



## Development of in-country live food production for amphibian conservation: The Mountain Chicken Frog (*Leptodactylus fallax*) on Dominica, West Indies

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**Abstract.**—Amphibian populations are in global decline. Conservation breeding programs (CBPs) are a tool used to prevent species extinctions. Ideally, to meet biosecurity, husbandry and other requirements, CBPs should be conducted within the species' geographic range. A particular issue with in-country amphibian CBPs is that of live food supply. In many areas, such as oceanic islands, commonly cultured food species used by zoos throughout the world cannot be used, as escapes are certain to occur and could lead to the introduction of alien, and potentially highly destructive, invasive species. Here, we describe the establishment of live food cultures for the Critically Endangered Mountain Chicken Frog (*Leptodactylus fallax*) at a conservation breeding facility on the Caribbean island of Dominica. Not all invertebrate species were suitable for long-term culture and several species were rejected by captive *L. fallax*, making them unsuitable as food items. Despite the CBP being established within a range state, it was not possible to provide a diet of comparable variety to that of wild *L. fallax*. Our experiences may provide guidance for the establishment of live food culture systems for other conservation breeding programs elsewhere.

**Keywords.** Captive breeding, live food culture; invertebrate husbandry, conservation breeding program, Critically Endangered, diet

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### Introduction

Amphibian populations are in decline globally, with extinction rates now reaching over 200 times the estimated background rate (Collins 2010; McCallum 2007; Norris 2007). Conservation breeding programs (CBPs) are one of the tools used to mitigate amphibian extinctions (Griffiths and Pavajeau, 2008). In order to be successful, these programs should aim to maintain genetically-representative populations of amphibians in captivity for future conservation translocations (Baker 2007; Browne et al. 2011; Shishova et al. 2011). Establishing amphibian CBPs outside the native range of a species is considered suboptimal due to the risk of transferring novel pathogens to the target species or from the target species into the local environment (Cunningham et al.

2003; Walker et al. 2008; Zippel et al. 2011). Establishing a CBP within the range of the target species reduces this risk, facilitates the provision of natural environmental cycles with relative ease, is often more cost effective and can also instill pride and confidence in the public and other stake holders in the range country (Edmonds et al. 2015; Gagliardo et al. 2008; Tapley et al. 2015a). Amphibian husbandry capacity, however, is often limited in the countries with the most diverse and threatened amphibian faunas (Zippel et al. 2011). For programs in these countries to succeed, it is essential that amphibian husbandry methods, successful or otherwise, are disseminated for the combined benefit of amphibian conservation.

Suboptimal husbandry or nutrition in CBPs can produce maladapted amphibians that are unsuitable for

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release (Antwis and Browne 2009; Mendelson and Altig 2016; Ogilvy et al. 2012). As the nutritional requirements of most amphibians are unknown, suboptimal diets, nutrition, and nutritional disease can be barriers to the implementation of successful amphibian CBPs (Antwis and Browne 2009; Dugas et al. 2013; Gagliardo et al. 2008; King et al. 2010; Ogilvy et al. 2012; Tapley et al. 2015b; Verschooren et al. 2011). Even when the diet is known, it is often not possible to replicate in captivity, as diets for captive amphibians are limited by the commercial availability of food species and the ability to establish breeding colonies of appropriate species, as well as difficulties in providing the prey species themselves with suitable diets. This could have significant repercussions for the success of amphibian CBPs (Tapley et al. 2015a).

The Critically Endangered Mountain Chicken Frog (*Leptodactylus fallax*) is the largest native amphibian species in the Caribbean and one of the world's largest species of frog (Adams et al. 2014; Fa et al. 2010). *Leptodactylus fallax* is endemic to the Caribbean islands of Montserrat and Dominica, although it once occurred on at least five other islands before being lost from those through a combination of habitat loss and degradation, introduced predators, and over-collection for food (Adams et al. 2014; Fa et al. 2010; Malhotra et al. 2007). More recently, the only two extant island populations have been driven towards extinction by the infectious disease, amphibian chytridiomycosis (Hudson et al. 2016a). The population of *L. fallax* on Dominica declined by more than 85% in the 18 months following the first identification of frog mortality due to chytridiomycosis on the island (Hudson et al. 2016a).

