

Kelley J. Kissner · Patrick J. Weatherhead ·
H. Lisle Gibbs

Experimental assessment of ecological and phenotypic factors affecting male mating success and polyandry in northern watersnakes, *Nerodia sipedon*

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Abstract To resolve conflicting field observations regarding the action of sexual selection, we used breeding experiments and paternity analysis of the 927 resulting offspring to assess how male size, condition, tail length, genetic similarity to the female, and variation in operational sex ratio (OSR) affected male reproductive success and the incidence of polyandry in northern watersnakes (*Nerodia sipedon*). Only size affected male mating success. Large males were more successful, but only when male size varied substantially and competition among males was intense (i.e., male-biased OSR). The conditional nature of the size advantage may explain why studies of free-living watersnakes have produced inconsistent results regarding the relationship between male size and mating success. Size differences between males did not affect the proportion of offspring each male sired within multiply sired litters.

We found positive size-assortative mating, but only when the OSR was female biased, suggesting that smaller males had improved access to females when competition among males was reduced, but that competition with larger males still restricted mating opportunities of small males to less preferred, smaller females. Most litters (58%) were multiply sired and larger females were more likely to produce multiply sired litters, similar to free-living watersnakes. There was no association between the incidence of multiple paternity and OSR, however, suggesting that polyandry is not simply a function of opportunity, with females passively waiting for males to court them.

Keywords Reproductive success · Multiple mating · Assortative mating · Operational sex ratio · Snakes · Genetic paternity analysis · Sexual selection

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K. J. Kissner · P. J. Weatherhead (✉)
Department of Biology, Carleton University,
Ottawa, Ontario K1S 5B6, Canada
e-mail: pweather@uiuc.edu
Tel.: +217-244-0319

H. L. Gibbs
Department of Biology, McMaster University,
Hamilton, Ontario L8S 4K1, Canada

Present address:
P. J. Weatherhead
Program in Ecology and Evolutionary Biology, University of
Illinois,
606 E. Healey Street,
Champaign, IL 61820, USA

H. L. Gibbs
Department of Evolution, Ecology and Organismal Biology,
The Ohio State University,
300 Aronoff Laboratory, 318 W. 12th Avenue,
Columbus, OH 43210-1293, USA

K. J. Kissner
59 Hidden Green NW,
Calgary, AB, T3A 5K6, Canada

Introduction

Sexual selection is relatively poorly studied in snakes, despite their biology making snakes interesting subjects for this field (Shine and Bonnet 2000). Polyandry appears to be the norm in snakes (e.g., Gibson and Falls 1975; Stille et al. 1986; Schwartz et al. 1989; Barry et al. 1992). Polyandry occurs in circumstances ranging from those in which males search for dispersed females (Madsen et al. 1993; Blouin-Demers et al. 2005) to those in which males “scramble” for access to aggregated females, with scrambling varying from mild (Weatherhead et al. 1995) to extreme (Gregory 1974; Mason and Crews 1985).

In addition to their potential as subjects for investigating polyandry (Madsen et al. 1992; Prosser et al. 2002), snakes are also promising subjects for studying factors that affect male mating success. Male snakes provide no resources to females and no parental care. Furthermore, snakes’ simplified external morphology limits male attributes on which sexual selection can act (Shine 1993). With fewer factors potentially affecting male mating success, identifying important factors should be more straightforward.

Studying sexual selection in snakes has been facilitated by using DNA-based methods to assign paternity (Gibbs and Weatherhead 2001) and thus assess mating success, even when mating is not readily observed (Blouin-Demers et al. 2005) or poorly predicts true success (Prosser et al. 2002). Also, some snake species are tractable subjects for mating experiments (e.g., Joy and Crews 1988; Shine et al. 2000). Here we combine both approaches to resolve issues concerning which male attributes affect mating success in northern watersnakes (*Nerodia sipedon*) and to determine how breeding sex ratios affect mating success and polyandry.

