

Factors Associated with Fecal Glucocorticoids in Alaskan Brown Bears (*Ursus arctos horribilis*)

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ABSTRACT

The aims of this study were to validate a radioimmunoassay (RIA) for quantifying glucocorticoid metabolite concentrations in the feces of Alaskan brown bears (*Ursus arctos horribilis*) and to investigate whether any of the following factors are associated with those concentrations: the presence of humans or other bears, fishing difficulty, sex-age class, diet, and season. We tested an established corticosterone RIA for assay sensitivity, similarity, precision, and sample matrix effects of brown bear feces, and it proved satisfactory. We collected fecal samples from brown bears along salmon-spawning streams and assessed fecal glucocorticoid (FG) concentrations. We observed that the factors explaining the most variation in measured concentrations were date and diet type and that there was a significant interaction between the two. We did not observe a significant effect of human and bear activities or sex-age class on FG concentrations. This study demonstrates that although FG concentrations may be assessed in brown bears, complex dietary patterns and seasonal variations must be taken into consideration in the study design in order to make inferences regarding stress.

Introduction

An adverse stimulus is known to initiate a physiological cascade of responses, which provides resources necessary to cope with

a stressor (reviewed in Moberg 1987). One such response is the activation of the hypothalamic-pituitary-adrenocortical (HPA) axis, resulting in synthesis and secretion of glucocorticoids by the adrenal cortex. Concentrations of these hormones have been used by many studies as a physiological index of stress in animals.

Traditionally, quantitative measurements of physiological stress have involved assessing glucocorticoid concentrations in blood plasma. The use of physiological stress measures on free-ranging animals has been limited due to the invasiveness and potential for bias of capturing and withdrawing blood from wild animals. Moreover, corticosteroid secretion into blood varies diurnally and has pulsatile secretory patterns (Monfort et al. 1993), causing sample plasma glucocorticoid concentrations to be highly variable. Fecal steroid measures now provide an appealing alternative to serum sampling. Samples for fecal glucocorticoid (FG) analysis are relatively easy to collect and can be gathered without disturbing study subjects. This approach also provides a smoothed estimate of glucocorticoid concentrations over a longer time period than serum sampling, because of the pooling effect of adrenocorticosteroids in feces (Harper and Austad 2000). This new technique is being applied to an increasing number of mammalian species (e.g., Monfort et al. 1998, African wild dog; Goymann et al. 1999, spotted hyenas; Strier et al. 1999, muriquis; Wallner et al. 1999, barbary macaques; Harper and Austad 2000, mice and voles; Wasser et al. 2000, multiple species; Millspaugh et al. 2001, elk).

This study extends the technique to brown bears (*Ursus arctos horribilis*). For bears, environmental factors such as human activities, presence of other bears, and food availability are potential sources of cognitive stress that may affect FG concentrations. In elk, human disturbance, as measured by road density and use, has been shown to correlate with FG levels (Millspaugh et al. 2001). However, factors independent of cognitive stress, such as circannual physiological rhythms and diet type, may also affect these concentrations. Seasonal changes in serum glucocorticoid concentrations have been demonstrated in bears (Palumbo et al. 1980; Harlow et al. 1990) and other hibernators (Shivatcheva et al. 1988, European ground squirrel; Armitage 1991, yellow-bellied marmots; Boswell et al. 1994, golden-mantled ground squirrels). Similarly, diet type may alter both serum and FG concentrations through a variety of channels. Diet can affect gut transit time, which in turn influences reabsorption of steroid hormones (Lewis et al. 1997). Also, FG concentrations can be augmented by ingestion and excretion of glucocorticoids from the diet (Cooper et al. 1996). Finally,

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sex-age class may affect measured FG concentrations through both status-related stress (Sapolsky et al. 1997) and innate physiological differences (Taylor 1971; Brooks 1979). There has been little investigation of nonstress processes as variables affecting FG concentrations.