In response to these disease-mediated declines on Dominica and Montserrat, a safety net population was established, together with a global partnership, to ensure the survival of *L. fallax* (Hudson et al. 2016b). In 2007, the Zoological Society of London (ZSL), in partnership with the Dominican Forestry, Wildlife and Parks Division, established a captive breeding facility in the botanical gardens of Roseau, the capital of Dominica (Fig. 1A, 1B; Adams et al. 2014; Tapley et al. 2014). A particular issue with regards to the keeping of mountain chickens in captivity is that of food. Mountain chickens have voracious appetites. The commonly cultured food species used by zoos and hobbyists throughout the world could not be used in Dominica as escapees could lead to the introduction of alien (and potentially highly destructive) invasive species onto the island. Therefore, prior to acquiring founding stock of *L. fallax* for the facility, therefore, it was imperative to establish live food cultures of sufficient quantity to provide adequate nutrition for the captive animals. Brooks Jr (1982) investigated the diet of *L. fallax* on Dominica and additional prey items were reported by Rosa et al. (2012) for the species on Montserrat. This knowledge was used to inform the species' captive diet.

Herein we describe the methods used to establish sustainable live food cultures for *L. fallax* on Dominica.

This may provide guidance for the establishment of subsequent live food culture systems for other range state amphibian conservation breeding.

## Methods

### Initial considerations

All species selected for culture were harvested from Dominica. Local species were chosen because: 1) accidental release would not lead to introductions of non-native species; 2) acclimatization to local environmental conditions would not be necessary; 3) purchasing and importation costs would be eliminated; 4) availability of stock would not be affected by delayed importation due to tropical storms or other unforeseen circumstances; 5) restocking of depleted cultures would be relatively simple and cost-effective (at the cost of culture adapted species). As well as being local, one of the criteria for choosing a species to trial for live food culture was a perceived ability to rapidly reproduce. Preference was given to those species that had been documented to form part of the wild diet of *L. fallax* (Brooks Jr 1982). In addition to the species initially selected for live food culture, further species were harvested from the wild to include more variation in the captive diet. All substrate was purchased from agricultural suppliers in order to reduce the likelihood of contaminating agents/animals being brought into the facility.

