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RESEARCH ARTICLE

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Development of a body condition score for the mountain chicken frog (*Leptodactylus fallax*)

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The Critically Endangered mountain chicken frog (*Leptodactylus fallax*) has undergone drastic population decline due to habitat loss, hunting, invasive species, and chytridiomycosis. In response, several partner institutions initiated a conservation breeding program. It is important to maintain the captive population in good health. Therefore the program partners have recommended establishment of protocols for health examination of the species, including body condition assessment. Visual body condition scoring is a useful means to assess body condition in zoo animals for which regular bodyweight measurements are impractical or associated with capture-related stress. In this study, the authors developed a visual body condition score for the mountain chicken frog based on an ordinal categorical scale from 1 to 5 (1 = lowest body condition, 5 = highest body condition) using anatomical features that vary with total body energy reserves. Veterinary staff, animal managers, keepers, researchers, and students subsequently used the body condition score to assign scores to 98 mountain chicken frogs (41 male, 57 female) aged between 8 months and 12 years housed in five zoos in the UK and Jersey between February and March 2016. Body condition scores showed moderate (rho = 0.54; males) to strong (rho = 0.6; females) correlation with the scaled mass index, an objective measure of total energy reserves. The majority of pairwise comparisons between scores showed slight to substantial intra-observer agreement (93.8%) and slight to almost perfect inter-observer agreement (97.2%). Cases of poor agreement were likely due to limited observer experience working with the species.

KEYWORDS
amphibian, conservation, health assessment, zoo

1 | INTRODUCTION

The mountain chicken frog (*Leptodactylus fallax*) is a large Critically Endangered anuran native to the Caribbean islands of Dominica and Montserrat (IUCN SSC Amphibian Specialist Group, 2017). The species recently underwent one of the fastest population declines recorded, with 85% loss of the population in less than 18 months on Dominica and almost complete extirpation on Montserrat due to the fungal disease chytridiomycosis (Hudson et al., 2016). Consequently, several institutions established a captive population for conservation breeding (Adams et al., 2014; IUCN SSC Amphibian Specialist Group, 2017). The immediate future of the species on Montserrat is uncertain with the most realistic chance of success being through captive breeding and release (Adams et al., 2014).

The partners of the conservation breeding program are committed to ensuring the highest health standards are maintained. Therefore, they have recommended development of common protocols to allow comparable health assessments across the captive population, including methods for assessment of body condition (Adams et al., 2014). For the purposes of this study, body condition is defined as an individual's total body energy reserves, the measurable component of which is fat mass plus lean body mass (Clancey & Byers, 2014). As poor body condition is often reported with many diseases of amphibians, development of a method to assess body condition for use by animal husbandry and veterinary staff could improve health monitoring of species in this taxonomic group (Chai, 2015; Densmore & Green, 2007). Veterinarians commonly observe thin to emaciated body condition within the captive population of mountain chicken frogs at post mortem examination, often in specimens affected by intestinal adenocarcinoma which is frequently diagnosed in this species (Barbon, Flach, & Lopez, pers. obs.; Jaffe et al., 2015). With experience and training, regular body condition assessment may provide an earlier warning of certain diseases in the captive population (Schiffmann, Clauss, Hoby, & Hatt, 2017).

There are several possible methods to estimate body condition. The gold standard direct measure is chemical analysis of carcass tissues for lipid content (Mawby et al., 2004). However, stored whole carcasses may not be readily available and euthanasia of animals for this purpose is not usually justifiable in a zoo setting, particularly for threatened species such as the mountain chicken frog. Indirect methods include dual-energy X-ray absorptiometry (DEXA), deuterium oxide (D_2O) dilution, quantitative magnetic resonance (QMR), body condition indices based on morphometric measurements (such as the scaled mass index), and body condition scores which are based on visual assessment, with or without manual palpation, of key anatomical features (Clancey & Byers, 2014). Apart from visual body condition scores, all of these techniques require some form of manual or chemical restraint. Therefore, body condition scores based on visual assessment may be considered a more appropriate method for regular measurement of body condition of captive and free-ranging wild animals for which repeated manual or chemical restraint is impractical or associated with capture-related stress (Clements & Sanchez, 2015; Schiffmann et al., 2017; Thomson, Burkholder, Heithaus, & Dill, 2009).

Body condition scores should be: 1) Well described; 2) Relevant to the species to which they are applied; 3) Repeatable (low intra-observer variability); 4) Reproducible (low inter-observer variability); and 5) Correlate well with objective measures of body fat content (Laflamme, 1997; Summers, Clingerman & Yang, 2012). It has been recommended that body condition scores are tested for the latter three points (Schiffmann, Clauss, Hoby, & Hatt, 2017). Although body condition scores have been described in a number of captive and free ranging wild mammals, birds and reptiles, few scoring systems have been assessed for repeatability, reproducibility, and correlation with an objective measure of body fat content and to the authors' knowledge there are no published body condition scores for an amphibian species (Burton, Newnham, Bailey, & Alexander, 2014; Lamberski, 2013; Schiffmann et al., 2017; Thomson, Burkholder, Heithaus, & Dill, 2009).