Northern watersnakes mate during the month following emergence from hibernation (Weatherhead et al. 1995). Mating occurs in aggregations of one female and one to several males. Males compete by attempting to align their bodies next to females. The large size of females and loose structure of aggregations appear to allow females to control mating, although whether they exercise mate choice remains unknown. The opportunity for sexual selection is 5 times higher for males than females (Prosser et al. 2002). Observational studies have provided evidence for and against large size and higher body condition increasing male mating success (Weatherhead et al. 1995; Brown and Weatherhead 1999; Weatherhead et al. 2002). Dispersion of females, which affects operational sex ratios (OSR; Emlen and Oring 1977), may also influence male mating competition (Brown and Weatherhead 1999). Our first goal was to determine whether size or condition affected male mating success, and whether these traits were more advantageous when OSR was male biased, and thus competition among males more intense. We also assessed whether relative tail length affected male mating success because it has been shown to be important in gartersnakes (Shine et al. 1999), although not in watersnakes (Weatherhead et al. 2002).

In addition to mating with more females and siring more of those females' offspring, males can improve their success by mating with larger, more fecund females. Size-assortative mating has been documented in snakes (Shine et al. 2000; 2001) and lizards (Olsson 1993; Cooper and Vitt 1997). In free-living northern watersnakes, Weatherhead et al. (2002) found size-assortative mating, but only where females were dispersed and courted by fewer males. Our second goal was to investigate whether OSR affected assortative mating in watersnakes. Assuming all males prefer larger females (Shine et al. 2001), and large males can out-compete smaller males (i.e., objective 1 above), male-biased OSR should provide small males few mating opportunities. Thus, size-assortative mating would not occur. Conversely, with female-biased OSR, large females should be monopolized by large males, allowing small males to mate with small females by default, producing size-assortative mating (Cooper and Vitt 1997).

Genetic similarity to females could also affect male reproductive success. Among inbred sand lizards (*Lacerta agilis*), males genetically most different from females sired more offspring in multiply sired litters, despite having no

mating advantage (Olsson et al. 1996). Although the watersnakes we studied are not inbred, gene flow is restricted (Prosser et al. 1999), making mating between close relatives possible. Weatherhead et al. (2002) found no evidence that genetic similarity influenced mating, but did not examine whether paternity within litters was influenced by relatedness. If discrimination against related males occurs after mating, it would be apparent only in polyandrous females. Our third objective was to determine whether paternity in multiply sired litters was biased in favor of males least related to females.

Our final objective was to identify factors associated with multiple paternity. Multiple paternity occurs in 54–86% of watersnake litters, with larger females more likely to be polyandrous (Barry et al. 1992; Prosser et al. 2002). Larger females may have the opportunity to mate with more males because they are more attractive (Prosser et al. 2002). Females in better condition could also mate with more males if they are better able to afford any associated costs. In addition to testing whether greater size and condition increase the incidence of multiple paternity, we examine effects of OSR. When more aggregated, females are courted by more males (Brown and Weatherhead 1999) and weak evidence suggests this increases multiple paternity (Prosser et al. 2002). Thus, we determine whether multiple paternity is more prevalent when OSR is more male-biased.

Methods

Capturing and handling of snakes

Adult male (SVL > 42 cm) and female (SVL > 55 cm) watersnakes were captured in 1997–1999 from marshes within 10 km of the Queen's University Biological Station in eastern Ontario, Canada (45° 37'N, 76° 13'W). Snakes were captured by hand in late April and early May after they emerged from hibernation and before we observed mating in the wild. Snout–vent length (SVL), tail length and mass of each individual were measured and a blood sample taken for paternity analysis. Because tail length and SVL were correlated ($r=0.51$, $n=42$, $p=0.0006$), we used residuals from a regression of tail length and SVL as our measure of tail length. Each individual was marked using a passive integrated transponder (PIT) tag (Anitech Identification Systems, Markham, ON).

We established small mating groups in tanks indoors and larger mating groups in outdoor enclosures (details below). In mid to late June after mating ended, males were removed and released at their capture sites. Females in outdoor enclosures were brought indoors to give birth beginning in late July. Females mated indoors were housed in the same tanks until they gave birth. After giving birth, females were released at their capture sites. Females that did not give birth by mid-September were unlikely to be gravid and were released at their capture sites.

Mating experiments

Practical considerations required that we conduct experiments in which only one trait was varied systematically. We controlled effects of other traits either statistically or by minimizing variation in those traits.

