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DESMOGNATHUS QUADRAMACULATUS **(Black-bellied Salamander). MAXIMUM CLUTCH SIZE.** *Desmognathus quadramaculatus* is a semi-aquatic plethodontid of the southern Appalachians in the southeastern USA. The reproductive ecology of this species has been studied in sites throughout its range (Petranka 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, DC. 587 pp.; Camp et al. 2000. Can. J. Zool. 78:1712–1722; Camp et al. 2002. Herpetologica 58:471–484). Average clutch size is typically 31–54, depending on locality (Petranka 1998, *op. cit.*). A previous note reported a maximum clutch size of 82 from northeastern Georgia, USA (Camp et al. 2010. Herpetol. Rev. 41:330). In the late morning of 01 June 2013 we discovered two *D. quadramaculatus* clutches attended by females (both escaped and were not captured) under small rocks (~15 × 8 × 5 cm) in a tributary of the Toccoa River in Union Co., Georgia, USA (34.7589°N, 84.0310°W; WGS84). We counted 71 eggs in the smaller clutch and 105 eggs in the larger clutch, which now represents the maximum clutch size for this species. We returned the rocks carefully to their position in the stream and returned to check the rocks five hours later to determine if the females returned to their clutch. The female attending the larger clutch had returned, but the other had not.

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EURYCEA NAUFRAGIA **(Georgetown Salamander). BEHAVIOR.** *Eurycea naufragia* is a neotenic spring and cave dwelling plethodontid salamander. This species is known from approximately 15 sites from the San Gabriel River watershed in Texas, an area that is undergoing rapid urbanization. The limited range of the species and its restricted habitats led to its recent listing as a threatened species. From 22 March to 03 May 2012, salamanders were trapped at a permanent spring in Williamson Co., Texas, USA (precise locality withheld due to conservation concerns). Each trap consisted of a fine mesh net 76.2 cm long with a 16.5 cm opening supported by wire at one end and the other end attached to a capped 5.08 × 29.2 cm PVC pipe, with a mesh covered opening on the side to allow water flow. Each week we conducted one diurnal and one nocturnal sampling event separated by 24 to 48 h. We randomized whether diurnal or nocturnal trapping was conducted first within each week. Three traps were placed at the same locations within the spring run (at the spring outflow, 1 m and 7 m downstream) for each sample. Diurnal sampling was conducted from 0700 h to 1900 h and nocturnal sampling from 1900 h to 0700 h. Based on a visual estimate of total length (following Bowles et al. 2006. Hydrobiologia 553:111–120; Pierce et al. 2010. Southwest. Nat. 55:296–301; Bendik et al. 2014. Herpetol. Conserv. Biol. 9:206–222), each salamander was assigned to one of two size classes: juvenile (<5.1 cm total length) or adult (>5.1 cm total length). After processing, all salamanders were released back into the spring run at point of capture. For comparison of nocturnal and diurnal samples, we combined all salamanders captured in the three traps during a sampling event.

We captured more salamanders during the nocturnal sampling events (5.71 ± 2.56 salamanders, mean ± SD) than during diurnal sampling events (1.14 ± 1.07). These differences, however, were not consistent across the two size classes. We captured more juvenile salamanders in nocturnal sampling events (4.57 ± 2.30) than in diurnal sampling events (0.14 ± 0.38). There was no significant difference in number of adult salamanders captured during nocturnal (1.14 ± 1.46) and diurnal sampling events (1.00 ± 1.15). The total number of salamanders that we captured in this study (N = 48) was relatively low, but the results were nevertheless straightforward and suggest that juvenile *E. naufragia* salamanders are more active at night.