The aim of this study is to develop a visual body condition score (BCS) for the mountain chicken frog that could be used by animal husbandry and veterinary staff to monitor body condition of individuals of this species in captivity.

2 | MATERIALS AND METHODS

2.1 | Development of the body condition score (BCS)

The authors requested photographs of captive mountain chicken frogs at rest in their enclosures from institutions housing this species and subsequently excluded images of restrained mountain chicken frogs due to distortion of anatomical landmarks when held. The primary author, experienced mountain chicken frog keepers, and veterinary staff at the Zoological Society of London reviewed the photographic database, alongside personal observations of the species, and selected potential anatomical features for the BCS that: 1) Could be assessed visually with the animal in a standard resting position without requiring handling; 2) Were expected to vary with body energy reserves; and 3) Would not be directly affected by other variables (e.g. gender, sex-related seasonal changes, age, and posture). They initially selected the following anatomical features: a) the soft tissue covering the sacrum, suprascapulae, ilia, and urostyle; b) the crus at its widest part (compared to the maximum width of the palpebral opening); c) the dorsolateral dermal ridge; and d) the soft tissue in the gular region (Figure 1). A BCS was developed with drawings and written descriptions of the selected anatomical landmarks at different grades of condition using an ordinal categorical scale ranging from 1 (lowest condition) to 5 (highest condition). A comment on the overall shape of the body (for example, angular or rounded) was also included in the written descriptions.

2.2 | Assessment of the BCS

The authors performed a pilot study to assess for intra- and inter-observer variability when observers used the BCS to score photographs of mountain chicken frogs. The primary author selected 19 photographs of captive mountain chicken frogs which included frogs representing the full range of body condition scores from 1 to 5, in a natural resting position, with the selected anatomical landmarks clearly visible. Eight observers (four veterinary surgeons, three keepers, and one veterinary nurse) were each provided with a copy of Figure 1, as well as the BCS, and the nineteen photographs which were randomly ordered. The observers assigned a score from 1 to 5 to each photograph based on the BCS. Four observers repeated the process 1 week later. Observers were asked to give written feedback on difficulties encountered when using the BCS. Observers were blinded to their previous scores and to those assigned by other observers.

Intra- and inter-observer agreement between scores was assessed using weighted κ statistics and expressed using cut-off values between 0 and 1 whereby $\kappa < 0.00$ represents poor agreement, $0.00 \le \kappa \le 0.20$ slight agreement, $0.20 < \kappa \le 0.40$ fair agreement, $0.40 < \kappa \le 0.60$ moderate agreement, $0.60 < \kappa \le 0.80$ substantial agreement and $0.80 < \kappa \le 1.00$ almost perfect agreement (Cohen, 1968; Landis & Koch, 1977). Tables

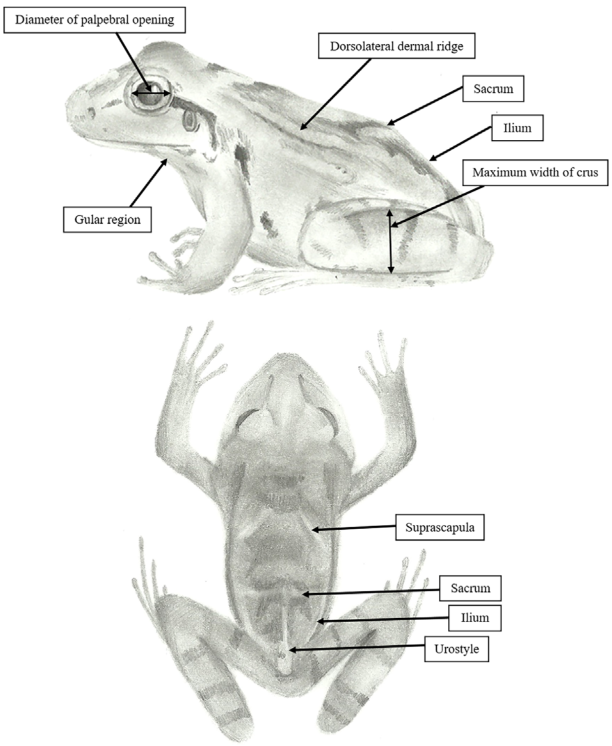


FIGURE 1 Anatomical features initially selected for use in development of the mountain chicken frog (*Leptodactylus fallax*) body condition score (BCS). The dorsolateral dermal ridge was not used in the final BCS (Figure 2) following observer feedback that it appeared to vary inconsistently with body condition

consisting of two columns containing the scores for each combination of two observers were inputted into an online calculator using the Cohen's Kappa for two raters setting and the weighted κ value reported (StatsToDo, 2014).

