

# REPRODUCTIVE CHARACTERISTICS OF THE MALE GRIZZLY BEAR IN THE CONTINENTAL UNITED STATES

DON WHITE, JR., Department of Biology, Montana Tech of the University of Montana, Butte, MT 59701, USA, email: dwhite@po1.mtech.edu

JAMES G. BERARDINELLI, Department of Animal and Range Sciences, Montana State University-Bozeman, Bozeman, MT 59717, USA, email: jgb@gemini.oscs.montana.edu

KEITH E. AUNE, Montana Department of Fish, Wildlife, and Parks, Wildlife Laboratory, Montana State University-Bozeman, Bozeman, MT 59717, USA, email: fwpka@trout.oscs.montana.edu

**Abstract:** We evaluated testicular growth, seminiferous tubule development, and age of sexual maturity in 20 male grizzly bears (*Ursus arctos horribilis*) killed in Montana and Wyoming between 1978 and 1992. Seminiferous tubule diameter did not differ among the regions of each testicle sampled. Testicular mass was related linearly to age. Seminiferous tubule diameter was related non-linearly to age. Mean testicular mass, volume, and seminiferous tubule diameter were smaller in immature bears than in mature bears. Based upon the presence or absence of spermatozoa in the lumen of the seminiferous tubules, sexual maturity in grizzly bears from the continental United States is attained at approximately 5.5 years of age.

*Ursus* 10:497-501

**Key words:** grizzly bear, Montana, reproduction, sexual maturity, testicular growth, *Ursus arctos*, Wyoming.

Bears in northern temperate environments have a well-defined breeding season extending from May until early July. During the breeding season, testicular weights are higher than any other time of year. By September, testicles regress in size and by late September azoospermia occurs, although spermatozoa may be present in the epididymides (Erickson et al. 1964, 1968; Pearson 1975; Horan et al. 1993; Garshelis and Hellgren 1994). Testicular regression continues throughout the non-breeding season. Although the pattern or extent of regression differs among individual bears, by 1 October, the diameter of the seminiferous tubules is markedly decreased (Erickson et al. 1964, 1968; Pearson 1975; McMillin et al. 1976; Reynolds and Beecham 1980; Tsubota and Kanagawa 1989). By mid- to late October or early November, testicular regression is nearly complete; by mid-November the testicles are infiltrated by adipose tissue and loose, fibrous, connective tissue (Erickson et al. 1964, 1968; Pearson 1975). The tubules at this time are small and show no lumen, and the germinal epithelium consists only of Sertoli cells (Erickson et al. 1964, 1968). Testicular weights in the early winter months are the lowest found in the mature bear throughout the year (Erickson et al. 1964, 1968; Pearson 1975).

Testicular recrudescence, which is well underway before emergence from winter denning (Erickson et al. 1964, Palmer et al. 1988, Garshelis and Hellgren 1994), generally involves seminiferous tubule enlargement and increased Leydig cell activity (Erickson et al. 1964, 1968; Pearson 1975). Active Leydig cell development and spermatogenesis, with the presence of spermato-

zoa in the epididymides, have been noted in bears killed in late May and early June to the middle or end of July (Erickson et al. 1964). Fully formed spermatozoa are present in seminiferous tubules and epididymides at least 1 month before and several months after the seasonal period of estrus in females in the European brown bear (*U. a. arctos*, Dittrich and Kronberger 1963), American black bear (*Ursus americanus*, Erickson et al. 1964), Alaskan brown bear (*U. a. middendorffi*, Erickson et al. 1968), grizzly bear (Pearson 1975), and the Hokkaido brown bear (*U. a. yesoensis*, Tsubota and Kanagawa 1989).

Although we have a general understanding of male brown bear (*U. a. spp.*) gonadal activity, most of our knowledge regarding the breeding activity of male brown bears is based on empirical observations. Few studies have characterized male brown bear reproductive biology, including testicular growth and spermatogenesis. Currently, no data are available for testicular growth and age of sexual maturity of grizzly bears in the continental United States.

