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Sexual Dimorphism and Color Polymorphism in the Wallum Sedge Frog (*Litoria olongburensis*)

Sexual dimorphism describes intraspecific differences in size, shape or other traits between males and females. One of the most common secondary sexual dimorphisms of anuran amphibians is a disparity in size, with adult females commonly exceeding adult males in body size (Howard 1981; Lee 2001; McGarrity and Johnson 2009; Monnet and Cherry 2002). Fecundity selection is proposed to be the driving force behind female-biased sexual size dimorphism (SSD; Darwin 1871), as clutch size and/or egg and subsequently offspring size increase with increasing female body size (Kupfer 2007; Shine 1979; Wells 2007). Selection may not only favor larger females, but also small males, as demonstrated in *Litoria xanthomera* and *L. chloris*, where smaller males used less energy when calling, had increased chorus tenure, and were more successful than larger males (Morrison et al. 2001). Sexual differences in growth, maturation, population age structure, and life history have also been suggested as possible driving forces behind SSD in some species (Howard 1981; Monnet and Cherry 2002; Shine 1979).

Many amphibians exhibit sexual differences in morphology or coloration that allow males and females to be readily distinguished (Hoffman and Blouin 2000; Wells 2007). An example of a secondary sexual characteristic of anurans is the presence of male vocal sacs, used to create advertisement calls to females during breeding events (Ryan 1991). Additionally, males of many anuran species develop nuptial pads, which are predominantly located on the inner surface of the thumb and are used for gripping a female during amplexus, and during male-male competition (Duellman and Trueb 1994). Nuptial pads are generally more developed in aquatic-breeding species (where males need to maintain a strong grip on females in rapidly flowing waters), and in species that engage in aggressive male-male encounters (Duellman and Trueb 1994). As nuptial pads are usually densely pigmented with melanin, and thus easily observed, they are commonly used to determine the sex of amphibians in the field (Duellman and Trueb 1994; Greene and Funk 2009). Additionally, some species display intersexual differences in forelimb size, allowing males to retain a firm grip on the female in amplexus (Lee 2001). Occasionally, males and females possess obvious differences in body coloration, which also facilitates sex identification (Hoffman and Blouin 2000). However, for some species, there are no obvious size or color differences and no nuptial pads, making identification of sex difficult.

The Wallum Sedge Frog, *Litoria olongburensis*, is a small (~25 mm) hylid restricted to coastal swamps of eastern Australia (Barker et al. 1995; Cogger 1995; Ingram and Corben 1975). The species is listed as vulnerable under the Commonwealth *Environmental Protection and Biodiversity Conservation Act 1999*, Queensland *Nature Conservation Act 1992*, New South

Wales *Threatened Species Conservation Act 1995*, and by the International Union for the Conservation of Nature (IUCN 2010). *Litoria olongburensis* is known to be polymorphic for dorsal coloration, including gray-brown, beige or bright green, occasionally with dark flecking (Barker et al. 1995; Liem and Ingram 1977) (Fig. 1). However, little is known of the color and morphological differences between the sexes and field sex identification in this species can be difficult, predominantly due to their small size. Identification of sexes is essential to determine different habitat use, population sex ratios, and for captive husbandry. We examined sexually dimorphic characteristics of museum specimens and wild populations of *L. olongburensis* to expand our knowledge on the morphology of the species and sex identification in the field. We hypothesize that females will have a larger body length than males, and males will have proportionately larger forearms. Also, we hypothesize that males possess secondary sexual characteristics useful for field identification all year round, such as nuptial pads, vocal sacs, and different coloration relative to females. The results from this study may also assist with our understanding of sexual dimorphism and color polymorphism in congeneric species.



FIG. 1. Brown (A) and green (B) color morphs of *Litoria olongburensis*.

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Materials and Methods.—Male (N = 40) and female (N = 62) *Litoria olongburensis* held in the Queensland Museum (QM) and originally collected from throughout their distribution in Queensland and New South Wales (NSW), were examined. Snout–urostyle length (SUL), right tibia length (TL), and forearm length (FL) were measured with vernier calipers to the nearest 0.1 mm. Frogs were sexed by inspection of nuptial pads, vocal sac slits under the tongue, and gonads. The numbers of eggs of four preserved females were counted and the mean egg diameter (MED) of ten eggs per female was measured with digital vernier calipers. This was recorded to fill a gap in our knowledge of fecundity in this species and may be used in future comparisons with congeners. Eggs of some females had been previously removed and kept aside, and others still remained within the female's abdomen. Thus, egg numbers may be underestimated due to possible loss of eggs during preservation. As color is greatly affected by preservation, no dorsal color analysis was undertaken on museum specimens.