Observers noted in written feedback that the dorsolateral dermal ridge appeared to vary inconsistently with body condition therefore the authors removed the dorsolateral dermal ridge as an anatomical feature of the BCS, resulting in the final BCS shown in Figure 2. The authors subsequently assessed the BCS for intra- and inter-observer variability, as well as correlation with an objective measure of body condition (scaled mass index), using live mountain chicken frogs. Four to six observers at each of five zoos (A-E) used the BCS to assign a body condition score to the live mountain chicken frogs held at each zoo. The study population comprised the total population of mountain chicken frogs in captivity in the UK and Jersey, excluding juveniles under 6 months of age (nine individuals) as they were not due to be handled for bodyweight and snout-vent length measurements due to their young age. This resulted in a total study population of 98 frogs (41 male, 57 female) aged between 8 months and 12 years, including 12 frogs aged 3 years at Zoo A, 37 frogs aged 8 months—11 years at Zoo B, 14 frogs aged 4–12 years at Zoo C, eight frogs aged 2 years at Zoo D and 27 frogs aged 3 years at Zoo E. Observers scored the frogs just prior to the species' breeding season (between late February and late March 2016). Observer 5 at zoo C, and 3, 4, and 5 at zoos D and E consisted of three work experience students, a probationary keeper, a trainee keeper, a seasonal keeper, and a conservation researcher. They were considered less experienced working with mountain chicken frogs than other observers which included full time veterinarians, veterinary nurses, keepers, and animal management staff. Three of the authors involved in designing the BCS participated as observers (Observer 1 at Zoos A-E and Observers 2 and three at Zoo A); as such these observers were more familiar with the anatomical features of the BCS. Other observers were sent a copy of Figure 1 (with the dorsolateral dermal ridge removed) and the BCS (Figure 2) 1 week prior to scoring; however, observers were not required to view the BCS prior to use. No training in use of the BCS or opportunities to practice its use with captive mountain chicken frogs were provided. Each observer viewed each mountain chicken frog at rest in their enclosure prior to handling (Zoos A, C, D, and E) or at rest in an open transparent plastic box immediately after being moved from their enclosure (Zoo B) for a period of up to five minutes and assigned a score based on the BCS. At Zoos B, C, D, and E this was repeated 24 or 48 hr later. Individual identification of the mountain chicken frogs was confirmed by scanning the electronic microchip of each individual immediately following scoring and was not known to observers at the time of scoring.

Following one scoring event for each frog, the snout-vent length (mm to nearest 1 mm) was measured by the primary author from the most rostral edge of the snout to the most proximal edge of the vent using electronic calipers (LCD Digital Electronic Caliper Vernier Gauge Micrometer Tool, LUPO, UK) with the frog in hand, held around the waist. Bodyweight (grams to nearest 1 g) was measured using electronic scales (Tare and Fine Digital Kitchen Scale, Tanita, NL) immediately following body condition scoring. The authors calculated the scaled mass index for each mountain chicken frog using the formula described by Peig and Green (2009): scaled mass index $\widehat{M}_i = M_i \left[\frac{L_o}{L_i}\right]^{b_{SMA}}$ where M_i and L_i are the body mass and the snout-vent length of individual i respectively; b_{SMA} is the scaling exponent estimated by the standardised major axis (SMA) regression of M on L; L_0 is the mean value of snout-vent length L for the study population; and $\widehat{M}_i$ is the predicted body mass for individual i when the snout-vent length is standardized to L_0.

The authors used Spearman rank correlation in Excel to assess for correlation between the mean body condition score assigned to an individual mountain chicken frog by all observers and its scaled mass index, and weighted κ statistic to test for agreement between body condition scores assigned by independent observers and by the same observer (Cohen, 1968; Millar, 2001; StatsToDo, 2014).

2.3 | Welfare considerations

Mountain chicken frogs are routinely weighed as part of the ongoing health monitoring of this species in captivity. To reduce unnecessary stress associated with handling, the authors chose the timing of the snout-vent length and bodyweight measurements to coincide with routine bodyweight checks of these individuals. Experienced animal husbandry and veterinary staff handled the subjects, with at least two people (one handler, one data recorder) present to keep the time

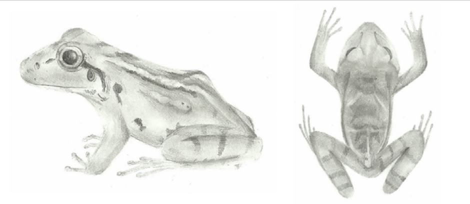
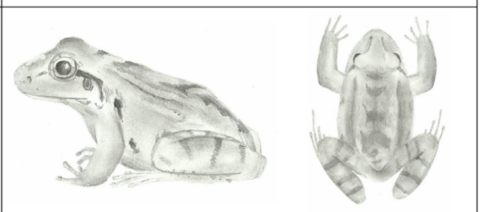
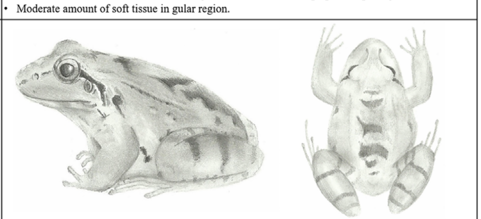
SCORE	PICTURES & DESCRIPTION
1	 • Body has a markedly angular appearance. • Sacrum, ilia, urostyle and suprascapulae markedly prominent with sharp edges and a thin layer of soft tissue covering. • Maximum width of crus similar to diameter of palpebral opening. • Minimal soft tissue in gular region.
2	 • Body has mildly angular appearance. • Sacrum, ilia and urostyle prominent with small amount of soft tissue cover. Suprascapulae only just visible. • Maximum width of crus greater than diameter of palpebral opening. • Minimal soft tissue in gular region.
3	 • Body has mildly rounded appearance. • Sacrum, ilia and urostyle easily visible but not prominent and have softened edges due to moderate amount of soft tissue cover. Suprascapulae not visible. • Maximum width of crus at least 1.5 times greater than diameter of palpebral opening. • Fair amount of soft tissue in gular region.
4	 • Body has moderately rounded appearance. • Sacrum, ilia and urostyle visible but rounded edges as marked amount of soft tissue cover. • Suprascapulae not visible. • Maximum width of crus at least 2 times greater than diameter of palpebral opening. • Moderate amount of soft tissue in gular region.
5	 • Body has markedly rounded appearance. • Ilia and sacrum difficult to distinguish or not visible and urostyle difficult to distinguish as covered by large amount of soft tissue. • Suprascapulae not visible. • Maximum width of crus at least 2 times greater than diameter of palpebral opening. • Moderate amount of soft tissue in gular region.

