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ROMANCE WITHOUT RESPONSIBILITIES: THE USE OF THE IMMUNOCONTRACEPTIVE PORCINE ZONA PELLUCIDA TO MANAGE FREE-RANGING BISON (*BISON BISON*) ON CATALINA ISLAND, CALIFORNIA, USA

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Abstract: Prior to 2010, the introduced population of American bison (*Bison bison*) on Santa Catalina Island, California, was managed through the shipment of surplus bison to private ranches, Native American reservations, and livestock auctions on the mainland. In response to escalating costs, transport-induced stress to the animals, and ecologic impacts associated with high bison numbers on-island between shipments, the use of the immunocontraceptive vaccine porcine zona pellucida (PZP) as a fertility control option for managing the population was investigated. Between 2009 and 2012, a total of 64 bison cows (≥ 1 yr old) received primer inoculations of 100 μ g PZP emulsified with 0.5 ml Freund's modified adjuvant (FMA) delivered through a combination of intramuscular injections by hand (50 bison cows) during roundups and via field darting (14 bison cows). Pre-rut booster inoculations of 100 μ g PZP emulsified with 0.5 ml Freund's incomplete adjuvant (FIA) were administered exclusively via field darting in 2010, 2011, and 2012 to 45, 48, and 61 bison cows (≥ 1 yr old), respectively. During the present study, 38 adult cows (marked and unmarked) received one or more PZP inoculations during their first, second, or third trimesters of pregnancy, and of these individuals, 35 successfully produced calves. Low pregnancy values detected in the remaining three cows have been attributed to residual progesterone associated with unsuccessful fertilization. The 2010 pretreatment calving rate (calves born per cow) determined via direct observation was 67.4% (29 calves from 43 cows). Through the use of PZP, the calving rate was reduced to 10.4% by 2011 and to 3.3% by 2012. Considering the annual mortality rate of 2–5% documented during this study, the results demonstrate the potential of PZP use as an effective nonlethal tool for controlling population growth in free-ranging bison.

Key words: Bison, contraception, fertility control, immunocontraception, porcine zona pellucida.

INTRODUCTION

In 1924, a herd of 14 American bison (*Bison bison*) was introduced to Catalina Island, California, USA, to provide the backdrop for the production of a film (Gingrich, unpublished document, 1974, *The Buffalo of Catalina*) (*The Catalina Islander, 1924, Fourteen Buffalo Are Free on Catalina Island*). Forty-five additional bison were transported to the island between 1934 and 1996 to increase genetic variation, and at its highest recorded estimate, the herd contained more than 500 individuals.²⁹ The presence of bison on the island was embraced by the island's residents as bison-viewing opportunities quickly generated tourism dollars from the nearly 1 million annual visitors, but growing concerns about overgrazing by livestock, bison, and other

nonnative herbivores present at the time led to the implementation of a culling program in 1969.¹⁹

After its founding in 1972, the Catalina Island Conservancy (hereafter, Conservancy) inherited the responsibility of managing approximately 88% (170 km²) of the island, including the bison herd. From 1970 to 2009, bison population management was achieved through periodic roundups and shipment of bison to mainland livestock auctions, private dealers, and most recently to economically struggling Native American reservations in South Dakota. Because of the cultural and economic significance of bison to the island community, the Conservancy investigated management options that would incorporate both the ecologic and socioeconomic consequences of maintaining a free-ranging bison herd on the island.²⁹

A multi-year research project aimed at quantifying the ecologic impacts of bison on Catalina's flora and fauna was completed in 2002,²⁸ in which it was determined that approximately 142 bison could be maintained within their current range of nearly 16,200 ha on the island's East End, while reserving adequate vegetation for native organisms. In attempt to establish a balance between

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the potential ecologic impacts and the demands for wildlife-viewing opportunities, the Conservancy set a goal of maintaining the herd at approximately 150–200 individuals, while protecting sensitive plant species through selectively placed fenced enclosures.

Maintaining this target herd size through removal became unsustainable as a result of high costs, concerns over potential animal stress during transport, and the expansion of the herd beyond ecologically sustainable numbers between shipments. Additional management challenges came to light in 2004 after genetic testing confirmed a relatively high prevalence of cattle gene integration within the Catalina herd as compared with mainland herds.²⁴ Bison geneticists raised concerns about shipping excess bison from the Conservancy herd to supplement mainland bison herds that had no historical or genetic evidence of cattle hybridization.²⁵ Given these circumstances, the Conservancy investigated available methods of fertility control as a possible alternative to culling.

Since it was first reported to the American Association of Zoo Veterinarians in 1992, the immun contraceptive agent porcine zona pellucida (PZP) has been used for fertility control in 112 species in 103 zoos.⁶ The application of PZP to captive zoo species was initiated based on its previous success in controlling reproduction of wild horses (*Equus caballus*)^{22,30} and white-tailed deer (*Odocoileus virginianus*).^{15,18,24,31,32} The vaccine works by stimulating the production of anti-PZP antibodies in the treated animal, which in turn target the zona pellucida surface of the ovum and alter its shape, preventing sperm attachment and subsequent pregnancy.¹⁵

Conservation efforts involving the American bison continue throughout much of North America, but the growth of localized wild populations in many areas has created conflict with human interests and management objectives. In several of these areas, managers have considered the use of contraceptive agents, but to date no study has been completed examining the efficacy or practicality of fertility control among free-ranging bison.

While several investigations have demonstrated successful fertility inhibition among treated individuals, the ultimate expression of wildlife fertility control is at the population scale. Because PZP has already proven effective among bison in captive populations of 1–12 treated individuals in zoos (K. M. Frank, pers. com.), the potential use of a contraceptive as a safe and cost-effective

means of regulating large, free-ranging bison populations by administering PZP to all bison cows within the Catalina Island population was investigated.

STUDY AREA

Santa Catalina Island (194 km²), located approximately 40 km west of coastal Los Angeles, California, has a rugged mountainous terrain, with a central ridge running its length (Fig. 1). The elevation ranges from sea level to 640 m, and a narrow isthmus (<800 m wide) located at the small community of Two Harbors separates the island into two geographically distinct sides: the larger East End, comprising 84% of the entire island, and the smaller West End, comprising the remaining 16%.²³ The island has a Mediterranean climate, with average annual precipitation recorded from six weather stations across the island during this study (2009–2012) of 324.57 mm.²⁵ The majority of rainfall occurs between November and April. Common plant communities include coastal sage scrub, coastal bluff scrub, island chaparral, island woodland, riparian woodland, and coastal grassland.^{17,20} Catalina is home to more than 50 endemic species of plants and animals.