In this study, FG concentrations were assessed in brown bears that frequented salmon streams in Katmai National Park and Preserve, Alaska. This study had two objectives. The first was to biochemically validate the use of a radioimmunoassay (RIA) for quantifying cortisol metabolite concentrations in the feces of brown bears by evaluating assay sensitivity, similarity, precision, and sample matrix effects. An adrenocorticotrophic hormone (ACTH) challenge in grizzly bears has been performed subsequently (Hunt and Wasser 2003). Second, we sought to investigate whether any of the following factors are associated with FG concentrations: human presence, the presence of other bears, fishing difficulty, sex-age class, diet, and season.

Material and Methods

Data Collection in the Field

This study was carried out at Katmai National Park and Preserve during the 1999 salmon-spawning season in one location where human use varied dramatically over the course of the season (Brooks River) and in one location with consistently low human activity (Margot Creek). These locations are approximately 11 mi apart, and the river segments have been found comparable in flow velocity, suspended solid content, and bear activity (W. Troyer, unpublished manuscript; La-Perriere 1996). In addition, observational studies have shown that different populations of bears frequent these two areas (Olson et al. 1990).

We randomly selected ten 50 × 15-m plots at each river location, for a total of 20 plots. On each day from June through September of 1999, we sampled and thoroughly cleared all feces from all plots at one river location. By alternating river locations daily, we ensured that all samples collected were less than 2 d old, thereby avoiding bias associated with sample age. Each sample was mixed thoroughly with a gloved hand, and a random subsample was collected. All fecal samples were stored in a freezer at -20°C and remained frozen until analyzed.

During collection, the following factors were recorded at each plot: plot number in which the sample was found, date of collection, numbers of bears and humans visible from the plot, and fishing difficulty. Fishing difficulty was measured by dividing the total number of minutes of bear fishing effort over the total number of fish caught during a 20-min observation period. Diet type of each sample was also noted on collection and recorded as "grasses," "berries," or "flesh" when the sample visibly consisted of only one of these three components or "mixed" if more than one of these components were present. Dietary composition of samples was confirmed in the laboratory by observation while sifting feces through a wire mesh.

In a concurrent sampling effort, we opportunistically collected samples from bears of known sex-age groups (adult male, adult female, subadult, yearling, cub) when they were observed defecating. These data were collected for investigation of sex-age class differences in FG concentrations. With each of these samples, we recorded the same covariate data described previously.

Fecal Steroid Extraction

Fecal samples were extracted and analyzed at the Wasser laboratory in the Woodland Park Zoo, Seattle, Washington. Fecal samples were lyophilized for 130–140 h at -20°C to control for variable water contents of diets (Wasser et al. 1993). Dried feces were then pulverized, and the resulting powder (0.200 ± 0.015 g) was weighed to the nearest 0.0001 g and extracted with 4.00 mL of 90% methanol. After vortexing for 30 min in a pulsing vortexer, the sample was centrifuged (2,500 g, 20 min, 4°C), and the methanol supernatant was collected and stored frozen (-20°C) in labeled cryovials until assayed. The percent extraction efficiency of tritiated cortisol of grizzly bear feces for this extraction method at this laboratory is 89.5% (K. E. Hunt, unpublished observation).

Fecal Radioimmunoassay

Fecal samples were analyzed for cortisol metabolites using a double-antibody ¹²⁵I RIA kit for corticosterone (ICN Biomedicals, Costa Mesa, Calif.) with high affinity for ursid fecal cortisol (S. K. Wasser, personal communication). Samples and standards were analyzed in duplicate and results averaged. Methanol-extracted samples were diluted 1 : 32 in steroid diluent (phosphosaline gelatin buffer with rabbit gamma globulins). The manufacturer's assay protocol was followed except that half-volumes were used. The resulting precipitate was counted for 2 min in a Crystal Multi-Detector Gamma System (United Technologies, Packard). The standard curve was generated with six standards spanning 0.125–5.0 ng/mL, using a four-parameter logistic curve fit. Results are expressed in nanograms of hormone per gram dry fecal matter.

