MARK-RECAPTURE POLAR BEAR POPULATION ESTIMATES USING TETRACYCLINE AS A BIOMARKER

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ABSTRACT

Polar bears and apparently most mammals may be efficiently and reliably marked by remote injection of tetracycline without restraint or immobilization. The dose level required to leave a permanent and reliably detectable tetracycline deposit in the teeth depended on the time between inoculation and tooth sampling. A minimum dose of 25 mg/kg body weight was required when the interval to sampling was short (7 days). However, all dose levels at or greater than 2 mg/kg were detected after 1 year.

The year that the mark was given may be determined from the tetracycline deposition position in the annular ring of the teeth. Conventional mark-recapture models which do not require identification of individual animals may be employed to give population estimates from the ratio of marked to unmarked animals. Similarly the survival of marked animals may be determined from the ratio of animals marked 1,2,3,... n times if a multiple year marking program is implemented.

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INTRODUCTION

The first indication of the calciphilic character of tetracycline came from the observation that children's teeth sometimes became pigmented following clinical administration of tetracycline. Subsequent investigation revealed that the brown stain came from photo-reduction of tetracycline that was permanently bonded into the tooth enamel (Sande and Mandell 1985). Prior to degradation, the tetracycline pigment exhibits a yellow florescence with an absorption peak at 270nm when exposed to UV light.

Tetracycline is deposited in growing bones and teeth at the front of mineralization (Ellenton and Johnston 1979). It may be incorporated into the hydroxyapatite crystals of bones for months or years; however, it is eventually lost through the process of remodelling (Sande and Mandell 1985). If tetracycline is given when the crowns of the teeth are being formed, the tetracycline may be deposited in the enamel. However, after tooth eruption, the tetracycline is only deposited in the root dentin and cementum. Tetracycline that is deposited in the teeth and not subject to photo-oxidation appears to persist indefinitely (Johnston et al. 1987).

Tetracycline has been exploited as a method of placing a time-specific mark in the cementum to aid in the interpretation of the cementum annuli (Yagi et al. 1963, Cleall et al. 1964, Diehl 1964, Kiel 1965, Suzuki and Mathews 1966, Linhart and

Kennelly 1967, Linhart and Knowlton 1967, van Coillie 1967, Evans and Griffith 1973, Allen 1974, Best 1976, Ellenton and Johnston 1979, Domning and Myrick 1980, Gurevich et al. 1980, Myrick et al. 1984, Day and Carrel 1986, Geraci et al. 1986). The required dose levels for reliable marks ranged from 2 mg/kg to 60 mg/kg.

Although both mark-recapture techniques and methods of permanently marking animals with tetracycline biomarkers are well known, we were unable to find any documented use of tetracycline to mark animals for mark-recapture population estimates. Our application was a population of polar bears that are concentrated in early autumn along coastal areas in the northern Hudson Bay and Foxe Basin area. These animals are difficult and dangerous to immobilize because of warm temperatures, high relief and open water. The feasibility of remote injection of tetracycline as a biomarker for the purpose of estimating population numbers with mark-recapture statistics is investigated.

For the tetracycline mark to be useful for multiple year mark-recapture population estimates it must be permanent and be recognized on recovery. The number of bears marked must be known for each year of marking. For our target population, the recapture sample is from approximately 160 polar bears per year that are harvested. The lower jaw of each harvested bear is collected providing the required tooth for laboratory examination.

METHODS

Two issues were of interest. The first was the dose level required to mark the cementum layers of polar bear teeth reliably. The second was to develop a delivery system that could administer the required dose safely and reliably.

The dose level was evaluated experimentally and on opportunity. Thirty-one polar bears were injected with tetracycline at dose levels ranging between 2 mg/kg and 25 mg/kg and recaptured 7 to 21 days later. Twenty of the 31 were relocated using an ear-tag radio transmitter and the remaining 11 were relocated by a dye mark made remotely with a PNEU-DART marker dart. An additional 111 teeth from animals injected with tetracycline were obtained from harvested animals and other research programs. The teeth collected opportunistically were from animals with dose levels ranging from 2 mg/kg to 25 mg/kg and were collected between 1 week and 4 years post injection. Thirty unmarked teeth were used as controls.