Although no large native grazers occurred on the island, several introductions have taken place over the last 2 centuries. Feral goats (*Capra hircus*) were established on the island in the early 1800s, and domestic cattle (*Bos taurus*), horses (*Equus caballus*), and sheep (*Ovis aries*) were present in the 1890s. Feral pigs (*Sus scrofa*) were initially established in the 1930s.²⁶ The pigs and goats were removed through an extensive eradication program that was conducted from 1990 to 2004.^{26,29} Domestic cattle operations ceased by the 1950s,²⁸ and the island is currently considered to be in a state of recovery. Mule deer (*Odocoileus hemionus*) were introduced to the island in 1928 and have been managed since 1998 through a Private Lands Management hunting program²⁷ in partnership with the California Department of Fish and Wildlife.

Approximately 88% (17,051 ha) of Catalina is owned and managed by the Conservancy, a nonprofit 501(c)(3) public charity and land trust dedicated to the protection and management of Catalina Island's ecosystem through a combination of conservation practices, environmental education, and outdoor recreation. Catalina is home to a resident population of more than 4,500 (most of whom reside in Avalon) and welcomes an average of 800,000 visitors per year (Catalina

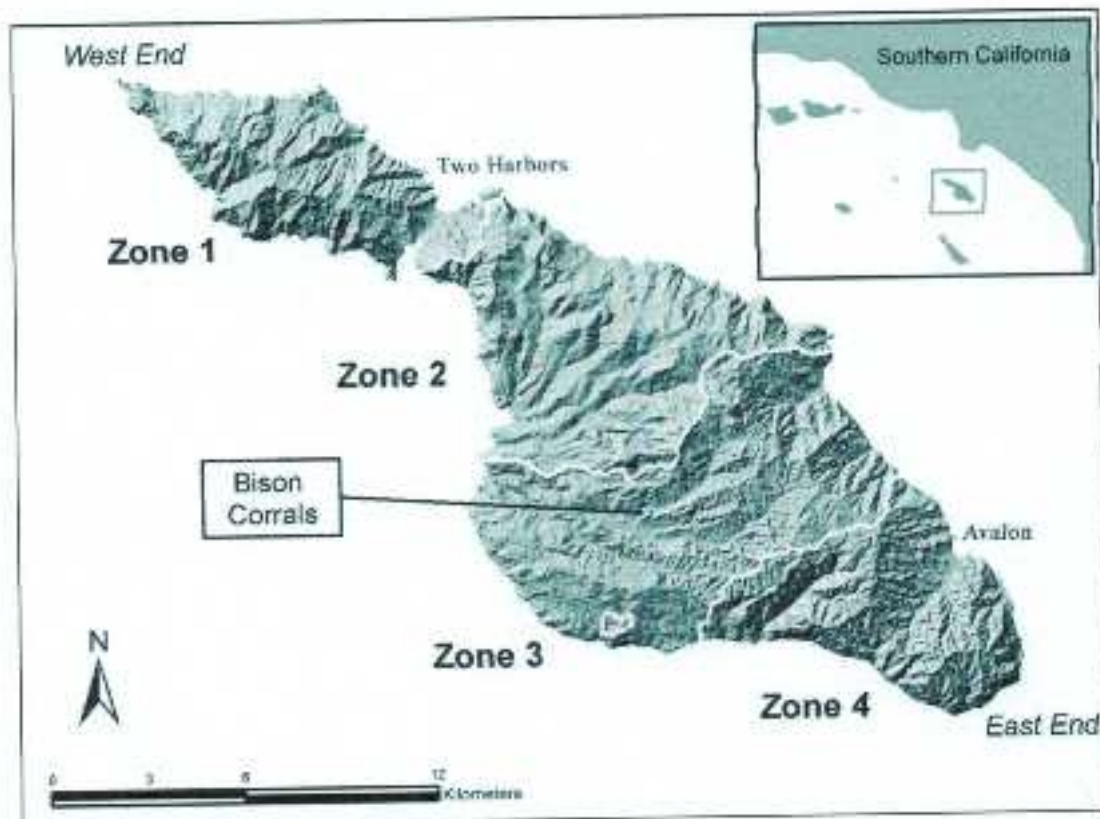


Figure 1. Map of Catalina Island illustrating locations of three cross-island fences that partition the island into four zones. Zone 1 is bison free, whereas zones 2, 3, and 4 are occupied by bison. Inset in the upper right shows the location of Catalina Island in relation to mainland Southern California and several nearby Channel Islands. Figure modified from Schuyler et al.²⁶

Chamber of Commerce, pers. com., 2008), making it the most populous of the California Channel Islands.

METHODS

The native PZP antigen used in this study was prepared from porcine oocytes at the Science and Conservation Center in Billings, Montana.² The antigen was shipped to the Conservancy on dry ice and stored frozen (-10°C) until time of use, under the authority of the Food and Drug Administration Investigational New Animal Drug Exemption (No. 8840-G003-004).

Bison cows treated during this study were given intramuscular inoculations of 100 μg PZP (in phosphate buffered saline) in the caudal thigh or gluteus muscle either by hand injection or remotely by 1.0-ml dart. Following the application protocol recommended for bison, we emulsified the PZP antigen with Freund's modified adjuvant (FMA; Calbiochem, La Jolla, California 92037, USA) for the initial (primer) inoculation and used Freund's incomplete adjuvant (FIA; Sigma, St.

Louis, Missouri 63103, USA) for all subsequent booster inoculations.^{4,7} FMA is a mixture of non-metabolizable oil (mineral oil), a surfactant (Ar-lacel A), and freeze-dried fractionated cell walls of *Mycobacterium butyricum*. The use of FMA for primer inoculations is necessary in order to elicit an adequate immune response and raise antibody levels initially. FIA is a weaker adjuvant, consisting of the same oil/surfactant mixture but lacking any mycobacteria. It is sufficient in maintaining elevated antibodies, is less expensive than FMA, and has a lower potential for causing injection-site reactions.^{4,7}

Approximately 50 of 64 primer inoculations (78%; PZP + FMA) were completed in conjunction with bison roundups conducted by the Conservancy on 30 September and 20 November 2009, and on 2 December 2011. Each individual was temporarily restrained in a squeeze chute to facilitate blood sample collection, marking, and PZP vaccine administration by hand injection. The remaining 22% (14 of 64) were inoculated by darting. Darting was accomplished with 1.0-ml

Table 1. Annual treatment schedule and delivery method of porcine zona pellucida to free-ranging bison on Catalina Island, California, during 2009–2012.

Year	Month	Activity	Delivery method	Primer/Booster	Adjuvant ^a	Cows
2009	Sept–Nov	Roundup	Syringe	Primer	FMA	35
2010	Feb–May	Field darting	Dart	Primer	FMA	12 of 13 ^b
2010	Apr–July	Field darting	Dart	Booster	FIA	45 of 47 ^c
2011	Apr–June	Field darting	Dart	Booster	FIA	48 ^d
2011	Dec	Roundup	Syringe	Primer/Booster	FMA/FIA	13/4
2012	Jan	Field darting	Dart	Primer	FMA	2
2012	Apr–May	Field darting	Dart	Booster	FIA	61 ^e

^a FMA, Freund's modified adjuvant; FIA, Freund's incomplete adjuvant.