Nearly all grizzly bears killed in the continental United States are necropsied at the Montana Department of Fish, Wildlife, and Parks (MDFWP) Wildlife Laboratory in Bozeman, Montana. Due to the lack of information on male grizzly bear reproductive physiology and the fortuitous, although limited, availability of male reproductive tracts, we sought to: (1) evaluate testicular growth and seminiferous tubule development in grizzly bears  $\leq 15$  years of age, and (2) estimate age of sexual maturity in male grizzly bears from Montana and Wyoming. Although our conclusions are limited

by small sample sizes, we present the only information available on testicular histology of male grizzly bears in the continental United States.

This study was funded by the Peter W. Busch Family Foundation, Montana Department of Fish, Wildlife, and Parks, and the Department of Animal and Range Sciences, Montana State University—Bozeman. We thank M. Ellingson, P. Hardiman, and A. Allingham for their technical assistance.

## METHODS

Testicles from 20 male grizzly bears were collected from bears killed by Federal and State wildlife personnel or hunters in Montana and Wyoming from 31 May 1978 to 27 August 1992. Age of each bear was estimated by counting the cementum annuli of extracted premolars (Willey 1974, Coy and Garshelis 1992). As testicle collections were fortuitous, it was not possible to obtain specimens for all ages and seasons.

Usually within a few hours after death, the testicles of each bear were removed from the scrotum, dissected free of the tunica vaginalis, fixed in neutral-buffered formalin for several days, then stored in either 10% formalin or 70% ethanol at the MDFWP Wildlife Laboratory for  $\leq 10$  years. Tissue shrinkage and suboptimal staining are known to occur by prolonged storage in fixative (G. Callis, Dep. Vet. and Molecular Biol., Mont. State Univ., Bozeman, pers. commun., 1996). No corrections for shrinkage were made on our data.

Each testicle was weighed without the epididymis to the nearest 0.001 g using a Mettler PJ 300 digital balance (Mettler Instrument Corp. Hightstown, N.J.). Testicle length and diameter were measured without the epididymis with vernier calipers to the nearest 0.1 cm. Using a dial scale, body mass was determined to the nearest pound and converted to kg; body length was measured with a tape to the nearest inch and converted to cm.

One of the 2 testicles from each bear was selected (right and left side were unknown) and cut transversely into 3 approximately equal sections that represented the top, middle, and bottom. Two 3-mm<sup>3</sup> blocks were cut from each section (6 blocks of tissue/testicle), dehydrated in ethanol, and embedded in paraffin. Five sections (5  $\mu$ m thick) were cut from each block of embedded tissue at 3 locations within the block (i.e., front, middle, and back) and placed on separate glass microscope slides. Within each block, the 5 sections from each location within each block were separated by 150  $\mu$ m of tissue. Sections were removed from the paraffin and stained with Shandon hematoxylin 1 (Shandon, Inc., Pittsburgh, Pa.) and eosin Y.

Three of the 5 sections from each location within each block (front, middle, and back) were chosen randomly and the diameters of seminiferous tubules were measured using a Zeiss standard microscope 20 (Zeiss, West Germany) (100X) and an image analyzer (AUSKey 2.0, Animal Ultrasound Services, Inc., Ithaca, N.Y.). Tubular diameters were estimated by taking the mean of 3 linear measurements at different axes that intersected at the center of the tubule. This procedure was repeated for each slide from each block taken from the top, middle, and bottom of each testicle. This represented 18 slides from each testicle with 30 seminiferous tubule diameters measured/slide.

Seminiferous tubules from each grizzly bear were examined visually under a Zeiss standard microscope 20 (400X) for the presence of fully formed spermatozoa. A bear was considered sexually mature if there were spermatozoa in the lumen of the seminiferous tubules.