### Environmental conditions

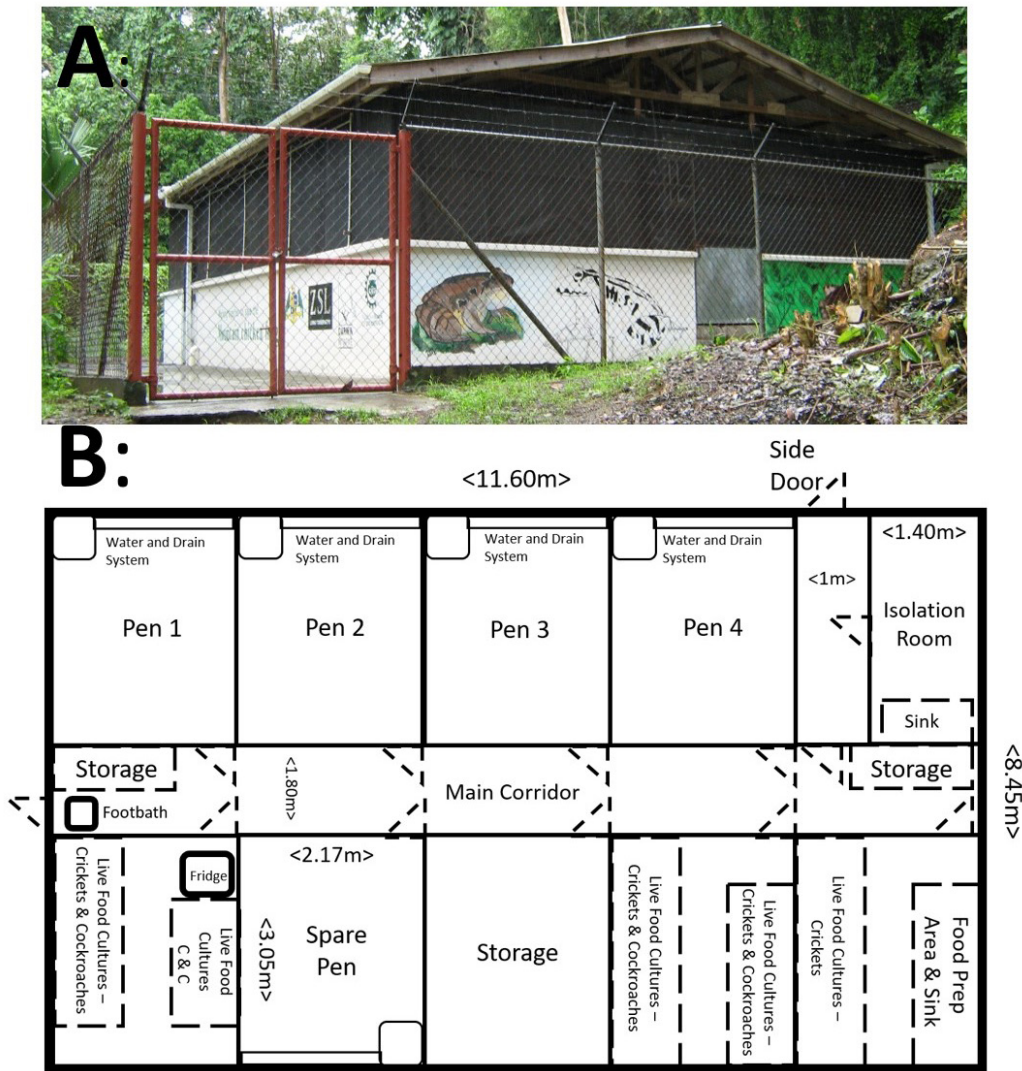
The facility in Dominica is open-sided, using a combination of metal wires and mesh netting. This allows the facility to closely match the ambient temperature and humidity of Dominica without the use of climate control methods. The facility itself therefore matches the local temperature range of 20–30 °C throughout the year.

### Species used

Since the facility's opening in 2007, live food culture of eight species has been attempted: three species of cricket (*Gryllodes sigillatus*, Fig. 2A; *Gryllus assimilis*, Fig. 2B; *Caribacusta dominica*, Fig. 2C), one cockroach (*Blaberus discoidalis*, Fig. 2D), one beetle (*Zophobas atratus*, Fig. 2E), one slug (*Veronicella sloanii*, Fig. 2F), one snail (*Pleurodonte dentiens*, Fig. 2G), and an assortment of unidentified millipede species (one species represented in Fig. 2H).

### Orthoptera

Orthopterans represent a large proportion (44%) of the known diet of *L. fallax* on Dominica (Brooks Jr 1982). Cultures of two cricket species were established at the start of the project: *G. sigillatus* (Fig. 2A), and *C. dominica* (Fig. 2C). A colony of *G. assimilis* (Fig. 2B) was



**Fig. 1. (A)** The Dominican mountain chicken project captive breeding and research facility, Roseau, Dominica. **(B)** Layout of the conservation breeding facility. Photo: D. Nicholson.

formed four years after the facility was set up in order to increase the variety of live food being offered to captive *L. fallax*. The founding population of *C. dominica* was collected from forested areas around the island. *Gryllus assimilis* colonies were established from just two founders that were collected using baited bottle traps. No other individuals of *G. assimilis* have been observed on the island since the original opportunistic encounter. *Gryllus assimilis* and *C. dominica* are native to Dominica and the West Indies (Orthoptera Species File 2016, Weissman et al 2009). *Gryllodes sigillatus* is a southeast Asian native but is now globally distributed (Otte 2006). Individuals used for culture were wild-caught in-country.

