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## Short Notes

# Sexual dimorphism in the cane toad *Bufo marinus*: a quantitative comparison of visual inspection methods for sexing individuals

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A study was conducted to determine whether simple morphological characteristics could be used to rapidly determine the sex of cane toads. We found that four characteristics reliably allowed rapid assessment of sex: skin texture on the dorsal surface, skin colour on the dorsal surface, the presence of a creamy-coloured stripe along the dorsal margin and the presence of vocal sac openings. These criteria were tested by an assessment of use by both experienced and novice operators and were shown to be reliable for sexing cane toads that were large enough to assess morphological characteristics reliably (individuals with a snout–vent length exceeding 50 mm). Of the four techniques, the presence of vocal sac openings proved to be the most reliable. Such techniques may be used for a number of purposes, and are particularly useful during conservation projects that attempt to eradicate or reduce the effects of this invasive alien species on local ecosystems.

*Key words:* Fiji Islands, morphological characteristics, sexing, vocal sac

The Fiji Islands (18°00'S, 175°00'E) have been identified as a “hotspot” in the Pacific region because of their high biological diversity and rates of endemism (Ryan, 2000). However, a number of invasive alien species, including the cane toad *Bufo marinus* (also named *Chaunus marinus*; Frost et al., 2006; Smith & Chiszar, 2006), are considered to be a major threat to this unique native fauna. The cane toad is a prolific breeder and since its intentional introduction in 1936 (in an unsuccessful attempt to control insect pests in cane fields) it has become a major pest and is thought to compete with endemics such as the Fiji ground frog (*Platymantis vitianus*). Attempts to eradicate cane toads are now being made in order to protect such unique species (Morley et al., 2004). Eradication

plans propose various methods such as the use of chemicals, traps and drift-net fences to remove individuals from a selected site. As part of this labour-intensive process it is essential that appropriate census data are available with respect to the numbers of males, non-gravid and gravid females to provide information on the success of the operation. Further, cane toad eradication protocols also include a focus on the removal of gravid females and adult males to initiate a rapid population decline. The presence of even a few gravid females after eradication attempts will result in failure due to their reproductive capacity (Cameron, 2002).

This study was done to develop rapid sexing criteria for use in cane toad eradication projects within the Fiji Islands but has general applicability. In view of the common use of cane toads as a model amphibian species for the elucidation of various biological concepts, and to help develop ecologically sustainable and effective eradication projects for the species, we felt the need to develop a rapid sexing technique to help the inexperienced in the collection of cane toads during field sampling. Zug & Zug (1979) demonstrated that visual characteristics, primarily skin pattern and coloration, could be used to sex cane toads. Similarly, Lee (2001) demonstrated strong sexual dimorphism in the musculoskeletal system of the forelimbs (from a single breeding congregation). However, no quantitative assessments of the reliability of these methods were done in either study. The present study quantitatively assesses methods for rapidly sexing cane toads.

Field work for our study was undertaken in March and April 2006 at a breeding pond on the University of the South Pacific (USP) campus, Suva. During the field study both juvenile and adult cane toads were found *in situ*, indicating that breeding was occurring.

The sampling of cane toads was done in two phases. During phase one, ten cane toads sufficiently large to reliably assess morphological characteristics, with a snout–vent length exceeding 50 mm (Zug & Zug, 1979), were collected from the edge of the breeding pond at dusk following the method of Lauron et al. (2003). The following morphological measurements were then recorded: body weight, g; snout–vent length, mm; skin texture on the dorsal surface; skin texture on the ventral surface; creamy stripe along dorsal margin; skin coloration on the dorsal surface; skin coloration on the ventral surface; texture and coloration of thumb pads; robustness of musculoskeletal system of the forelimbs; abdominal spreading; presence of vocal sac openings. To definitively confirm the sex, dissection of each individual was done. Dissection was carried out humanely under laboratory conditions using a double-pithing technique adopted from Costanzo et al. (1998). Three individuals were male and seven female. A subjective assessment of the recorded characteristics was then done.

