Kuiu Island Black Bear Pilot Study:
Population Estimation and Sexual Segregation

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SUMMARY

Studies to evaluate the population size of black bears have not been conducted in Southeast Alaska, where black bear harvest has increased at about 10% annually over the past decade. Hunting pressure has increased to the point where local hunters and big game guides are concerned about declines in the number of bears on Kuiu Island. Since estimating the population size of bears in forested habitats is notoriously difficult, we have used this 1 year pilot study to evaluate the efficacy of novel population estimation techniques along with testing the assumptions of these methods. The first objective was to use tetracycline biomarking to estimate the size of the North Kuiu Island bear population, and secondly to examine alternate statistical approaches for such a mark-recapture study. The tetracycline biomarking study was successful. We were able to biomark a sample of bears with and recapture some of these marked bears through hunter harvest. This will result in a population estimate for part of Kuiu Island with increased precision as more of these bears are harvested in subsequent hunting seasons. Statistical and logistical assessment of this approach enables us to plan a more extensive biomarking estimation study for the entire island. The other objectives involved noninvasive genetic sampling of black bears, in an attempt to use this method for population estimation and to study black bear social structure along salmon streams. Over 800 hair samples were successfully collected along five salmon streams on Kuiu Island. These microsatellite loci are currently being used to establish genetic individual and sexual identification of the samples; this information will be used for population estimation and social structure documentation. The final

objective of this pilot study was to examine black bear vigilance behavior in relation to riparian habitat and bear distribution. Behavioral data have been collected and analyzed, and suggests that there were differences in vigilance behavior between habitats.

CONTENTS

BACKGROUND

A pilot study on Kuiu Island black bears (*Ursus americanus)* was conducted to collect preliminary population, genetic and behavioral data. Tetracycline baits were tested as a marking technique to estimate population size. Although tetracycline baiting has been used successfully in mark-recapture studies of black bears, this marking method had to be refined given logistical considerations and bear density on Kuiu Island.

Noninvasive DNA sampling was also tested as a method of population estimation and to describe black bear spatial social structure. This pilot study served to evaluate various field methods for noninvasive DNA sampling. Hair-snags are a fairly recent field tool to obtain DNA from free-ranging bears; this technique had to be refined for Kuiu Island, where wet weather, high bear density and clumped food-resources complicate the design of a study. Various single-capture hair snag prototypes and traditional hair snags were tested.

Before noninvasively sampled DNA could be used for individual identification, background genetic analyses had to be performed to estimate the probability of genetic identity (PI) and to determine population genetic variation. The genetic data from hair samples gathered during this pilot season are being used in a capture-recapture fashion to estimate the number of bears using five salmon streams. They are also being used to preliminarily describe sexual segregation of black bears along and between salmon-spawning streams.

Finally, preliminary observational data were collected on vigilance behavior of black bears on salmon streams. My hypotheses regarding black bear behavior focus on stress of black bear females with dependent young on the salmon streams. In this study I assume that the salmon stream is a risky-habitat for bears, and is especially risky for sows with dependent young, due to

the threat of injury or infanticide demonstrated by adult male bears. I hypothesized that black bear sows in upstream areas and tidal areas would differ in the degree of stress they exhibited. This hypothesis is based on the assumption that male bear distribution differs between these two habitats, as well as the distance to escape cover (climbable trees). Male bear density is expected to be higher in upstream areas than in tidal areas, based on anecdotal reports of bear biologists and hunting guides. Since black bear sows defend their cubs by encouraging them to climb trees, foraging in tidal areas, where fishing is done further away from escape cover, may result in additional behavioral trade-offs. These vigilance data collected during this pilot study have been used to refine hypotheses concerning black bear stress behavior on salmon streams.

