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CLUTCH SIZE IN THE GREAT BASIN SPADEFOOT (*SPEA INTERMONTANA*), SOUTH OKANAGAN VALLEY, BRITISH COLUMBIA, CANADA

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Key words: anthropogenic breeding site, clutch size, fecundity

Breeding wetlands and upland terrestrial habitat used by the Great Basin Spadefoot (*Spea intermontana*) are under intense pressure from the expansion of agriculture, urban development, and loss of grasslands throughout the species range (AmphibiaWeb 2012). At the northern edge of the *Spea intermontana* geographic range, the British Columbia population of the Great Basin Spadefoot is designated as a threatened Species At Risk (BC Southern Interior Reptile and Amphibian Recovery Team 2007; Committee on the Status of Endangered Wildlife in Canada [COSEWIC] 2007). In the South Okanagan Valley, British Columbia, approximately 90% of wetlands have been lost (Lea 2008). In the lowland Valley (<450 m elevation), metamorphic success of *Spea intermontana* has been observed in natural ephemeral wetlands, small agricultural ponds, and diverse anthropogenic sites (Ashpole and others 2012). Spadefoot developmental adaptation (either a delay or an increased rate of metamorphosis) in response to the larval habitat has been attributed to resource availability and consequently fitness (see overview by Morey and Reznick 2004). Morey and Reznick (2004) report wild populations of *Spea intermontana* development ranging from 36 to 60 d. In a comparative resource study, *Spea intermontana* metamorphic development was slowest (41 d) in individuals with the lowest food availability (Morey and Reznick 2004). *Spea intermontana* clutches are described in standard field guides as containing few eggs (5 to 40 eggs/clutch) in a randomly shaped grape-sized cluster, where 1 egg can easily be detached from the group (Corkran and Thoms 1996; Cannings and others 1999; Jones and others 2005; Matsuda and others 2007). Here we describe large clutch sizes of *Spea intermontana* not previously documented in the scientific literature or field guides. Egg clutches were oviposited in 3 human-made 'ephemeral' breeding sites, including a swimming pool (Site A), a constructed agricultural retention pond (Site B), and a cement irrigation ditch (Site C). Additionally, road kill samples of *Spea intermontana* provide a broader range of possible fecundity rates, and skeletocronology suggests that reproductive maturity occurs at 1 y of age.

Site A

In 2004, *Spea intermontana* was observed breeding in a rural residential in-ground cement-walled swimming pool (depth = 2 m) north of Oliver, BC. The surrounding habitat includes vineyards to the east and native shrub-steppe habitat to the west. The pool partially fills with rainwater and woody debris each spring, and since 1994 the landowner, with assistance of the primary author in 2003–2004, has been counting the number of adults and emerging metamorphic Spadefoot Toads. The adults bury themselves in provided leafy debris at the dry shallow end of the pool and are removed by hand several nights after calling ceases. Metamorphic individuals are captured by net and then counted by hand and placed into temporary storage containers while the pool is drained using a filter (Shkrabuik, landowner, pers. comm.). The number of adults observed in the pool from 1994 to 2000 ranged from 4 to 24 individuals (Table 1). The number of *Spea intermontana* metamorphs in the pool has ranged from 1500 to 9500 with the exception of the years 1999–2000, when egg developmental success was observed by the landowner as being very low (<10 metamorphs annually), which was believed to be due to predacious invertebrates (Table 1).

On 26 April 2004, 48 *Spea intermontana* egg clutches were observed in the swimming pool (Gosner stage 9–17, dorsal lip to tail bud stage; embryo staging according to Gosner 1960) (Table 1). Egg clutches could easily have been misidentified using field guide small- egg-number and loose-cluster shape criteria. Unlike descriptions in field guides (Corkran and Thoms 1996; Cannings and others 1999; Jones and others 2005; Matsuda and others 2007), all egg clutches observed were tightly packed in elongated row-like bundles. Eggs were firmly attached along submerged woody stems not easily detached from each other as is suggested in the guides. At the time of egg clutch observation, 7 adult *Spea intermontana* were observed in the pool. The following morning (27 April), 6 newly deposited egg clutches were counted ($N_{clutches} = 6$; $mean_{eggs/clutch} = 76$, min = 51, max = 106, std dev = 20) (Table 1). After 27 April, no new clutches or adults were detected. Within 48 h of oviposition, all eggs reached Gosner stage 17 (tail bud development and muscular twitch), and Gosner stage 25 (free swim stage) was reached by the fifth day. Water temperature was recorded until 5 d after the egg-laying period using maximum-minimum thermometers (Vee Gee, model 84001), and temperature ranged from 15 to 21°C. Over the period 3 to 5 July 2004, 600 metamorphosed *Spea intermontana* individuals were counted by hand using nets as the pool was drained (Table 1). Time from egg laying to metamorphic emergence (larval period) of *Spea intermontana* at the pool site was approximately 68 to 71 d, with an estimated minimum metamorphic success of 14.6% (number metamorphs/(mean no. eggs/clutch × no. egg clutches) × 100).