FIGURE 2 Body condition score (BCS) for the mountain chicken frog (*Leptodactylus fallax*)

in-hand to a minimum. Handlers wore moistened powder-free nitrile gloves to minimize the risk of skin damage during handling (Wright, 2001). Research proposal forms were completed, reviewed, and authorized by each institution as required by the institution's individual research department. As the procedures involved were part of routine husbandry and management procedures for the species, the institutions did not require formal ethical review.

3 | RESULTS

3.1 | Pilot study using the initial BCS and photographs

All pairwise comparisons of scores recorded by the same observer for the same photograph of a mountain chicken frog at different times (intra-observer agreement) were substantial to almost perfect ($0.60 < \kappa \leq 1.00$). The majority (92.9%) of pairwise comparisons of scores recorded by different observers of the same photograph of a mountain chicken frog at the same time (inter-observer agreement) were fair to substantial ($0.20 < \kappa \leq 0.80$) and 7.1% showed slight agreement ($0.00 \leq \kappa \leq 0.20$).

3.2 | Live animal study using the final BCS

Four of the scores assigned to mountain chicken frogs at Zoo D were excluded from analysis (one by Observer 3 and three by Observer 4) as half scores, either 2.5 or 3.5, had been assigned. One frog at Zoo C was not handled to obtain snout-vent length and bodyweight measurements as it had a pre-existing leg fracture.

The distribution of mean body condition scores assigned using the BCS to the captive population of mountain chicken frogs aged between 8 months and 12 years in the UK and Jersey just prior to the breeding season in 2016 is shown in Figure 3. The majority (88%) of captive mountain chicken frogs had a mean body condition score between 3 and 5, with only 12% of individuals having a mean body condition score <3.

κ values for each pairwise comparison of the scores assigned to live mountain chicken frogs by the same observer (intra-observer agreement) and different observers (inter-observer agreement) are shown in Tables 1 and Table 2,. Intra-observer agreement was slight to substantial ($0.00 \leq \kappa \leq 0.80$) for 93.8% of pairwise comparisons, while 6.25% of pairwise comparisons showed poor agreement ($\kappa < 0.00$). Inter-observer agreement was slight to almost perfect ($0.00 \leq \kappa \leq 1.00$) for 97.2% of pairwise comparisons, with 2.78% showing poor agreement ($\kappa < 0.00$). Most scores assigned by two different observers to the same frog on the same day were within 1 score of each other, with only 4.22% of scores assigned being >1 score apart (Figure 4).

When the authors removed the less experienced observers' scores from the dataset (Observer 5 at zoo C, and 3, 4, and 5 at zoos D and E), intra- and inter-observer agreement improved such that 100% of κ values for pairwise comparisons of scores recorded by the same observer were slight to substantial ($0.20 < \kappa \leq 0.8$) and 100% of pairwise comparisons of scores recorded by different observers were slight to almost perfect ($0.20 < \kappa \leq 1.00$). The proportion of scores within one score of each other assigned by two different observers to the same frog on the same day also improved, with only 0.70% of scores assigned being >1 score apart.

The effect of age of the frogs on agreement between scores was evaluated at Zoo B as this was the only zoo which housed both juvenile (individuals aged less than 3 years) and adult (individuals aged greater than 3 years) frogs. For the 17 juveniles, there was fair to moderate

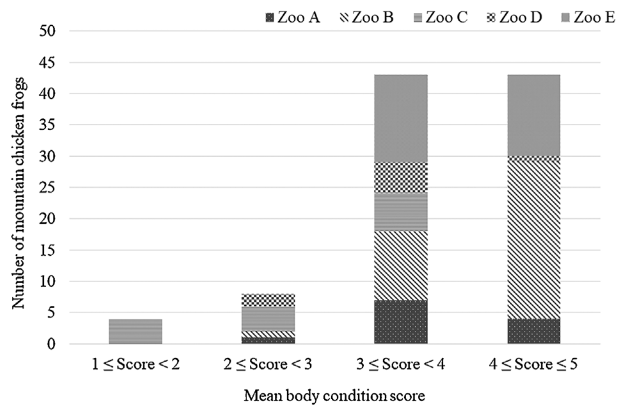


FIGURE 3 Distribution of mean body condition scores assigned to the captive mountain chicken frog population aged between 8 months and 12 years in the UK and Jersey in February and March 2016 using the body condition score (BCS) in Figure 2