OBJECTIVES

1. Evaluate the use of tetracycline biomarkers as a method of marking black bears; obtain a preliminary population estimate of north Kuiu Island black bears.
2. Evaluate the alternate statistical approaches for mark-recapture studies with small sample sizes and limited recapture opportunities.
3. Optimize microsatellite loci for the north Kuiu Island black bear population.
4. Estimate allelic diversity and probability of identity using microsatellite loci for the north Kuiu Island black bear population.
5. Estimate the number of bears using four streams during July and August through noninvasive DNA sampling. Complete genetic laboratory work on approximately 125 hair samples including:
    i. Sex determination of samples
    ii. Individual identity using microsatellite signatures
6. Obtain preliminary sexual segregation data on four salmon streams using genetic information.
7. Obtain preliminary data on black bear stress behavior on salmon streams.

STUDY AREA

The islands of the Alexander Archipelago in Southeast Alaska are distinguished by interesting distributions of mammalian taxa, and feature 27 endemic mammalian subspecies. Kuiu Island (56° 35' N, 134° 00' W) is biogeographically considered a 'middle and southern-inner' island of the archipelago (MacDonald & Cook 1996). The mammalian fauna of these islands is differentiated by the presence of the endemic subspecies of the Southern red-backed vole, (*Clethrionomys gapperi solus)* and the absence of brown bears (*Ursus arctos*) (Table 1). Southeast Alaska's temperate rainforest consists largely of Sitka spruce (*Picea sitchensis)*, mountain and western hemlock (*Tsuga mertensiana, T. heterophylla*), and western red and yellow cedar (*Thuja plicata, Chamaecyparis nootkatensis*). The undergrowth and riparian vegetation consists of berries (*Vaccinium* spp. and *Rubus* spp.), Sitka and red alder (*Alnus sinuata* and *A. rubra*), devil's club (*Oplopanax horridum*), and yellow skunk cabbage (*Lysichitum americanum*). Five species of Pacific salmon (*Oncorhynchus* spp.)—chum, king, pink, sockeye and coho—run in the creeks and rivers of Southeast Alaska and are an important, irruptive food source for at least 40 vertebrate species (Willson *et al.* 1998), including black bears. Where black bears are allopatric with brown bears on the Kenai Peninsula, meat (*i.e.*, salmon) constitutes $56 \pm 25\%$ of their diet (Jacoby *et al.* 1999).

Kuiu Island is part of the Tongass National Forest, the largest national forest in the United States. Kuiu Island north of the Port Camden and Bay of Pillars isthmus has been intensively logged and roaded; the extensive Tebenkof Bay and watershed in southern Kuiu Island is a federal wilderness area. This pilot study focused on north Kuiu Island due to accessibility and tractability.

Table 1. Documented mammalian fauna of Kuiu Island (MacDonald & Cook 1997)

Sorex cinereus	Masked shrew
Sorex monticolus	Dusky shrew
Tamiasciurus hudsonicus	Red squirrel
Castor canadensis	Beaver
Peromyscus keeni	Keen's mouse
Microtus longicaudus	Long-tailed vole
Ondatra zibethicus	Muskrat
Canis lupus	Gray wolf
Ursus americanus pugnax	Black bear
Martes americana	Marten
Mustela erminea	Ermine
Mustela vison	Mink
Gulo gulo	Wolverine
Lontra canadensis mira	River otter
Odocoileus hemionus sitkensis	Sitka black-tail deer
Alces alces	Moose
Additional taxa present in the "Middle and Southern Inner Islands" biogeographic region of the Alexander Archipelago. These have not (yet) been documented on Kuiu Island. (MacDonald & Cook 1997).	
Myotis lucifugus	Little brown myotis
Myotis volans	Long-legged myotis
Myotis keenii	Keen's myotis
Lasionycteris noctivagans	Silver-haired bat
Glaucomys sabrinus	Northern flying squirrel
Clethrionomys gapperi solus	Southern red-backed vole (endemic to this region)
Microtus pennsylvanicus	Meadow vole
Zapus hudsonius	Meadow jumping mouse
Erethizon dorsatum	Porcupine