The tetracycline (200 mg/ml and 400 mg/ml) was administered by syringe after the animal had been immobilized, by remote injection from a helicopter, and by jab-pole for some bears held temporarily in captivity. The 400 mg/ml slurry was only used for 12 bears because of difficulties in injection. Several varieties of darts were used for remote injections: Cap-Chur (Palmer Chemicals Ltd., Douglasville, Ga.) and PNEU-DART (PNEU-DART Inc., Williamsport, Pa.). Polar bears were immobilized according to techniques described by Lentfer (1968), Larsen (1971), and Schweinsburg et al. (1982) using the drug Telazole (Haigh et al. 1985, Stirling et al. 1985).

Aluminum Cap-Chur darts were employed initially for remote injection. However, these were replaced by disposable 6cc aluminum (needle length 4.5 cm), and 15cc plexiglass (needle length 4.5, 5, and 6.35 cm) darts from PNEU-DART. The 6cc darts (.50 calibre) were barbed with 0.1 cm diameter gauge stainless steel wire. The injection was accomplished by a powder charge that detonated on impact. The 15cc darts (.75 calibre) used barbless 0.3 cm diameter gauge needles. Barbs were not required for the 15cc darts because the contents was discharged from ports drilled in the sides of the needle near the plugged tip. The ports are covered by a plastic sleeve which is pushed back when the dart strikes. The pressure for injection comes from a reservoir of vinegar-bicarbonate of soda that is mixed just before the dart is fired.

All polar bears that were remotely injected with tetracycline were also marked with a PNEU-DART marker dart filled with "Nelspot" paint ball dye (Nelson Paint Company, Iron Mountain, MI). This non-toxic, oil based dye was available in black, blue, green, orange and yellow. The colours varied in viscosity. Application of the dye was to ensure that double marking did not occur.

The tooth extracted for examination for tetracycline was typically the first lower premolar. Occasionally the first upper premolar was taken because the first lower premolar had already been extracted. Longitudinal sections of 60-120 microns were cut at the root tip and examined under ultraviolet illumination at 200X with a Ziess microscope system. To avoid contamination between teeth marked with tetracycline and teeth not marked with tetracycline, the sectioning saw was rinsed frequently. Observations of both dentine and cementum were made. Teeth were independently classified as positive or negative with respect to the mark by 2 observers. The only animals counted as positive were those where both observers agreed that the mark was unambiguous.

To determine the effect of age on marking teeth sampled within 180 days and at dose levels less than 16 mg/kg, samples were stratified into subadult (age 0-5) and adult (age 6+). Similarly, dose levels were stratified into lower (0-7 mg/kg) and upper (7-15 mg/kg) categories to determine the effect of dose level on samples collected within 180 days of injection. The 2X2 contingency tables were examined with Fischer's exact test (Kendall and Stewart 1979).

RESULTS

Seventy-three of 74 potential tetracycline marks examined 180 days or longer after injection of tetracycline were read as positive in the tooth cementum. Potential marks included bears that had been given tetracycline in separate years. A triple marked bear was treated as 3 observations of the tetracycline mark. The only animal that failed to register a mark after 180 days was inoculated with 8 mg/kg of oxytetracycline as a yearling. The tooth was recovered 2 years later when the bear was harvested.

All bears inoculated at 25 mg/kg were found to have been marked. Although lower dose levels did produce marks in all instances over 180 days, they were not as bright as higher dose levels. Older marks were as or more apparent than younger marks because they were concentrated in the cementum annuli. Marks observed in samples taken shortly after injection were seen as a florescent sheen in the pulp cavity on the surface of the dentine nodules. However, up to 60 days, there seemed to be little or no visible tetracycline florescence in the outer layer of cementum. The tetracycline florescence may occur at the outer layer of the cementum, but may be masked by the bright blue-white florescence surrounding the outside of the tooth surface. This blue-white glow was evident in all the teeth examined and is probably from the periodontal membrane.

When only short-term samples (<180 days) at dose levels less than 16 mg/kg were considered, higher dose levels (>7mg/kg) resulted in 50% more positive marks although the increase was not significant ($p=0.135$). Younger animals were significantly more likely to be marked than older animals ($p=0.046$).

In 1986 one of the bears that had been hand injected with tetracycline in the rump (4.5 cm needle) died of cardiac arrest during immobilization 8 days after the injection. The field autopsy revealed a non-septic cyst in the subcutaneous fat at the site of tetracycline injection. The cyst was approximately 8 cm long and 5 cm in diameter. It appeared that the tetracycline had not reached the underlying muscle. The tooth from this animal was read as negative. Only 4 of 9 adults (6+) that received tetracycline in the rump were observed to be marked within 180 days of injection. Five of 6 young animals (<6), also in the <180 day group, that had been injected in the rump were observed to be marked. The fat layer of older animals is substantially thicker than the fat layer of smaller, younger animals. After 1986 all bears were injected in the neck or shoulder muscles.