^b Additional unmarked adult cow was present but not detected in 2010.

^c One geriatric cow died early in the year.

^d One additional adult bison cow was discovered in the population.

^e Two geriatric cows died.

Pneu-Dart® type "P" darts with 1.27 cm barbless needles (Pneu-Dart, Williamsport, Pennsylvania 17701, USA). The tail fin of each dart was painted red or orange using Rust-Oleum® (Rust-Oleum, Vernon Hills, Illinois 60661, USA) specialty fluorescent spray, and then labeled numerically to facilitate dart recovery and improve immunization recordkeeping. A small dab of petroleum jelly was applied to the tip of each dart to prevent PZP leakage.

Up to 20 ml whole blood was collected by coccygeal or jugular puncture during roundups. Blood samples collected for pregnancy testing were allowed to clot at ambient temperature for 30 min, and then placed into an ice chest. Serum was separated, and tubes were refrigerated until shipment for testing. Analysis of blood serum samples collected from cows >1 yr old in 2009 ($n = 31$) and in 2011 ($n = 47$) was conducted by BioTracking, LLC (Moscow, Idaho 83843, USA) using commercially available bioPRYN®, a ruminant-specific pregnancy test that measures the level of placental pregnancy-specific protein B (PSPB) in serum.⁶ Females were considered pregnant when optical density (OD) readings exceeded the cutoff value of 0.152 at ≥ 40 days post breeding and confirmed pregnant when OD exceeded 0.175.

All bison cows sampled during the roundup were marked with an Allflex® (Allflex USA, Inc., Dallas, Texas 75203, USA) United States Department of Agriculture (USDA) electronic identification tag and a Ritchey® universal jumbo cow tag (Ritchey Livestock ID, Brighton, Colorado 80601, USA) heat stamped with a unique four-digit number, then photographed to facilitate field identification. Additional unmarked bison that had eluded the roundup efforts were identified, photographed, and cataloged based on horn

growth, pelage color, natural markings, or colored fly tags affixed during roundups prior to this study.

Bison cows do not usually start calving until they are 3–4 yr old¹² but have been documented to initiate breeding as early as 1.3 yr of age.⁷ For this reason, this study considered any bison cow ≥ 1 yr to be of breeding age and therefore targeted them for contraception treatment with PZP. Taking into consideration a gestation period of 262–292 days,^{2,3} calving age for this study was defined as ≥ 2 yr when calculating annual calving rates.

RESULTS

During roundups conducted in 2009, 35 bison cows (three calves, 6–7 mo old, and 32 >1 yr) were marked and inoculated with a primer dose of PZP. Thirty-one of 32 cows >1 yr were sampled, and 25 (78.1%) were confirmed pregnant by bioPRYN OD >0.175 (range, 0.197–1.245). Through bi-weekly field monitoring, successful calving by 23 of 25 known pregnant cows between February and June 2010 was confirmed. One of the two cows that did not calve showed a decrease in OD values from September (OD = 0.569) to November (OD = 0.257), suggesting a first-trimester fetus loss. The second cow exhibited low test values when sampled in November 2009 (OD = 0.260), yet no calf was observed during subsequent monitoring.

In addition to the 35 cows inoculated during the roundup, 13 unmarked cows ≥ 1 yr that were not part of the roundups required primer doses of PZP. Of these 13 individuals, 12 were identified and successfully inoculated via field-darting efforts during February–May 2010, while one cow was mistaken for a recorded individual and subsequently did not receive a primer dose (Table 1). Six of the 13 unmarked cows subsequently

Table 2. Annual calving and mortality rates calculated for a free-ranging bison herd on Catalina Island, California, prior to porcine zona pellucida (PZP) vaccination (2010) and post PZP vaccination (2011 and 2012).

Year	Catalina bison population ^a	Calving age cows (≥2 yr)	Calves produced (F:M) ^b	Calving rate (%)	Mortalities (F:M) ^c	Mortality rate (%)
2010	120	43	29 (15:14)	67.4	5 (3:4)	4.2
2011	144	48 ^d	5 (5:0)	10.4	7 (2:5)	4.9
2012	142	61 ^e	2 (0:2)	3.3 ^e	3 (2:1)	2.1 ^e

^a Island bison population estimate prior to calving season.

^b F:M, ratio of females to males.

^c Four juvenile cows from 2010 reached calving age and one unmarked adult cow was discovered.

^d Two geriatric cows died, and 15 female calves from 2010 reached calving age.

^e As of December 2012.

produced calves between February and May 2010. Taking into consideration the death of one geriatric study animal early in the year, the combined population calving rate for 2010 was 67.4% (29 calves from 43 cows ≥2 yr; Table 2). Pre-rut boosters were given via darting to 45 of 47 cows (≥1 yr) that we believed remained in the population.

During field-monitoring efforts conducted in 2011, one additional unmarked adult cow was discovered, increasing the total number of cows (≥2 yr) to 48. From these 48 individuals, five calves were produced in 2011, for a calving rate of 10.4% (5 of 48). Four of five calves were produced by unmarked cows that we could not confirm had received two inoculations in 2010, and the fifth calf was born to a marked and properly inoculated individual. All 48 cows received booster inoculations during field-darting efforts completed between April and June 2011.

In December 2011, 51 cows (47 ≥1 yr and four calves) of the total 63 cows ≥1 yr (48 from 2011 plus 15 born in 2010) determined to be in the island population at that time were corralled during a roundup. Fourteen of the 47 cows were approximately 19 mo old and had not previously been vaccinated or marked, while four additional unmarked adults represented individuals that were believed to have received at least one dose of PZP prior to the 2011 roundup. Thirteen of 14 juvenile cows and the four unmarked adults were given primer and booster doses of PZP, respectively, via syringe. Of the 47 cows (≥1 yr) that were tested for pregnancy, two young cows (both previously unmarked and unvaccinated) were confirmed to be pregnant (OD = 0.655 and 0.576), for a pregnancy rate of 4.3% (2 of 47). Only one of these two individuals successfully calved in 2012.

In early 2012, two unvaccinated adult cows ≥1 yr (one marked from roundup, one unmarked)

received primer doses of PZP via field darting, and two geriatric cows within the study died. Between April and May 2012, all 61 identified cows (≥1 yr) within the population were given a booster dose of PZP. By October 2012, only two calves had been born, for a calving rate of 3.3%.

During the first year of the study, all darting efforts were accomplished by two biologists working together during 24 field-days and required approximately 97 hr each. In 2011 and 2012, the field effort associated with darting decreased to 12 days (54 hr) and 11 days (63 hr), respectively.