Site B

An agricultural pond (63 × 45 × 4 m) constructed for irrigation runoff in the Osoyoos-Oliver area, BC, was surveyed in 2004. Surrounding habitat included soft fruit and apple orchards with native shrub-steppe habitat to the west. Vegetation within the open-water area and the submergent zone was sparse. However, some bulrushes, non-native grasses, and invasive plants occurred in the non-emergent vegetation zone. A riparian or

TABLE 1. Total number of individuals, by life stage, of *Spea intermontana* in anthropogenic breeding sites, South Okanagan Valley, BC.

Site	Date	Adult	Metamorph	Tadpole	Number clutches	Individual eggs/ clutch(mean, range)	Metamorphic survival (%)	Fecundity estimate (no.eggs/female)[1]	Larval period(days)
Site A:	1994 to 2000	4 to 24	1500 to 9500[2]	-	-	-	14.6	-	-
Swimming Pool	26–27April 2004	7	-	-	54	76[3], 51 to 106	-	1173	68 to 71
	3–5 July 2004	-	600	-	-	-	-	-	-
Site B:	12 May 2004	65	-	-	-	74[4]	0.08	512	41
Constructed	15 May 2004	-	-	-	225	-	-	-	
Pond	7 June 2004	-	-	449	-	-	-	-	-
	16 June 2004	-	-	339	-	-	-	-	-
	24 June 2004	-	14		-	-	-	-	-
Site C:	24 April 2007	30	-	-	125	72[5], 38 to 95	-	600	45 to 67
Cement Ditch									

[1] Estimate using 50:50 sex ratio of observed adults.

[2] 1999 and 2000 metamorphic success <10 individuals.

[3] Based on 6 clutches collected on 27 April 2004.

[4] Average taken from swimming pool and cement ditch site.

[5] Based on a subsample of 15 clutches; - data not available.

buffer zone did not exist between the pond and the surrounding orchard. In the agricultural pond, the first measurable amount of water, originating from overhead spray irrigation, was on 12 May 2004, when an 8 × 4 m area and 0.18 m of water filled the northwest end of the pond. At dusk on this date, 65 adult *Spea intermontana* were observed in the pond, with 23 pairs in amplexus (Table 1). Precipitation had not occurred within 10 d prior to or during this breeding activity. On 15 May 2004, a total of 225 *Spea intermontana* egg clutches were counted (Table 1). On 3 occasions, as part of time-constrained active searching and trapping inventories by the authors (subset of relevant data presented here), the pond was dip-netted by 2 observers for 20 min each to determine relative species abundance. On 7 June 2004, 449 *Spea intermontana* tadpoles (3 with hind limb bud development) were captured, counted, and returned to the pond. A similar relative abundance was recorded on 16 June 2004, with 339 *Spea intermontana* tadpoles captured, including 30 with rear limb bud development. On the third sampling occasion, 3 floating minnow traps were set at 18:10, and the following morning (09:13) on 24 June 2004, no tadpoles were detected and 14 metamorphosed *Spea intermontana* were observed along the shoreline (Table 1). *Spea intermontana* development from egg laying to metamorphic emergence at the agricultural site was approximately 41 d; the minimum estimated metamorphic rate of survival, using the average number of eggs/clutch recorded from site A and C (74 eggs/clutch: 76 and 72 eggs/clutch respectively), was 0.08% (number metamorphs/(mean no. eggs/clutch × no. egg clutches) × 100).

Site C

Our 3rd anthropogenic site was a cement irrigation ditch (1 × 6 × 0.5m) no longer used to distribute irrigation water to farmlands (Fig. 1). The ditch is adjacent to vineyards to the east and native shrub-steppe habitat to the west, northwest of Oliver. Since the early 1980s, the landowners, each spring, have filled the ditch with irrigation water and observed breeding *Spea intermontana*. Two constructed sand berms retain the water, and provide burrowing habitat for *Spea intermontana* and exit points for metamorphs. On the afternoon of 23 April 2007, the ditch was filled with water and twigs were added as egg laying material. The following morning, 30 adults in amplexus and approximately 125 egg clutches were observed (Table 1). A subsample of 15 distinct egg clutches was used to estimate clutch size (mean $_{\text{eggs/clutch}}$ = 72, min = 38, max = 95, std dev = 26) (Table 1). *Spea intermontana* development from egg laying at the end of April to metamorphic emergence, 7 and 29 June 2007, occurred over a period of 45 to 67 d at this ditch (Firman, landowner, pers. comm.). Precise numbers of emerging metamorphic individuals have not been accurately estimated; however, in some years the landowners report a 'carpet' of recently metamorphosed spadefoots around the site.