METHODS

OBJECTIVES 1 & 2. TETRACYCLINE STUDY

In June 2000, 200 wooden bait boxes (46 cm x 9 cm x 9 cm) were made to bait and mark black bears with tetracycline (Garshelis & Visser 1997); wooden boxes prevented other species from taking the bait. The boxes also facilitated identification of the baited animal, because the boxes had to be broken to access the bait. In late June 2000, 188 tetracycline bait boxes were set out on Kuiu Island, north of the Bay of Pillars and Port Camden isthmus. Skiff crews placed 134 baits along the coastline at stream estuaries, and 54 baits were placed on the road system. Baits were set out at approximately 0.6-km intervals. Baits consisted of bacon, suet, molasses and fish and shrimp parts. Baits and broken boxes were retrieved approximately eight days after bait distribution.

Bone samples serving as the capture sample were collected from Kuiu Island hunters in Fall 2000 and spring 2001. Forty-one bone (n = 38) or tooth (n = 3) samples were collected from Kuiu Island black bears harvested in Fall 2000. In spring 2001, 126 bone or tooth samples were collected from harvested Kuiu bears. Toe bones were requested, as it is possible to detect a bear taking multiple baits only from bone samples. Bone and tooth samples were processed for tetracycline analysis at the Minnesota Department of Natural Resources laboratory. A Lincoln-Petersen mark-recapture model has been used to estimate the North Kuiu Island black bear population size.

OBJECTIVES 3 & 4. BACKGROUND GENETIC VARIATION

Tissue samples were collected from livers of harvested bears at the time of hide sealing, and from meat and cartilage of submitted toe bones. Nuclear DNA from these roughly 280 tissue samples has been extracted using the Qiagen method. Polymerase Chain Reactions (PCR) (Palumbi 1996) and genotyping has been performed at ten microsatellites and two sex identifiers. Microsatellites are neutral nuclear DNA regions (Jeffreys *et al.* 1985), commonly used as molecular markers for analysis of population parameters. Laboratory methods for these objectives, which involve tissue samples, are described below with Objective 5.

OBJECTIVE 5 & 6. NONINVASIVE GENETIC SAMPLING

Barbed wire hair snags were set up in five independent watersheds (Table 2). A total of 191.72 m of barbed wire was strung across bear trails. The average height of the barbed wire snags was 0.55 ± 0.02 m. Hair samples were collected in 6-day intervals. Hair samples were placed in coin envelopes and kept dry in a pelican case with DRIERITE to absorb moisture. All possible hair samples from barbs were not collected; hair was systematically sub-sampled from barbs.

Table 2. Noninvasive DNA Sampling Effort

Creek	# of hair - snags	Extent of Sampling	Dates of Sampling	# of Sampling Intervals	# of Samples Possibl	# of Samples Taken

					e	
Cabin	13	Entire spawning stretch	08/09/00 – 09/03/00	5	256	87
Portage	11	Entire spawning stretch	08/09/00 – 09/09/00	6	178	66
Saginaw	32	Tidal area; upstream area	07/20/00 – 08/31/00	8	903	346
Security	19	Tidal area; upstream area	07/20/00 – 09/01/00	8	556	207
Kadake	32	Tidal area; two upstream areas	07/21/00 – 08/27/00	6	292	119
Total number of hair samples taken in field season 2000:						825

DNA from hair and tissue samples has been extracted using the Qiagen Spin Column Method. The basic principal of this method is as follows: DNA is separated from protein and other cellular material during incubation in a lysis buffer and a protein enzyme. Then a series of washes are used with centrifugation, which bring all cellular material except the DNA through the filtering column; all this is considered waste. A final buffer is used to elute the DNA through the column. This buffer binds the DNA and brings it through the charged column upon centrifugation.

