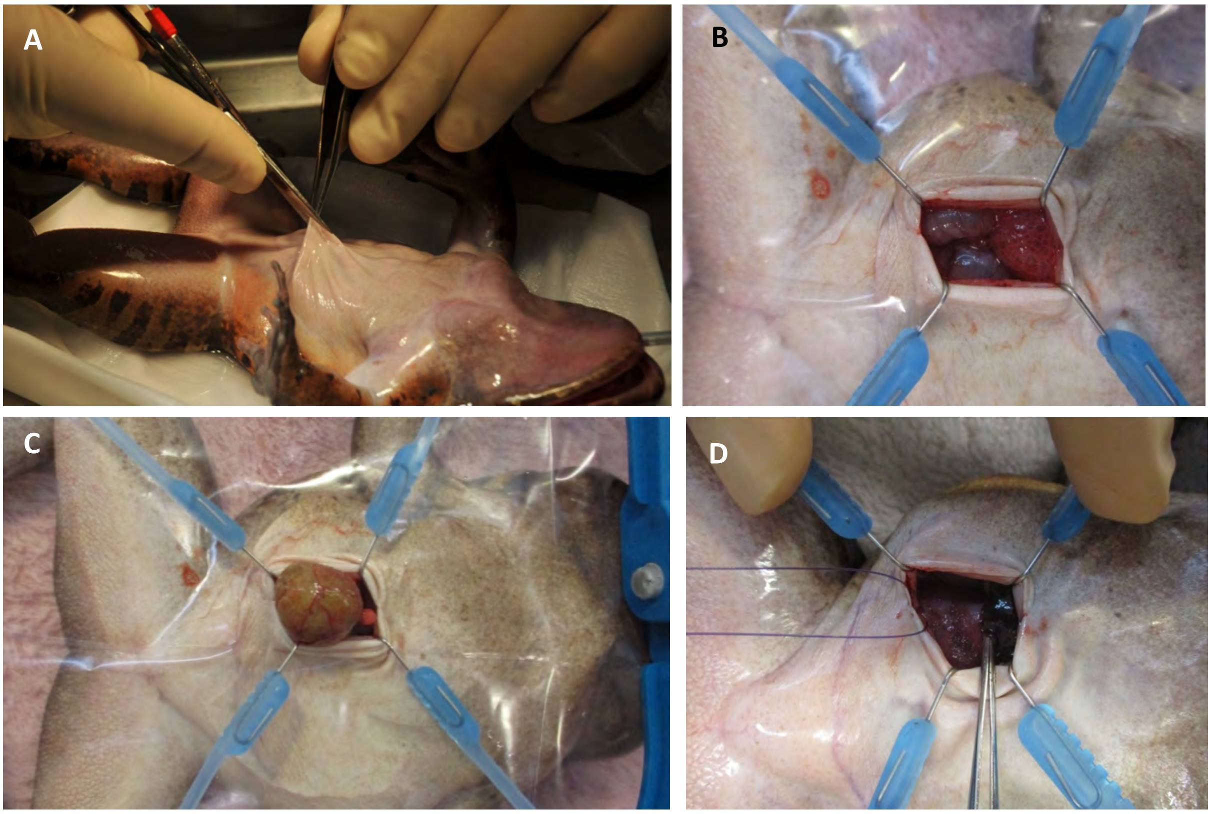

Figure 53. Coeliotomy of *Leptodactylus fallax*: (A) Paramedial skin incision to perform coeliotomy; (B) coeliotomy showing caudal aspect of right lung at cranial edge of incision; (C) coeliotomy showing right lobe of urinary bladder containing a foreign body; (D) coeliotomy to obtain an intestine biopsy, note inflammation in the colon serosa. (A. Barbon/ J. Lopez).

2. Cholecystectomy: A standard coeliotomy is carried out in the right cranial quadrant. The gall bladder is located in the right upper quadrant of the coelom, caudal to the cardiac apex. Once located, blunt dissection of the gall bladder from the surface of the liver is performed, any blood vessels ligated; the cystic duct is double clamped, ligated, and transected.

3. Orthopaedics: Tibiofibula and femur fractures have been surgically repaired in *Leptodactylus fallax* by using a single intramedular pin (Fig. 54).