To test whether seminiferous tubule diameter differed between the top, middle, and bottom of each testicle sampled, data for seminiferous tubule diameters were analyzed by analysis of variance for a split-split plot design using the general linear models (GLM) procedure (SAS Inst., Inc. 1987). Variables in the model included: part of testicle (top, middle, and bottom), block (2 blocks, 3-mm<sup>3</sup>), and slide (front, middle, and back), and all interactions. The error term for testing part of testicle was block within testicle and the error term for testing block was slide within block. Pearson correlation coefficients were calculated between age, body mass, body length, and selected testicular traits in all animals. We used least-squares linear regression analysis to investigate the relationship between age and mean testicular mass for each bear. The effects of age on seminiferous tubule diameters were examined by fitting a nonlinear regression model to these data using the PROC NLIN procedure (SAS Inst., Inc. 1987). The proportion of grizzly bears 4.5 years old that lacked fully formed spermatozoa found in the lumen of seminiferous tubules and  $\geq 5.5$  years old that had fully formed spermatozoa were analyzed using contingency chi-square. Testicular volume was estimated using the formula for a prolate spheroid (Beyer 1991). Data for testicle mass and volume and mean seminiferous tubule diameter were examined by an analysis of variance using the GLM procedure (SAS Inst., Inc. 1987). The model included age class and date of kill. Means were compared using the LSD procedure (SAS Inst., Inc. 1987). Data for mature bears were grouped by season (the breeding season [May through mid-Jul] and the nonbreeding season [mid-Jul through Nov]) and analyzed using a *t*-test.

**RESULTS**

There were no effects ( $P > 0.10$ ) of blocks and sections, and there were no interactions ( $P > 0.10$ ) of these variables among the other independent variables. Therefore, data for seminiferous tubule diameters were pooled to yield a single estimate for each bear (Table 1).

Age, body mass, and body length were correlated positively ( $P < 0.01$ ) with testicle mass ( $r = 0.76, 0.62,$  and  $0.69,$  respectively), testicle length ( $r = 0.70, 0.65,$  and  $0.74,$  respectively), testicle diameter ( $r = 0.69, 0.68,$  and  $0.77,$  respectively), and mean seminiferous tubule diameter ( $r = 0.60, 0.54,$  and  $0.62,$  respectively). Testicle mass was related linearly to age ( $r^2 = 0.80, P = 0.05;$  Fig. 1), whereas the relationship between age and seminiferous tubule diameter was non-linear (Fig. 2). Seminiferous tubule diameter increased rapidly in bears between 1.5 and 5.5 years of age, then increased less rapidly in bears older than 6.5 years (Fig. 2). Seminiferous tubule diameter appeared to reach an asymptote at about 10–12 years of age (Fig. 2).

The youngest bear in which fully formed spermatozoa were found in the lumen of seminiferous tubules was 3.5 years old and killed in July. However, 2 other 3.5-year olds (both killed in September) and three 4.5-

year-old bears (killed in July, August, and November) did not have spermatozoa in their tubules. Therefore, we considered the grizzly bears in our study that were  $\leq 4.5$ -years-old to be reproductively immature. Only 1 of 11 bears that were  $\leq 4.5$ -years-old had spermatozoa in the seminiferous tubules, whereas 8 of 9 bears  $\geq 5.5$ -years-old did. The single bear  $> 4.5$  years of age without spermatozoa was a 7.5-year-old bear killed in October. The proportion of bears  $< 5.5$  years old with and without spermatozoa was significantly different than the proportion in bears  $\geq 5.5$  years old ( $\chi^2 = 13.5, 1 \text{ df}, P < 0.005$ ).

Testicular mass, testicular volume, and seminiferous tubule diameter were smaller ( $P = 0.05$ ) in immature bears than in mature bears (Table 2). Mature males had testicles that weighed more than 25 g each ( $\bar{x} = 32.63 \text{ g}$ ) and seminiferous tubule diameters of at least 150  $\mu\text{m}$  ( $\bar{x} = 158.87 \mu\text{m}$ ). Mean testicular mass and seminiferous tubule diameter of immature male grizzly bears were 12.39 g and 116.29  $\mu\text{m}$ , respectively.