For the hair sample extractions, I have modified this general approach to increase the amount of eluted DNA. I use a Poly-A carrier, which is an RNA polymer that binds to the DNA. In this way, there is a larger mass of nucleotide polymers, which can be brought through the column more effectively. I have also increased the efficacy of the elution buffer by incubating it with the DNA before centrifugation. Finally, I have increased centrifugation speeds, and I use a smaller elution volume to increase the final concentration of DNA. DNA is extracted only from hair root follicles; ten follicles from each sample are used. These ten hairs are selected on the basis of similarity of length, texture and color. DNA from hairs shorter than 5 cm are not extracted to avoid sampling cubs of the year. Follicles are clipped using flame sterilized scissors and forceps. The simplified steps of the modified Qiagen extraction are as follows:

I. Vortex 180 µl of tissue lysis buffer ATL and 20 µl of Protinase-K enzyme in a 1.5 ml microcentrifuge tube for each sample.
II. Add the ten cut follicles to these tubes, and incubate overnight in 56°C water bath.
III. The next day, take samples out of bath and vortex thoroughly.
IV. Add 200 µl of buffer AL. Incubate at 70°C for 10 minutes.
V. Add 2 µl of Poly-A carrier* and 210 µl* of 90% ethanol to tube. Vortex thoroughly.

VI.   Pipette out entire contents of tube and place in spin column. Centrifuge at 10,000 rpm* for 1 minute.

VII.   Discard flow through, and add 500 µl of AW1 wash. Centrifuge at 10,000 rpm* for 1 minute.

VIII.   Discard flow through, and add 500 µl of AW2 wash. Centrifuge at 14,000 rpm for 3 minutes.

IX.   Discard flow through, and add 300 µl* of pre-heated (70°C)* AE buffer to the spin-column. Incubate in 70°C water bath* for 10 minutes.*

X.   Centrifuge at 14,000 rpm for 3 minutes. Final elution volume is 50 µl*.

    * Indicates deviation from Qiagen Protocol

PCR is used to amplify the black bear DNA at microsatellite regions. In short, PCR is an *in vitro* replication of DNA. It is necessary to amplify the DNA sequence of interest (*i.e.,* microsatellite locus) to have enough template to genotype the region. During this reaction, the DNA double helix is first denatured at a high temperature (95°C). Primers then anneal to the flanking regions of microsatellite locations on the single-stranded DNA (annealing temperatures range between 48 and 58°C), and then a polymerase works (at 72°C) to replicate the DNA by synthesizing the nucleotide sequence (*e.g.,* GTGTGT) between the primers. This process is repeated for 35 or 45 cycles, depending on the nature of the DNA template (*i.e.* hair or tissue DNA). In this way, the sequence between the primers, the microsatellite region, is replicated exponentially. I use the heat-stable Ampli*Taq* Gold polymerase, which is a naturally occurring DNA polymerase from the hot-pool bacteria, *Thermus aquaticus,* to extend the sequences. A heat-stable polymerase is needed, as a high temperature is required to denature the double-stranded DNA helix. Such a polymerase insures that a chain-reaction can proceed in which denaturation and nucleotide annealing can cycle without additional input of polymerase.

PCR reactions contain a buffer to keep the pH of the reaction at an optimal acidity for *Taq*Gold polymerase. Deoxynucleotide triphosphates (dNTPs) are the nucleotide bases (Guanine, Adenine, Thymine and Cytosine) used to synthesize fragment sequences. $MgCl_2$ catalyzes the synthesis reaction. The primers are added in excess to direct the synthesis of the microsatellite region. The conditions for a favorable PCR reaction are unique to the microsatellite locus and the quantity and quality of DNA template. It is therefore necessary to optimize the PCR conditions for each locus and population. Optimization maximizes the stringency and efficiency of the reaction and is a process where varying amounts of $MgCl_2$, primer concentration, and *Taq*Gold polymerase are tested at varying annealing temperatures. In general my PCR reactions contain: 1.5 µL of 10X Buffer Cetus II; 0.2 µL *Taq*Gold; 1.0-1.5 µL each of 10 µM forward and reverse primers; 0.5 µL of 10 mM dNTPs; and 1.5-2.0 µL of 25 mM $MgCl_2$. In addition 2 µL of DNA template is added for tissue PCRs with dH_2O enough to bring up the total reaction volume to 15 µL. In PCRs using hair as the DNA template, no dH_2O is used, instead the amount of template DNA varies (~9 µL) to bring up the total reaction volume to 15 µL.