Throughout the entire study, no injuries or abscesses were detected as a result of either syringe or dart-delivered injections. A total of 175 darts were fired and 142 (81%) were successfully recovered and examined. Of the 142 recovered darts, five delivered partial doses (≥0.5 dose), and one dart broke apart on impact. In the case of the broken dart, the individual cow was re-darted the same day.

DISCUSSION

Through the administration of PZP, the calving rate of the Catalina Island bison population was effectively reduced from 67.4% to 10.4% within the first year of application and declined to 3.3% by year two, despite an increase in the number of breeding-age cows. With annual calf production at or below total mortalities within the first year of treatment, it was clearly demonstrated that PZP can be an effective nonlethal tool used to reduce fertility and control population growth in free-ranging bison.

Annual precipitation and competition can influence the amount of resources available for the island bison herd, and nutritional constraints associated with drought conditions has the potential to affect calf production. This phenomenon was documented between 2001 and 2002

when the annual precipitation on Catalina decreased from 466.9 mm to 124.7 mm and the calving rate subsequently declined from 45% to 35%.²⁰ The bison herd size during this period was estimated to be over 300 animals, and the island resources were also being consumed by free-ranging feral pigs and feral goats. Throughout the duration of this current study, the bison herd was maintained at approximately 150 individuals (goats and pigs had been removed 5 yr prior), and annual precipitation documented between 2009 and 2012 ranged from 220.4 mm to 399.7 mm.²¹ These conditions suggest that resource availability was not a factor in the reduced calving rate demonstrated during this study.

Calf production documented during this study was the result of insufficient doses of the PZP vaccine or poor timing of delivery. It was documented in only one instance in which a cow produced a calf after receiving a primer inoculation and booster prior to pregnancy. After receiving a subsequent booster, this individual did not produce a calf the following year. Although other factors, such as advanced age, may be to blame, variation in the ability of individuals to produce adequate antibody levels to inhibit fertilization has been documented by several investigators.^{13,14,22} In some cases, repeated annual boosters have increased the duration of each treatment,²² and infertility has been achieved among individuals that had initially been unresponsive.²¹

The safety of PZP use during pregnancy has been documented in more than 18 different species including bison.²⁰ During this current study, 38 adult cows (marked and unmarked) received one or more PZP inoculations during their first, second, or third trimesters of pregnancy, and of these individuals, 35 successfully produced calves. Low pregnancy values detected in the remaining three cows were most likely attributed to residual progesterone associated with unsuccessful fertilization following estrus. Additionally, this study documented two instances in which female calves produced by cows that had received a primer dose of PZP while pregnant, had subsequently produced their own calves, and had similarly received a primer dose of PZP during pregnancy. This outcome further demonstrates that PZP use in pregnant animals does not interfere with the progress of gestation or with the future fertility of offspring produced by treated mothers.

Identification of unmarked closely aged cows posed a challenge in confirming treated individuals from year to year. The use of the jumbo ear

tags greatly enabled accurate identification of treatment animals at several hundred yards, and the custom brown color of the tags was found to be less obtrusive with respect to wildlife viewing. The long-term retention of the dangle tags was a concern at the initiation of the study, and the decision to apply the additional USDA electronic tags during the roundups proved to be advantageous when two tags were lost. In one instance, the use of an oil-based wildlife marker paint pellet (Nelson Paint Company, Kingsford, Michigan 49802, USA) applied in conjunction with a primer inoculation was utilized, and it was found that the paint mark could be detected for several months. This marking technique will be utilized more frequently in the future as additional unmarked cows reach breeding age, or dangle tags are lost.

The primary goal of the Conservancy's contraception program is to maintain the island bison population at the current size of approximately 150 animals and eliminate the need for costly and potentially stressful removals. In order to be considered successful, efforts required to accomplish this goal through the use of fertility control must be less than that associated with periodic shipments and must be sustainable. Through this research, the versatility of PZP use in a wild, free-ranging population was demonstrated, and it was shown that it can provide the desired results in a relatively short period of time. It was also demonstrated that the application of PZP can be accomplished opportunistically and that there is flexibility in the treatment process. During the first year of this study, primer inoculations were delivered 1–8 mo prior to booster doses. Booster inoculations were initiated annually in mid-April, approximately 4–6 wk prior to the onset of the breeding season and delivered opportunistically over a period of 4 mo. Despite this deviation from the recommended administration protocol, calving rates were successfully reduced from 67.4% to 10.4%.

In order to maintain a constant island bison population, the number of calves produced each year must be in balance with potential mortalities. Based on previous studies of PZP application in wild horses, mares that had been treated for up to 5 consecutive years returned to fertility once treatments ceased.¹¹ Beginning in 2013, a sample of breeding age cows will be removed from treatment each year and monitored for subsequent calving success. The length of time required for individuals to return to fertility will be recorded based on consecutive years of treatment,

and the number of cows that will remain untreated each year will be adjusted as needed.

Contrary to initial expectations, behavioral aversion by bison to the darting activities was most apparent during the first year of the study and decreased considerably during subsequent seasons. Although bison on Catalina Island are somewhat accustomed to human presence, this study attributed their increased darter tolerance to the absence of dependent calves and an increase in the experience of the field staff. As a result, the darting efforts required to maintain and/or further reduce the calving rate declined substantially during the second and third years.

Although the collective bison population in the United States remains significantly reduced in comparison to historic numbers, culling activities have been required within several public and privately managed herds as a result of habitat loss, ownership issues, disease spread, and cattle gene introgression. Population management through lethal means or hazing methods has become less acceptable, and these activities tend to target the symptoms of the problem rather than the source.

Several studies have demonstrated the safety and efficacy of PZP use among treated individuals, but the effective application of this agent in the management of entire populations has only been documented in a few species. Because of this, many people question whether fertility control agents offer a cost-effective and sustainable alternative to other forms of population control. The use of contraception for management purposes has the potential to reduce management costs by reducing the frequency of roundups, and in the case of Catalina's bison, the additional costs associated with transporting them off of the island. Contraception can also enhance the humaneness of management, since roundups and transport are stressful for the individuals, and herd social structure is changed dramatically when many individuals are removed during a single event. As demonstrated within this study, a substantial financial and organizational commitment may be required to initiate a successful contraceptive program using PZP if an initial roundup is required for ear tagging, but the efforts required for maintenance via field darting decrease dramatically during subsequent years. This study has also shown that an opportunistic approach to the administration of the vaccine can still provide desired results and that PZP may be more versatile as a population management tool than previously considered.