Radioimmunoassay Validation

This corticosterone assay was biochemically validated for brown bear feces by testing assay similarity, sensitivity, sample matrix effects, and precision (Grotjan and Keel 1996). Each test was performed on brown bear fecal samples from the three representative diet types (grasses, berries, flesh) but not on the mixed diet type, since this group of samples was not homogeneous. Similarity was assessed by assaying serial dilutions of the fecal samples (1 : 4–1 : 2048), calculating slopes for the linear portion of the curves and comparing these with the standard slope. Sensitivity was assessed by extrapolating to dose from

Table 1: Mean fecal glucocorticoid concentrations log (ng/g) at each month by diet-type combination

| Month | Diet Type \pm SEM (N) ^a | | | |
|-----------|--|---|--------------------------------------|--|
| | Flesh | Mixed | Berries | Grasses |
| June | NA | 2.209 \pm .317 (4) | NA | 1.494 \pm .082 (25) ^{j,k} |
| July | 1.445 \pm .045 (64) ^{a,b,c,d} | 1.956 \pm .048 (112) ^{c,f} | 1.269 \pm .000 (1) | 1.792 \pm .039 (147) ^{d,j,l} |
| August | 1.661 \pm .030 (153) ^{a,e} | 1.771 \pm .035 (107) ^{f,g,h} | 1.538 \pm .038 (54) ^{g,i} | 2.183 \pm .135 (13) ^{e,h,i,k,l} |
| September | 1.793 \pm .056 (34) ^b | 1.705 \pm .063 (32) | 1.621 \pm .092 (3) | NA |

Note. NA = Samples at this month by diet-type combination were not found. See footnotes for significant comparisons and 95% confidence intervals for the differences.

^a $P < 0.05$; 95% CI, 0.016–0.416.

^b $P < 0.01$; 95% CI, 0.063–0.633.

^c $P < 0.001$; 95% CI, 0.301–0.722.

^d $P < 0.001$; 95% CI, 0.146–0.548.

^e $P < 0.01$; 95% CI, 0.134–0.910.

^f $P < 0.05$; 95% CI, 0.004–0.367.

^g $P < 0.05$; 95% CI, 0.008–0.457.

^h $P < 0.05$; 95% CI, 0.018–0.807.

ⁱ $P < 0.01$; 95% CI, 0.230–1.060.

^j $P < 0.05$; 95% CI, 0.008–0.589.

^k $P < 0.001$; 95% CI, 0.230–1.148.

^l $P < 0.05$; 95% CI, 0.002–0.780.

the lower 95% confidence limit of percent bound at 0 dose for these same slopes. Sample matrix interferences with the antibody, reagents, or detection system were evaluated for the same diet types by assaying samples containing 50 μ L of each of the standards and 50 μ L of pooled fecal samples and testing homogeneity of regression. Assay precision was assessed by determining intra-, inter-, and extract coefficients of variation for the three representative diet types. Intraassay and interassay coefficients of variation were assessed by running eight duplicates of pools from each of these diet types in one assay and duplicates of the pools through eight different assays. The extract coefficients of variation were determined by running assays in duplicate with 10 unique glucocorticoid extractions from fecal pools of each of these diet types and subtracting the respective intraassay coefficient variation.

Statistical Analysis

Statistical analyses were conducted using SPSS 10.0 (SPSS, Chicago). All hormone metabolite concentrations were log transformed to normalize the data and to decrease heterogeneity of variances. Similar analyses were conducted for samples collected from plots and for the smaller number of samples collected from known individuals. For the first set of samples, mean daily FG levels from each plot served as the experimental unit. Effects on these plot log FG concentrations of the two locations and of the corresponding covariates date, human presence, bears presence, fishing difficulty, and diet type were analyzed using a nested ANCOVA with the random factor (plot) nested within the fixed factor (location). For the second set of samples, where sex-age class of the individual was known, ef-

fects on log FG concentrations of sex-age class and the covariates date, location, human presence, bear presence, fishing difficulty, and diet type were also analyzed using ANCOVA.

Variable selection was performed using a variety of criteria: F -tests of model and parameter significance at $\alpha = 0.05$, lowest mean square error, R^2 jump, highest adjusted R^2 , and the lowest difference between Mallows' C_p (Mallows 1973) and p . The need for higher-order terms of selected variables was determined using residual plots. Multiple comparisons between means were made using the Tukey-Kramer procedure.