Genotyping the microsatellite regions of each PCR product is done using ABI (Applied Biosystems, Inc.) 310 and 3700 Genetic Analyzers. Genotyping determines the lengths of the two alleles at a locus for each sample; microsatellite alleles differ in terms of the number of

nucleotide base pairs, in turn determined by the number of tandem repeats (*e.g.,* GTGTGT = 3 tandem repeats, 6 base pairs). Since microsatellites are hyper-variable molecular markers, there will be multiple alleles at a single locus within a population; there also can be two different alleles within an individual (*i.e.,* a heterozygote).

The forward primers used in the PCRs are labeled with a florescent dye, which is detected by the genotyping machines. This florescent dye tags the beginning of the microsatellite fragment to be analyzed. Samples are brought up through capillary tubes, which are filled with a liquid polymer, through which electrophoresis of the DNA fragment will occur. A voltage is applied across the capillary and the fragments, which are negatively charged, migrate; the rate of migration depends on the length of the allele. A laser detects the florescent fragment when it reaches the end of the capillary. The machine converts this information into an electropherogram, which plots dye concentration against time for each sample. In this way, alleles are differentiated.

Each DNA sample (both hair and tissue) will be genotyped at ten microsatellite loci. Since the length of an allele may vary within one base pair (*e.g.,* 165.34 – 166.26), actual fragment lengths will be binned into an allele size. A microsatellite signature, consisting of genotypes at each locus, will be determined for each sample; a unique microsatellite signature will identify individuals.

Analysis of hair samples will require filtering mechanisms due to problematic PCR and genotyping resulting from using picogram quantities of DNA template (Gagneux *et al.* 1997, Waits *et al.* 1998, Taberlet & Luikart 1999, Mills *et al.* 2000). Filters, which require re-amplification (re-PCR) of certain genotypes and microsatellite signatures, will be used to detect the appearance of false alleles and allelic dropout (false homozygotes) (Roon *et al.* 2001). These genetic artifacts are the two most common genotyping errors when using PCR with low quantity and quality DNA. These filters are necessary to determine the genotyping error rate, as this error must be incorporated with the PI in the overall error rate of the population estimation models. The following filters will be used to decrease and estimate genotyping error rate:

1. **Shadow Effect Filter**. Only samples that can be successfully genotyped at least seven loci will be used in analysis. Seven loci are deemed sufficient to decrease the shadow effect (Mills *et al.* 2000), the chance that the same microsatellite signature represents more than one bear, to an acceptable level. The shadow effect increases with increasing PI, and can greatly increase the error in population models (Taberlet & Luikart 1999, Mills *et al.* 2000, Waits *et al.* 2001).
2. **Relatedness Filter**. Samples with microsatellite signatures, which differ in genotype at only one locus, will be re-amplified (Roon *et al.* 2001).
3. **Singles Filter**. Samples with alleles that occur only once or are rare, will be re-amplified (Roon *et al.* 2001).

To complete objective five, every hair sample must be genotyped and assigned a microsatellite signature. This is not yet completed, due to the great number of samples collected in the 2000 field season. When all samples are assigned an individual identity they will be used in an open population model to estimate the number of bears using each stream.

Open population models, generally referred to as Jolly-Seber models, do not have assumptions of demographic and geographic closure. In fact, they are specifically designed to assess the degree of 'emigration' and 'immigration' in the population. In this case, the models will be used for each salmon stream independently, and the number of bears using the stream, and the degree of movement to and from the stream can be evaluated. The program MARK (White & Burnham 1999) will be used to assess these population parameters. MARK uses recapture histories of individuals over the course of several sampling intervals and calculates the recapture probability. Model parameters will be chosen which can distinguish between male and female bears, as the sexes may have different capture and recapture probabilities. Other parameters, such as heterozygosity (G. White, pers. comm.) can also be used to model individual differences in capture and recapture probabilities. MARK works in such a way that it evaluates all the different biological models that I create which attempt to explain differences in capture probabilities between groups of bears or individual bears. Akaike Information Criteria (AIC) (Anderson *et al.* 2000) are assigned to the various models depending on the fit of the data to the model; model averaging will be used to determine the best fit population parameters.