A number of other studies have concluded that terrestrial salamanders are more active at night (e.g., Hairston 1949. Ecol. Monogr. 19:47–73; Connette et al. 2011. Southeast. Nat. 10:109–120). However, only a few studies have examined daily activity in aquatic life stages and none have examined permanently aquatic species. A study on activity patterns of aquatic stream salamander larvae (*Desmognathus fuscus* [Northern Dusky Salamander], *Eurycea cirrigera* [Southern Two-lined Salamander], *E. guttolineata* [Three-lined Salamander], *Pseudotriton montanus* [Mud Salamander], and *P. ruber* [Red Salamander]) found higher nighttime activity (Connette et al. 2011, *op. cit.*). In a study of *E. bislineata*, aquatic larvae were more active at night (Petranka 1984. J. Herpetol. 18:48–55). Contrary to the current study, densities of *D. fuscus* aquatic larvae were similar during day and night (Orser and Shure 1975. Am. Midl. Nat. 93: 403–410).

Our observations suggest that juvenile *E. naufragia* are more active at night and that nocturnal surveys will result in higher abundance observations for this life stage. These conclusions are limited by only sampling during a single season and using a single survey method (traps). Nevertheless our observations suggest that investigators surveying larval or aquatic salamanders should be aware of potential differences in diurnal and nocturnal activity patterns that may affect survey results.

We thank the property owners for permission to survey salamanders on their land. The Texas Parks and Wildlife provided a scientific research permit for study of the salamanders. This study complied with all applicable institutional animal care guidelines.

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PLETHODON CINEREUS **(Eastern Red-backed Salamander). MORPHOLOGY.** Two color morphs are common in *Plethodon cinereus*, striped and unstriped (Petranka 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, D.C. 587 pp.). Both morphs are present in Ohio, with the northwestern portion of the state containing a high frequency of the unstriped morph, which is typically far less common than the striped morph. South Bass Island, part of the Lake Erie archipelago, contains a well-documented monomorphic unstriped population (Pfingsten and Walker 1978. J. Herpetol. 12:163–167;

Fig. 1. Striped *Plethodon cinereus* found within a monomorphic unstriped population on South Bass Island, Ohio. This individual represents the only striped individual collected from the island.

Hantak, pers. obs.). To date, a single striped individual has been discovered on South Bass Island (Reichenbach 1981. Herpetol. Rev. 12:53) among hundreds of unstriped *P. cinereus* collected over decades (Pfingsten and Walker 1978, *op. cit.*; Reichenbach 1981, *op. cit.*; Hantak, pers. obs.). Thirty-six years after the sighting of the first striped individual on South Bass Island (Reichenbach 1979, pers. obs.), a second individual has been found (Fig. 1). It was discovered on 29 April 2015 on the Heineman property, South Bass Island, Ottawa Co., Ohio, USA (41.6465°N, 82.8234°W, WGS84). The individual was a juvenile (SVL = 22.75 mm; 0.26 g), and was found with three unstriped juveniles under a decaying log. This specimen was collected and preserved at Ohio University (MMH459), unlike the previous individual, which was only photographed. The genetics underlying the striped/unstriped color polymorphism of *P. cinereus* are currently not well understood; however, discordant results by Highton (1959. Copeia 1959:33–37; Highton 1975. Genetics 80:363–374) suggest that the genetics of the polymorphism may be geographically variable. Whether the striped morphs occur on South Bass Island as a consequence of a recent, novel mutation, or whether these individuals reflect the retention of an ancestral polymorphism, is not known.

We thank the Heineman family for allowing us to work on their property.

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ANURA — FROGS

***HYLA CHRYSOSCELIS* (Cope's Gray Treefrog) × *HYLA CINEREA* (Green Treefrog). PUTATIVE NATURAL HYBRID.** Naturally occurring hybrid treefrogs have been occasionally found in the eastern United States. However, these hybrids are almost always between members of the same species group (e.g., Gerhardt 1974. Behaviour 49:130–151; Gerhardt et al. 1980. Copeia 1980:577–584; but see Anderson and Moler 1986. Copeia 1986:70–76). When *Hyla cinerea* group individuals were artificially crossed with *H. versicolor* group individuals, little reproductive success was achieved, and those tadpoles that survived to metamorphosis showed severe phenotypic abnormalities, and abnormal or incomplete sexual development (Mecham 1965. Am. Midl. Nat. 74:269–308).