Data for mature bears were grouped into 2 seasons: May through mid-July (the breeding season) and mid-July through November (the post-breeding season). Testicle mass and volume and seminiferous tubule di-

**Table 1. Age, date killed, and testicular characteristics of 20 male grizzly bears from Montana and Wyoming, 1978–1992.**

Age (yr)	Date killed	Testicle mass (g) <sup>a</sup>		Testicle length (cm)		Testicle diameter (cm)		Mean STD <sup>b</sup> ( $\mu\text{m}$ )
		A	B	A	B	A	B	
1.5	7 Jul	5.26	5.53	4.2	4.3	1.4	1.4	80.4
1.5	19 Sep	4.83	4.40	4.5	4.2	1.31	1.3	83.8
2.5	31 May	10.77	9.86	5.3	4.9	1.75	1.3	106.9
2.5	4 Aug	10.21	10.62	4.7	4.8	1.85	1.9	144.1
2.5	27 Aug	13.76	13.68	4.8	4.5	1.78	1.8	105.0
3.5	30 Jul	18.55	17.67	5.3	5.2	2.74	2.6	141.5
3.5	13 Sep	11.12	11.98	4.5	4.3	2.01	2.1	94.6
3.5	24 Sep	21.50	23.71	5.3	5.4	2.93	3.0	112.9
4.5	7 Jul	5.83	5.89	3.6	3.5	1.66	1.7	108.1
4.5	17 Aug	19.83	19.44	5.5	5.6	2.80	2.8	120.4
4.5	8 Nov	14.61	14.08	5.4	ND <sup>c</sup>	2.39	2.5	181.5
5.5	25 Jul	26.45	26.66	5.8	6.3	2.96	2.9	125.9
5.5	25 Jul	22.61	21.10	5.7	5.6	2.93	2.9	169.3
5.5	12 Aug	31.47	30.76	6.2	6.5	3.03	3.0	157.2
7.5	18 May	36.53	ND	6.3	ND	3.34	ND	151.1
7.5	18 Aug	27.77	ND	5.8	ND	3.03	ND	146.6
7.5	19 Oct	18.45	ND	6.0	ND	2.71	ND	133.3
11.5	20 Sep	31.15	31.74	6.1	6.1	3.18	3.2	167.2
12.5	26 Jun	45.41	46.60	6.8	7.0	3.54	3.5	212.6
14.5	31 Jul	52.28	53.08	7.6	7.6	3.63	3.5	167.3

<sup>a</sup> Testicles for each bear labeled A and B because side was unknown.

<sup>b</sup> STD = Seminiferous tubule diameter.

<sup>c</sup> ND = No data.

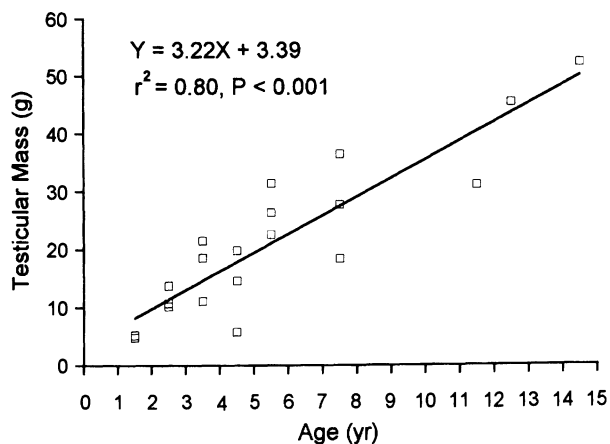


Fig. 1. Age variation in testicle mass for male grizzly bears from Montana and Wyoming, 1978–92.

ameter did not differ ( $P < 0.05$ ) between the seasons (Table 3), although our sample sizes were small.

## DISCUSSION

Effects of season on gonadal activity in black bears (Erickson et al. 1964, McMillin et al. 1976, Reynolds and Beecham 1980, Horan et al. 1993, Garshelis and Hellgren 1994) and brown and grizzly bears (Erickson et al. 1968, Pearson 1975, Tsubota and Kanagawa 1989) are well documented. Few studies, however, have investigated testicular and seminiferous tubule growth with age. Our analysis of data in Tsubota and Kanagawa (1991) indicated that testicle mass in Hokkaido brown bears is not

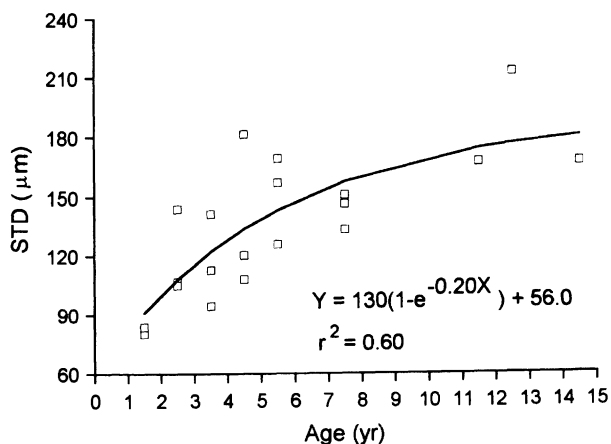


Fig. 2. Growth curves of seminiferous tubule diameters (STD) in male grizzly bears from Montana and Wyoming, 1978–92.