   Femur fractures: surgical approach is done from the cranioventral aspect of the thigh; the pin is advanced into the distal fragment and through the skin cranial to the stifle, allowing pushing the pin in a retrograde fashion into the proximal fragment (Fig. 54A). Pin should be measured in advance to prevent going into the coxofemoral joint. A pin of the same size can be used to push the intramedular pin proximally, once it is cut as close to the bone as possible. Minor migration of the pin through the skin cranial to the stifle has been observed in one case requiring additional pin trimming and manipulation in order to maintain the pin intraosseous.

   Tibiofibula fractures: the fracture site is approached from the ventral aspect of the limb; a single intramedular pin is driven into the proximal fragment and through the skin cranial to the stifle, allowing to push the pin into the distal fragment (Fig. 54B). As in the previous case, measuring the pin to prevent damage to the joint whilst advancing the pin is required.

Tibiofibula fractures have been managed conservatively allowing healing by second intention (Fig. 54C and D). This may cause a shortening of the affected bone, but not obvious ill effects have been observed due to this post-operative complication.

Figure 54. Orthopaedics of *Leptodactylus fallax*: (A) Post-op radiograph of femur fracture after placing intramedular pin; (B) post-op radiograph of tibiofibula fracture after placing post intramedular pin; (C) tibiofibula fracture; (D) tibiofibula fracture 12 months later with no surgical intervention. (A. Barbon/ J.Lopez).

2.7.6 Euthanasia

A 0.3 - 0.4% MS222 bath is suitable for euthanasia. Alternatively, the frog can be placed in a small plastic bag with a ball of cotton wool soaked in isoflurane. Sectioning of the spinal cord at the base of the head and destruction of the brain with a wire or a needle (pithing) should be carried out if post-mortem examination is to be carried out immediately.

2.7.7 Post mortem examination

It is essential to carry out a thorough post mortem examination in all dead *Leptodactylus fallax* including animals where the cause of death is known. This is essential to understand the epidemiology of serious diseases (like adenocarcinoma) that threaten the viability of the captive population.

Dead amphibians decompose quickly so specimens must be placed in the refrigerator immediately and the post mortem must be carried out as soon as possible, ideally within a few hours. If an animal is very ill and prognosis is doubtful it is recommended to euthanise and perform a fresh post-mortem. Waiting for the frog to die on its own will seriously compromise welfare and reduce the diagnostic quality of the specimen afterwards. It is advised to examine all frogs, even if in poor state of preservation, as valuable information (such as body condition, presence or absence of a large intestinal lump, or faeces in bladder) can still be gained from severely autolytic specimens.

Post-mortem reports should be sent to EEP coordinator as soon as possible. A step-by-step protocol for post mortem examination and sampling and pictures of normal anatomy and common post-mortem lesions are provided in appendix 4.

2.8 Specific problems

While *Leptodactylus fallax* readily engages in reproductive behaviour on an annual basis, one problem in many institutions with breeding this species is a high percentage of “infertile nests”. Here nests are produced and eggs are present in the foam but no tadpoles hatch.

As an example, 333 foam nests were recorded at Jersey Zoo between May 2000 and June 2015. 62 of these were produced in group situations in larger communal enclosures with an average range of 2.4 to 4.7 animals, 177 in group situations (>6 frogs) without recorded sex ratio, and 94 were produced by animals kept in breeding enclosures with only 1.1 animals per enclosure. Of these 94 nests, 12 (12.7%) were fertile and yielded tadpoles whereas from the 239 nests of the combined group situations only 12 (5%) nests were fertile. Interestingly, 19 of the total of 24 “good” nests were produced early in the breeding season, i.e. in April and May (Durrell Wildlife Conservation Trust unpublished data).

Whether this is a case of infertility on the males’ part or some other problem or a combination of various factors is not confirmed nor has it been thoroughly investigated. Permanent infertility is unlikely though, since males that were engaged in production of such nests did also produce successful offspring before and after (M. Goetz pers. obs.).

Current thoughts, although unproven, are that this situation might be due to a lack of essential nutritional components; research is underway to shed light onto this question. Environmental toxins (e.g. plasticisers leaching from enclosures) are also considered, however, this would lead to a permanent "infertile" situation and "infertile" nests are also common in enclosures without plastic components. Another possible source of potentially problematic components is the very commonly, nearly universally used pine bark-chip substrate (e.g. Odynets et al. 1991) although these might be more significant as carcinogens. It has also been suggested that high levels of infertile nests may relate to the age of the individuals involved. Many of individuals repeatedly producing infertile nests are among the oldest currently present in captivity, nearing the maximum life expectancy for *Leptodactylus fallax* at over 10 years old. As such, these individuals may have reached the end of their reproductive life.