(0.20 < κ ≤ 0.60) inter-observer agreement for 50% of pairwise comparisons and no to slight (0.00 ≥ κ ≤ 0.20) inter-observer agreement for 50% of pairwise comparisons. Inter-observer agreement was far higher for the 20 adults, with moderate to substantial agreement (0.40 < κ ≤ 0.80) for 91.7% of pairwise comparisons and fair agreement (0.20 < κ ≤ 0.40) for 8.33% of pairwise comparisons. Intra-observer agreement showed a similar pattern, with no to moderate agreement (0.00 ≥ κ ≤ 0.60) for juveniles, versus moderate to almost perfect agreement (0.40 < κ ≤ 1.00) for adults. For both juveniles and adults, all scores assigned by two different observers to the same frog on the same day were within one score of each other.

In the whole study population, mean body condition score showed moderate (rho = 0.54; males) to strong (rho = 0.6; females) positive correlation with scaled mass index. Mean body condition score was more strongly correlated with scaled mass index in juveniles than adults in both sexes (rho in males = 0.67 (strong) in juveniles, 0.44 (moderate) in adults; rho in females = 0.91 (very strong) in juveniles, 0.55 (moderate) in adults).

TABLE 1 κ values to show intra-observer agreement when the body condition score (BCS) in Figure 2 was used to assign body condition scores to the captive mountain chicken frog population aged between 8 months and 12 years housed at four zoos in the UK and Jersey in February and March 2016

	Observer				
Zoo	1	2	3	4	5
B	0.70	0.48	0.32	0.57	
C	0.77	0.50	0.52		
D	0.42	0.20	−0.25	0.09	
E	0.51	0.29	0.04	0.16	0.39

κ < 0.00 represents poor agreement, 0.00 ≤ κ ≤ 0.20 slight agreement, 0.20 < κ ≤ 0.40 fair agreement, 0.40 < κ ≤ 0.60 moderate agreement, 0.60 < κ ≤ 0.80 substantial agreement, and 0.80 < κ ≤ 1.00 almost perfect agreement (Landis & Koch, 1977). Observer 1 was the same person at all zoos. Observers 2–5 were different people at each zoo. Observers 3 and 4 at zoo D and 3, 4, and 5 at zoo E were less experienced working with mountain chicken frogs and included two work experience students, a probationary keeper, a trainee keeper and a conservation researcher. All other observers were full time veterinarians, veterinary nurses, keepers, and animal management staff.

4 | DISCUSSION

In this study a body condition score (BCS) was developed for the mountain chicken frog (*Leptodactylus fallax*) and assessed for intra- and inter-observer variability, as well as correlation with an objective measure of body condition (scaled mass index), in February and March 2016 just prior to the beginning of the breeding season. To the author's knowledge this is the first BCS to be developed and assessed for any amphibian species.

Historically, herpetologists have determined body condition in amphibians using body condition indices based on morphometric measurements, typically snout-vent length, and bodyweight (Băncilă, Hartel, Plăiaşu, Smets, & Cogălniceanu, 2010; Bell, Carver, Mitchell, & Pledger, 2004; Denoël, Hervant, Schabetsberger, & Joly, 2002; Gendron et al., 2003; Leary, Jessop, Garcia, & Knappa, 2004; MacCraken & Stebbings, 2012; Pope & Matthews, 2002; Yahnke, Grue, Hayes, & Troiano, 2012). Fulton's index (based on the formula: $k = \frac{M}{L^3}$ where k = Fulton's factor, M= body mass, and L = length) has been used in leopard frogs (*Rana pipiens*) to assess the effect of an agricultural pesticide on lungworm infection (Gendron et al., 2003; Peig & Green, 2010). Relative condition, relative mass and the residual index all use ordinary least squares regression of the linearized power equation relating mass and length and one or more of these methods have been used in the mountain yellow-legged frog (*Rana muscosa*), paedomorphic, and metamorphic Alpine newts (*Triturus alpestris*), and the yellow-bellied toad (*Bombina variegata*) (Băncilă et al., 2010; Denoël et al., 2002; Pope & Matthews, 2002). More recently, Peig and Green (2010) favored the scaled mass index over other indices as it is not affected by the change in relationship between mass and snout-vent length as growth occurs. In one study, MacCracken and Stebbings (2012) validated the scaled mass index in larval and juvenile bullfrogs (*Lithobates catesbeianus*) and rough-skinned newts (*Taricha granulosa*) by correlation with scaled fat mass and scaled lean mass values obtained via carcass analysis. A key advantage of using the BCS of this study over body condition indices based on morphometric measurements is that manual restraint is not required. There have been anecdotal reports of reduced food intake and reduced weight gain following manual restraint for husbandry and veterinary procedures in captive mountain chicken frogs and the frequency of handling has been suggested as a potential factor that may be affecting breeding success within the captive population (Harding, Michaels, & Tapley pers. obs.). Handling has been associated with increased corticosterone levels in a number of anurans and handling-related stress for 5, 15, or 30 min was associated with decreased testosterone excretion in non-breeding male cane toads (*Rhinella marina*) (Narayan, Hero, & Cockrem, 2012; Narayan, Molinia, Christi, Morley, & Cockrem, 2010). Using the BCS developed in this study to monitor body condition of the captive mountain chicken frog population during the breeding season