The completion of the sixth objective concerning social spatial structure of bears on salmon streams also requires data that will be obtained upon completion of the genetic sex and individual determination. Once the sex of all individuals using the salmon streams are known, I will use logistic regression analysis to determine whether there is sexual segregation within salmon streams.

OBJECTIVE 7. SOCIAL BEHAVIOR OF BLACK BEARS ON SALMON STREAMS

Five treestands were constructed in tidal and upstream areas on North Kuiu Island to conduct behavioral observations on black bears. Tidal treestands were put up on Port Camden Cabin Creek, Port Camden Portage Creek and Security Creek. Of the tidal treestands, only the Port Camden Cabin Creek platform was used this year, because late salmon runs coincided with the hunting season and due to additional logistical considerations. Observations were also recorded from the bridge at the tidal area of Port Camden Cabin Creek. A temporary stand was put up in the tidal flats of Kadake Creek, but flooding and late timing of the run resulted in no observations from this stand. Upstream treestands were constructed and used on Saginaw Creek and Security Creek. Treestands were built with plywood and 2 x 4's and were designed for single-person use. It was necessary to put lag-bolts in some of the trees to facilitate climbing. All treestands were equipped with safety ropes; safety equipment was used to ascend to and descend from the treestands.

Approximately 214 hours of behavioral observations were recorded from July 23rd – September 3rd 2000. Data were recorded with sample sheets and audio tape recorders. Behavioral categories recorded included: Vigilance; traveling; searching for fish; pursuing fish; scavenging fish; consuming fish; and consuming vegetation.

RESULTS AND DISCUSSION

OBJECTIVES 1 & 2. TETRACYCLINE STUDY

Black bears consumed 144 (76%) baits. Of the baits placed along the coast, 63% were taken; 82% of the baits placed on the road system were taken. Six of the 144 taken baits probably did not result in a marked bear, as greater than half of the nine 50-mg tetracycline pills were found in the vicinity of these baits. Thus, I estimate that there were 138 marks in the population, before the fall 2000 hunting season. Furthermore, hair samples were collected from 68% of the stations, where bait was consumed. DNA has been extracted from these hair samples to obtain a genetic and sex identity of baited bears.

Tetracycline marks were found in six of the 41 Kuiu Island bone samples collected in fall 2000. Marks were found in five of the 126 bones collected from Kuiu Island in the spring of 2001. One of the eleven marked samples (9.1%) had a double mark. Since we assume an equal probability of capturing marked and unmarked bears, we must assume that 9.1% of the 138 marks in the population occur are double marked. Thus, adjusting for double marks, there were 126.5 marked bears on North Kuiu, prior to the fall 2000 hunting season. The ratios of marked to unmarked bears in the capture samples are being used to estimate the population size of black bears on North Kuiu. We are currently developing several different models, which incorporate emigration (loss of marks from the population), and effects of differential marking probabilities of female and male bears. We are also using various averaging techniques to combine the fall and spring samples (Bartmann *et al.* 1987).

OBJECTIVES 3 & 4. BACKGROUND GENETIC VARIATION

Tissue samples from 280 black bears harvested in Southeast Alaska have been collected; DNA has been extracted from all of these samples. Individuals at five loci (G1A, G10B, G10C, G10L and G10L) have been sufficiently characterized (average of n = 37) to make some preliminary generalizations about the allelic diversity, and other population genetics parameters of the Kuiu black bears (Table 3). The average number of alleles per locus is 5.2, and no locus has fewer than 4 alleles. The number of alleles at these loci is less than in other bear populations (Paetkau & Strobeck 1994, Paetkau *et al.* 1998, Paetkau *et al.* 1999, Waits *et al.* 2000); however, a reduced number of alleles is to be expected in an insular population and in a preliminary survey. The number of alleles per locus is likely to increase when more tissue samples are examined during the continuation of this project. The number of alleles (*k*) and range of allele sizes (*r*) are important parameters for individual identification. The more alleles a locus has, the more useful it is for individual identification.