Measurements of male (N = 94) and female (N = 82) *L. olongburensis* in wild populations were recorded in Queensland: Great Sandy National Park (Cooloola section; N = 73), Beerburrum Scientific Area 1 and Mooloolah River NP (N = 53); and from NSW: Yuraygir NP (N = 33) and Tyagarah Nature Reserve (N = 37). These locations represent the presumed current range of the species and were utilized as four latitudes in analyses (Fig. 2). The four

latitudes used in decimal degrees were 26.01, 26.78, 28.61, and 29.85 (datum: WGS 84). Frogs were measured in March, April, and November, 2010. Up to 20 frogs first observed at each site were caught and the SUL was measured as described above. The presence of nuptial pads, vocal sacs, and skin folds, as well as coloration of the dorsum, thigh, and throat, was recorded. Frogs were categorized as green or brown based on the dominant dorsal color as observed by KL. A third category of partial green included any frogs that had approximately equal proportions of green and brown dorsal coloration. Males were identified by the presence of nuptial pads (where discernible), vocal sacs, and/or folds in the throat skin. Females were identified by the presence of thick, granular throat skin (opaque in males), and a lack of nuptial pads. Additionally, any metamorphs observed were measured to ascertain size at metamorphosis. Frogs were captured using small plastic bags and were released following measurements. Bags were not re-used, and aquatic equipment was treated with Virkon (fungicide) between sites to prevent disease transmission.

For museum frogs, linear regression analyses were used to analyze the relationships among SUL, TL, and FL. For both museum and wild frogs we developed a Generalized Linear Model, using a Gaussian family of SUL, with the explanatory variables sex and origin (wild or museum) as fixed factors. For wild frogs, we again used Generalized Linear Modelling, using a Gaussian family. The response variable was SUL, with the explanatory variable sex as a fixed factor, and latitude as a covariate. We also examined data to detect whether a latitudinal trend existed with SUL or SSD. Data were analyzed using PASW Statistics 18, Release Version 18.0.0 (SPSS, Inc., 2009, Chicago, Illinois, www.spss.com) and the statistical significance was set at $\alpha = 0.05$.

Results.—Both museum and wild (all latitudes) frogs displayed female biased SSD. Snout–urostyle length for each sex overlapped up to 28.7 mm, with all individuals greater than 28.7 mm being female. Female SUL (range = 20.0–34.1 mm, mean = 26.7, N = 144), was significantly larger than males (range = 19.3–28.7 mm, mean = 24.2 mm, N = 134; Wald $\chi^2 = 78.98$, $P < 0.001$, Fig. 3). Origin (wild or museum) had a significant effect on SUL (Wald $\chi^2 = 14.94$, $P < 0.001$). There was no significant interaction between origin and sex (Wald $\chi^2 = 0.25$, $P = 0.614$; Fig. 3). Female TL (range = 11.2–18.4 mm, mean = 14.8) was also found to be significantly larger than male TL (range = 7.2–16.0, mean = 12.7; $P < 0.001$); as was female FL (range = 3.9–6.8 mm, mean = 5.1) compared to male FL (range = 2.4–5.8 mm, mean = 4.6; $P < 0.01$). Snout–urostyle length and TL of museum frogs were highly correlated ($P < 0.001$, $r^2 = 0.797$), as were SUL and FL ($P < 0.001$, $r^2 = 0.641$). Additionally, male and female FL and TL relative to SUL did not differ significantly ($P = 0.466$, $P = 0.382$, respectively). Thus, only SUL was recorded for frogs measured in the wild. For wild measured frogs, SUL was significantly affected by sex (Wald $\chi^2 = 4.26$, $P = 0.039$) and latitude (Wald $\chi^2 = 15.98$, $P < 0.001$). There was no significant interaction between sex and latitude (Wald $\chi^2 = 2.85$, $P = 0.091$; Fig. 4). Although latitude had a significant effect, there did not appear to be a latitudinal cline in SUL or SSD.

The smallest male with discernible nuptial pads on the inner side of the thumb had a SUL of 20.4 mm, and most males (93.2%) larger than this possessed nuptial pads (Fig. 5). Nuptial pad pigmentation was greater in the breeding periods, and occasionally indiscernible outside these periods. The average number of eggs from preserved females was 121 (range 75–160), and average MED was 1.03 mm (range 0.9–1.2 mm). The smallest size at metamorphosis recorded in the wild was 13.8 mm.