related linearly with age ( $n = 24$ ,  $r^2 = 0.47$ ); rather, testicular mass appeared to reach an asymptote in 8–10 years. In contrast, we found a linear increase in testicle mass for bears  $\leq 14.5$  years old (Fig. 1). Two bears, ages 15 and 18, greatly reduced the slope of the line in Tsubota and Kanagawa's data. The lack of bears older than 14.5 years could explain why testicular mass did not reach an asymptote in our study. Parenthetically, our results indicate that studies designed to investigate age or seasonal variation in grizzly bear reproductive biology could biopsy testicular parenchyma without regard to testicular location.

Our findings do not support the general trend of testicular growth in spring and regression in autumn. However, only 3 of our bears were killed in May and June, and only 6 were killed in September through November (Table 1). Low sample sizes and temporally limited data made it difficult to interpret seasonal trends.

In our study, mean testicular mass, volume, and seminiferous tubule diameters of immature and mature grizzly bears differed (Table 2), which is consistent with data reported by Tsubota and Kanagawa (1991) for Hokkaido brown bears. Mean testicular mass and seminiferous tubule diameters in the immature grizzly bears of our study were consistently larger than those of immature Hokkaido brown bears. However, in our study, mean testicular mass and seminiferous tubule diameters in mature grizzly bears were similar to those in mature Hokkaido brown bears. The reason for the discrepancy in the immature bears is not readily apparent except that Tsubota and Kanagawa (1991) used a different method than we did for measuring testicular size.

Tsubota and Kanagawa (1991) studied age of sexual maturity in male Hokkaido brown bears and concluded that sexual maturity, based on the presence of fully formed spermatozoa in the lumen of seminiferous tubules, occurred between 2 and 5 years of age. Erickson et al. (1968) and Pearson (1975) reported that fully formed spermatozoa first appeared in seminiferous tubules of brown bears in Alaska and grizzly bears in the Yukon at approximately 4.5 and between 5 and 7 years of age, respectively. These data generally agree with our results, except for the captive 2 year-old Hokkaido brown bears showing active spermatogenesis (Tsubota and Kanagawa 1991). The youngest bear in our study that showed spermatogenesis was 3.5 years old. Jonkel and Cowan (1971), Rogers (1976), and Elowe and Dodge (1989) have implicated nutrition as an important influence on reproduction in the American black bear. They found that reproductive rates of female black bears declined when mast or berry crops were poor the previous fall. Age of sexual maturity, breed-

**Table 2. Mean (SE), testicle mass, volume, and seminiferous tubule diameter (STD) for immature and mature grizzly bears in Montana and Wyoming, 1978–92.**

Age class <sup>a</sup>	<i>n</i>	Testicle mass (g)	Testicle volume (cm <sup>3</sup> )	STD (μm)
Immature (≤4.5 years old)	11	12.4 (1.8A) <sup>b</sup>	96 (18.2A)	116 (8.9A)
Mature (≥5.5 years old)	9	32.6 (3.2B)	267 (26.1B)	158 (7.5B)

<sup>a</sup> Based on the presence (mature) or absence (immature) of spermatozoa in the lumen of the seminiferous tubules.

<sup>b</sup> Different capital letters within a column indicate a significant difference ( $P < 0.05$ ).