TABLE 2 κ values to show inter-observer agreement when the body condition score (BCS) in Figure 2 was used to assign body condition scores to the captive mountain chicken frog population aged between 8 months and 12 years housed at five zoos in the UK and Jersey in February and March 2016

		Observer				
Zoo	Observer	2	3	4	5	6
A	1	0.17	0.39	0.80		
	2		0.17	0.17		
	3			0.39		
B	1	0.41, 0.69	0.36, 0.57	0.36, 0.44		
	2		0.50, 0.53	0.31, 0.34		
	3			0.44, 0.34		
C	1	0.93, 0.76	0.35, 0.5	0.54	0.34	
	2		0.31, 0.57	0.61	0.65	
	3			0.45	0.49	
	4				0.69	
D	1	0.20, 0.56	0.38, 0.40	0.59, 0.40	0.55	0.38
	2		0.20, 0.25	0.53, 0.75	-0.11	0.20
	3			0.70, 0.50	0.62	0.47
	4				0.17	0.30
	5					1.00
E	1	0.44, 0.63	0.17, 0.15	0.26, 0.01	0.35, 0.27	
	2		0.19, 0.10	0.14, −0.01	0.53, 0.13	
	3			0.06, 0.06	0.09, 0.03	
	4				0.09, 0.03	

κ < 0.00 represents poor agreement, 0.00 ≤ κ ≤ 0.20 slight agreement, 0.20 < κ ≤ 0.40 fair agreement, 0.40 < κ ≤ 0.60 moderate agreement, 0.60 < κ ≤ 0.80 substantial agreement and 0.80 < κ ≤ 1.00 almost perfect agreement (Landis & Koch, 1977). Where two scores were recorded by an observer 24–48 hr apart, κ values for pairwise comparisons between observers' scores on each day are shown as x, y where x is at 0 hr and y at 24–48 hr. Observer 1 was the same person at all zoos. Observers 2–5 were different people at each zoo. Observer 5 at zoo C, and 3, 4, and 5 at zoos D and E were less experienced working with mountain chicken frogs and included three work experience students, a probationary keeper, a trainee keeper, a seasonal keeper, and a conservation researcher. All other observers were full time veterinarians, veterinary nurses, keepers, and animal management staff.

instead of manual restraint for snout-vent length and weight measurements may reduce the effects of handling-related stress during this critical time.

The authors developed an overview format of visual BCS in this study, in which observers assign a score based on overall appearance, in contrast to a composite BCS in which observers score individual body regions and calculate a sum or an algorithm BCS in which observers follow a flow chart to assign a score (Schiffmann et al., 2017). The authors preferred the overview format in this case due to its practicality and simplicity (Schiffmann et al., 2017). When developing a BCS, researchers typically select anatomical features based on appraisal of photographs of the species, advice from experienced keepers, nutritionists or veterinary medical staff, and adaptation of previously published body condition scores in the same or closely related species if available (Audigé, Wilson, & Morris, 1998; Cook et al., 2001; Ezenwa, Jolles, & O'Brien, 2009; Franzmann, 1977; German et al., 2006; Morfeld, Lehnhardt, Alligood, Bolling, & Brown, 2014; Pettis et al., 2004; Reppert, Treiber, & Ward, 2011; Rudman & Keiper, 1991; Schiffmann et al., 2017; van der Jeugd & Prins, 2000; Wemmer et al., 2006). As a BCS had not been described in similar species, the authors selected anatomical landmarks based on the experience of keepers and veterinary staff and appraisal of photographs of the species. Intra-coelomic fat bodies are well described as a fat storage site in anurans (Pond, 1978); however, there is little published literature regarding the relative importance of externally visible fat storage sites in anurans and how they vary at different grades of condition. Amphibians lack subcutaneous fat; however fat storage has been demonstrated in the somatic musculature (Pond, 1978). The variation in anatomical sites selected for the BCS in this study with body condition likely reflect changes in energy reserves in skeletal musculature (Pond, 1978). The authors selected anatomical features that were considered to not be directly affected by other variables (e.g. gender, sex-related seasonal changes, age, and posture). For example, male mountain chicken frogs, like males of some other species of the family Leptodactylidae, develop forelimb muscular hypertrophy in the breeding season (Tapley, Acosta-Galvis, & Lopez, 2011), therefore the authors did not use forelimb size as a site in the BCS. The BCS may therefore be advantageous over body condition indices based on mass and length as these two measures may be influenced by factors other than energy reserves such as age, sex, developmental stage, hydration