Experiment 1 was designed to determine the effect of male size on reproductive success while minimizing variation in condition. Twelve females were housed individually in fiberglass tanks, each with three males that varied in size but were similar in condition. Two additional females were housed individually with two males that differed in size but were similar in condition. Condition was estimated using residuals from a regression of \ln mass on \ln SVL at the time of capture. Maximum difference in SVL between males in a mating group was 22 cm (equivalent to 85% of the range in SVL among mature males). Maximum difference in condition between males in a mating group was equivalent to 32% of the range in condition among mature males.

Experiment 2 was designed to determine whether tail length influenced male reproductive success, while minimizing effects of male size and condition. By minimizing size differences, this experiment also tested whether any size effects observed in experiment 1 were lost when there was less variation in male size. Each of 12 females was housed with 3 males that were similar in size and condition. Because size and tail length are correlated, controlling male size also reduced variation in tail length. Thus, we increased variation in tail length by using males with tails shortened due to previous injury in as many groups as possible (Shine et al. 1999). Poor performance by these males could result from having been injured rather than from having short tails per se, but if they perform well, long tails clearly would not be advantageous. Tail lengths varied between 8.3 and 22.9 cm (49% of the range among mature males). Maximum difference in condition between males in a mating group was 25% of the range in condition among mature males. Maximum difference in SVL was 30% of the range among mature males.

In the third experiment we varied adult sex ratios in mating groups in outdoor enclosures to determine how OSR affects male reproductive success and multiple mating. Four to twelve females (total $N=100$) were housed together with two to twelve males (total $N=120$), resulting in the OSR varying between 0.33:1 and 3:1 (total groups = 18). Males were randomly assigned to mating groups, without consideration of their phenotypes. A potential problem is that OSRs might not reflect breeding sex ratios because females usually skip years between reproductive bouts, so >50% of females in experimental groups were not likely to reproduce (Prosser et al. 2002). Furthermore, some mature males appear not to attempt to mate in some years (Brown and Weatherhead 2004). We considered neither of these issues to be problematic. Males regularly court females that are not reproductive (Prosser et al. 2002), so males appear to treat all females as reproductive, and all sexually mature males appear capable of reproducing (i.e., they produce sperm), so effort expended on mating may depend on the intensity of mating competition (Brown and Weatherhead 2004).

Besides these experiments, we housed females with males indoors to obtain offspring for another study. In multiple paternity analyses we use data from six of these litters produced by females housed with from two to five males each, and these plus an additional seven litters from females housed with one male each for analysis of stillbirths (see results section).

Housing conditions

Indoors, snakes were maintained in fiberglass tanks (74 cm \times 59 cm \times 55 cm) with carpeting or wood shavings. Each tank contained a water dish large enough to allow snakes to submerge. Heating sources were provided in all tanks and room temperature was maintained above 22°C (mean \pm SE = 24.4 \pm 0.4°C). Females were fed fish (minnows and smelts) ad libitum 1–3 times per week.

Six outdoor enclosures 5 m \times 6 m were constructed of sheet metal (75 cm high) painted to reduce reflection. Each enclosure had a pond (3 m \times 3 m \times 1 m) stocked with minnows from nearby marshes. Natural vegetation in and around ponds provided habitat similar to that used by watersnakes in the wild to thermoregulate (Brown and Weatherhead 2000). Perimeter electric fencing discouraged mammals from entering enclosures. Birds were deterred by flagging tied to monofilament line strung above enclosures. Details of construction can be found in Kissner and Weatherhead (2005). To reduce disturbance, we did not observe mating behavior, but instead used paternity assignments to determine mating patterns and reproductive success.

Genetic analysis of paternity

Blood samples were taken from the caudal vein using a 0.5 ml insulin syringe with a 28 gauge needle. Approximately 100 μ l of blood was taken from adults and 10 μ l from live neonates and stored in 800 and 500 μ l of Queen's Lysis Buffer (Seutin et al. 1991), respectively. A small sample of tissue was taken from stillborn neonates.

We assigned paternity to offspring using seven microsatellite DNA loci (Ns μ 2, 3, 6, 9b, 10, 110 and 119) used by Prosser et al. (2002). Probabilities of exclusion for each locus ranged between 0.21 and 0.82. We first genotyped each offspring, its mother, and all potential fathers at a single locus. After determining which offspring could be assigned to a single father, we genotyped remaining offspring, their mother, and potential fathers at other loci until all offspring could be assigned to a single father. Typically, two to four loci were sufficient to assign paternity.

