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SHORT NOTE

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Records of multiple clutching in captive mountain chicken frogs *Leptodactylus fallax*

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ABSTRACT - Multiple clutching, with two or three successful clutches raised in a single breeding season, is reported from two females of *Leptodactylus fallax* in two European zoos. Previously, only single clutches were known to be raised by this species. Multiple clutching is perhaps unexpected in this species due to its resource-heavy parental care behaviour. Potential drivers of multiple clutching, including food resourcing and timing and size of initial clutches, are discussed.

INTRODUCTION

The mountain chicken *Leptodactylus fallax* (Müller, 1926) is the largest native species of anuran in the eastern Caribbean, and a vital part of the islands' ecology as a native apex predator (Hudson et al., 2016). Historically occurring on six - possibly eight - Lesser Antillean islands, its range is now restricted to Montserrat and Dominica, due to anthropogenic pressures (Hudson et al., 2016), including the arrival of the fungal pathogen *Batrachochytrium dendrobatidis*, one of the causative agents of the disease amphibian chytridiomycosis (Hudson et al., 2016). Now assessed as Critically Endangered (IUCN SSC, 2017), approximately 130 individuals of *L. fallax* remain on Dominica and the species is likely extinct in the wild on Montserrat (Hudson et al., 2016). Until the threat of emerging infectious disease can be ameliorated, a bio-secure, captive metapopulation has been established in European zoos, alongside a non-biosecure population from the same gene pool, with the aims of producing viable stock for future translocation purposes, as well as for research and public engagement initiatives (e.g. Tapley et al., 2015; Jayson et al., 2018a,b; Michaels et al., 2021). Captive breeding success and translocation planning requires further life history data to inform refinement and to tackle current limitations (Jameson et al., 2019) and for this reason, it is important to document new information when unique observations are made.

The reproductive mode of *L. fallax* is unique to the species and was first described from captive animals. Larvae hatch from eggs laid within a terrestrial foam nest created by the male and female frog. The female guards the nest throughout tadpole development and feeds the tadpoles by releasing unfertilised eggs into the nest (Gibson & Buley, 2004). The male likely defends the surrounding area (Gibson & Buley, 2004). Once the tadpoles are ready to metamorphose, they leave the nest and at this point, parental care stops (Gibson & Buley, 2004).

Although *L. fallax* has repeatedly reproduced in captivity (e.g. Gibson & Buley, 2004; Tapley et al., 2015; Jameson et al., 2019), reproduction is inconsistent between individual animals and between institutions, only single viable clutches per female per annum have been recorded in ex situ populations and there is very little information on reproduction in nature (e.g. Davis et al., 2000). Captive female *L. fallax* are known to produce multiple foam nests containing eggs within a single season (Jameson et al., 2019), but have not been documented to rear more than one of these; others are typically abandoned soon after oviposition (Jameson et al., 2019).

Here we describe the first incidences for the species of multiple successful clutching and rearing events in two European institutions.

METHODS AND RESULTS

Animals involved in observations are referred to by institution (ZSL or NA), sex (F or M) and number, where more than one individual of a given sex is involved. For detail of the backgrounds of each animal and husbandry conditions refer to Table 1S in the Supplementary Material. Multiple clutching was observed separately at the Zoological Society of London, London Zoo in 2017 and 2019, and at Nordens Ark in 2020 and 2021. Environmental conditions are an important determinant of fecundity in amphibians; frogs in both institutions were housed according to best practice husbandry guidelines (Table S1; Jameson et al., 2019). Briefly, animals were kept in large pens of at least 2 x 2 m with a substrate of damp organic mulch, various refugia and nest boxes for reproduction (see below). Temperatures were between c. 21 °C (night) and 30-35 °C (day) with seasonal variation and spatial gradients. Reproduction was primarily stimulated by small increases in temperature, especially at night, combined with increasing frequencies of spraying, simulating the warm, rainy period triggering reproduction in nature (Jameson et al., 2019). Nests were exclusively produced in nest boxes constructed according to Jameson et al. (2019), essentially opaque plastic storage boxes with entrance tunnels and lined with damp clay. Boxes were checked c. weekly for new nests. Nests were left under