Although the Catalina bison population remains disease free, an effective contraceptive agent such as PZP may also provide an additional tool in the effort to reduce the transmission of bovine brucellosis (*Brucella abortus*) within isolated areas. Transmitted primarily through contact with infected aborted fetuses, preventing pregnancies in brucellosis-infected bison would potentially aid in preventing the spread of the disease.¹⁴

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ROMANCE WITHOUT RESPONSIBILITIES: THE USE OF THE IMMUNOCONTRACEPTIVE PORCINE ZONA PELLUCIDA TO MANAGE FREE-RANGING BISON (*BISON BISON*) ON CATALINA ISLAND, CALIFORNIA, USA

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Abstract: Prior to 2010, the introduced population of American bison (*Bison bison*) on Santa Catalina Island, California, was managed through the shipment of surplus bison to private ranches, Native American reservations, and livestock auctions on the mainland. In response to escalating costs, transport-induced stress to the animals, and ecologic impacts associated with high bison numbers on-island between shipments, the use of the immunocontraceptive vaccine porcine zona pellucida (PZP) as a fertility control option for managing the population was investigated. Between 2009 and 2012, a total of 64 bison cows (≥ 1 yr old) received primer inoculations of 100 μ g PZP emulsified with 0.5 ml Freund's modified adjuvant (FMA) delivered through a combination of intramuscular injections by hand (50 bison cows) during roundups and via field darting (14 bison cows). Pre-rut booster inoculations of 100 μ g PZP emulsified with 0.5 ml Freund's incomplete adjuvant (FIA) were administered exclusively via field darting in 2010, 2011, and 2012 to 45, 48, and 61 bison cows (≥ 1 yr old), respectively. During the present study, 38 adult cows (marked and unmarked) received one or more PZP inoculations during their first, second, or third trimesters of pregnancy, and of these individuals, 35 successfully produced calves. Low pregnancy values detected in the remaining three cows have been attributed to residual progesterone associated with unsuccessful fertilization. The 2010 pretreatment calving rate (calves born per cow) determined via direct observation was 67.4% (29 calves from 43 cows). Through the use of PZP, the calving rate was reduced to 10.4% by 2011 and to 3.3% by 2012. Considering the annual mortality rate of 2–5% documented during this study, the results demonstrate the potential of PZP use as an effective nonlethal tool for controlling population growth in free-ranging bison.

Key words: Bison, contraception, fertility control, immunocontraception, porcine zona pellucida.

INTRODUCTION

In 1924, a herd of 14 American bison (*Bison bison*) was introduced to Catalina Island, California, USA, to provide the backdrop for the production of a film (Gingrich, unpublished document, 1974, *The Buffalo of Catalina*) (*The Catalina Islander, 1924, Fourteen Buffalo Are Free on Catalina Island*). Forty-five additional bison were transported to the island between 1934 and 1996 to increase genetic variation, and at its highest recorded estimate, the herd contained more than 500 individuals.²⁹ The presence of bison on the island was embraced by the island's residents as bison-viewing opportunities quickly generated tourism dollars from the nearly 1 million annual visitors, but growing concerns about overgrazing by livestock, bison, and other

nonnative herbivores present at the time led to the implementation of a culling program in 1969.¹⁹

After its founding in 1972, the Catalina Island Conservancy (hereafter, Conservancy) inherited the responsibility of managing approximately 88% (170 km²) of the island, including the bison herd. From 1970 to 2009, bison population management was achieved through periodic roundups and shipment of bison to mainland livestock auctions, private dealers, and most recently to economically struggling Native American reservations in South Dakota. Because of the cultural and economic significance of bison to the island community, the Conservancy investigated management options that would incorporate both the ecologic and socioeconomic consequences of maintaining a free-ranging bison herd on the island.²⁸

A multi-year research project aimed at quantifying the ecologic impacts of bison on Catalina's flora and fauna was completed in 2002,²⁸ in which it was determined that approximately 142 bison could be maintained within their current range of nearly 16,200 ha on the island's East End, while reserving adequate vegetation for native organisms. In attempt to establish a balance between

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the potential ecologic impacts and the demands for wildlife-viewing opportunities, the Conservancy set a goal of maintaining the herd at approximately 150–200 individuals, while protecting sensitive plant species through selectively placed fenced enclosures.

Maintaining this target herd size through removal became unsustainable as a result of high costs, concerns over potential animal stress during transport, and the expansion of the herd beyond ecologically sustainable numbers between shipments. Additional management challenges came to light in 2004 after genetic testing confirmed a relatively high prevalence of cattle gene integration within the Catalina herd as compared with mainland herds.²¹ Bison geneticists raised concerns about shipping excess bison from the Conservancy herd to supplement mainland bison herds that had no historical or genetic evidence of cattle hybridization.²² Given these circumstances, the Conservancy investigated available methods of fertility control as a possible alternative to culling.

Since it was first reported to the American Association of Zoo Veterinarians in 1992, the immun contraceptive agent porcine zona pellucida (PZP) has been used for fertility control in 112 species in 103 zoos.⁴ The application of PZP to captive zoo species was initiated based on its previous success in controlling reproduction of wild horses (*Equus caballus*)^{23–25} and white-tailed deer (*Odocoileus virginianus*).^{12,18,24,21,25} The vaccine works by stimulating the production of anti-PZP antibodies in the treated animal, which in turn target the zona pellucida surface of the ovum and alter its shape, preventing sperm attachment and subsequent pregnancy.¹²

Conservation efforts involving the American bison continue throughout much of North America, but the growth of localized wild populations in many areas has created conflict with human interests and management objectives. In several of these areas, managers have considered the use of contraceptive agents, but to date no study has been completed examining the efficacy or practicality of fertility control among free-ranging bison.

While several investigations have demonstrated successful fertility inhibition among treated individuals, the ultimate expression of wildlife fertility control is at the population scale. Because PZP has already proven effective among bison in captive populations of 1–12 treated individuals in zoos (K. M. Frank, pers. com.), the potential use of a contraceptive as a safe and cost-effective

means of regulating large, free-ranging bison populations by administering PZP to all bison cows within the Catalina Island population was investigated.

STUDY AREA

Santa Catalina Island (194 km²), located approximately 40 km west of coastal Los Angeles, California, has a rugged mountainous terrain, with a central ridge running its length (Fig. 1). The elevation ranges from sea level to 640 m, and a narrow isthmus (<800 m wide) located at the small community of Two Harbors separates the island into two geographically distinct sides: the larger East End, comprising 84% of the entire island, and the smaller West End, comprising the remaining 16%.²⁶ The island has a Mediterranean climate, with average annual precipitation recorded from six weather stations across the island during this study (2009–2012) of 324.57 mm.²² The majority of rainfall occurs between November and April. Common plant communities include coastal sage scrub, coastal bluff scrub, island chaparral, island woodland, riparian woodland, and coastal grassland.^{17,21} Catalina is home to more than 50 endemic species of plants and animals.