Of 120 offspring for which paternity could not be assigned, 96 were from 7 litters in which none of the males housed with the females sired the litters. This probably resulted from those females mating prior to capture despite efforts to capture females before mating began. We estimated the minimum number of males that sired these

litters by counting paternal alleles at each locus for a litter and using the locus with the most paternal alleles (cf. [Blouin-Demers et al. 2005](#)). We excluded these litters from analyses requiring data on exact paternity or male reproductive success, but included them in analyses involving multiple paternity where appropriate. It is unlikely that offspring assigned to males from mating groups were sired by wild males with similar genotypes because the average exclusion probability for the four most commonly used loci was ≈ 0.99 ([Prosser 1999](#)).

For 12 offspring for which more than one male in the mating group could not be excluded as potential sires, we assigned paternity to the male that sired all other offspring in the same litter. Two other offspring could not be assigned because two males could not be excluded and both had sired offspring in the litter. We arbitrarily assigned these two offspring to the male that sired the most offspring in their litters. Ten offspring from four litters could not be assigned because their DNA samples could not be genotyped at most or all loci. For two of the four litters, paternity of unassigned offspring would not alter which male had sired the majority of the litter, so we left the offspring unassigned but included males from these two litters in analyses requiring the proportion of offspring sired. We excluded males from the other two litters from analysis.

Genetic relatedness

We determined relatedness of males and females after experiments were completed because there was inadequate time to produce genetic profiles between capturing snakes in the spring and establishing mating groups. We generated relatedness scores (r values; [Queller and Goodnight 1989](#)) using Kinship 1.2 software ([Goodnight and Quellar 1999](#)) for every male and female in each mating experiment pooled across years. In total, calculations were based on 194 individuals genotyped at four loci (2, 3, 6, and 10). We excluded the other loci because samples for many individuals did not resolve at all of them. Failure to resolve genotypes at one of those four loci precluded calculation of relatedness values for two individuals.

Analyses

We used paired t -tests to compare mean body sizes or tail lengths of successful and unsuccessful males within groups in experiments 1 and 2. We used groups rather than individuals because in small mating groups it was most appropriate to compare successful males to those they had competed against. In experiment 3 we used individual females as our unit of replication and used step-wise multiple logistic regression to compare attributes of males with which each female mated (successful) or did not mate (unsuccessful). Because numbers of males in mating groups varied, larger mating groups could bias our analysis of mating success by contributing more observations. To equalize contributions we used mean attributes of successful and unsuccessful

males in analyses. Because results were qualitatively the same using means for raw attribute values and using values relative to those of other males in a mating group, we present results only using raw values.

We restricted analysis of size-assortative mating to enclosure mating groups because both males and females had access to more than one potential mate. Within groups, we ranked each individual on the basis of its size relative to other individuals of the same sex. To control for variation in group size, we converted raw ranks to rank percentages with the formula: $[(x-1)/(n-1)] [100]$, where x is the raw rank score and n is the number of snakes ranked. Thus, the smallest snake always received a rank percentage of 0, the largest snake a rank percentage of 100, and the modal individual a rank percentage of 50, regardless of the number of snakes ranked. For polyandrous females, we scored the size of her mates as the mean of their adjusted ranks. We calculated the Pearson Product-moment correlation between adjusted ranks of females and their mates ([Iman and Conover 1979](#)).

An association between female size and polyandry could result from large females mating with more males, or their larger litters creating opportunities for more males to sire offspring. We assessed these alternatives by including both litter size and maternal SVL in an ANOVA with number of sires as the dependent variable. We first included all females to test the mating hypothesis and then included only females that produced multiply sired litters to test the fertilization opportunity hypothesis ([Prosser et al. 2002](#)).

Analyses were carried out using SAS ([SAS 1999](#)) software. We assessed whether data met assumptions of tests and transformed variables when necessary. If transformations did not allow data to meet assumptions of parametric tests, we used non-parametric analyses. We report only Type III SS for variables of interest. Goodness-of-fit of logistic models to the data was examined using the Hosmer and Lemeshow test ([Hosmer and Lemeshow 2000](#)). Significance was assessed at $\alpha=0.05$.

