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Sex ratio shifts within litters of meadow voles (*Microtus pennsylvanicus*)

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Summary. Changes in the sex ratio of juvenile recruits into a population of meadow voles (*Microtus pennsylvanicus*) were correlated with shifts in the weight and mortality of pups within the population. The biased recruitment of female juveniles in the spring was reflected in differential allocation of energy within the litters, as measured by female pups being heavier than male pups ($n = 245$). In the fall, the shift in recruitment to male juveniles was reflected within litters by male pups being heavier than female pups ($n = 139$). Nestling mortality showed a similar gender bias. Skewed sex ratios were most evident within the litters of larger mothers, indicating the gender bias was not triggered by energy limitations. We postulate that gender differences in social spacing and behavior result in spring/fall fluctuations in the reproductive success of offspring, based on their gender.

Introduction

A facultative sex ratio has been predicted in response to maternal energy stress, resulting in a bias for the "less expensive" sex (Myers 1978), or the most "reproductively successful" sex (Trivers and Willard 1973). Clutton-Brock et al. (1981) proposed that examples of sex ratio biases are not the result of maternal manipulation, but rather the outcome of differential energy expenditures by males and females, which result in differential mortality during periods of energy limitations.

Some examples of sex ratio biases have been reported within populations of small mammals (Kalela 1971; Kaufman and Kaufman 1982). Michener (1980) reported differences in the fitness of female Richardson's ground squirrels that were correlated with the sex ratio of the offspring they produced. Pregnant bank voles (*Clethrionomys glareolus*) brought into the lab have shown litter sex ratios that shifted from a female to male bias with increasing population density (Nanmov et al. 1969). Within the genus *Microtus,* female-biased recruitment has been reported prior to periods of population increase (Myllymaki 1977; Hansson 1978), while male-biased populations have been noted during population highs (Myers and Krebs 1971; Chelkowska 1978; Jannett 1981). Fall populations have shown male-biased recruitment (Madison et al. 1984) and increased survival of juvenile males (Getz 1972).

Field data collected to date are not necessarily good evidence for facultative sex ratios in small mammals because these skewed sex ratios could have resulted from parental manipulation, differential mortality after dispersal, or simply differential trappability. In fact, Williams (1979) found no evidence for adaptive shifts in the prepartum sex ratio of mammals. Myers and krebs (1971) found no evidence for sex ratio biases at birth in *Microtus pennsylvanicus* or *Microtus ochrogaster*. They attribute reports of sex ratio biases for these two species as the result of differential mortality, growth, and trappability after weaning.

Laboratory data, on the other hand, indicate the potential for facultative sex ratios in the field. McClure (1981) demonstrated, in wood rats (*Neotoma floridanus*), a postpartum maternal preference for female offspring during periods of energy limitations. Golden hamsters (*Mesocricetus auratus*) have demonstrated a prepartum female-biased sex ratio under an energy-restricted diet (Labov et al. 1985). To test whether maternal manipulation of sex ratio occurs in microtines in the field, the allocation of energy to offspring before they disperse from the nest must be measured. We report the occurrence of gender-biased recruitment into a population of *Microtus pennsylvanicus*. We also examine both pre- and postpartum periods of maternal care for signs of gender preference, as indicated by differences in the relative weight of the pups within litters, and by the mortality suffered by each gender.

Methods

A 0.63 ha trapping grid was set up within a 100 ha field in Brackney, Pa., 25 km south of Binghamton, N.Y., in the spring of 1983. The trapping grid was 70 × 90 m (0.63 ha) with trap stations placed at 10 m intervals. During July 1983 the trapping grid was shifted 150 m within the field due to mowing operations. The second grid contained the same trap spacing and was 50 × 130 m (0.65 ha).

Live trapping was conducted one day a week from March to July, and from mid-August to late October during 1983 and 1984. Each trap station consisted of two single capture traps (Spencer), and all stations were checked four times over a 10-h period. Captured animals were weighed, sexed, marked (toe-clipped), and checked for reproductive condition (testes scrotal, vagina perforate, teats swollen, visible pregnancy). Since the body weight of female voles fluctuates over the course of each pregnancy, the maternal weight of the female, for each litter located, was considered the weight at first capture after parturition.

During the trapping periods, between 15 and 25 reproductively active females were impanted with radiotransmitters using standard techniques (Madison et al. 1985; McShea 1985). These implanted females were radiotracked hourly, for a 10-h period, one day a week. After each weekly telemetry session, the located nests were opened and the pups were removed. The pups were marked (toeclipped), weighed, counted, and their anal-genital length recorded. The pups were returned to the nest usually within 2 min of removal. Females found to be pregnant during the weekly trapping census were located on additional days of the week, in order to record each litter as soon after birth as possible. Due to the pattern of trapping and telemetry, we will refer to "spring" and "fall" to denote the major periods of observation each year. Trapping was conducted on a once-a-month basis between these periods. Because of the similarity in habitats and proximity of the two trapping grids, the data collected for the two are combined for analysis.