2.9 Recommended research

Table 4. Recommended research topics required to progress and improve the *Leptodactylus fallax* recovery program and ex-situ husbandry. Recommendations taken from the outcome of the Mountain Chicken Project partners meeting (2018) and from discussion resulting from the compilation of this document.

Project type	Project	Priority	Status	Person responsible
In-situ conservation	Development of trial reintroduction programs on Montserrat	High	Ongoing research	Michael Hudson Michael.Hudson@ioz.ac.uk
In-situ conservation	Development of camera trap monitoring protocols ex-situ for use in-situ	High	Not Started	Michael Hudson Michael.Hudson@ioz.ac.uk
In/ex-situ conservation	Development of facilities on Martinique and Guadeloupe	High	Project proposal under review and awaiting funding	Gerardo Garcia g.garcia@chesterzoo.org
Husbandry/population management	Investigation of genetic structure of the captive population	High	Currently part of PhD program	Nina White WhiteN8@cardiff.ac.uk Michael Hudson Michael.Hudson@ioz.ac.uk
Husbandry	Investigation of effect of handling on	Medium	Project started	Tom Jameson t.jameson@cheserzoo.org

	welfare and health		within Chester Zoo	Gerardo Garcia g.garcia@chesterzoo.org
Husbandry	Investigation of effect of replicating seasonal environment and diet affects fertility in captivity	High	Not started	N/A
Husbandry	Investigation of nature of "fertility". Identify if fertility drop-offs due to sperm viability/ production, egg viability/ production, synchronisation of gamete production, or combination of the above.	High	Not started	N/A
Husbandry	Investigate options for feeding vertebrate prey	Medium	Not started	N/A
Husbandry	Investigate optimum protocol for raising juveniles. Compare separation from adult enclosures to being left in adult enclosures.	Low	Not started	N/A
Husbandry/ veterinary	Investigation of optimal nutrition and related health problems	High	Ongoing research: King et al. 2011; Tapley et al. 2014 Nicholson et al. 2017;	N/A

			Jayson et al. 2018; Jameson et al. in press	
Veterinary	Establish reference values for haematology and biochemistry	Medium	Not started	N/A
Veterinary	Investigation of Cholelithiasis	Medium	Currently part of PhD program	Ian Ashpole i.ashpole@chesterzoo.org Javier Lopez j.lopez@chesterzoo.org
Veterinary	Investigation of gastrointestinal carcinoma	High	Currently part of PhD program	Ian Ashpole i.ashpole@chesterzoo.org Javier Lopez j.lopez@chesterzoo.org
Veterinary	Investigation of parasite burden and relationship to fitness	Low	Currently part of PhD program	Ian Ashpole i.ashpole@chesterzoo.org Javier Lopez j.lopez@chesterzoo.org
Veterinary	Refinement of body condition scoring	Low	Not started	N/A

Section 3 – Acknowledgments, References, and Appendix

3.1 Acknowledgments

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3.3 Appendix

Appendix I – Invasive species in the current and historic range of *Leptodactylus fallax*
Table showing the presence (y) and absence (n) of invasive species relevant to *Leptodactylus* fallax as predators, prey, competitors, and habitat modifiers on the islands representing the current and historic range of the species (Global Invasive Species Database).

Species	Dominica	Montserrat	Antigua	Guadeloupe	Martinique	St. Kitts	St. Lucia
Mammals							
Bos taurus	y	y	n	n	n	n	n
Canis lupus	y	y	y	n	n	n	y
Cercopithecus mona	n	n	n	n	n	y	n
Capra hircus	y	y	y	y	n	n	y
Equus asinus	y	y	n	n	n	n	n
Felis catus	y	y	y	y	n	n	y
Herpestes auropunctatus/javanicus	n	n	y	y	y	y	y
Mus musculus	y	n	n	y	y	n	y
Ovis aries	n	n	n	n	n	n	y
Rattus norvegicus	y	y	n	y	y	n	y
Rattus rattus	y	y	y	y	y	n	n
Sus scrofa	y	y	n	n	n	n	y
Procyon lotor	n	n	n	y	y	n	n
Didelphis marsupialis	y	n	n	n	n	n	n
Birds							
Bubulcus ibis	n	n	n	n	n	n	y
Columba livia	y	n	n	y	y	y	y
Gallus gallus	y	y	n	n	n	n	n
Passer domesticus	n	n	n	y	n	n	n
Anas platyrhynchos	n	n	y	y	y	n	n
Molothrus bonariensis	n	n	n	n	y	n	n
Monomorium floricola	y	y	y	n	y	y	y
Streptopelia decaocto	y	n	y	y	y	y	y
Reptiles							
Anolis cristatellus	y	n	n	n	n	n	n
Anolis extremus	n	n	n	n	n	n	y
Anolis wattis	n	n	n	n	n	n	y
Elaphe guttata	n	n	y	n	n	n	n
Geochelone carbonia	y	y	n	n	n	n	n
Hemidactylus mabouia	y	n	n	n	y	n	n
Iguana iguana	y	y	n	y	y	n	y
Trachemys scripta elegans	n	n	n	y	y	n	y
Norops sagrei	n	n	n	n	n	n	y