Results

Over 3 years, 53 litters produced 835 live offspring, 92 offspring that were either stillborn or partly developed, and 100 unfertilized follicles. We assigned paternity to 821 of the 927 offspring (88.6%; see methods section).

Male mating success

In experiment 1, in which male size was varied, 6 of 14 females gave birth. All males in one mating group were successful, precluding comparison of successful and unsuccessful males, and in another, none of the males in the group sired the offspring (see methods section). In the remaining groups, successful males were larger than unsuccessful males (paired $t=3.71$, $df=3$, $p=0.03$, [Fig. 1](#)).

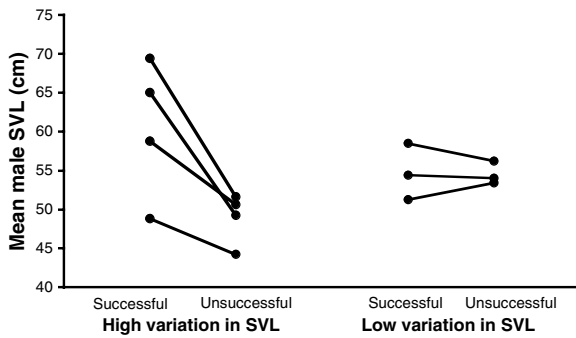


Fig. 1 Mean size (SVL) of male northern watersnakes that were successful or unsuccessful in siring offspring. Values are means of males in the same mating group and lines join males from the same mating group. Results are shown for Experiment 1 (see text) in which size variation among males was high and for Experiment 2, where size variation was low

In experiment 2, three females produced litters sired by group males. Mean relative tail length did not differ between successful and unsuccessful males (paired $t = -1.95$, $df = 2$, $p = 0.19$). Because variation in SVL was much lower in this experiment than in experiment 1 (30% vs. 85% of the range in SVL among all mature males), it also provided a test of whether the size advantage observed in experiment 1 occurred when variation in SVL was reduced. Successful males were not bigger than unsuccessful males (paired $t = 0.14$, $df = 2$, $p = 0.90$, Fig. 1). Thus, when size variation was reduced, size no longer affected male success.

In experiment 3 (enclosure matings), mean male SVL was not correlated with mean relative tail length ($r = -0.05$, $n = 50$, $p = 0.75$) or mean condition ($r = -0.01$, $n = 50$, $p = 0.96$). However, mean relative tail length was negatively correlated with mean condition ($r = -0.35$, $n = 50$, $p = 0.01$). Given results from experiment 2 showing no effect of tail length, we removed tail length from this analysis. Step-wise multiple logistic regression indicated that only SVL affected male success (mean SVL: $\beta = 0.12$, $X^2 = 4.34$, $df = 1$, $p = 0.04$; mean condition: $X^2 = 0.67$, $df = 1$, $p = 0.41$). Again, successful males were larger than unsuccessful males.

Results from experiment 3 supported the prediction that male traits should have a greater effect on male success when competition was more intense. In groups with male-biased OSR (3:1), SVL but not condition of males was important in determining success (mean SVL: $\beta = 0.43$, $X^2 = 7.92$, $df = 1$, $p = 0.005$; mean condition: $X^2 = 0.47$, $df = 1$, $p = 0.49$, Fig. 2). In groups with female-biased OSR (0.33–0.40: 1), neither SVL nor condition was associated with the probability of success (mean SVL: $X^2 = 0.01$, $df = 1$, $p = 0.90$; mean condition: $X^2 = 0.20$, $df = 1$, $p = 0.66$, Fig. 2).