Pups within each nest were ranked by weight. The rank of pups, for those pups seen more than once over the nesting period, was consistent between checks ($r = 0.72$). A rank of one was given to the heaviest pup, a rank of two to the second heaviest pup, and so on. For pups that were equal in weight, one pup was randomly assigned the higher rank. When the tied pups differed in their gender, we alternated the gender assigned the higher ranking. Occasionally, data were combined for two adjacent ranks of pups to increase sample size for analysis (all these occurrences are mentioned in the text).

For 36 litters, we rexamined the pups 4–9 days following their initial examination. All reexamined litters contained pups prior to eye-opening, and were still completely dependent on their mothers.

In order to estimate prepartum sex ratio, each month of the study on the live-capture grids was matched with the snap-trapping of females on capture lines set up within similar habitats between 60 and 100 m from the study grid (see McShea 1985). For mature embryos (greater than 13 days gestation) it was possible to determine their sex through the positioning and morphology of the embryo reproductive tract. The weight of the mothers was determined by subtracting the weight of embryos and uterus from whole body weight.

The anal to genital distance (A–G length) is used for sex identification of juvenile meadow voles. Females have a significantly shorter A–G length than males (Twigg 1975). In neonates the A–G length changes as the pups mature, so the A–G length was divided by the weight of the pup to give a relative measure. A total of 199 measurements taken of pups were later verified by capture of the marked juveniles in the weekly census. The A–G lengths of these pups of known sex were used to generate a discriminate function equation ($F_{1,190} - 70.4$, $P < 0.0001$, average squared canonical correlation $= 0.27$) to classify pups by gender based on their relative A–G length. The designation of an incorrect sex to a pup was random with respect to the weight and sex of that pup (see McShea 1985).

Results

The sex ratio of juvenile voles (< 25 g) recruited into the trappable population changed significantly over the course of the study (Table 1). More female juveniles were recruited in the spring, while more male juveniles were recruited in the fall. This difference was not due to shifts in the prepartum sex ratio, as there was no significant shift from spring to fall in embryo sex ratio (% males $= 45$ and 40 respectively; $n = 155$ and 125; χ^2 test, $P > 0.1$). The shifts must therefore occur between birth and first capture.

If the skewed sex ratio of the recruits arises before weaning, then differences in energy allocation to the young should appear before weaning. Energy allocation was measured through comparisons of the relative weight of pups within litters. A comparison of heavy vs. light pups within litters showed seasonal differences, with females being heavier than males in spring litters and lighter than males in fall litters (Table 2).

It is also possible to measure energy allocation by examining litters which have a sex ratio that is "naturally" biased. If the prepartum sex ratio is random, then by chance some females may give birth to litters of predominately one gender. The "value" of a particular sex to the female can be determined by reviewing litters with just one male

Table 1. The sex ratio (% males) of all juveniles (less than 25 g) recruited into the study population. Sex ratios of the spring and fall seasons (1983 and 1984 combined) were significantly different ($\chi^2 = 6.76$, $df = 1$, $P < 0.01$). The 509 juveniles captured included 153 pups that were marked as pups (15 of which were captured at over 24 g)

	1983		1984	
	Spring	Fall	Spring	Fall
% males	38.5	70.5	33.0	50.5
No. of recruits	131	157	50	161

Table 2. The sex ratio (% males) of the pups within each rank for litters of the fall and spring season. In parentheses is the number of pups within each rank. For this analysis the ranks of pups were combined, with the highest rank representing a combination of the heaviest and second heaviest within each litter. The second rank is the third and fourth heaviest pup within each litter, and the third rank represents all pups ranked below the fourth pup within each litter. Excluded from analysis were all litters where one sex made up less than 25% of the pups. Females are more likely to be the heaviest pups in the spring ($\chi^2=4.22$, $df=1$, $P<0.05$) and the lightest pups in the fall ($\chi^2=5.07$, $df=1$, $P<0.05$)

	Rank 1	Rank 2	Rank 3
Spring	31 (92)	46 (81)	66 (72)
Fall	57 (62)	52 (51)	22 (26)