FIG. 2. Occurrence of *Litoria olongburensis* records along the east coast of Australia and the four latitudinal collection locations. Map of Australia shows enlarged area. Records sourced from the Australian Museum, Queensland Museum, South Australian Museum, Environmental Protection Agency/Queensland Parks and Wildlife Service WildNet database, New South Wales Dept of Environment and Conservation Wildlife Atlas database, and various biologists.

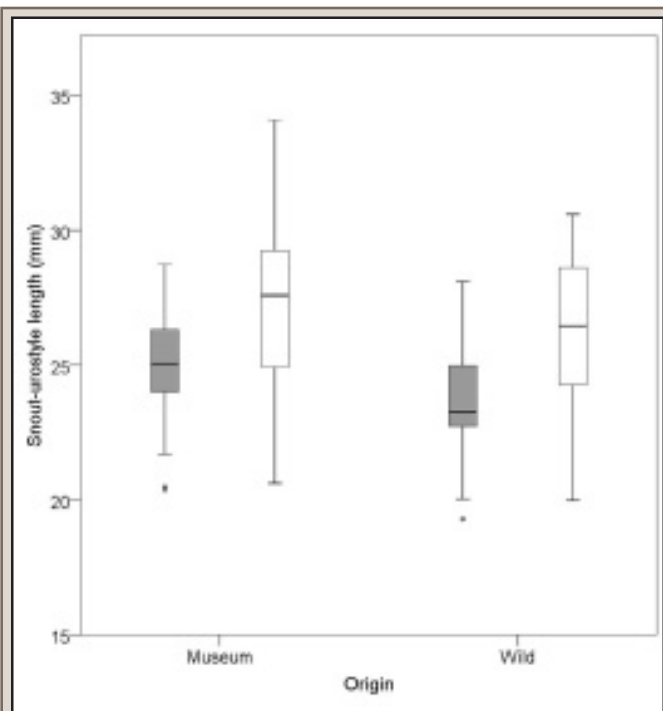


FIG. 3. Box plot of snout–urostyle length measurements of male ($N = 134$, gray bars) and female ($N = 144$, white bars) *L. olongburensis* taken from museum specimens ($N = 102$) and wild populations ($N = 176$). Significant female biased sexual size dimorphism was detected ($P < 0.001$), with no significant interaction between origin and sex ($P = 0.614$).

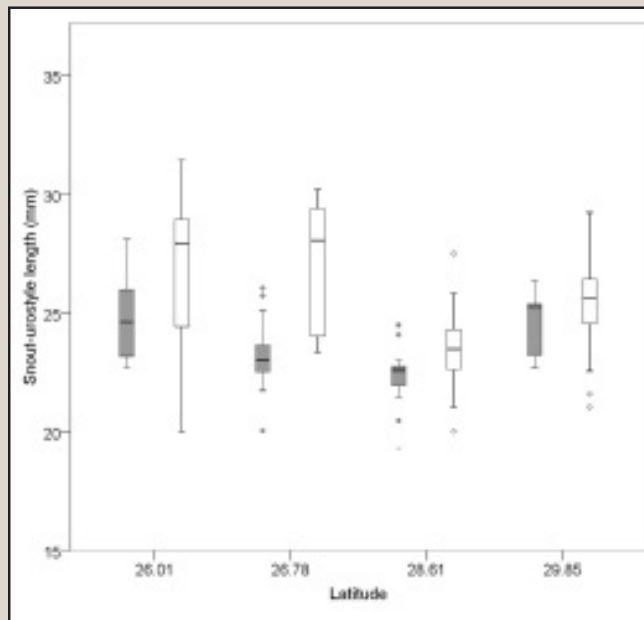


FIG. 4. Box plot of snout–urostyle length of wild male ($N = 102$, gray bars) and female ($N = 94$, white bars) *L. olongburensis* from four latitudes across the distributional range of the species (C, $N = 73$; MB, $N = 53$; T, $N = 37$; W, $N = 33$). Significant female biased sexual size dimorphism was detected ($P = 0.039$), with no significant interaction between origin and sex ($P = 0.091$).