In contrast to our prediction that body size and tail length might influence the proportion of offspring sired in multiply sired litters, proportion of offspring sired did not vary with male size ($r = 0.14$, $n = 38$, $p = 0.39$) or with relative tail length ($r = 0.12$, $n = 38$, $p = 0.48$) in experiment 3. Sur-

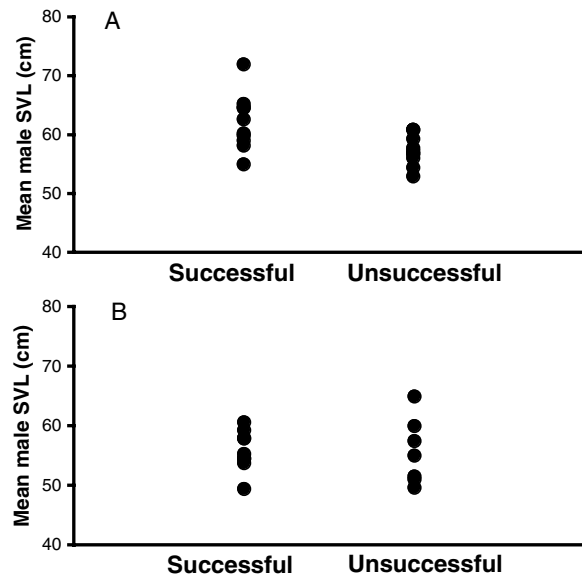


Fig. 2 Mean size (SVL) of male northern watersnakes that were successful or unsuccessful in siring offspring in mating groups in outdoor enclosures when operational sex ratios (OSR) were (A) male biased or (B) female biased. Values are means of males in the same mating group

prisingly, males in poorer condition sired a greater proportion of offspring ($r = -0.32$, $n = 38$, $p = 0.05$). Absolute number of offspring sired was not associated with male size ($r = 0.16$, $n = 38$, $p = 0.35$), residual tail length ($r = 0.06$, $n = 38$, $p = 0.71$), or condition ($r = -0.24$, $n = 38$, $p = 0.15$). Sperm depletion did not appear to conceal associations between male traits and relative success in multiply sired litters because we found no association between the proportion or absolute number of offspring sired in one litter and that in a second litter (proportion: $r = 0.21$, $n = 9$, $p = 0.58$; absolute number: $r = 0.45$, $n = 9$, $p = 0.23$), for males siring young in two litters.

Genetic similarity did not influence male success ($X^2 = 0.13$, $df = 1$, $n = 48$, $p = 0.71$). Similar ranges of relatedness between females and successful (-0.30 – 0.42) and unsuccessful (-0.30 – 0.52) males suggests that failure to find an effect of relatedness was not simply a consequence of all mated pairs being outbred. In multiply sired litters, the proportion of offspring sired ($r = 0.04$, $n = 38$, $p = 0.79$) and number of young sired ($r = -0.12$, $n = 38$, $p = 0.46$) also did not vary with genetic similarity between parents.

Size-assortative mating

Across all mating groups from enclosures, male size was not associated with the size of females with which they mated ($r = 0.07$, $n = 26$, $p = 0.73$). As predicted, however, there was no evidence for size-assortative mating when OSR was male-biased ($r = -0.55$, $n = 10$, $p = 0.10$, Fig. 3), but snakes mated assortatively when OSR was female-biased ($r = 0.66$, $n = 9$, $p_{1-tailed} = 0.026$, Fig. 3).

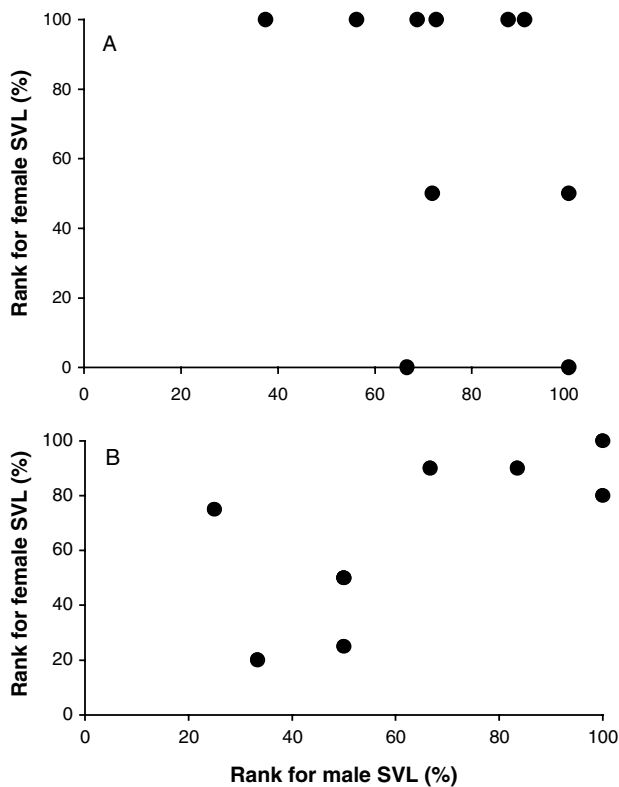


Fig. 3 Relationship between size of female northern watersnakes and the size of their mates at (A) male-biased and (B) female-biased operational sex ratios (OSR). Sizes are shown as ranks (based on SVL) of individuals of the same sex in the same mating group (see methods section)