Table 3. The rank of pups within 35 litters (based on weight) where they represent the only pup of one gender. Excluded are all litters with less than three pups. The expected distribution of pups within each rank was calculated by summing the number of pups within each rank and dividing by the total number of pups. The observed ranks of the only sex pups were significantly different from random expectation ($\chi^2=6.49$, $df=2$, $P<0.05$). Eight of the eleven males ranked first occurred in the fall and six of the eight males ranked last occurred in the spring. The observed female ranking did not differ significantly from that predicted (χ^2 test, $P>0.05$). Mean litter size for this sample was 4.71 ± 0.33 SE

	Expected	Observed		
		Total	Male	Female
Ranked first	0.30	0.46	11	5
Ranked middle	0.43	0.23	2	6
Ranked last	0.27	0.31	8	3

Table 4. The sex ratio (% males) of pups for each season based on the weight of the mother. The division between heavy and light females was determined by the median maternal weight for all litters. The sample set for each sex ratio is enclosed in parentheses. Fall litters show a significant difference in sex ratio based on the weight of the mother ($\chi^2=9.6$, $P<0.01$)

	Light females (≤ 43 g)	Heavy females (>43 g)
Spring	36.0 (131)	39.5 (215)
Fall	52.0 (139)	23.5 (119)

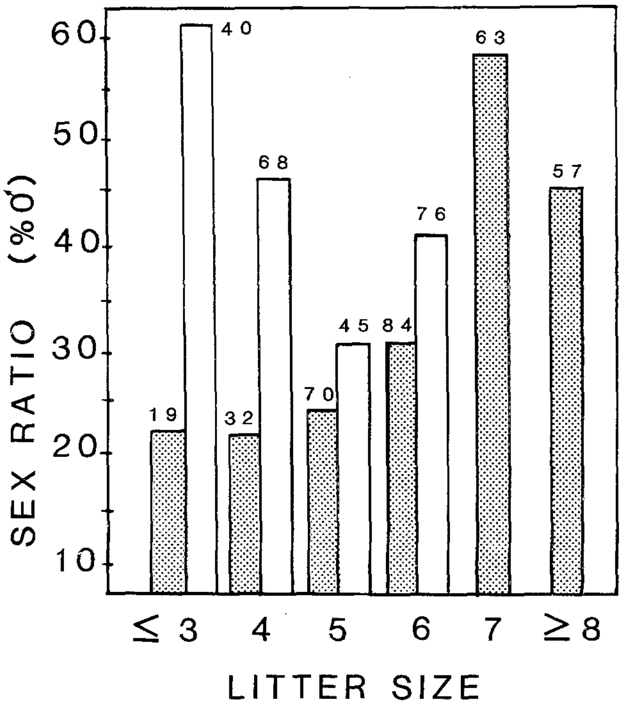

Fig. 1. The sex ratio (% males) of litters from large (dark bars) and small (light bars) mothers (division at 43 g). The sample size is represented within each bar. For litter sizes less than 5, there is a significant difference between the pup sex ratio for large and small mothers ($\chi^2=7.12$, $P<0.01$). The sample set for litter size 6 includes larger litter sizes for the small mothers

or female, but several offspring of the opposite sex. The single offspring was ranked significantly higher or lower within the litter than would be predicted by chance (Table 3). This difference was due to the male offspring being ranked either higher or lower than predicted. The difference in rank was related to season: males were generally ranked lower than expected in the spring population and higher than expected in the fall population.

The ultimate measure of "value" placed on an offspring would be the degree of mortality experienced by each sex prior to dispersal form the nest. Of the 11 litters that experienced mortality between nest examinations, and were originally composed of individuals from both sexes, the majority of the fatalities (10 out of 15 pups) involved the lightest member of the litter. The remaining five fatalities involved one female pup that disappeared in the fall and four male pups that disappeared in the spring. For all the disappearances, seven of the eight male fatalities occurred in the spring (when female recruitment was higher), while four of the seven female fatalities occurred in the fall (when male recruitment was higher).

The data indicate that variations in recruitment into the trappable population are consistent with the weight and mortality of each sex within its litter. The variation in recruitment appears to follow a seasonal trend of increased female recruitment in the spring and increased male recruitment in the fall (Table 1). If energy shortages to lactating females determine the onset of these sex ratio biases, a division of the data set based on the median maternal weight (43 g) should show "light" females have a more biased litter than "heavy" females. During the postpartum period, a comparison of heavy and light females showed no significant difference in the spring. During the fall, the heavy females demonstrated a sex ratio bias toward females that was not evident in the litters of the light females, an apparent contradiction to the prediction that lighter females should respond sooner to energy shortages (Table 4).