The 15cc darts were tested on adult males, adult females, and subadults. The 4.5 cm needles were adequate for subadults and adult females. Five cm needles were adequate for adult males. The 6.35 cm needles could be used safely on large males, but were not required.

The larger diameter of the 15cc dart needles and the non-explosive injection provided by the vinegar and bicarbonate of soda allowed the dart to remain in the bear long enough for the injection to occur without requiring the needle to be barbed. The 15cc darts typically fell free of the bear within 2 minutes after the injection. No bears were found with 15cc darts still attached in subsequent surveys. The clear plexiglass dart body allowed each injection to be visually verified.

In 8 of 89 15cc darts employed, partial rather than complete injection occurred. The principle cause of incomplete injection was the angle of the dart to the surface of the bear. Low angle shots tended to be more susceptible to bouncing than more perpendicular shots. The number of incomplete injections and bounced darts decreased when increased discipline in shot selection was exercised.

The 10cc darts obtained from the PNEU-DART company had defective charges and tail pieces. Their use was discontinued as soon as these problems were noticed. These darts were originally manufactured by the Distinject company. Subsequent testing indicated that the tail pieces required pliers to tighten them.

The 6cc and 3cc disposable PNEU-DARTs performed well. However, the needles of these darts (as well as the 10cc darts) are barbed so that the dart is not expelled by the explosive injection of the drug. Only 1 bear (a cub accompanied by its mother) was observed to have retained the dart on surveys 7 days post injection. The cub was observed with a 3cc dart still in its shoulder, captured, and the dart was removed. The reason the dart had not fallen out or been removed by the mother appeared to be that the dart needle had been bent into a fishhook shape. It is not possible to indicate the extent of this problem from our sample because not all bears that were injected with barbed darts were seen later.

Although the paint marking equipment performed well, the marker paint proved to be unreliable. The paint was apparent on only a few of the bears after 7 days of field application and wear. It was apparent that all the colours of paint had been "weathered" off. Some bears could be identified by dart wounds as having been marked with tetracycline. In 2 instances 2 of 3 members of family groups had lost paint marks.

DISCUSSION

The fraction of animals marked with tetracycline when sampled 180 days or longer after injection was 0.987. The only bear that was not positive for the tetracycline mark after 180 days was injected as a yearling and harvested as a 3 year old. The age at death of the bear was confirmed by aging a first premolar (Stirling et al. 1977). The age and reported sex of the bear matched the age and sex expected from the initial handling data; however, several animals of the same age and sex were taken by the community. The ear tags of marked bears are sometimes kept separate from the lower jaws by the hunters. The possibility of misidentification exists, but cannot be demonstrated.

The tendency for younger animals to exhibit tetracycline marks when sampled less than 180 days after injection is probably due to more active dental tissue in younger animals. The age chosen to stratify younger from older bears (i.e., age 6) was the mode age of first reproduction of females. Male polar bears continue to grow at a slower pace after age 6 and most females are close to maximum adult weight by that age (Kingsley 1979).

The failure to detect differences in tetracycline marking between sexes suggests that the physiology of calcium deposition in dental tissue does not differ between males and females. It appears that tetracycline is an equally effective biomarker for both sexes.

Although all bears injected at 25 mg/kg were read as positive, the increase in the fraction of animals marked with progressively higher dosages below 25 mg/kg was not significant. Higher doses did appear to result in brighter marks in all samples and more distinct marks in samples taken less than 180 days post injection. Failure to reject the null hypothesis of equal effect of dose level may have been mainly due to the high variability in results within the initial 180 day period.

The highest concentration that was practical for field delivery was found to be 200 mg/ml for both tetracycline hydrochloride and oxytetracycline. At 200 mg/kg for dose levels of 25 mg/kg a large male bear (550 kg) would require 68.75 ml or 4-5 15cc darts. At 15 mg/kg the same bear would require 41.25cc or 3 15cc darts. We did not wish to give more than 15cc per injection site and not more than 3 darts per bear. The recommended protocol for tetracycline marking given in Table 1 is based on the mean autumn weights of Hudson Bay polar bears.