FIGURE 1. Site C is an abandoned agricultural ditch artificially filled with water by the landowners that acts as an ephemeral *Spea intermontana* breeding site. After a length of 6 m, sand berms are constructed in the ditch for amphibian entrance to and exit from the water. Photo credit: R Firman 2010.

Fecundity and Age Estimates

To determine the fecundity and estimate the age of *Spea intermontana*, 2 intact road-killed females were collected on 29 June and 24 July

2011, with a snout vent length (SVL) of 48 and 42 mm, respectively. The mean SVL of local *Spea intermontana* females (52.7 mm, $n = 613$, range = 44.5–67.2 mm; J Crosby, University of Waterloo, unpubl. data) has a similar adult size range (20 to 65 mm SVL) reported by field guides (Corkran and Thoms 1996; Cannings and others 1999; Jones and others 2005; Matsuda and others 2007). The 2 road-killed females were heading east to breeding ponds from upland terrestrial habitat along highway 97, south of Osoyoos, BC. The specimens were frozen whole immediately after collection and defrosted prior to egg removal. Eggs were counted using a dissecting stereomicroscope (16× magnification; Zeiss Stemi, Model 3919021628). To ensure accurate egg counts, the same observer completed blind counts of each mass on 3 occasions. The age of each frog was estimated using skeletochronology by counting the number of distinct lines of arrested growth (LAG) on the hind phalanges (histological methods by Kusano and others 1995). The total number of eggs for the specimen collected on 29 June was 1386, and 2 LAG estimated the individual to be 2 y of age and in the 3rd growing season. The specimen collected on 24 July, which was only marginally smaller in size, had a total of 285 eggs and a single LAG estimating the individual to be 1 y of age and in the 2nd growing season. Our field observations of 72 to 76 eggs/clutch suggest that a female such as the road-killed animal with 1386 eggs could lay up to 19 clutches/season.

Our observations of 38 to 106 eggs/clutch are clutch sizes considerably higher in number than previously described in field guides (5 to 40 eggs/clutch). Gravid *Spea intermontana* will lay several distinct egg clutches; however, it is not known if eggs are deposited in a single evening or over a period of time. Our data estimates that the number of eggs that can be produced by a single female in a season ranges conservatively from 512 to 1173 (mean no. eggs/clutch × no. egg clutches observed/no. of females assuming a 50:50 sex ratio), and is within a similar magnitude found in our road-kill specimens: 285 and 1386 eggs, respectively. Previous field estimates suggesting a single female can lay 300 to 800 eggs in a season (Stebbins 1985, 2003; Leonard and others 1993; Cannings and others 1999; COSEWIC 2007; Matsuda and others 2007) may be an underestimate by a factor of 1.7 times. In captivity, pairs of *Spea intermontana* have been recorded to produce up to 1000 eggs (S Morey, unpubl. data cited by AmphibiaWeb 2012).