Results

Radioimmunoassay Validation

Serial dilutions of pooled samples from the three representative diet types yielded antibody displacement curves parallel to that of the standards at a statistical threshold of 0.05 [(sample slope – standard slope)/SE for grasses: $t_4 = 1.70$; berries: $t_4 = 1.41$; flesh: $t_4 = 1.72$], demonstrating similarity of reactivity. Assay sensitivity at $\alpha = 0.05$ was 0.079 ng/mL extract. Homogeneity of regression for additive effects of analyte to a given amount of each sample matrix revealed that slopes did not differ from unity at a statistical threshold of 0.05 [(observed slope – 1)/SE for grasses: $t_4 = 2.72$; berries: $t_4 = 1.36$; flesh: $t_4 = 0.24$], demonstrating that sample matrix effects were not significant. The intraassay coefficients of variation (% SD/mean, eight replicates from the same pools in one RIA) for pooled samples from each of the three representative diet types were 4.95% for grasses, 4.86% for berries, and 4.26% for flesh. Estimates of interassay coefficients of variation (% SD/mean, eight replicates in different RIAs) were 8.30% for grasses, 10.51% for

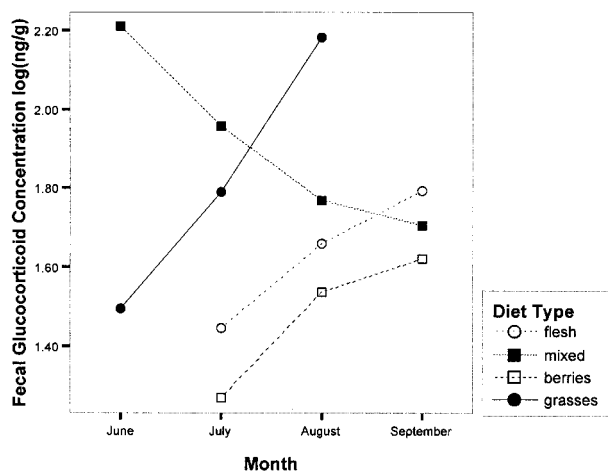


Figure 1. Date \times diet type interaction depicted by mean fecal glucocorticoid concentrations log (ng/g) for each month by diet-type combination.

berries, and 10.63% for flesh. The extract coefficients of variation (% SD/mean, 10 replicates from the same pools in one RIA) were 0.17% for grasses, 1.44% for berries, and 1.95% for flesh.

Summary Data

The following medians and ranges describe our covariate data to give an overall sense of the study areas. The number of people seen per plot in the variable human-use area ranged from 0 to 43 with a median of 7. No visitors were observed in the low visitor-use area over the course of the season. Numbers of bears visible per plot ranged from 0 to 25 with a median of 4 in the variable human-use area and 1 to 17 with a median of 6 in the low visitor-use area. The minimum time it took bears to catch fish was 0.33 min in the variable human-use area and 1.75 min in the low visitor-use area. Maximum fishing time cannot be stated because bears were not always successful at catching fish in the 20 min per plot observation period. Samples from all of the four diet types were collected, although changing food availability resulted in no flesh and berry samples during June and no grass samples during September (Table 1).

Factors Associated with Glucocorticoid Concentrations

A total of 749 samples within plots were collected. The nested ANCOVA did not detect any variability among the replicated plots ($F_{38,694} = 1.115$; $P = 0.294$). Thus, the values from the plots were pooled post hoc (Underwood 1997). The majority of model selection criteria concurred that both date and diet type explained variation in log FG concentrations ($R^2 = 0.223$) and that the other variables, including location, measures

of human and bear presence, and fishing difficulty, did not explain much of the variation beyond that explained by date and diet type. Inspection of residual plots of the selected variables date and diet type revealed that an interaction term between date and diet type was appropriate, and this was confirmed by F -tests of significance of the interaction ($F_{81,604} = 1.406$; $P = 0.015$). R^2 for the final model including date, diet type, and an interaction between these two variables was 0.346. Significance of main effects of date and diet type on log FG concentrations could not be assessed because of the presence of the higher-order interaction between these two variables.