**Housing:** Orthopteran colonies were housed in clear plastic containers measuring 52 × 36 × 38 cm, with an open top covered with fine fly mesh to prevent escape (Fig. 3A). Refugia, including cardboard (hens') egg boxes and cardboard tubes, were provided. Housing containers were cleaned monthly (for *G. sigillatus*) or twice

monthly (for *G. assimilis* and *C. dominica*) to remove faecal waste; uneaten food was removed three times per week.

**Feeding:** Orthopteran colonies were fed fresh food three times per week. A number of different fruits and vegetables were provided, including pumpkin (1 cm cubes), lettuce (diced), cabbage (diced), and carrots (0.5 cm thick discs, halved). Also, a teaspoon each of Seminole Feed® Premium Performance Dog Food (Seminole Feed, Florida, USA) and Pentair® Colour Mix Fish Flake Food (Pentair Aquatic Eco-Systems, North Carolina, USA) were provided to each container three times per week. These were used due to their high protein content (dog food: 26% protein, fish food: 45% protein) and ease of storage.

**Breeding:** Oviposition sites were created using a 1:1 mix of compacted sand and sphagnum peat moss placed into (10 × 5 × 5 cm) plastic containers (margarine tubs).





**Fig. 2.** Cultured species at the CBP in Dominica. (A) *Gryllodes sigillatus*. (B) *Gryllus assimilis*. (C) *Caribacusta dominica*. (D) *Blaberus discoidalis*. (E) *Zophobas atratus*. (F) *Veronicella sloanii*. (G) *Pleurodonte dentiens*. (H) *Leptogoniulus* sp. Photos: D. Nicholson.

These were removed from housing units after two weeks, or sooner if hatchlings were observed (Fig. 3B). After removal, oviposition sites were placed into separate housing units until all 1<sup>st</sup> instar crickets hatched and exited the nest box. The substrate in the oviposition sites was kept moist at all times.

**Rotation:** All housing units were arranged and rotated depending on instar. Once the oldest adult crickets had been given sufficient time to lay eggs in the allocated oviposition site and provided with a respite and feeding period, they were fed to the captive *L. fallax* population. The associated oviposition sites were then placed in the first housing unit of the rotation and the remaining crickets at the most advanced stage of development were provided with an oviposition site.

### Blattodea

Cockroaches are not known to be a natural prey item for *L. fallax* (Brooks Jr 1982). They were, however, selected for culture due to their durability, high fecundity, large size, suitability to wide scale propagation and because they are readily consumed by captive *L. fallax* in Europe (B. Tapley, pers. obs.). It is not known if *B. discoidalis* (Fig. 2D) is native to Dominica, but it is native to Central America and distributed across the West Indies (Cockroach Species File 2016). The founding stock was collected from a chicken shed on the island.

**Housing:** Cockroaches were housed in large plastic dustbins (51 × 69 cm) with an open top covered with mesh lining to prevent escape (Fig. 3A). The bins were 1/3 filled with a sphagnum peat moss substrate to facilitate burrowing and cardboard boxes were added as refugia (Fig. 3C). Once per month, the containers were cleaned and the substrate was replaced.

**Feeding:** Cockroach colonies were fed potatoes (1 cm cubed, approx.), citrus fruits (quartered) and dry dog food (Seminole Feed® Premium Performance Dog Food) *ad lib*, with fresh food provided three times per week.