Species	Dominica	Montserrat	Antigua	Guadeloupe	Martinique	St. Kitts	St. Lucia
Amphibians							
Eleutherodactylus johnstoni	y	y	n	y	y	n	n
Dasyprocta antillensis	y	y	n	n	n	n	n
Osteopilis septentrionalis	y	n	y	y	n	n	n
Rhinella marina	n	y	y	y	y	y	y
Scinax ruber	n	n	n	n	y	n	y
Scinax x-signatus	n	n	n	y	n	n	n
Invertebrates							
Achatina fulica	n	n	n	y	y	n	y
Acromyrmex octospinosus	n	n	n	y	n	n	n
Aedes aegypti	y	y	y	y	y	y	y
Bemisia tabaci	n	n	n	n	n	y	y
Cactoblastis cactorum	n	y	y	n	n	y	n
Diaphorina citri	n	n	y	n	n	n	n
Icerya purchasi	n	y	n	n	n	n	n
Maconellicoccus hirsutus	y	y	y	n	n	y	y
Nylanderia (=Paratrechina) pubens	n	n	y	n	y	n	n
Paratrechina longicornis	y	y	y	n	y	n	y
Raoiella indica	y	n	y	n	y	n	y
Solenopsis geminata	y	n	y	n	y	y	y
Solenopsis invicta	n	n	y	n	n	n	n
Tapinoma melanocephalum	y	n	y	n	y	y	y
Varroa destructor	n	n	n	n	n	n	y
Wasmannia auropunctata	n	n	y	n	y	n	n

Appendix 2 – Amphibian ringer's solution (Wright 2001)

Distilled water: 1 litre, NaCl 6.6 g, KCl 0.15 g, $CaCl_2$ 0.15 g, $NaHCO_3$ 0.2 g.

This solution has been used extensively in *Leptodactylus fallax* as supportive care in sick specimens or during surgery to maintain the body surface moistened without any obvious deleterious effects but detailed studies about plasma osmolality and the effects in this specific species need to be carried out.

Appendix 3 - Example of template for pre-import health requests

«AddressBlock»

<Date>

Dear Sir or Madam

I understand we are due to receive X.X Mountain chickens from you in brief.

Please see below for the list of pre-import health requirements that we would like to request for this move. I understand that in some instances not all the tests requested will be possible but I will be grateful if you could carry these out as far as you can, letting us know which you will not be able to comply with so we can plan ahead for the quarantine requirements on arrival.

Please note that we will need the information requested and confirmation of the extent and results of the health screening before we can accept the animals, and that the lack of this information may lead to delays or cancellation of the move.

- Isolation (from new contacts for duration of screening period, suggested minimum 21 days).
- Faecal parasitology and treatment if appropriate (not required for tadpoles).
- PCR test for Chytrid or evidence of testing negative in the last 2 years for the group.
- Declaration free of rana virus in the collection for the last 5 years.

In addition, to this, can you please provide information on the following:

- Health problems in the amphibian collection which could be of relevance to the amphibians exported. In particular I am interested in the ranavirus and chytrid fungus testing status of the collection.
- Relevant health problems that you have observed in the species.
- Routine treatments given or required
- We will appreciate if you can make available a copy of the clinical history and relevant veterinary inspection/investigations/results and Medical notes printouts before the transport date.

Do not hesitate to contact us on ----------------------- if you have any questions or concerns.
Thank you very much for your help

Yours faithfully,

«Signature»

Appendix 4 – *Leptodactylus fallax* post mortem examination form

See supplementary material.