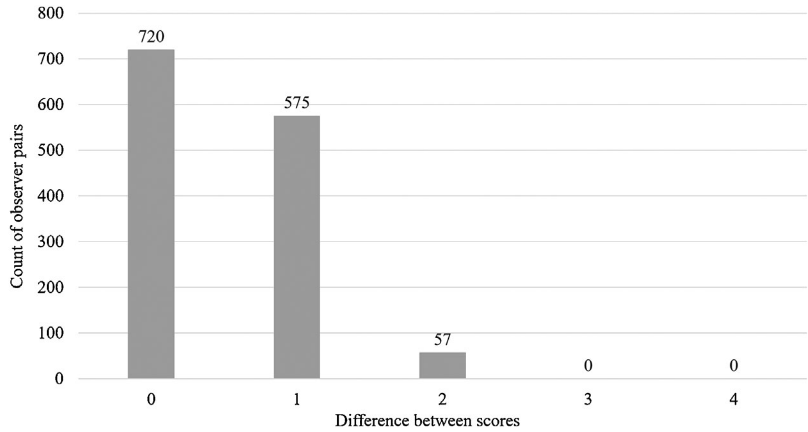


FIGURE 4 Distribution of diffrerences between scores across all pairs of observers per mountain chicken frog per scoring session when the body condition score (BCS) in Figure 2 was used to assign body condition scores to the captive mountain chicken frog population aged between 8 months and 12 years housed at five zoos in the UK and Jersey in February and March 2016

status, gut fill, structural deformities, and reproductive status (MacCracken & Stebbings, 2012). However, scores assigned using the BCS would likely still be affected by major structural deformities of the key anatomical landmarks, for example, caused by trauma or metabolic bone disease, although the latter is no longer a common problem in the captive population as mountain chicken frogs are routinely fed a variety of invertebrates supplemented with a high-calcium multivitamin and mineral supplement containing vitamin D_3 and provided with appropriate levels of UV-B radiation (Tapley et al., 2014).

Evaluation of body condition scores for intra- and inter-observer variability is important given the subjective nature of these systems, resulting in variability in scores assigned by independent observers (Clancey & Byers, 2014). The BCS of this study was assessed for intra- and inter-observer agreement in a similar way to BCS assessment in other species, with multiple observers assigning condition scores at least twice and a length of time apart which would be unlikely to result in a change in condition (Burton et al., 2014; Clingerman & Summers, 2005; Kristensen et al., 2006; Morfeld et al., 2014). In a previous study evaluating the effect of training on intra- and inter-observer agreement between body condition scores assigned to Holstein dairy cattle by practicing dairy veterinarians, the authors showed that veterinarians who had received a 2 hr theoretical lecture regarding use of a BCS prior to its use had slight to substantial inter-observer agreement ($\kappa = 0.17$–0.78) (Kristensen et al., 2006). Agreement improved to moderate to substantial after a further 2.5 hr practical training session ($\kappa = 0.41$–0.82) and intra-observer agreement between the first and second scoring sessions was fair to substantial ($\kappa = 0.22$–0.75). Far higher agreement was achieved when highly trained instructors who had worked closely together in a formal network over at least 3 years used the same BCS ($\kappa \geq 0.86$) (Kristensen et al., 2006). Observer experience also appears important in other species, for example, Pettis et al. (2004) found almost perfect inter-observer agreement ($\kappa = 0.86$–0.87) when a 3-point visual BCS was applied by three experienced right whale biologists, one of whom developed the BCS, to photographs of North Atlantic right whales, and Morfeld et al. (2014) demonstrated stronger inter-observer agreement between two observers who developed a five-point visual BCS for female African elephants than between these observers and an observer with no prior experience of using a BCS ($\kappa = 0.89$ compared to 0.62–0.67). Given that no specific theoretical or practical training in use of the BCS was provided in this study but observers had the opportunity to view the BCS in advance, the authors predicted that intra- and inter-observer agreement for veterinarians, veterinary nurses, animal managers, and keepers working with the species would be slight to substantial, as Kristensen et al. (2006) observed with practicing dairy veterinarians scoring Holstein dairy cattle after a 2 hr theoretical lecture. As some of the observers in this study had no or minimal experience working with mountain chicken frogs, the authors expected intra- and inter-observer agreement to be lower than that observed by Kristensen et al. (2006) for practicing veterinarians working with dairy cattle (i.e. poor to slight). Slight to substantial agreement was achieved in 93.8% of intra-observer pairwise comparisons and slight to almost perfect agreement in 97.2% of inter-observer pairwise comparisons in this study. When the authors removed individuals with no or minimal prior experience working with mountain chicken frogs (work experience students, a conservation researcher, a probationary keeper, and a seasonal keeper) agreement increased, such that 100% of pairwise comparisons showed slight to substantial intra-observer agreement and 100% of pairwise comparisons showed slight to almost perfect inter-observer agreement. When using a BCS in other species, agreement between scores can be improved with training and experience and it has become a useful component of routine clinical examination to estimate body energy reserves in many species (Bewley & Schutz, 2008; Clancey & Byers, 2014; Clements & Sanchez, 2015; Houston & Radostits, 2000, Kristensen et al., 2006). With further training in use of the mountain chicken frog BCS, the authors expect that agreement between scores assigned at zoos with less experienced staff would improve over time.