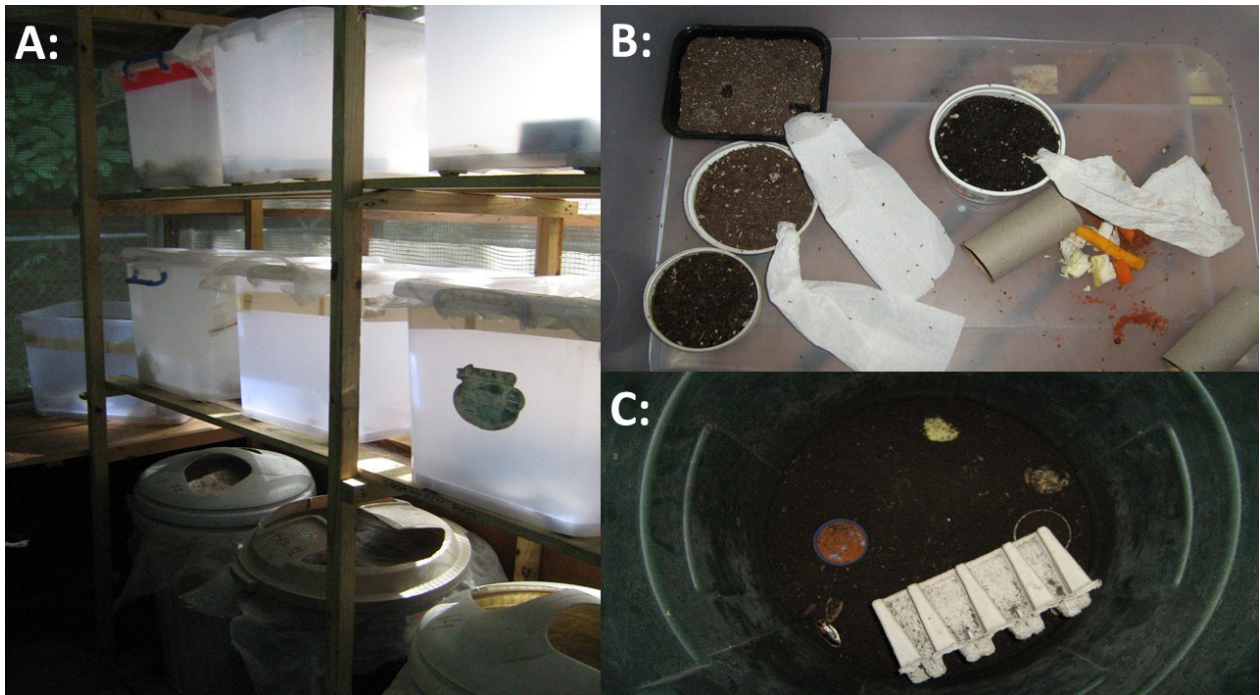
**Breeding:** The substrate used (sphagnum peat moss) provided a sufficient breeding medium.

### Coleoptera

Coleoptera comprise 7% of the known diet of wild *L. fallax* (Brooks Jr 1982). Beetles were incorporated into the culture process at the facility after the giant mealworm beetle (*Zophobas atratus*, Fig. 2E) was found to be breeding in the cockroach containers and was noted to be eaten by the captive *L. fallax*. *Zophobas atratus* is native to Central and South America, and it is believed to be naturally occurring in Dominica (Peck 2006). Separate colonies of this beetle were established using the method and housing described above for the cockroaches. Both beetle larvae and adult beetles were offered to *L. fallax*.

### Gastropoda

Gastropods make up 18% of the known diet of wild *L. fallax* (Brooks Jr 1982), which have been observed consuming them (D. Nicholson, pers. obs.). Slugs (*V. sloanii*, Fig. 2F) and snails (*P. dentiens*, Fig. 2G) were selected for culture as they are highly abundant and widespread across Dominica, readily observed on nocturnal transects and easy to capture. *Veronicella sloanii* was first discovered on Dominica in 2009 and is believed to have been introduced. *Pleurodonte dentiens* is endemic to Dominica, Martinique, and Guadeloupe (Robinson et al. 2009). **Housing:** Both gastropod species were housed in clear plastic containers (52 × 36 × 38 cm) with open tops covered with mesh to prevent escape (Fig. 3A). All housing



**Fig. 3.** (A) Two rows of cricket breeding containers and cockroach breeding bins below. (B) Inside of a cricket breeding container, including refugia, food items, and several egg laying containers, transplanted into an empty container to allow eggs to hatch. (C) Inside view of a cockroach breeding bin, including substrate, refugia, and several food items. Photos: D. Nicholson.

units contained refugia such as cardboard egg boxes and sections of tree bark; sphagnum peat moss substrate was also added. Housing containers were cleaned weekly to remove faecal waste and un-eaten food. High humidity was maintained by misting the substrate with water, as required to keep it damp.