The results here contradicted Lee (2001) since neither the thumb pad character nor visual assessment of the robustness of the musculoskeletal system of the forelimbs

**Table 1.** The characteristic morphological features of skin texture on dorsal surface (STDS), skin coloration on dorsal surface (SCDS), vocal sac (VS) and creamy stripe on dorsal margin (CS) used in the rapid sexing criteria for cane toads.

Morphological feature	Male	Female
STDS	An evenly distributed pattern of small round warts.	Presence of larger and unevenly distributed warts on dorsal surface and two rows of evenly sized round warts present along the dorsal margin.
SCDS	Yellowish-green dorsal surface with brown warts.	Distinctive patches of brown mottled patterns with a dark green coloration.
CS	No creamy stripe observed along the dorsal margin.	Distinctive creamy stripe along the dorsal margin from head to vent region.
VS	Pair of vocal sac openings present on both sides of the floor of the buccal cavity of males. Calling may also be heard during handling. To locate a vocal sac opening, gently open the buccal cavity (mouth) of the cane toad wide and lift the tongue outward using a sterilized forcep. A vocal sac opening is a thin cut of the inner membrane on the floor of the lower jaw, at the junction of the upper jaw and the lower jaw.	No vocal sac present.

was able to separate the sexes of the cane toads that we studied. It was evident that coloration of thumb pads, abdominal spreading and ventral surface characters did not provide useful measurements as no distinction could be made between male and female individuals; hence these characters were discarded and four morphological features retained: skin texture on the dorsal surface, STDS; skin colour on the dorsal surface, SCDS; vocal sac openings, VS; and presence of creamy stripe on dorsal margin, CS. Clearly defined criteria were formulated for these characteristics, gained from experience during this phase of the study (Table 1).

During phase two of the study, these four characteristics were used to sex individuals. First, 50 individuals collected from the same breeding pond were sexed by two “expert” individuals who conducted this study. This was done by evaluating the sex of each individual by the four methods followed by definitive sexing by dissection. The accuracy of each of the four methods compared to the actual sex was assessed using Kappa analysis (Chuang, 2001), which provides a measure of the degree to which

two judges using different techniques (i.e. a rapid method versus definitive sexing by dissection) concur in their respective sortings of individuals into two mutually exclusive categories. Hence, the reliability of STDS, SCDS, VS and CS were assessed by calculating a *K* value (which varies from 0 to 1, 1 being perfect correspondence) and described using the agreement chart adopted by Landis & Koch (1977).

Each of the four morphological characteristics was useful for sexing purposes (Table 2). However, STDS, SCDS and CS were not as accurate as VS. Since VS showed perfect correspondence to sexing by dissection, it may be considered to be the most useful characteristic, although it was slightly more time consuming than the other methods tested.

Second, in order to test the suitability of these methods for field sampling, the techniques were tested by “novice” surveyors. In this case, another sample ( $n=30$ ) was assessed, drawn from the cane toad population of Viwa Island (a 60 ha island off the east coast of the main island of Viti Levu). Sexing was done by local field work-

**Table 2.** Number of cane toads identified as male or female by the four morphological features (STDS, SCDS, VS and CS) and corresponding Kappa statistics. Sexing by dissection recorded 15 males and 35 females.

Character	No. of individuals sexed as male	No. of individuals sexed as female	No. of individuals incorrectly sexed as male	No. of individuals incorrectly sexed as female	Kappa value ( <i>K</i> )	Strength of agreement
STDS	12	35	0	3	0.8	Substantial
SCDS	12	35	0	3	0.8	Substantial
CS	12	35	0	3	0.8	Substantial
VS	15	35	0	0	1.0	Perfect

ers from the Viwa village community, who were trained immediately prior to the experiment. In this case, all four methods achieved 100% accuracy. Hence, the techniques appear to be robust and applicable to sub-populations within the Fiji Islands.

In conclusion, this study demonstrates that morphological characteristics may be used to rapidly sex cane toads. Whilst the vocal sac opening criterion was the most reliable, it is also more time consuming than the other three methods. Hence, the combined use of SCDS, STDS and CS may also be used to aid in the sexing process, which would have the benefit of rapidity.

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