FG concentrations were grouped by month to facilitate both display and discussion of results. Estimates of mean log FG concentrations were calculated at each month by diet-type combination (Table 1); the interaction is depicted in Figure 1.

Some differences emerged among months within each diet type (Fig. 1). Mean log FG levels in flesh samples increased over the course of the season, with statistically significant increases taking place between July and both August ($P < 0.05$; 95% CI, 0.016–0.416) and September ($P < 0.01$; 95% CI, 0.063–0.633). Similarly, the FG levels in grass samples increased over the season, with significant increases occurring between June and July ($P < 0.05$; 95% CI, 0.008–0.589), June and August ($P < 0.001$; 95% CI, 0.230–1.148), and July and August ($P < 0.05$; 95% CI, 0.002–0.780). Finally, although the seasonal trend in FG concentrations for the berry diet type is suggestive, the sample sizes were too small to confirm a trend. On the other hand, samples from the mixed diet type showed a distinct downward trend, with mean log FG concentrations in August exhibiting a statistically significant decline from those in July ($P < 0.05$; 95% CI, 0.004–0.367).

Some differences also emerged among diet types within each month (Fig. 1). Mean log FG levels in flesh samples were consistently less than in grass samples in months in which both were available, July ($P < 0.001$; 95% CI, 0.146–0.548) and August ($P < 0.01$; 95% CI, 0.134–0.910). The effect size of the latter comparison is large. Furthermore, samples from the berry diet type had lower concentrations than those in both grass and flesh diet type. Among those differences, the increment between berry and grass was significant in August ($P < 0.01$; 95% CI, 0.230–1.060), and this difference was large. By contrast, levels within the mixed diet type were higher than any others in the beginning of the season, with a large and significant increment over flesh in July ($P < 0.001$; 95% CI, 0.301–0.722), continued to be higher than berry ($P < 0.05$; 95% CI, 0.008–0.457), but dropped below grass in August ($P < 0.05$; 95% CI, 0.018–0.807), and finally approximated those of the flesh diet type at the end of the season.

Sex-Age Patterns in Fecal Glucocorticoid Metabolite Levels

A total of 57 samples from individuals of known sex-age class were collected and analyzed. The majority of variable selection

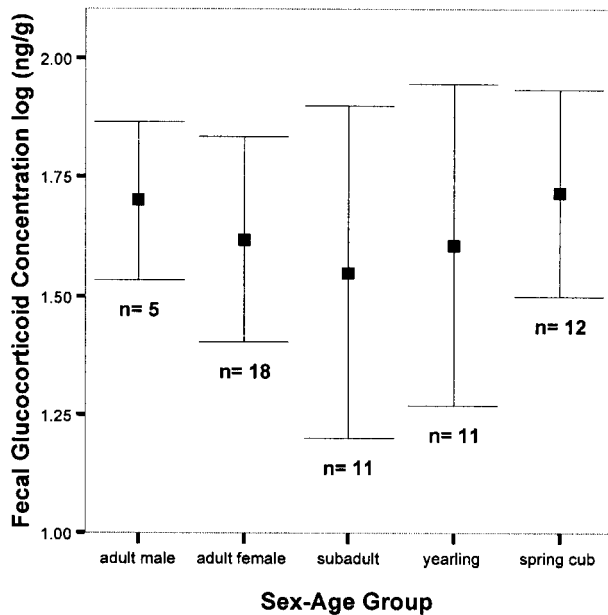


Figure 2. Mean \pm 95% confidence interval of fecal glucocorticoid concentrations log (ng/g) for each sex-age group.

criteria concurred that, as in the previous model, date and diet type explained variation in log FG concentrations ($R^2 = 0.510$) and that the remaining variables did not explain much of the variation beyond that explained by date and diet type. *F*-tests of parameter significance ($\alpha = 0.05$) indicated that none of the variables tested explained a significant amount of the variation of log FG concentrations in feces. Thus, model selection criteria used indicated that sex and age did not explain a significant amount of the variation of log FG concentrations both in a model with other covariates, as well as without any measured covariates ($F_{4,52} = 0.257$; $P = 0.904$). Adult males ($N = 5$) and spring cubs ($N = 12$) had the highest mean log FG concentrations, and subadults showed the lowest levels ($N = 11$), although none of these differences were statistically significant at $\alpha = 0.05$ nor were the magnitudes of the differences large (Fig. 2).