Average heterozygosity across these loci is 54% and ranges between 45 and 69%. In comparison, average heterozygosity of brown bear (*U. arctos*) populations range between 26% (Kodiak Island) and 75% (W. Brooks Range) (Strobeck 2001). For three black bear populations at four microsatellite loci, heterozygosity ranged between 36% (Newfoundland; insular) and 80% (Paetkau & Strobeck 1994). Heterozygosity is a useful measure of genetic diversity of a population. If a population has undergone a demographic bottleneck, heterozygosity will likely be reduced. If a population is subject to non-random mating due to an insular nature or inbreeding, heterozygosity can also be reduced. In extreme cases, low genetic heterozygosity at

neutral markers (*e.g.*, microsatellites) can be an indicator of phenotypic problems such as low reproductive success (Roelke *et al.* 1993).

The probability of identity (PI) is the probability that two randomly chosen individuals from the same population have the same microsatellite signature. PI is important to estimate, as individual identification using molecular markers is a probability of, not an absolute, identity. The lower the PI, the more confident one is at correctly assigning individual identities. My initial estimate of PI over these five loci is 0.0008. In other words, there is a chance of 1 in 1,152 that two randomly chosen Kuiu black bears have the same microsatellite signature. For comparison, other bear populations have a PI that range between 1 in 93 (brown bears on Kodiak Island) and 1 in 210,000,000 (brown bears in Richardson, Canada) (Strobeck 2001). Since PI decreases with increasing number of loci surveyed, my initial estimates of PI are likely to decrease as more individuals and loci are surveyed. It is important to note, however, that bears are not randomly distributed on salmon streams; it is likely that females on salmon streams are more related than a random pair of individuals. Therefore, for application to the noninvasive sampling study conducted along salmon streams, PI for siblings and parent-offspring pairs will also be calculated.

Another aspect involving allelic diversity which has been superficially investigated is the degree of population bottleneck that the Kuiu Island black bears have, or have not, undergone. Garza & Williamson (Garza & Williamson 2001) have recently devised an index that detects reductions in population size using allelic diversity at microsatellite loci. Their index, *M*, is the ratio between the total number of alleles and the range in allele size (base pairs) at a locus:

$$M = k/r$$

This index is based on the principle that rare alleles are lost first when a population is reduced, *i.e.* the number of alleles can be reduced without a concomitant reduction in the range of alleles. The average *M* for the five loci in the Kuiu bear population is 0.30. Garza & Williamson (2001) remark that "any data set with seven loci or more and a value of *M* < 0.68 can be assumed to have gone through a recent reduction in size." For comparison, the authors have found an *M* = 0.641 for the insular Newfoundland black bears at eight loci (Paetkau *et al.* 1997, Garza & Williamson 2001). It is likely that this initial average *M* for Kuiu bears will change with more individuals and loci surveyed. However, *M* is just an average parameter, unlike PI, and therefore does not necessarily increase or decrease with increased sampling. From this preliminary analysis, it appears that the Kuiu black bear population may have undergone a population bottleneck.

The program BOTTLENECK (Piry *et al.* 1999) detects effective population size reductions using allele frequency data. A bottleneck is likely to have occurred if a significant number of loci show heterozygosity excess (Cornuet & Luikart 1996, Luikart *et al.* 1998). Under the mutation-drift equilibrium model (assumes no bottlenecks) heterozygosity excess is no more likely than heterozygous deficiency. In a population that has undergone a bottleneck, allelic diversity is lost faster than heterozygosity, and thus the heterozygosity calculated from the allelic diversity should be lower than the observed heterozygosity, *i.e.* there is excess observed heterozygosity. In these first simulations, with the Kuiu allele frequency data, none of the statistical tests found

heterozygosity excess at these loci, *i.e.* no population bottleneck was detected for the Kuiu black bears.