Although no large native grazers occurred on the island, several introductions have taken place over the last 2 centuries. Feral goats (*Capra hircus*) were established on the island in the early 1800s, and domestic cattle (*Bos taurus*), horses (*Equus caballus*), and sheep (*Ovis aries*) were present in the 1890s. Feral pigs (*Sus scrofa*) were initially established in the 1930s.²⁶ The pigs and goats were removed through an extensive eradication program that was conducted from 1990 to 2004.^{26,27} Domestic cattle operations ceased by the 1950s,²⁶ and the island is currently considered to be in a state of recovery. Mule deer (*Odocoileus hemionus*) were introduced to the island in 1928 and have been managed since 1998 through a Private Lands Management hunting program in partnership with the California Department of Fish and Wildlife.²⁷

Approximately 88% (17,051 ha) of Catalina is owned and managed by the Conservancy, a nonprofit 501(c)(3) public charity and land trust dedicated to the protection and management of Catalina Island's ecosystem through a combination of conservation practices, environmental education, and outdoor recreation. Catalina is home to a resident population of more than 4,500 (most of whom reside in Avalon) and welcomes an average of 800,000 visitors per year (Catalina

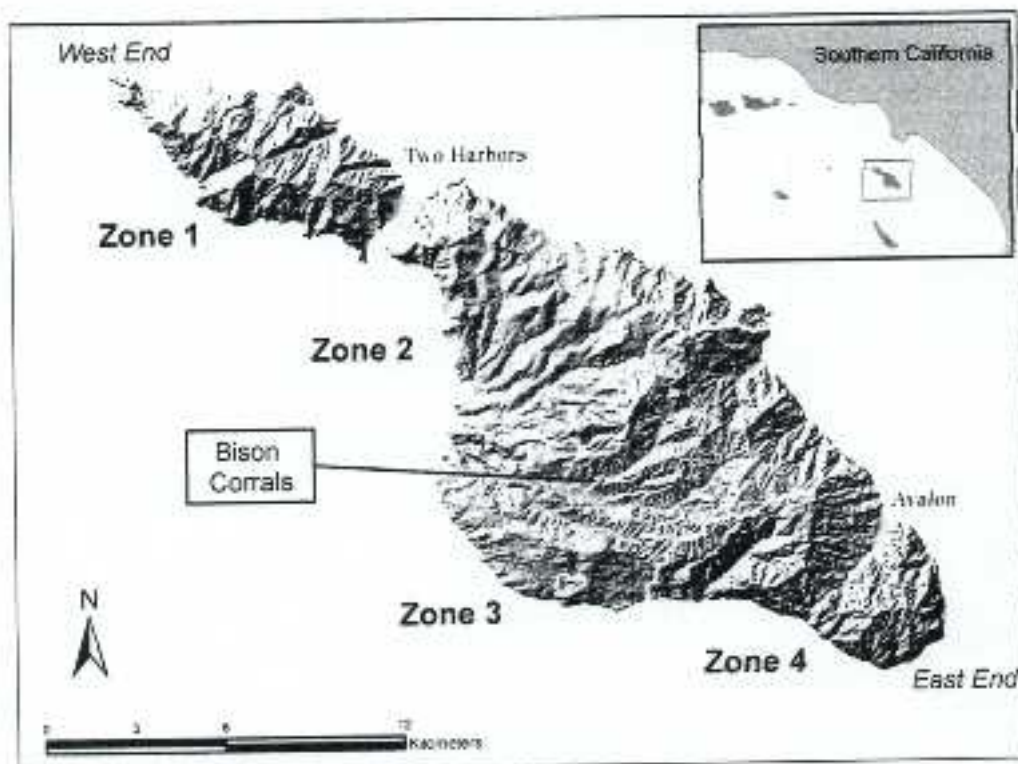


Figure 1. Map of Catalina Island illustrating locations of three cross-island fences that partition the island into four zones. Zone 1 is bison free, whereas zones 2, 3, and 4 are occupied by bison. Inset in the upper right shows the location of Catalina Island in relation to mainland Southern California and several nearby Channel Islands. Figure modified from Schuyler et al.²⁸

Chamber of Commerce, pers. com., 2008), making it the most populous of the California Channel Islands.

METHODS

The native PZP antigen used in this study was prepared from porcine oocytes at the Science and Conservation Center in Billings, Montana.³ The antigen was shipped to the Conservancy on dry ice and stored frozen (-10°C) until time of use, under the authority of the Food and Drug Administration Investigational New Animal Drug Exemption (No. 8840-G003-004).

Bison cows treated during this study were given intramuscular inoculations of 100 μg PZP (in phosphate buffered saline) in the caudal thigh or gluteus muscle either by hand injection or remotely by 1.0-ml dart. Following the application protocol recommended for bison, we emulsified the PZP antigen with Freund's modified adjuvant (FMA; Calbiochem, La Jolla, California 92037, USA) for the initial (primer) inoculation and used Freund's incomplete adjuvant (FIA; Sigma, St.

Louis, Missouri 63103, USA) for all subsequent booster inoculations.⁴⁷ FMA is a mixture of non-metabolizable oil (mineral oil), a surfactant (Ar-lacel A), and freeze-dried fractionated cell walls of *Mycobacterium butyricum*. The use of FMA for primer inoculations is necessary in order to elicit an adequate immune response and raise antibody levels initially. FIA is a weaker adjuvant, consisting of the same oil/surfactant mixture but lacking any mycobacteria. It is sufficient in maintaining elevated antibodies, is less expensive than FMA, and has a lower potential for causing injection-site reactions.⁴⁷

Approximately 50 of 64 primer inoculations (78%; PZP + FMA) were completed in conjunction with bison roundups conducted by the Conservancy on 30 September and 20 November 2009, and on 2 December 2011. Each individual was temporarily restrained in a squeeze chute to facilitate blood sample collection, marking, and PZP vaccine administration by hand injection. The remaining 22% (14 of 64) were inoculated by darting. Darting was accomplished with 1.0-ml

Table 1. Annual treatment schedule and delivery method of porcine zona pellucida to free-ranging bison on Catalina Island, California, during 2009–2012.

Year	Month	Activity	Delivery method	Primer/Booster	Adjuvant ^a	Cows
2009	Sept–Nov	Roundup	Syringe	Primer	FMA	35
2010	Feb–May	Field darting	Dart	Primer	FMA	12 of 13 ^b
2010	Apr–July	Field darting	Dart	Booster	FIA	45 of 47 ^c
2011	Apr–June	Field darting	Dart	Booster	FIA	48 ^d
2011	Dec	Roundup	Syringe	Primer/Booster	FMA/FIA	13/4
2012	Jan	Field darting	Dart	Primer	FMA	2
2012	Apr–May	Field darting	Dart	Booster	FIA	61 ^e

^aFMA, Freund's modified adjuvant; FIA, Freund's incomplete adjuvant.