Discussion

We validated the use of an RIA for quantifying glucocorticoid metabolites in brown bear feces by evaluating assay sensitivity, similarity, precision, and sample matrix effects of the three representative diet types. This assay also has been biologically validated for grizzly bear fecal samples with an ACTH challenge (Hunt and Wasser 2003). We observed that the variables that best explained variation in brown bear FG concentrations were date and diet type, with date having an effect on these concentrations that differed across the diet types. Neither human nor bear activities explained a statistically significant portion

of this variation. The resulting R^2 for the model was very low, which may be due to the inherent difficulty of predicting such a complex physiological outcome.

Model selection suggested that sex-age class did not explain a significant portion of the variation of FG concentrations. Gender differences in patterns of glucocorticoid synthesis and excretion have been demonstrated in other mammals (Taylor 1971; Brooks 1979) and are likely due to sex hormone modifications of the HPA axis (Kitay 1963; Colby and Kitay 1972a; Rodier and Kitay 1974; Bell et al. 1991) and hepatic and intestinal metabolism of glucocorticoids (Eriksson and Gustafsson 1970; Colby and Kitay 1972b). In addition, sex-age class differences in glucocorticoid concentrations may be anticipated due to potentially disparate social stressors placed on these different groups (Sapolsky et al. 1997). Our failure to observe differences among these sex-age groups may be due to the small magnitude of these differences and our inability to measure these differences precisely because of sample size limitations.

Many studies of this type have reported a link between FG concentrations and stress (e.g., Goymann et al. 1999; Wallner et al. 1999; Harper and Austad 2000), and indeed, the interaction between date and diet type in this study could be explained by stress. For example, a bear eating grass at the start of the season may experience less stress than one forced to eat grass near the end of the season. However, it is more difficult to explain in terms of cognitive stress why a mixed diet type would be associated with decreasing FG concentrations over the course of the season, whereas a diet consisting of flesh would be associated with increasing FG concentrations. Although there is clearly much room for further research in this area and cognitive explanations may emerge, the remainder of this discussion will focus on potential physiological factors unrelated to stress that may explain this statistically significant interaction. Because many of these reasons have not been addressed in studies of FG monitoring, this finding identifies an important area for future research.

Effects of high-fiber diets on fecal excretion of steroid hormones may explain why samples from the grass diet type showed higher FG levels than the flesh diet type, with a statistically significant increment in July and August, and why bears consuming a mixture of flesh and vegetation showed significantly higher FG levels than those eating only flesh in July. The influence of vegetarian diets on another steroid hormone, estrogen, has been documented in women by Goldin et al. (1981, 1982), with a vegetarian diet leading to both increased fecal weight and to a two- to threefold increase in fecal excretion of the hormone. Similarly, Anderson et al. (1987) demonstrated that the ratio of protein to carbohydrate intake influenced plasma concentrations of cortisol in humans, with lower plasma cortisol levels found during the high carbohydrate diet than the high protein diet. These dietary effects are likely caused by decreased intestinal reabsorption of steroid hormones due to the increased gut transit time of high fiber diets (Lewis et al.

1997). Although these studies support the argument that high dietary fiber intake by bears may increase the concentration of glucocorticoid metabolites recovered in the feces, two points may run contrary to this result. First, the berry diet, which is relatively high in fiber (Pritchard and Robbins 1990), showed the lowest FG concentrations of all diets in August, the one month in which sample sizes were large enough to enable comparison. Second, Wasser et al. (1993) showed that an increase in fecal bulk from a high-fiber diet outweighs the increase in fecal progesterone concentrations in baboons and that lyophilizing can eliminate some, although not all, of this variation. Clearly, further research is needed to confidently predict the influence of dietary fiber on fecal excretion of glucocorticoids in brown bears.