At 2145 h on 10 June 2014 we heard an unusual anuran advertisement call along Bayou Manual Road in Sherburne Wildlife Management Area in the Atchafalaya Basin of south-central Louisiana, USA (30.4026°N, 91.6748°W, WGS84). The call resembled that from a *Hyla*, but did not sound like any of the *Hyla* species known from the area (*H. cinerea*, *H. chrysoscelis*, and *H. squirella* [Squirrel Treefrog]). The dominant calls at that time in the immediate vicinity were from *H. chrysoscelis*, with *H. cinerea*, *H. squirella*, *Incilius nebulifer* (Gulf Coast Toad), and *Gastrophryne carolinensis* (Eastern Narrow-mouthed Toad) each vocalizing to lesser degrees.

We collected the individual and brought it to the laboratory for further study. The treefrog measured 45 mm (SVL), with an elongated body shape, appearing more like *H. cinerea* than *H. chrysoscelis* in that regard. The subocular spot typical of *H. chrysoscelis* and the lateral stripe typical of *H. cinerea* were both absent. The overall pattern was mottled, and his coloring changed dramatically from predominantly green to predominantly brown. Even while green, however, his lower legs remained gray. The inner thighs did not exhibit bright flash colors typical of *H. chrysoscelis* (Fig. 1). Mecham (1965, *op. cit.*) described similar physical characteristics to our hybrid in his laboratory–crossed female *H. cinerea* and male *H. chrysoscelis* hybrid treefrogs. Reciprocal crosses between male *H. cinerea* and female *H. chrysoscelis* produced different patterns and resulted in a lack of one or both eyes (Mecham 1965, *op. cit.*).

The frog did not respond to calls of recordings of *H. cinerea* but did vocalize in response to the recorded call of *H. chrysoscelis*. We made a recording of the call of the putative hybrid in the laboratory at an air temperature of 24.6°C. We analyzed only four calls for measurements of the pulse duration, pulse interval, and pulse period by using the measurement tool in Sound Ruler ver. 0.9.6 (Gridi-Papp [ed.]. 2007. SoundRuler: Acoustic Analysis for Research and Teaching. http://soundruler.sourceforge.net). The dominant frequency was found using the dfreq function in the R package seewave (Sueur et al. 2008. Bioacoustics 18:213–226). The analyses produced the following means: pulse duration 0.0703 s, pulse period 0.1171 s, pulse interval 0.0473 s, and pulse rate 8.782 pulses/s. The mean dominant frequency of the calls was 2169 Hz (Fig. 2) which is higher than the 1906 Hz mean dominant frequency (at 25.0°C air temperature) of a laboratory-reared 38.2 mm hybrid resulting from a cross between a female *H. cinerea* and a male *H. chrysoscelis* (Fortman and Altig 1974. Herpetologica 30:221–234). The call had a frequency spectrum within the range of frequencies by the putative parent species (Fig. 2). The pulse rate, however, lay intermediate between the putative parent species (much faster than *H. cinerea*, and much slower than *H. chrysoscelis*). The full audio recording has been accessioned into the Macaulay Library with catalog number ML 148096 (http://macaulaylibrary.org/audio/148096).

Flow cytometry was used (Jenkins et al. 2011. Reproduction 141:55–65) to determine whether this individual had produced viable spermatozoa. The putative hybrid showed 3.4% (SE = 0.009) viable testicular cells in a suspension of the macerated testis compared to a normal male *H. chrysoscelis*, which had 44.3% (SE = 0.014) viable testicular cells. The testes of the putative hybrid were smaller (3.67 mm length, 1.44 mm width) compared to the normal *H. chrysoscelis* (4.5 mm length, 2.37 mm width). No