^bAdditional unmarked adult cow was present but not detected in 2010.

^cOne geriatric cow died early in the year.

^dOne additional adult bison cow was discovered in the population.

^eTwo geriatric cows died.

Pneu-Dart[®] type "P" darts with 1.27 cm barbless needles (Pneu-Dart, Williamsport, Pennsylvania 17701, USA). The tail fin of each dart was painted red or orange using Rust-Oleum[®] (Rust-Oleum, Vernon Hills, Illinois 60661, USA) specialty fluorescent spray, and then labeled numerically to facilitate dart recovery and improve immunization recordkeeping. A small dab of petroleum jelly was applied to the tip of each dart to prevent PZP leakage.

Up to 20 ml whole blood was collected by coccygeal or jugular puncture during roundups. Blood samples collected for pregnancy testing were allowed to clot at ambient temperature for 30 min, and then placed into an ice chest. Serum was separated, and tubes were refrigerated until shipment for testing. Analysis of blood serum samples collected from cows >1 yr old in 2009 ($n = 31$) and in 2011 ($n = 47$) was conducted by BioTracking, LLC (Moscow, Idaho 83843, USA) using commercially available bioPRYN[®], a ruminant-specific pregnancy test that measures the level of placental pregnancy-specific protein B (PSPB) in serum.⁶ Females were considered pregnant when optical density (OD) readings exceeded the cutoff value of 0.152 at ≥ 40 days post breeding and confirmed pregnant when OD exceeded 0.175.

All bison cows sampled during the roundup were marked with an Allflex[®] (Allflex USA, Inc., Dallas, Texas 75203, USA) United States Department of Agriculture (USDA) electronic identification tag and a Ritchey[®] universal jumbo cow tag (Ritchey Livestock ID, Brighton, Colorado 80601, USA) heat stamped with a unique four-digit number, then photographed to facilitate field identification. Additional unmarked bison that had eluded the roundup efforts were identified, photographed, and cataloged based on horn

growth, pelage color, natural markings, or colored fly tags affixed during roundups prior to this study.

Bison cows do not usually start calving until they are 3–4 yr old¹² but have been documented to initiate breeding as early as 1.3 yr of age.⁷ For this reason, this study considered any bison cow ≥ 1 yr to be of breeding age and therefore targeted them for contraception treatment with PZP. Taking into consideration a gestation period of 262–292 days,^{13,14} calving age for this study was defined as ≥ 2 yr when calculating annual calving rates.

RESULTS

During roundups conducted in 2009, 35 bison cows (three calves, 6–7 mo old, and 32 >1 yr) were marked and inoculated with a primer dose of PZP. Thirty-one of 32 cows >1 yr were sampled, and 25 (78.1%) were confirmed pregnant by bioPRYN OD >0.175 (range, 0.197–1.245). Through bi-weekly field monitoring, successful calving by 23 of 25 known pregnant cows between February and June 2010 was confirmed. One of the two cows that did not calve showed a decrease in OD values from September (OD = 0.569) to November (OD = 0.257), suggesting a first-trimester fetus loss. The second cow exhibited low test values when sampled in November 2009 (OD = 0.260), yet no calf was observed during subsequent monitoring.

In addition to the 35 cows inoculated during the roundup, 13 unmarked cows ≥ 1 yr that were not part of the roundups required primer doses of PZP. Of these 13 individuals, 12 were identified and successfully inoculated via field-darting efforts during February–May 2010, while one cow was mistaken for a recorded individual and subsequently did not receive a primer dose (Table 1). Six of the 13 unmarked cows subsequently

Table 2. Annual calving and mortality rates calculated for a free-ranging bison herd on Catalina Island, California, prior to porcine zona pellucida (PZP) vaccination (2010) and post PZP vaccination (2011 and 2012).

Year	Catalina bison population ^a	Calving age cows (≥ 2 yr)	Calves produced (F:M) ^b	Calving rate (%)	Mortalities (F:M) ^c	Mortality rate (%)
2010	120	43	29 (13:14)	67.4	5 (1:4)	4.2
2011	144	48 ^d	5 (5:0)	10.4	7 (2:5)	4.9
2012	142	61 ^e	2 (0:2)	3.3 ^f	3 (2:1)	2.1 ^g

^a Island bison population estimate prior to calving season.

^b F:M, ratio of females to males.

^c Four juvenile cows from 2010 reached calving age and one unmarked adult cow was discovered.

^d Two geriatric cows died, and 15 female calves from 2010 reached calving age.

^e As of December 2012.

produced calves between February and May 2010. Taking into consideration the death of one geriatric study animal early in the year, the combined population calving rate for 2010 was 67.4% (29 calves from 43 cows ≥ 2 yr; Table 2). Pre-rut boosters were given via darting to 45 of 47 cows (≥ 1 yr) that we believed remained in the population.

During field-monitoring efforts conducted in 2011, one additional unmarked adult cow was discovered, increasing the total number of cows (≥ 2 yr) to 48. From these 48 individuals, five calves were produced in 2011, for a calving rate of 10.4% (5 of 48). Four of five calves were produced by unmarked cows that we could not confirm had received two inoculations in 2010, and the fifth calf was born to a marked and properly inoculated individual. All 48 cows received booster inoculations during field-darting efforts completed between April and June 2011.

In December 2011, 51 cows (47 ≥ 1 yr and four calves) of the total 63 cows ≥ 1 yr (48 from 2011 plus 15 born in 2010) determined to be in the island population at that time were corralled during a roundup. Fourteen of the 47 cows were approximately 19 mo old and had not previously been vaccinated or marked, while four additional unmarked adults represented individuals that were believed to have received at least one dose of PZP prior to the 2011 roundup. Thirteen of 14 juvenile cows and the four unmarked adults were given primer and booster doses of PZP, respectively, via syringe. Of the 47 cows (≥ 1 yr) that were tested for pregnancy, two young cows (both previously unmarked and unvaccinated) were confirmed to be pregnant (OD = 0.655 and 0.576), for a pregnancy rate of 4.3% (2 of 47). Only one of these two individuals successfully calved in 2012.

In early 2012, two unvaccinated adult cows ≥ 1 yr (one marked from roundup, one unmarked)

received primer doses of PZP via field darting, and two geriatric cows within the study died. Between April and May 2012, all 61 identified cows (≥ 1 yr) within the population were given a booster dose of PZP. By October 2012, only two calves had been born, for a calving rate of 3.3%.

During the first year of the study, all darting efforts were accomplished by two biologists working together during 24 field-days and required approximately 97 hr each. In 2011 and 2012, the field effort associated with darting decreased to 12 days (54 hr) and 11 days (63 hr), respectively.