It is unclear if the number of eggs deposited in a clutch is related to the larval environment, metamorphic rate, or individual fitness. Similarly, it is not known if South Okanagan Valley *Spea intermontana* deposit substantially greater numbers of eggs compared to other populations throughout the species range. Ephemeral anthropogenic wetland sites in the South Okanagan Valley provide breeding habitat for the threatened *Spea intermontana*. The 3 sites described here, compared to 108 wetland sites we inventoried in 2003 to 2012 in the Okanagan Valley, had the highest density of *Spea intermontana* eggs and tadpoles recorded in the lower South Okanagan (Ashpole and others 2012). However, the high density of eggs and tadpoles provides a poor indicator of overall reproductive success or habitat quality. Both private landowners have direct observations and photo documentation of complete failure of the eggs to develop through to metamorphosis in some years due to predatory species, specifically aquatic invertebrates (suspected dragonfly larvae) at the pool site, and Gopher Snakes (*Pituophis catenifer*) and American Crows (*Corvus brachyrhynchos*) at the ditch site. Additionally, *Spea intermontana* at these 2 artificial sites rely strictly on landowner stewardship for water and feeding (using dry cat kibble). Characteristics of anthropogenic wetland sites, including prey and predator dynamics, in the South Okanagan Valley are highly variable, but many prove to be viable sites for *Spea intermontana*. However, the metapopulation dynamics of *Spea intermontana* in the South Okanagan is unknown; consequently the nature of anthropogenic sites as possible sink populations has yet to be addressed. Due to the overall lack of available breeding habitat, it is unlikely that individuals are selecting anthropogenic sites over natural ones. Sites with impervious substrates, such as cement, are unpredictable as amphibian breeding locations. The water temperature may be unnaturally elevated due to the lack of vegetation and the shallow depth of the site. Artificially constructed agricultural wetland sites, such as site B, are also vulnerable to changes in water dynamics from extraction, contamination, modification, and as irrigation practices move toward resource conservation and the instillation of drip irrigation. The consequence is increased wetland disturbance and possibly a loss of amphibian breeding sites due to significantly less agricultural run-off, altering the hydroperiod and timing of inundation. Reductions in surface water availability are predicted for the South Okanagan Valley in response to demographic, land-use, and climate change (Harma and others 2012). Currently, agricultural wetlands in the South Okanagan Valley have a high annual probability of inundation due to land-use practices, and while this may become unpredictable, agricultural wetlands may provide the greatest availability of breeding habitat. Natural ephemeral wetlands in the South Okanagan Valley demonstrate variable inundation, and may in the future experience longer periods of drought, higher temperatures, and increased rates of evapotranspiration (Neilsen and others 2006). The future of amphibian population reliance on natural and anthropogenic ephemeral habitats and the hydrodynamics of these habitats require further investigation.

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FIRST RECORDS OF THE NIGHT SMELT, *SPIRINCHUS STARKSI*, IN THE SALISH SEA, WASHINGTON

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Key words: COI, distribution, genetics, Longfin Smelt, Night Smelt, Osmeridae, *Spirinchus starksi*, *Spirinchus thaleichthys*, Washington

Ten species of smelts (Osmeridae), including 1 introduced species, have been found in the eastern North Pacific (Love and others 2005). Of these 10 species, 5 have been recorded from the inland marine waters of the Salish Sea, including Puget Sound and the Straits of Juan de Fuca and Georgia (Hart 1973): Whitebait Smelt (*Allosmerus elongatus*), Surf Smelt (*Hypomesus pretiosus*), Capelin (*Mallotus villosus*), Longfin Smelt (*Spirinchus thaleichthys*), and Eulachon (*Thaleichthys pacificus*). Results from a study using molecular markers to distinguish smelt remains found in the stomach contents of fishes (Paquin and others 2014) indicated that a specimen collected from Discovery Bay, Washington, an embayment at the eastern terminus of the Strait of Juan de Fuca, was misidentified as *S. thaleichthys* in the online Barcode of Life Database (BOLD; Ratnasingham and Hebert 2007). Subsequent examination of the preserved specimen has confirmed its identity as Night Smelt (*Spirinchus starksi*) and constitutes a new record for this species in the Salish Sea. Since this discovery, additional specimens of *S. starksi* have been collected in the area for inclusion in this study.

To confirm the identification of the Discovery Bay specimen, both morphological and nucleotide sequence data from *S. starksi* were compared with data from the closely related species *S. thaleichthys*. Whole specimens and tissue samples (frozen or preserved in ethanol) from 11 *S. starksi* and 5 *S. thaleichthys* were obtained from the Northwest Fisheries Science Center (NWFSC) and the University of Washington Fish Collection (UW). Specimens of *S. starksi* were collected from Discovery Bay, Washington ($n = 1$), Green Point, Washington ($n = 5$), and north of Monterey Bay off the California coast ($n = 5$). Specimens of *S. thaleichthys* were collected from the Gulf of Alaska ($n = 1$), San Juan Islands ($n = 1$), and Puget Sound ($n = 3$) (Table 1).

DNA extractions were performed on fin clips using a QIAGEN[1] DNeasy kit (QIAGEN, Valencia, CA) and the manufacturer's animal tissue protocol. A 750 base-pair region of the mitochondrial genome, cytochrome *c* oxidase I (COI), was amplified using PCR with universal fish primer cocktail C_FishF1t1-C_FishR1t1 (Ivanova and others 2007). The COI gene region has been used as the barcode for biodiversity to distinguish among species, whether they are distantly related species such as mammals and insects, or closely related congenerics (Ward and others 2005, 2009; Holloway 2006; Ward and others 2009). Nucleotide sequence data were collected for 16 samples (including the misidentified specimen of *S. starksi* from Discovery Bay) in both forward and reverse directions with Big Dye chemistry using EXO-SAP purified PCR products. Sequences were read using an ABI 3730 automated sequencer

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[1] Reference to trade names does not indicate endorsement by NOAA-Fisheries or the US Government