The darts were found to be both reliable and efficient. Individual polar bears could be marked with both the marker dye and the tetracycline within 2-4 minutes in most instances. Bears were marked in the ocean, and along cliff faces. No bears encountered were abandoned without marking. The 15cc darts were particularly effective because they were transparent. Each injection could be visually verified.

Although the smaller volume (<15 cc) darts worked well, they required barbs to remain in the bear during injection.

Additionally, injection could not be visually verified because the bodies of the darts were opaque. Darts similar to the .75 caliber 15cc model could be developed in .50 caliber for volumes up to 10cc.

Table 1. The number of darts of 4, 10, and 15 cc that are requiredto inoculate polar bears with 15 mg/kg of 200 mg/ml tetracycline are given for mean autumn weights in the Hudson Bay area.

TETRACYCLINE MARKING FOR POLAR BEARS
PROTOCOL FOR 15 mg/kg

AGE/SEX	4cc	10cc		15cc
CUBS	1			
YEARLINGS		1		
SUBADULTS (2-4)				1
ADULT FEMALE		1	+	1
ADULT MALE				2
LARGE MALE (>550 kg)				3

Dart placement was facilitated by driving the bears into water before darting. Additionally, polar bears sighted in the water could be quickly darted. This technique has obvious drawbacks for use with immobilization drugs, but can be safely utilized with tetracycline marking.

The marker darts deployed the paint with sufficient force that both guard hair and under fur were marked. The failure of the paint to last on free ranging polar bears may be due to several factors. Many of the bears entered the sea shortly after or during marking. Polar bear fur is oily in part to repel water during swimming. The combination of oily fur and sea water could have caused the paint to be washed from the fur before it had set. Some polar bears were observed licking at the paint. In any event it appears that Nelspot paint may be added to the long list of failed markers for polar bears.

Beagon (1983) has noted that field studies often have difficulty meeting the assumptions of mark-recapture models. A multiple year mark-recapture program is typically required for estimating the size of a polar bear population. The tetracycline marks appear to satisfy the 100% recognizability assumption of the mark-recapture models only after the initial year (180 days) of injection. An estimate of annual mortality is then required to determine the number of marks at risk in a given year.

The year that the marks were given can be determined from their position in the cementum annuli. In practice, the annuli of polar bear teeth are difficult to see on hard sections under the UV light source; however, under polarized light, the annuli are more apparent. The year of the mark may be determined by switching between light sources. To estimate the annual survival of marks, one must know how many years previous to the sample year the animal was marked. The annual survival rate of marks is required to determine the marks at risk in any given year. Multiple year marking will result in multiple marking of some animals. Each mark may be considered according to procedures described by Fisher and Ford (1947). Alternatively the mortality rate for marks may be assumed, estimated independently (DeMaster et al. 1980), or calculated from the distribution of animals marked 1,2,3 ...n times.

This application requires that the survival rate of all marks is equal and constant. The survival rate of young and subadult animals may not be the same as adults. A stratified design where the marks at risk are a function of the age strata of previously marked animals, age specific survival rates, and the time since marking occurred may be more appropriate. However, the assumption of constant survival may be reasonable for harvested polar bear populations that are not experiencing density effects (Taylor et al. 1987).

The recapture sample of teeth would come from the harvest. The harvest of polar bears is geographically non-random and sex-age selective. However, if the marks are distributed randomly, the recapture sample (harvest) may be drawn in an arbitrary manner without affecting the ratio of marked to unmarked animals. Operationally, randomness will be approximated by marking all individuals encountered and uniformly covering the population's geographic range.

All members of family groups will be marked. Although family groups may be encountered as groups by hunters, this type of bias will be lost when the cubs are weaned. Because 180 days are required for all marks to be apparent, the recapture sample (harvest) will come 1 year after the year of marking. Females marked with yearlings will have weaned their cubs by then in the Hudson Bay/Foxe Basin area (Ramsay and Stirling 1986). It is illegal to hunt females with cubs and two years after the mark all animals marked as family groups will no longer be associated. The only family groups that may be sampled as a group by hunters are those killed as a family unit when the cubs are yearlings.

CONCLUSION

Biomarking polar bears with tetracycline for the purpose of obtaining a mark-recapture population estimate of polar bears appears both feasible and practical. The tetracycline mark appears to be permanent, reliably recognized 180+ days post injection, and both the relative and absolute age may be determined for all marks observed in a given tooth. Several existing mark-recapture methods appear to be suitable for determining a population estimate from the ratio of marked to unmarked animals in the harvest.

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