Prepartum sex ratio was not affected by the weight of the female (% males $=48$ and 41 for light and heavy females; $n=96$ and 184 respectively; χ^2 test, $P>0.1$), so the sex ratio shifts probably occur postpartum. An examination of the sex ratio of litters belonging to heavy females shows that decreasing litter size results in an increase in the percentage of females in the litter (Fig. 1). Large females that were found to have small litters show a preference toward "saving" female offspring as opposed to male offspring. Light females do not show the same trend, indicating that energy limitations do not trigger the sex ratio biases seen in *Microtus pennsylvanicus.*

Discussion

This study suggests the existence of a facultative sex ratio in meadow voles, although without direct observations of maternal behavior, it is impossible to determine whether material manipulation occurs. However, sex ratio biases do arise between birth and weaning and these biases show seasonal shifts. Assuming maternal manipulation occurs, we are not suggesting maternal culling of one sex, but rather under conditions of limited energy resources, a selective allocation of that energy by the mother.

The observed biases are not in agreement with present theories based on energy limitations. If energy limitations result in a female bias due to increased male mortality (Clutton-Brock et al. 1981) or to the production of the "cheaper" sex (Myers 1978), then occurrence of a female bias in the litters of large mothers, and not in those of small mothers, can only be explained if large mothers are more energy limited. However, we have found no evidence for a negative correlation between pup growth rate and maternal weight (see McShea 1985). The existence of male-biased litters agrees with McGinley's (1984) proposal that decreased litter size may result in the "excess" energy being shunted into more costly male offspring, but his proposal would fail to explain the female bias in the spring. Theories based on energy costs do not predict sex ratio biases in both directions. Our results would agree with predictions based on changes in reproductive success (Trivers and Willard 1973) or gender-specific mortality (Werren and Charnov 1978).

Age-structured populations, with overlapping generations, should exhibit facultative sex ratios in response to changing mortality or reproductive patterns that are gender specific (Werren and Charnov 1978; Werren and Taylor 1984). We maintain that gender differences in social spacing and behavior will result in shifts in the onset of reproduction for each sex. Any gender-specific shifts in reproduction (without equal changes in mortality) should result in females "favoring" those pups that will breed sooner.

Male and female meadow voles differ with respect to social spacing. Field studies on *Microtus pennsylvanicus* indicate female territoriality and polygynous mating (Madison 1980; Webster and Brooks 1981;, Boonstra and Rodd 1983). Assuming a social system that involves non-overlapping female territories and male competition for females, a change in density should have different ramifications for each sex. Space limitations would prevent new females from settling within a population, while an inability to gain access to reproductive females should not necessarily hinder male settlement. Female offspring at the onset of the reproductive season (i.e. low densities) would have less difficulty acquiring territories than would female offspring later in the reproductive season, who would have to compete with their mother and other larger females for space. Male offspring produced under both high and low densities would not compete with their mother for space.

The social organization of the species may also result in shifts of breeding age for each gender. With the onset of reproduction after a period of winter mortality, female offspring produced will have access to vacant breeding territories, while male offspring produced will be competing against larger overwintering males and should be less successful at mating. Therefore, females produced at the onset of reproduction should reproduce early, while males produced should have delayed reproductive success.

During the fall this argument would be reversed. The predominance of spring matings by large overwintering males means that a female who produces a son in the fall with be the ancestor of more offspring the following year than if she had produced a daughter (assuming both sons and daughters survive the winter). Juvenile males may even have a higher overwinter survival rate, since it appears they are more tolerated within the winter communal groups (Madison et al. 1984). Thus, based on gender shifts in the time until breeding, females should demonstrate male-biased litter production in the fall and female-biased litter production in the spring.

Why don't large fall mothers produce more male offspring as predicted? Large fall females are most likely females that bred during the previous spring and are reaching the end of their lifespan, making future competition with daughters unlikely. Large females also continue reproducing into the winter (Krebs et al. 1973); if reproduction means the continuation of solitary nesting patterns, the production of male offspring for the formation of winter communal groups may not be important. Finally, territory quality has not been examined in meadow voles, but if increased body size means an increase in the size and quality of the female's territory, a large mother may compete less with the female offspring who remain within her territory than would a small mother. In summary, large fall females may not fit our predictions due to high energy resources and a low probability of surviving until spring breeding.

Several of the hypotheses we have developed to support a shift in nestling sex ratio involve population density. In general, we predict a low density should favor the production of females and a high density the production of males. Our data were not analyzed with respect to population density because, over the period of our study, density and season varied simultaneously. We do not know what effect a high density during the spring, or low density during the fall, would have on our results, but we feel at this point a better case can be made for an explanation based on seasonal shifts. Shifts in offspring sex ratio should affect population density by accelerating and decelerating population growth (see Myers and Krebs 1971). We have discussed nestling sex ratios from the standpoint of a female altering her maternal behavior in order to increase her fitness in a changing environment. It should be noted that the environment, in turn, may be altered by her response, resulting in oscillations of population growth and decline.

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