**Feeding:** All gastropod species were fed *ad lib* with the leaves of lettuce, cabbage, and spinach, with fresh food being provided three times per week.

### Diplopoda

Millipedes (Fig. 2H) are very common on Dominica and comprise 7% of the known diet of wild *L. fallax* (Brooks Jr 1982). Millipedes were, therefore, chosen for culture at the start of the project but this was soon abandoned as high numbers were readily available in the immediate area of the captive breeding facility. They were, therefore, collected from the wild and presented as a prey source shortly after capture. The different millipede species obtained were not identified to the species level.

### Provisioning of *L. fallax*

Up to 11 *L. fallax* were housed in the facility at any one time. The captive *L. fallax* were fed three times per week. Provisioning took place at night as this species is nocturnal (Adams et al. 2014). Night-provisioning increased the likelihood of successful predation and this allowed staff to monitor the behavior, feeding rate, and health of individual frogs. Prey items were placed in a plastic bag and dusted with a multivitamin and mineral supplement high

in calcium and containing vitamin D<sub>3</sub> Nutrobal® (Vetark Professional, Winchester, UK) before being released into the frog pens. The amount of prey offered at each feeding event varied depending on the condition of the frogs. Individuals with lower than expected body weight for their size were given more food items to encourage weight gain. Also, before and during the breeding season (February–September, Davis et al. 2000) the number of prey items offered was increased to provide for the additional energy expenditure associated with vocalizing, fighting (males), egg production, and nesting. During this period, 5–6 large prey items (cockroaches) or 10–12 small prey items (crickets) per frog were provisioned. The number of invertebrates offered to the frogs was reduced by 30% during the non-breeding season (October–January).

### Preventing metabolic bone disease

Metabolic bone disease (MBD) has been reported in captive *L. fallax* reared on diets supplemented with multivitamin and mineral supplements containing vitamin D<sub>3</sub> and calcium but not provided with ultraviolet B radiation (UV-B) (Tapley et al. 2015b). Animals on the same diet did not develop MBD when provided with UV-B, indicating that the disease was caused by vitamin D<sub>3</sub> deficiency (Tapley et al. 2015b). In most vertebrates, vitamin D<sub>3</sub> is synthesized via exposure to the UV-B present in sunlight. Uptake of ingested vitamin D<sub>3</sub> might not be sufficient in all species for optimal health and this appears to be the case for *L. fallax*. Vitamin D<sub>3</sub> plays a critical role in regulating calcium metabolism, as well as hav-

**Table 1.** Suitability of invertebrate species captured in the wild on Dominica for live food culture for captive Mountain Chicken Frogs.

Class or Order of live food item	Species of live food item	Sustainable population of food item cultured?	Food item readily consumed by <i>L. fallax</i> ?
Orthoptera	<i>Grylloides sigillatus</i>	Yes	Yes
Orthoptera	<i>Gryllus assimilis</i>	Yes	Yes
Orthoptera	<i>Caribacusta dominica</i>	No	Yes
Blattodea	<i>Blaberus discoidalis</i>	Yes	Yes
Coleoptera	<i>Zophobas atratus</i>	Yes	No
Gastropoda	<i>Veronicella sloanii</i>	No	Yes
Gastropoda	<i>Pleurodonte dentiens</i>	No	Yes
Diplopoda	<i>Leptogoniulus</i> sp.	Yes	No

ing important roles in organ development, muscle contraction, and the functioning of the immune and nervous systems (Wright and Whitaker 2001). To prevent MBD in the captive *L. fallax* all food items were dusted with a multivitamin and mineral supplement which is high in calcium and contains vitamin D<sub>3</sub> (Nutrobal®, Vetark Professional) before being released into *L. fallax* pens. Pens were also supplied with UVB emitting lamps (12% UVB D<sub>3</sub> 24 W Basking Lamp, Arcadia).