For all but the mixed diet type, FG concentrations tended to increase over the course of the season, with several statistically significant differences pointing to a potential seasonal cycle in circulating glucocorticoids. Although seasonal variations in FG levels have been observed in a variety of mammalian species (Harper and Austad 2001, mice and voles; Millspaugh et al. 2001, elk), such increases have been specifically associated with prehibernatory fattening in hibernators (Shivatcheva et al. 1988, European ground squirrel; Armitage 1991, yellow-bellied marmots; Boswell et al. 1994, golden-mantled ground squirrels). Circulating concentrations of glucocorticoids have also been shown to increase in adult black bears in the fall relative to summer (Palumbo et al. 1980; Harlow et al. 1990). Glucocorticoids play a central role in metabolism (Tataranni et al. 1996) and may be involved in prehibernatory fattening in two ways. First, glucocorticoids can stimulate lipogenic enzymes in the liver (reviewed in Berdanier 1989), resulting in an anabolic effect on adipose tissue (Mayer et al. 1956). Second, glucocorticoids have been shown in rodents to be necessary for appetite (Kumar et al. 1988) and to act directly on the central nervous system to enhance weight gain (Green et al. 1992). Thus, the seasonal FG increases in this study may have been due to the effects of predenning preparations on glucocorticoid levels.

A second possible explanation for the temporal pattern observed may relate to the increase in food intake documented in bears before denning (Nelson et al. 1980; Hissa 1997). Increasing consumption accelerates gastrointestinal transit time (Palme et al. 1996), which has been shown to decrease reabsorption of steroid hormones (Barbhaiya and Welling 1982; Lewis et al. 1997). Of course, this is likely to increase fecal bulk as well, so the net result of this effect would require further research.

A third possible explanation for the seasonal pattern observed is specific to the flesh diet type. Ingested glucocorticoids from dietary sources may influence glucocorticoid concentrations in bears due to potential absorption and excretion by the body. The excellent bioavailability and subsequent excretion of oral administration of glucocorticoids have been well documented for multiple species (Heazelwood et al. 1984; Cooper

et al. 1996) and may also hold for bears feeding on salmon, in which the predominant glucocorticoid is cortisol (Hane and Robertson 1959). A fivefold increase in cortisol concentrations has been demonstrated in salmon between the mouth of the river and the spawning grounds (Idler et al. 1959). Consistent with a hypothesized increase from ingested cortisol, peak FG concentrations in bear feces in this study corresponded to salmon-spawning months at both rivers. Bioavailability and excretion of glucocorticoids from ingested prey need to be better understood so that, if necessary, this influence can be accounted for in future field studies of FG concentrations.

As noted previously, the one diet type for which the seasonal trend is reversed is the mixed diet type. We did not perform detailed analyses of the contents of each sample in the mixed diet type. The mixture was likely composed predominantly of vegetation during June and July, when salmon were not as readily available as later in the season. Composition may have shifted in favor of flesh in September, when salmon were easy to catch because they were dead or dying due to spawning activities. The hypothesis that the decreased level of FG concentrations in the mixed diet type is due to the changing composition of the samples is supported by the fact that the mean FG levels of this diet type ultimately approximated those of the flesh diet type during salmon spawning months.

In sum, we observed that the variables that best explained variation in brown bear FG concentrations were date and diet type and that the effect of date on FG concentrations differed across the diet types. No other studies of FGs in free-ranging mammals have addressed such an interaction, perhaps because study subjects maintained a more consistent dietary mix over the study period. However, brown bears in the wild rely on a wide range of food sources, from plant matter to terrestrial and marine meat, and selection of specific foods changes seasonally (Pritchard and Robbins 1990; Mattson et al. 1991; Clevenger et al. 1992). This makes use of FG concentrations as an index of stress in brown bears and other similarly situated wildlife particularly challenging. Future designs for brown bear stress research using FG concentrations as an index should take into consideration potential complex seasonal and dietary influences. Further studies are required to assess the relative importance of dietary intake, temporal variations, and competitive access to resources on FG concentrations.

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