Throughout the entire study, no injuries or abscesses were detected as a result of either syringe or dart-delivered injections. A total of 175 darts were fired and 142 (81%) were successfully recovered and examined. Of the 142 recovered darts, five delivered partial doses (≥ 0.5 dose), and one dart broke apart on impact. In the case of the broken dart, the individual cow was re-darted the same day.

DISCUSSION

Through the administration of PZP, the calving rate of the Catalina Island bison population was effectively reduced from 67.4% to 10.4% within the first year of application and declined to 3.3% by year two, despite an increase in the number of breeding-age cows. With annual calf production at or below total mortalities within the first year of treatment, it was clearly demonstrated that PZP can be an effective nonlethal tool used to reduce fertility and control population growth in free-ranging bison.

Annual precipitation and competition can influence the amount of resources available for the island bison herd, and nutritional constraints associated with drought conditions has the potential to affect calf production. This phenomenon was documented between 2001 and 2002

when the annual precipitation on Catalina decreased from 466.9 mm to 124.7 mm and the calving rate subsequently declined from 45% to 35%.²⁴ The bison herd size during this period was estimated to be over 300 animals, and the island resources were also being consumed by free-ranging feral pigs and feral goats. Throughout the duration of this current study, the bison herd was maintained at approximately 150 individuals (goats and pigs had been removed 5 yr prior), and annual precipitation documented between 2009 and 2012 ranged from 220.4 mm to 399.7 mm.²⁴ These conditions suggest that resource availability was not a factor in the reduced calving rate demonstrated during this study.

Calf production documented during this study was the result of insufficient doses of the PZP vaccine or poor timing of delivery. It was documented in only one instance in which a cow produced a calf after receiving a primer inoculation and booster prior to pregnancy. After receiving a subsequent booster, this individual did not produce a calf the following year. Although other factors, such as advanced age, may be to blame, variation in the ability of individuals to produce adequate antibody levels to inhibit fertilization has been documented by several investigators.^{13,14,22} In some cases, repeated annual boosters have increased the duration of each treatment,²² and infertility has been achieved among individuals that had initially been unresponsive.²¹

The safety of PZP use during pregnancy has been documented in more than 18 different species including bison.²⁴ During this current study, 38 adult cows (marked and unmarked) received one or more PZP inoculations during their first, second, or third trimesters of pregnancy, and of these individuals, 35 successfully produced calves. Low pregnancy values detected in the remaining three cows were most likely attributed to residual progesterone associated with unsuccessful fertilization following estrus. Additionally, this study documented two instances in which female calves produced by cows that had received a primer dose of PZP while pregnant, had subsequently produced their own calves, and had similarly received a primer dose of PZP during pregnancy. This outcome further demonstrates that PZP use in pregnant animals does not interfere with the progress of gestation or with the future fertility of offspring produced by treated mothers.

Identification of unmarked closely aged cows posed a challenge in confirming treated individuals from year to year. The use of the jumbo ear

tags greatly enabled accurate identification of treatment animals at several hundred yards, and the custom brown color of the tags was found to be less obtrusive with respect to wildlife viewing. The long-term retention of the dangle tags was a concern at the initiation of the study, and the decision to apply the additional USDA electronic tags during the roundups proved to be advantageous when two tags were lost. In one instance, the use of an oil-based wildlife marker paint pellet (Nelson Paint Company, Kingsford, Michigan 49802, USA) applied in conjunction with a primer inoculation was utilized, and it was found that the paint mark could be detected for several months. This marking technique will be utilized more frequently in the future as additional unmarked cows reach breeding age, or dangle tags are lost.

The primary goal of the Conservancy's contraception program is to maintain the island bison population at the current size of approximately 150 animals and eliminate the need for costly and potentially stressful removals. In order to be considered successful, efforts required to accomplish this goal through the use of fertility control must be less than that associated with periodic shipments and must be sustainable. Through this research, the versatility of PZP use in a wild, free-ranging population was demonstrated, and it was shown that it can provide the desired results in a relatively short period of time. It was also demonstrated that the application of PZP can be accomplished opportunistically and that there is flexibility in the treatment process. During the first year of this study, primer inoculations were delivered 1–8 mo prior to booster doses. Booster inoculations were initiated annually in mid-April, approximately 4–6 wk prior to the onset of the breeding season and delivered opportunistically over a period of 4 mo. Despite this deviation from the recommended administration protocol, calving rates were successfully reduced from 67.4% to 10.4%.

In order to maintain a constant island bison population, the number of calves produced each year must be in balance with potential mortalities. Based on previous studies of PZP application in wild horses, mares that had been treated for up to 5 consecutive years returned to fertility once treatments ceased.¹⁴ Beginning in 2013, a sample of breeding age cows will be removed from treatment each year and monitored for subsequent calving success. The length of time required for individuals to return to fertility will be recorded based on consecutive years of treatment,

and the number of cows that will remain untreated each year will be adjusted as needed.

Contrary to initial expectations, behavioral aversion by bison to the darting activities was most apparent during the first year of the study and decreased considerably during subsequent seasons. Although bison on Catalina Island are somewhat accustomed to human presence, this study attributed their increased darter tolerance to the absence of dependent calves and an increase in the experience of the field staff. As a result, the darting efforts required to maintain and/or further reduce the calving rate declined substantially during the second and third years.

Although the collective bison population in the United States remains significantly reduced in comparison to historic numbers, culling activities have been required within several public and privately managed herds as a result of habitat loss, ownership issues, disease spread, and cattle gene introgression. Population management through lethal means or hazing methods has become less acceptable, and these activities tend to target the symptoms of the problem rather than the source.

Several studies have demonstrated the safety and efficacy of PZP use among treated individuals, but the effective application of this agent in the management of entire populations has only been documented in a few species. Because of this, many people question whether fertility control agents offer a cost-effective and sustainable alternative to other forms of population control. The use of contraception for management purposes has the potential to reduce management costs by reducing the frequency of roundups, and in the case of Catalina's bison, the additional costs associated with transporting them off of the island. Contraception can also enhance the humaneness of management, since roundups and transport are stressful for the individuals, and herd social structure is changed dramatically when many individuals are removed during a single event. As demonstrated within this study, a substantial financial and organizational commitment may be required to initiate a successful contraceptive program using PZP if an initial roundup is required for ear tagging, but the efforts required for maintenance via field darting decrease dramatically during subsequent years. This study has also shown that an opportunistic approach to the administration of the vaccine can still provide desired results and that PZP may be more versatile as a population management tool than previously considered.

Although the Catalina bison population remains disease free, an effective contraceptive agent such as PZP may also provide an additional tool in the effort to reduce the transmission of bovine brucellosis (*Brucella abortus*) within isolated areas. Transmitted primarily through contact with infected aborted fetuses, preventing pregnancies in brucellosis-infected bison would potentially aid in preventing the spread of the disease.¹⁶

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