**Table 3. Mean (SE), testicular mass, volume, and seminiferous tubule diameter (STD) in mature grizzly bears killed during May through mid-July (breeding season) and mid-July through November (post-breeding season) in Montana and Wyoming, 1978–92.**

Date	<i>n</i>	Testicle mass (g)	Testicle volume (cm <sup>3</sup> )	STD (μm)
May through mid-Jul	2	20.8 (4.1A) <sup>a</sup>	250 (30.0A)	131.8 (11.3A)
Mid-Jul through Nov	7	22.4 (2.5A)	327 (30.6A)	138.1 (6.3A)

<sup>a</sup> Same capital letters within a column indicate no significant difference ( $P > 0.05$ ).

ing interval, and litter size were all altered by nutritional factors. An increased nutritional plane may explain why male Hokkaido brown bears reach sexual maturity earlier than male grizzly bears in the continental United States. An examination of short-term gonadal responsiveness to nutritional factors in brown bears warrants further study.

## LITERATURE CITED

- BEYER, W.H. 1991. Standard mathematical tables and formulae. 29th ed. CRC Press, Boca Raton, Fla. 609pp.
- COY, P.L., AND D.L. GARSHELIS. 1992. Reconstructing reproductive histories of black bears from the incremental layering in dental cementum. *Can. J. Zool.* 70:2150–2160.
- DITTRICH, L., AND H. KRONBERGER. 1963. Biologisch-anatomische Untersuchungen über die Fortpflanzungsbiologie des Braunbären (*Ursus arctos* L.) und anderer Ursiden in Gefangenschaft. *Zeitschrift für Säugetierkunde* 28:129–192. (In German.)
- ELOWE, K.D., AND W.E. DODGE. 1989. Factors affecting black bear reproductive success and cub survival. *J. Wildl. Manage.* 53:962–968.
- ERICKSON, A.W., H.W. MOOSMAN, R.J. HENSEL, AND W.A. TROYER. 1968. The breeding biology of the male brown bear (*Ursus arctos*). *Zoologica*. 53:85–105.
- , J. NELLOR, AND G.A. PETRIDES. 1964. The black bear in Michigan. Michigan State Univ. Agric. Exp. Stn. Res. Bull. 102pp.
- GARSHELIS, D.L., AND E.C. HELLGREN. 1994. Variation in reproductive biology of male black bears. *J. Mammal.* 75:175–188.
- HORAN, K.T., R.A. NELSON, S.S. PALMER, AND J.M. BAHR. 1993. Seasonal response of the pituitary and testes to gonadotropin-releasing hormone in the black bear (*Ursus americanus*). *Comp. Biochem. Physiol.* 106:175–182.
- JONKEL, C.H., AND I.M. COWAN. 1971. The black bear in the spruce-fir forest. *Wildl. Monogr.* 27:57pp.
- McMILLIN, J.M., U.S. SEAL, L. ROGERS, AND A.W. ERICKSON. 1976. Annual testosterone rhythm in the black bear (*Ursus americanus*). *Biol. of Reprod.* 15:163–167.
- PALMER, S.S., R.A. NELSON, M.A. RAMSAY, I. STIRLING, AND J.M. BAHR. 1988. Annual changes in serum sex steroids in male and female black (*Ursus americanus*) and polar (*Ursus maritimus*) bears. *Biol. of Reprod.* 38:1044–1050.
- PEARSON, A.M. 1975. The northern interior grizzly bear. *Can. Wild. Serv. Rep. Ser.* 34, Ottawa. 86pp.
- REYNOLDS, D.G., AND J.J. BEECHAM. 1980. Home range activities and reproduction of black bears in west-central Idaho. *Inter. Conf. Bear Res. and Manage.* 4:181–190.
- ROGERS, L. 1976. Effects of mast and berry crop failures on survival, growth, and reproductive success of black bears. *Trans. North Am. Wildl. and Nat. Resour. Conf.* 41:431–438.
- SAS INSTITUTE, INC. 1987. Users guide: statistics. Fifth ed. SAS Institute, Inc., Cary, N.C. 956pp.
- TSUBOTA, T., AND H. KANAGAWA. 1989. Annual changes in serum testosterone levels and spermatogenesis in the Hokkaido brown bear, *Ursus arctos yesoensis*. *J. Mammal. Soc. Japan* 14:11–17.
- , AND ———. 1991. Sexual maturity of the Hokkaido brown bear. *Asiatic Bear Conf.* 1:1–9.
- WILLEY, C.H. 1974. Aging black bears from first premolar tooth sections. *J. Wildl. Manage.* 38:97–100.