Multiple paternity

We detected multiple paternity in 25 of 46 (54.3%) litters from mating groups in which females had the opportunity to mate with multiple males (41.2% for indoor females and 62.1% for enclosure females). All multiply sired litters were sired by two or three males. The mean proportion of offspring fathered by a single male in multiply sired litters was 0.42 (± 0.05) (range 0.03–0.97).

Larger females were more likely to produce litters sired by multiple males ($\beta=0.11$, $X^2=4.68$, $df=1$, $n=46$, $p=0.03$, Fig. 4), and females in poorer condition tended to produce multiply sired litters ($\beta=-4.76$, $X^2=3.04$, $df=1$, $n=46$, $p=0.08$). Multiply sired litters had an average of six more offspring than singly sired litters ($t=2.85$, $n=46$, $p=0.01$). When both litter size and maternal SVL were included in an ANOVA, neither maternal size nor litter size explained variation in number of sires (SVL: $F=1.39$, $df=1$, 40, $p=0.24$; litter size: $F=1.59$, $df=1$, 40, $p=0.22$). Similarly, when the analysis was restricted to females that produced multiply sired litters, neither maternal size nor litter size explained variation in number of sires (SVL: $F=0.79$, $df=1$, 19, $p=0.39$; litter size: $F=1.24$, $df=1$, 19, $p=0.28$). The proportion of litters that were multiply sired did not vary either with OSR ($r=-0.45$, $n=12$, $p=0.14$) or with the number of males in mating groups ($r=0.03$, $n=12$, $p=0.93$).

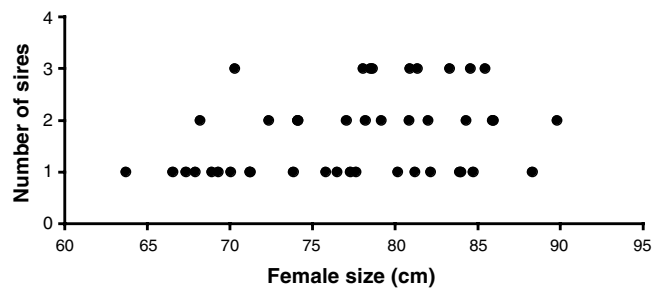


Fig. 4 Relationship between size (SVL) of female northern watersnakes and the number of males that sired their litters, for mating groups in outdoor enclosures

Of 1027 “offspring”, 100 (9.7%) were unfertilized ova and 92 (9.0%) were stillborn. The mean proportion of unfertilized follicles did not differ between females whose litters were singly or multiply sired ($t=-0.46$, $df=51$, $p=0.65$). The mean (\pm SE) proportion of stillborn offspring was higher ($t=2.43$, $df=51$, $p=0.02$) in multiply sired (0.13 ± 0.04) than singly sired (0.03 ± 0.01) litters. Even after removing two litters in which more than 50% of offspring were stillborn, there were more, though not significantly, stillborn offspring in multiply sired litters ($t=1.84$, $df=49$, $p=0.07$).

Discussion

Male mating success

Larger males were generally more successful than smaller males, but only when OSR was male biased and when variation in male size was extensive. Male size, tail length, genetic similarity with females or fertilization success in other litters did not affect the proportion of offspring sired by a male in multiply sired litters. Males in poorer condition had higher fertilization success. Because condition was based on measurements made at the start of the experiments, this result cannot be explained by mating effort (i.e., high-mating effort increasing fertilization success at the expense of condition). This result is inconsistent with results from free-living watersnakes (Weatherhead et al. 2002) and warrants further investigation.