These preliminary analyses regarding the probability of a population bottleneck provide conflicting results. The continuation of this pilot study will provide more confident conclusions on the population genetic status of the Kuiu Island black bear population.

Table 3. Genetic Parameters at Microsatellite Loci for the Kuiu Island Black Bear Population

Locus	A*	Range of Alleles (bp)	M**	Heterozygosity (H)	Kuiu H compared to other studies †	PI***	Kuiu PI compared to other studies ‡	# Individuals Surveyed
G10B	5	11	0.45	47.7%	Lower	0.21	Higher	44
G1A	5	24	0.21	45.0%	Lower	0.39	Higher	40
G10M	4	8	0.5	56.5%	Lower	0.33	-	23
G10L	7	57	0.12	69.7%	Higher	0.10	Higher	33
G10C	5	21	0.23	51.0%	Same/Lower	0.31	-	47
Overall	**5.2**	-	**0.30**	**53.98%**	**-**	**0.0008**	**-**	
G10P	Not fully optimized							
G10O	Not fully optimized							
G10D	Not fully optimized							
G10J	Not fully optimized							
G10X	Not fully optimized							

* Number of alleles at the locus
** $M = k/r$; a measure of population bottleneck (Garza & Williamson 2001)
*** Probability of Identity; the chance that two randomly chosen individuals from a population have the same genotype (microsatellite signature for "overall") at this locus (loci)
† (Paetkau *et al.* 1999, Waits *et al.* 2000)
‡(Paetkau & Strobeck 1994)

OBJECTIVE 5 & 6. NONINVASIVE GENETIC SAMPLING

Over 800 hair samples were collected from five watersheds on North Kuiu Island over the course of nine weeks. Approximately half of these samples have been extracted, and PCR'ed at seven loci. More laboratory work must be done before individual and sex identity of the samples can be established.

OBJECTIVE 7. SOCIAL BEHAVIOR OF BLACK BEARS ON SALMON STREAMS.

Thus far I have analyzed data collected on behavior of sows with dependent young. I analyzed data on thirteen known individual mothers; each datum was the mean of a behavioral category for the particular sow. The results I report here are for the behavioral categories of VIGILANCE and FOCUSED VIGILANCE. Vigilance is a common animal behavioral response, which researchers associate with anti-predator behavior, and usually negatively correlates with foraging. I defined VIGILANCE as any head-up posture; it is a mutually exclusive behavioral category. A subcategory of VIGILANCE I defined as FOCUSED VIGILANCE. FOCUSED VIGILANCE is a sharp, focused look and/or sniff at something in the bear's environment. I chose to distinguish between VIGILANCE and FOCUSED VIGILANCE because I believe these are different behaviors. The former may not be an accurate measurement of stress for bears, whereas the latter is certainly a reaction of a bear to its environment and detracts from foraging.

The data are not distributed normally; I used a Kruskal-Wallis test to assess the difference in VIGLANCE of mother bears between tidal and upstream areas (p = 0.11) and FOCUSED VIGILANCE (p = 0.04). In both of these categories, Levene's test suggested that there were unequal variances between the tidal and upstream areas. Variance in VIGILANCE in the tidal area is larger than that in upstream areas (p = 0.06); variance in FOCUSED VIGILANCE is larger in the tidal areas than in upstream areas (p = 0.03).

CONCLUSIONS AND RECOMMENDATIONS

The field component of the tetracycline study was successful; uncertainties regarding whether bears would take baits, time, and human effort required for baiting and whether we could prevent other animals taking the baits were resolved. I recommend using this tetracycline method to estimate the population size of the entire Kuiu Island, employing methods that have been successful and those that have been revised during this pilot study.

Noninvasive hair sampling was successful in that we could sample hair from black bears along salmon streams without using bait and we are able to extract DNA of sufficient quality and quantity from these samples. I am in the process of determining whether our sampling design during this pilot study was adequate to use as a population estimation and documentation of social structure method.

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