The coloration of males and females was highly variable and there were no distinguishing color patterns for either sex (Fig. 6). For all wild frogs, dorsal coloration was measured as brown (48.3%), green (40.1%), and partial green (11.6%). The majority of frogs with green dorsal coloration were female (60.0%). Dark flecking on the dorsal surface was uncommon (four individuals). Throat color was either green (52.9%) or white/beige and the majority of frogs with green throats were female (60.04%). The largest proportion of measured frogs contained at least some blue thigh coloration (femoral streak) (59%), which was more common in frogs with green dorsal coloration (83.1%) than brown dorsal coloration (33%); and was present in both males (61.2%) and females (56.4%). However, thigh color was highly variable, including blue, orange, and brown; and combinations such as: blue/purple/orange, blue/brown/orange, blue/orange, blue/brown, brown/orange, orange/purple, and brown/purple.

Discussion.—The larger size of females (compared to males) found in *Litoria olongburensis* is consistent with the general trend in anurans (Monnet and Cherry 2002; Shine 1979). For field identification, nuptial pads and vocal sacs present in males, and the size disparity between the sexes are the best tools for sex identification. However, it must be noted that nuptial pads may be indiscernible outside the breeding periods, with differences in throat morphology becoming a more useful identification tool. Both sexes exhibited similar body coloration, thus coloration is a poor measure for sex identification. Although there were significant differences in SUL and SSD at different latitudes, we did not detect any latitudinal cline in SUL or SSD. Greater replication or more samples from along the latitudinal distribution of the species would offer more information on any latitudinal cline.

Although the selective pressures leading to SSD may be clear in some species, it may not be so obvious in others, as many selective processes mentioned in the introduction, such as fecundity selection, sexual selection and differences in life history, may occur in the same taxon (Monnet and Cherry 2002; Shine 1989). Whether there is a higher fecundity in larger females, differences in life history strategies, or selection for smaller males in *L. olongburensis* is unknown and would require further investigation.

Occasionally, male body size exceeds that of female body size, particularly in species with aggressive male encounters (Shine 1979). However, this is not true for most small species of the family Hylidae (Wells 2007), which includes *L. olongburensis*. Aggressive male-male encounters were observed in this species and appeared to be a defense of calling sites, with vocalizing males attempting to dislodge other males from a high position (KL, pers. obs.). However, it is unclear whether physical combats influence male reproductive success in this species, as no females were observed near the fighting males on these occasions.

Nuptial pads and vocal sacs were a useful characteristic for identifying males in the field. Although not quantified, we had the impression that nuptial pads were darker at the start of the breeding season. Thus, to reduce the frequency of misidentification, sex identification should occur at the beginning of the breeding season, when nuptial pad pigmentation is heightened. During the non-breeding period, when nuptial pads were indistinct, males could be identified based on faint vocal sac inflation creases or folds visible on the throat. Also, the two inner vocal sac openings were frequently visible through the outer surface of the throat. Whereas males had rather smooth and sometimes opaque skin on the throat, female throat skin was much more granular in texture and appearance. Positive identification of

females may also be heightened during the breeding season, when eggs are more easily detected, however, outside this period, size and throat morphology are better tools for sex identification.

The majority of *L. olongburensis* in the wild had brown, or gray-brown, dorsal coloration, with a large subset having green dorsal coloration, and a small proportion with both. Although the majority of green frogs were female, there was no significant sexual color dimorphism in *L. olongburensis*. Juveniles with green dorsal coloration were occasionally observed, however, the degree of ontogenetic shift in color is unclear. Green dorsal coloration is most likely to appear after metamorphosis, but whether dorsal coloration is genetically programmed or environmentally determined, or both, remains to be examined. The majority of live specimens had some blue coloration in the thigh, characteristic of the species, but thigh coloration was highly variable, and the blue was distinctly absent in some individuals. Striking thigh coloration is thought to act as a possible anti-predator mechanism, serving to confuse an attacking predator or warn predators of the presence of toxins (Toledo and Haddad 2009). Although not quantified, field observations indicated that particularly bright dorsal and thigh coloration may be more prominent in females.

Color or pattern polymorphism has been described in many amphibians (Hoffman and Blouin 2000; Wente and Phillips 2003) and is, in some cases, due to females selecting for brightly colored males (Chunco et al. 2007; Roulin and Bize 2007; Todd and Davis 2007). However, many anuran amphibians exhibit fixed color or pattern polymorphisms in both sexes within natural populations, and some species exhibit seasonal color change (Croshaw 2005; Hoffman and Blouin 2000; Wente and Phillips 2003, 2005). Color polymorphism may be particularly advantageous in an environment consisting of spectrally heterogeneous microhabitats, and presumably makes it difficult for predators to form a reliable search image, which is more advantageous when individuals select microhabitats matching their pattern (Morey 1990; Zug et al. 2001). Indeed, *L. olongburensis* inhabits heterogeneous brown and green sedgeland microhabitats, and future studies should aim to determine whether individuals select microhabitats to match their dorsal coloration, and the extent of genetic and/or environmental influence on color polymorphism.