Reproductive events at ZSL London Zoo		
Date	**Event**	**Offspring**
24.05.2016	ZSLF and ZSLM1 paired	
06.04.2017	Sprays increased 2x/day	
28.04.2017	Nest 1 found (sire: ZSLM1)	4/6 metamorphosed
15.06.2017	First metamorphs moved from nest	2/4 metamorphs developed health conditions
16.06.2017	Last metamorph moved from nest	requiring euthanasia
30.06.2017	Nest 2 found (sire: ZSLM1)	31/33 metamorphosed
14.08.2017	All metamorphs moved from nest	31/31 metamorphs remained healthy
02.09.2017	Sprays decreased 1x/day	
16.04.2018	ZSLM1 moved out of enclosure	
30.03.2019	ZSLF and ZSLM2 paired	
01.04.2019	Sprays increased 2x/day	
07.04.2019	Nest 1 found (sire:ZSLM2)	6/7 metamorphosed
31.05.2019	First metamorphs moved from nest	6/6 metamorphs remained healthy
04.06.2019	Last metamorph moved from nest	
06.06.2019	ZSLM2 moved out of enclosure	
12.06.2019	ZSLF and ZSLM3 paired	
17.07.2019	Nest 2 found (sire: ZSLM3)	13/13 metamorphosed
30.09.2019	First metamorphs moved from nest	1/13 metamorphs developed health conditions
05.09.2019	Last metamorph moved from nest	requiring euthanasia
Reproductive events at Nordens Ark		
Date	**Event**	**Offspring**
29.08.2019	NAF, NAM1 and NAM2 paired	
01.10.2019	Sprays and photoperiod decreased	
01.11.2019	Sprays and photoperiod decreased	
01.03.2020	Sprays and photoperiod increased	
25.04.2020	Nest 1 found (sire: NAM1)	1/1 metamorphosed
01.05.2020	Photoperiod increased	
01.06.2020	Sprays increased	
17.06.2020	Last metamorph moved from nest	
22.06.2020	Nest 2 found (sire: NAM1)	4/4 metamorphosed
01.08.2020	Sprays decreased	1/4 metamorphs developed health conditions
15.08.2020	First metamorphs moved from nest	requiring euthanasia
16.08.2020	Last metamorph moved from nest	
30.09.2020	Nest 3 found (sire: NAM2)	10/10 metamorphosed
01.11.2020	Photoperiod decreased	
26.11.2020	Last metamorph moved from nest	
21.12.2020	NAF and NAM3 paired	
01.03.2021	Sprays and photoperiod increased	
01.04.2021	Sprays and photoperiod increased	
04.05.2021	Nest 1 found (sire: NAM3)	3/3 metamorphosed without further complications
01.06.2021	Sprays increase	
07.07.2021	2/3 metamorphs moved from enclosure	
10.07.2021	Nest 2 found (sire: NAM3)	6/6 metamorphosed frogs are kept with parents and sibling
01.08.2021	Sprays decrease	
19.08.2021	Metamorphs (6) are starting to leave the nest	

parental care; tadpoles were counted once they became individually discernible. Metamorphs were removed once the tail had shrunk considerably and mouthparts began to transform (Gosner stages 43-45; Gosner, 1960), indicating the cessation of oophagy.

One female, ZSLF, produced two viable nests in 2017 and 2018, while NAF produced three viable nests in 2020 and two in 2021. Temporal sequences of frog pairing, courtship and nest production, environmental manipulation and the metamorphosis of young are recorded in Table 1, along with the number of tadpoles produced and proportion surviving to metamorphosis. All other aspects of reproduction were unremarkable.

DISCUSSION

Multiple clutching has been reported in a number of other frog species, especially taxa that are asynchronous in oogenesis (Tsuji & Lue, 2000). However, multiple clutching is typically observed in species without extended female parental care such as those where eggs are abandoned at oviposition (Tsuji & Kue, 2000), or where male, rather than female, parental care is present (Townsend & Stewart, 1994; Rogowitz et al., 2001), although notable exceptions to this include dendrobatid frogs with both multiple clutching and female extended parental care (Meuche et al., 2011). Reproduction in L. fallax females, which are likely asynchronous in oogenesis, is particularly costly in that the animal must commit resources to nest construction and courtship, production of both developing and infertile feeder eggs (an estimated 10,000-25,000 feeder eggs per clutch; Gibson & Buley, 2004), defence and maintenance of the nest, and must also have sufficient energy stores to survive almost complete cessation of feeding during larval development. Females frequently lose up to one third of their pre-breeding body mass during the period of parental care (pers. obs., authors) and the short breeding season across c. 3 months of the year leaves little time for replenishment of energy reserves after an initial successful clutch.

Although we report multiple clutching from two separate individuals of *L. fallax*, this is still a rare occurrence in the captive population and has never been documented in the wild. A number of factors common to both individuals may have contributed to this phenomenon. Both females were in very good body condition (scoring 3-4 on the mountain chicken frog body condition score scale; Jayson et al., 2018b) and were well habituated to keeper routines, ZSLF having been established in captivity for more than a decade and NAF having been raised in the Nordens Ark facility since metamorphosis. This allowed animals to capitalise on feeding events to consume numerous prey items and maximise the nutritional benefits of prey items, which lose nutritional quality quickly after addition to an enclosure (Michaels et al., 2014). ZSLF was an especially large animal and had far exceeded average longevity for the species, being an adult when collected from the wild and then having lived for around 16 years in captivity, compared with an average longevity of around nine years (Guarino et al., 2014). Although this may have influenced reproductive capacity, the Nordens Ark female was not similarly large or aged. Both animals produced initial, small clutches early in the reproductive season. This may have resulted in minimal depletion of energy reserves, and sufficient time to replenish these reserves in time to produce further clutches. At least one clutch per animal was within normal numbers for this species in captivity, bearing in mind that clutch size in captivity is substantially smaller than seen in new founder animals (Gibson & Buley, 2004) and appears to be decreasing in time within and between generations (Jameson et al., 2019). Although both animals had access to only one (ZSLF) or two (NAF) males (Table 1), both facilities contained additional males, the presence of which may have stimulated reproductive events. Finally, handling of frogs was kept to an absolute minimum, with animals captured only for necessary veterinary interventions; as disturbance and capture is thought to reduce food intake (Jameson et al., 2019), this low-disturbance management may have facilitated frogs building energy reserves through feeding as well as avoiding the interruption of breeding events.

The ability of *L. fallax* to produce multiple clutches within one breeding season may have implications for captive management and for the recovery of populations in nature, which at least for the time being, is likely to be dependent on the translocation of captive bred individuals. A better understanding of drivers of multiple clutching and of the relatively small clutch sizes associated with some clutches would facilitate this and further research in this area is recommended.

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