The conditional nature of the size advantage in mating may explain inconsistency from non-experimental studies of northern watersnakes in which larger males had an advantage only in some cases (Weatherhead et al. 1995; Brown and Weatherhead 1999; Weatherhead et al. 2002). Weatherhead et al. (1995) found that large male watersnakes in the wild had higher mating success only when the OSR was more male-biased. Similar conditional effects of size on male mating success have been reported for other snake species in the wild (Madsen and Shine 1992; Shine et al. 2000). Experimental studies with red-sided garter-snakes (*Thamnophis sirtalis*) found that a large male mating advantage was affected by the OSR, but interestingly, was more apparent when the OSR was less male biased

(Joy and Crews 1988; Shine et al. 2000). Results for watersnakes and gartersnakes are not as different as they appear, however. For gartersnakes, the advantage of large size was weak or absent at extreme OSRs (e.g., 24–48 males per female), but was strongest when the OSR was similar to our male-biased treatments. As the number of males attempting to mate with a single female becomes extreme, stochastic factors presumably weaken or eliminate the advantage of large size (Shine et al. 2000).

As predicted from free-living snakes (Weatherhead et al. 2002), we found size-assortative mating when the OSR was female biased. We assumed that all males prefer large females (e.g., Shine et al. 2001) and that large males have a competitive advantage over small males, which we confirmed to be the case. Therefore, it appears that small males had reduced access to females when competition was intense, but gained access to smaller females under less-competitive conditions. In the wild small males may be less disadvantaged, however, because some very small males obtained paternity when competition among males was high (Weatherhead et al. 2002). Presumably, attributes such as highly active searching (Weatherhead et al. 2002), combined with chance, sometimes allow small males to locate unattended, receptive females.

Polyandry

Among females that reproduced that were housed with multiple males, 54% produced litters sired by more than one male, similar to the frequency (58%) in the wild (Prosser et al. 2002). Also as in the wild (Prosser et al. 2002), larger females were more likely to be polyandrous. We were unable to determine whether the association between female size and polyandry resulted from large females mating with more males or from their larger litters providing more opportunities for paternity, and thus for polyandry. Evidence from wild females suggests both factors contribute to the pattern (Prosser et al. 2002).

Variation in OSR did not affect the incidence of polyandry, consistent with results from free-living watersnakes (Prosser et al. 2002), suggesting that polyandry is not simply a function of opportunity, where females wait passively for males to court them. In free-living black ratsnakes (*Elaphe obsoleta*), males and females are dispersed during the mating season and reproductive females move more than non-reproductive females (Blouin-Demers and Weatherhead 2002). Consistent with movement functioning to promote polyandry, most ratsnake clutches are sired by multiple males (Blouin-Demers et al. 2005). If females actively promote polyandry, polyandry might increase with female size because larger females choosing to mate with more males, or because their larger litters provide more opportunities for multiple paternity. There is no reason to expect the former explanation to be true (i.e., polyandry would have to be more beneficial to large females), whereas there is some support for the latter explanation (Prosser et al. 2002).

In species with little opportunity for females to benefit directly from multiple mating, polyandry is hypothesized to enhance genetic quality of resulting offspring (Jennions and Petrie 2000). Among reptiles, such benefits have been observed in inbred populations of adders (Madsen et al. 1992) and lizards (Olsson et al. 1994), and Blouin-Demers et al. (2005) found higher hatching success in multiply versus singly sired clutches of black ratsnakes. Polyandrous mating may also involve costs, such as increased exposure to disease (e.g., Lombardo 1998, 2000; Westneat and Rambo 2000) that could be detrimental to developing offspring. Prosser et al. (2002) found no association between the incidence of stillborn young and multiple paternity, whereas we found more stillborn young in multiply sired litters. The higher incidence of stillbirths in our study is unlikely to be associated with rearing conditions because Weatherhead et al. (1998) found no difference in stillbirths between watersnakes housed indoors and in the wild. Thus, more stillbirths in multiply sired litters remains an interesting but unexplained pattern. If the pattern reflects a cost of polyandry, however, it is noteworthy that we found some evidence that offspring from multiply sired litters were more likely to survive winter (Kissner, Weatherhead and Gibbs, unpublished data), so polyandry may provide female watersnakes benefits that compensate for any costs.

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