Disruptive coloration and patterning can also be an important component of crypsis, as blotches, stripes, bands, or spots break up the general outline of an individual (Merilaita and Lind 2005; Toledo and Haddad 2009). This may make it difficult to detect the whole animal, especially against a background containing a mixture of color (Zug et al. 2001). *Litoria olongburensis* possesses a dark brown loreal streak from eye to nostril, as well as a brown and ventrally cream streak from behind the eye to halfway down the flank (Liem and Ingram 1977). These pronounced lateral stripes may aid in breaking up the lines of the body, which may be especially suitable in the preferred sedgeland habitat of *L. olongburensis* (Lewis and Goldingay 2005). Although the closely related *L. fallax* possesses similar lateral streaking, it is less pronounced. The increased intensity of lateral streaking in *L. olongburensis* may be due to a more specialized requirement for sedgelands, whereas *L. fallax* is a more generalist species.

We have presented data that adds to our knowledge of morphology and sexual dimorphism of *L. olongburensis*. It is unclear what selective pressures may be acting to maintain different color morphs or sexual size dimorphism in *L. olongburensis* and it is likely that multiple factors may act synergistically.



FIG. 5. Ventral surfaces of the hands of (A) a representative male *L. olongburensis*, showing the pigmented nuptial pad on the inner surface of the thumb, and (B) a representative female hand for comparison. Photos are of preserved specimens from the Queensland Museum (Male, QMJ34282; Female, QMJ81483).

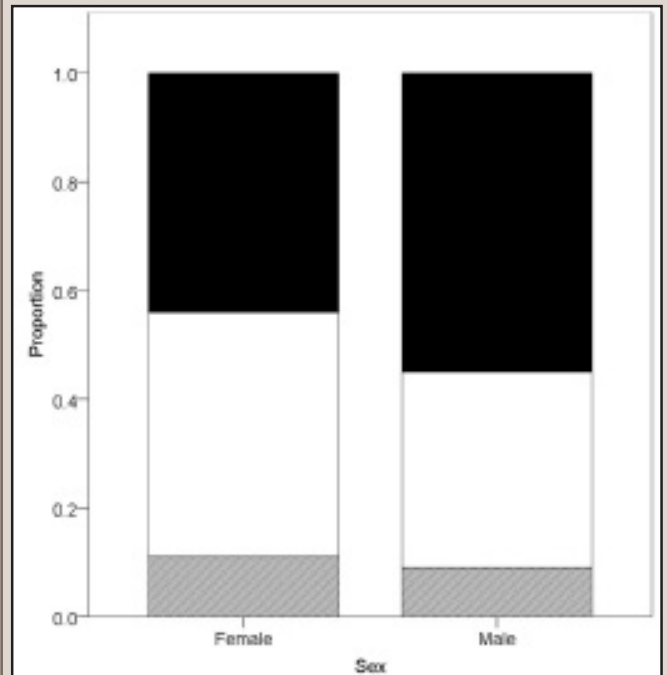


FIG. 6. The proportion of different color morphs of male ($N = 93$) and female ($N = 79$) *L. olongburensis*. Black bar, brown morph; white bar, green morph; striped bar, partial green morph.

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First Records of Limb Malformations in Wild Populations of the Endangered Balearic Midwife Toad, *Alytes muletensis*

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Limb deformities and developmental abnormalities in natural populations of amphibians have been reported for decades (Bishop 1947; Reynolds and Stephens 1984; Sessions and Ruth 1990; Woitkewitch 1961) but few reports involve deformities in rare and endangered amphibians.

The Balearic Midwife Toad *Alytes muletensis* (Sanchíz and Adrover 1977) is an endemic and threatened species of the Northeast Mallorca (Balearic Islands, Spain). Naturally, *A. muletensis* inhabits streams with vertical side walls and a bed with a stepped profile (Alcover et al. 1984). Thanks to conservation efforts, numerous anthropogenic constructions, such as fountains, and open and covered troughs, employed to provide water to livestock, have also been used as habitat for toads. These sites provide *A. muletensis* with new breeding locations, and may act as population reservoirs in the case of threats to natural