## Results

The ability to develop sustainable invertebrate cultures and the palatability of these as food items for *L. fallax* are summarized for each species in Table 1.

### Orthoptera

*Grylloides sigillatus* and *G. assimilis* cultures were successful and populations of both species have yielded approximately 50 adults per week to date (over a period of approximately seven and 2 years, respectively). Both species were readily consumed by captive *L. fallax*. However, although readily consumed by *L. fallax*, the live culture of *C. dominica* had a poor outcome. The reproductive output was consistently very low, hatchlings had high mortality rates, and adults had short lifespans. In 2015, five years after its establishment, the population finally collapsed when all surviving adults died without reproducing. The species is very common across Dominica, therefore restarting the culture was not deemed viable due to the ease of collecting animals from the wild and the unsuitability of the species for large scale production.

### Blattodea

Live culture of *B. discoidalis* was successful. To date, seven years after its establishment, the facility has maintained a yield of approximately 60 cockroaches per week. This food item was readily consumed by *L. fallax*.

### Coleoptera

Giant mealworm beetles were successfully cultured over six years, but consumption rates by *L. fallax* were low. While both life stages of *Z. atratus* were observed to be predated by the captive frogs (D. Nicholson, J. Spencer, pers. obs.), it was noted that adult beetles were promptly regurgitated. Laval forms were almost entirely ignored, apart from a few occasions. The culture of *Z. atratus* was, therefore, discontinued.

### Gastropoda

Culture attempts, while successful for both species, yielded low numbers (<10 per week) and were labor intensive: the enclosures required a disproportionate amount of cleaning and maintenance for the yield. Continuous cultures of gastropods were, therefore, stopped after approximately three years. Cultures of both gastropod species are, however, re-established during the breeding season to supplement the diet as they are readily consumed by the captive frogs.

### Diplopoda

The harvesting of millipedes was opportunistic, therefore the numbers offered to the frogs as food varied as a result. Despite being consumed by wild *L. fallax* (Brooks Jr 1982), observations of feeding behavior of captive *L. fallax* showed that all millipedes species were regurgitated after ingestion. The use of millipedes as a food item was therefore stopped at the facility. It is possible that the species of millipede provisioned in captivity is different to that observed as a wild food source by Brooks Jr (1982).

## Discussion

Provision of an appropriate diet is vitally important for amphibians in CBPs as nutrition influences health, longevity, and reproductive output (Li et al. 2009). The amount of space required for rearing invertebrates for a



relatively small number of frogs was considerable and accounted for 20% of the facilities footprint. When CBPs are conducted in-country, the risk of introduction of alien pest species used as live food is high, especially in island situations. In these cases, a culture of locally-caught species should be developed. A range of such species was trialled in Dominica, of which crickets *G. sigillatus* and *G. assimilis* and the cockroach *B. discoidalis* proved to be most successful. Some other species, such as gastropods, could be cultured successfully, but the labor and other costs of doing so outweighed the ease of harvesting from the wild. Together, the live food culture, augmented by harvesting from the wild, has provided a sustainable supply of food for the maintenance of captive *L. fallax* since their introduction into the facility on Dominica in 2011. Wild harvesting of live food might also provide trace nutrients not obtained from cultured live food, although this was not investigated in our study. The Mountain Chicken Frog CBP on Dominica has had no requirement for the import of food from overseas and no evidence of nutritional disease has been observed, although the frogs have not yet bred in the facility.