The second stage of assessment is correlation with an objective measure of total energy reserves. The gold standard objective measure is carcass analysis (Gerhart, White, Cameron, & Russell, 1996). However, the authors did not consider euthanasia for carcass analysis following condition scoring appropriate for the mountain chicken frog given the threatened status of the species and the importance of animals involved in this study for maintaining the captive population (Adams et al., 2014). Researchers may use other indirect objective measures such as dual-energy X-ray absorptiometry (DEXA), deuterium oxide (D_2O), dilution, and quantitative magnetic resonance imaging (QMR) in species for which these techniques have been validated; however, none of these methods have been validated in the mountain chicken frog or any amphibian species (Laflamme, 1997; Nixon et al., 2010; Rudolph, Stahly, & Cromwell, 1988). An alternative option used in certain mammalian species is measurement of subcutaneous fat by ultrasonography; however, anurans do not typically store fat subcutaneously (Pond, 1978; Schiffmann et al., 2017). Measurement of intra-coelomic fat bodies by ultrasonography could be considered; however, they can be difficult to differentiate on coelomic ultrasonography (Pond, 1978; Schildger, 2001). Therefore, the authors considered correlation with a body condition index based on morphometric measurements which had been validated in other amphibian species to be the most appropriate method to validate the condition score in this study. The authors used the scaled mass index as it is not affected by changes in snout-vent length and bodyweight as growth occurs (MacCracken & Stebbings, 2012; Peig & Green, 2009, 2010). In future studies, the authors recommend validation of both the BCS developed in this study and the scaled mass index by analysis of carcass energy reserves following use of the BCS and measurement of scaled mass index as carcasses become available.

The effect of age on agreement between scores was evaluated at Zoo B which housed 17 juvenile and 20 adult frogs. There was no to moderate intra- and inter-observer agreement for juvenile frogs, whereas there was moderate to almost perfect intra-observer agreement and fair to substantial inter-observer agreement for adults. Clingerman and Summers (2012) also found inter-observer agreement was poorer in juveniles than adults when a BCS was used in rhesus macaques, likely due to there being less muscle mass and fat reserves in normal juveniles, and juveniles may be experiencing growth and changes in body stature. Despite the apparent poorer agreement between scores assigned to juvenile frogs at Zoo B, all of the scores assigned to juvenile frogs by different observers on the same day at Zoo B were no greater than one score apart and correlation with the scaled mass index was strong to very strong in the total study population of juvenile frogs. Further evaluation of the BCS in juvenile frogs is recommended to determine its utility in this age group given the small number of juvenile frogs within this study population.

The authors assessed the BCS in this study at one time-point of the year, just prior to the breeding season. Previous studies with other species such as the Magellanic penguin (*Spheniscus magellanicus*) have demonstrated variation in BCS with season (Clements & Sanchez, 2015). Many anuran species, including the mountain chicken frog, expend a great deal of energy by engaging in reproductive behavior due to egg production, and parental care in females and vocalization and agonistic behavior in males (Fitzpatrick, 1976; Gibson & Buley, 2004; Mizell, 1965; Morton, 1981; Seymour, 1973; Smith, 1950). These changes will likely change the distribution of body condition scores within a population. Therefore the authors recommend that the mountain chicken frog BCS is evaluated with data collected at different times of the year, for example at the end of the breeding season once energy resources are most depleted.

The BCS of this study represents the range of body condition observed in the captive mountain chicken frog population from 1 (lowest condition) to 5 (highest condition). In mammals and birds, the lowest body condition score of a BCS is typically described as emaciated, the highest as obese, and the middle score considered normal or ideal (Bewley & Schutz, 2008; Clements & Sanchez, 2015; Laflamme, 1997). However, the ideal score depends on the context, for example in dairy cattle the ideal body condition score depends on the stage of lactation and the production system (Bewley & Schutz, 2008). Descriptive terms such as emaciated, normal, and obese were not assigned to the numerical scores in this study as insufficient information is known about which grade of condition constitutes normal. Obesity has been described in certain species of frog, such as the White's tree frog (*Pelodryas caerulea*), and is characterized by abdominal distension due to fat deposition in the coelomic fat bodies and enlargement of the supraocular skin folds in this species, which may impair vision (Wright & Whitaker, 2001). However clinical obesity, defined as fat accumulation which may impair health, has not been reported to date in captive mountain chicken frogs (Lopez, pers. obs.). In contrast, thin to emaciated body condition is commonly observed at post mortem examination (Barbon, Flach & Lopez, pers. obs). The majority (88%) of the mountain chicken frogs in the captive population in this study had a mean body condition score of 3–5 just prior to the breeding season, which likely represents normal condition, while 12% had a mean score of <3 which is likely abnormal, with two representing thin and one emaciated body condition. The authors recommend correlation between body condition scores assigned using the BCS and health parameters, as well as other parameters important to conservation breeding programs, such as reproductive output, longevity and post-release survival, to determine the ideal body condition of this species in different contexts (Bewley & Schutz, 2008; Pettis et al., 2004).

5 | CONCLUSIONS

1) A body condition score (BCS) has been developed for the mountain chicken frog (*Leptodactylus fallax*).
2) The BCS has been evaluated in February-March just prior to the beginning of the breeding season. Agreement between scores

assigned by the same and different observers is expected to be slight to substantial if observers have received no formal training with the BCS but have experience working with mountain chicken frogs. Agreement between scores may be poor if observers have received no formal training with the BCS and have no or minimal experience working with mountain chicken frogs.

3) Further assessment of the BCS is recommended for use in juveniles and in adults at other times of year, for example, at the end of the breeding season when energy supplies are likely to be most depleted.

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