The known diet of *L. fallax* in the wild is varied, comprising at least 30 different prey species. In the captive breeding facility on Dominica, however, only five prey species could be regularly provisioned. The depauperate captive diet was primarily due to three reasons: 1) several species were unsuitable for propagation either because of an inability to maintain large enough cultures or because of labor requirements; 2) certain species that could be cultured were not consumed by *L. fallax* in captivity; 3) species not known to be prey items were cultured (including a non-native cricket and cockroach, both of which were already established on Dominica). Even if the known wild diet of *L. fallax* could be matched, the diets used to culture live food are different to those eaten by the invertebrates in the wild. It is unlikely, therefore, that the nutritional content of cultured live food accurately represents that of the same invertebrate species in the wild. It is possible that the cultured diet supplied to the captive frogs is not optimal and therefore a wider range of food species should be harvested from the wild if captive animals are to be maintained and bred on the island in the future. Determining the nutritional content of the wild diet of *L. fallax*, rather than replicating the food items themselves, could inform a viable alternative of manipulating the nutritional content of cultured live food through supplementation or gut loading.

The orthopteran, *C. dominica*, is thought to be one of the key prey items for wild *L. fallax* and is very commonly encountered on Dominica (Brooks Jr 1982); however, we were unable to culture it successfully in large enough numbers to be a useful food item. Possible reasons for the unsuitability of *C. dominica* to the culture process could include inappropriate diet, territoriality, or naturally low reproductive rates. The orthopteran section

of the diet therefore relied on two species, *G. assimilis* and *G. sigillatus*, the latter believed to be a non-native species that has become established on Dominica.

A further limitation in our ability to provide a varied diet was the apparent unpalatability of the readily cultured *Z. atratus* and the various unidentified millipede species. These beetles and (certain) millipedes were reported as being key components of the wild diet of *L. fallax* (Brooks Jr 1982), but when offered to captive frogs they were either rejected (millipede sp. and adult *Z. atratus*) or ignored (larval *Z. atratus*). This might be due to the ability of these species to produce defensive chemicals (Gullan and Cranston 2005), which could affect prey preference in captivity in particular because the captive frogs are provided with a readily available food supply. It was not possible to ascertain the identity (even to the level of genus) of the three types of millipede offered as prey items, and only genus of consumed millipedes was reported by Brooks Jr (1982). Perhaps *L. fallax* is very species-specific regarding millipedes and the wrong prey items were being offered.

The unsuitability of certain invertebrate species as live food items left the facility on Dominica heavily reliant on non-native species which were not listed in the wild diet of *L. fallax* but were easier to culture, notably *G. sigillatus* and *B. discoidalis* (Brooks Jr 1982). *Gryllobates sigillatus* is native to Southwestern Asia but has spread rapidly across the globe and is used in other CBPs where it is non-native (Edmonds et al. 2012). Its arrival date and how well it is established on Dominica is not known. *Blaberus discoidalis* is native to Venezuela, a country which has exported live poultry and other agricultural products to Dominica since establishing a trade relationship in the late 1970s (A. James, pers. comm.; Cockroach Species File 2016). *Blaberus discoidalis* was cultured in the facility after being found in a local chicken coop. As with *G. sigillatus*, the original introduction time frame for *B. discoidalis* is unknown but it is reasonable to suggest the species has been present on Dominica for many years, at least since the trade agreement with Venezuela began.

An accurate replication of the wild diet for animals in CBPs, including those in range states, often is unachievable. For the *L. fallax* CBP, and programs like it, we recommend that the focus should be towards supplying a diversity of locally sourced prey species while, if possible, increasing an understanding of the nutritional make-up of the diet in the wild. It is important to study, wherever feasible, the wild diet of any species maintained as part of a CBP. In this case, comprehensive studies such as Brooks Jr (1982) and additional findings (e.g., Rosa et al. 2012) were important for ascertaining potential prey species for culture. Establishing the wild diet and subjecting this to detailed nutritional analyses should provide the data required to provide an optimal diet in captivity, possibly through manipulating the nutritional content of live food species via supplementation or gut loading.

## Conclusion

Sustainable colonies of invertebrates were established using locally caught species on Dominica. These colonies were productive enough to sustain a captive population of *L. fallax*. There was no need to import exotic species to use as live food, but the species most suitable for culture were locally collected, non-native species. The wild diet could not be fully replicated in captivity but frogs did not exhibit any evidence of nutritional disease over the six years of this study.

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