Report on surveying and catching Black-footed cats (*Felis nigripes*) on Benfontein Game Farm, 8 - 24 May 2007

Alexander Sliwa, Beryl Wilson, Nadine Lamberski, Jason Herrick

Study Area:

Benfontein Game Farm, owned by De Beers Consolidated Mines, lies 10 km SE of Kimberley on the border of the Northern Cape and Free State Provinces in central South Africa. The majority of the 11.400 ha of arid plant communities have been the subject of the first in-depth field study on the black-footed cat (Sliwa 1993, 1994, 2004; 2006, Olbricht & Sliwa 1997), which largely defines the present day knowledge on densities, habitat preference, ecology and behaviour of the species.

This project is part of a multidisciplinary effort to study the distribution, ecology, health, and reproductive status of black-footed cats. With the aim of capturing black-footed cats for biological sampling, and radio-collaring for subsequent observation, several methods were employed to survey areas, previously known to hold black-footed cats. In 2005 and 2006 a similar capture operation was conducted. Two reports are available on this period by the authors of this report.

Methods:

(A) Spot-lamp searching: For 9 nights a 4x4 vehicle (2.5 litre Diesel Isuzu vehicle, owned by Barend Vermeulen) drove a route of 20 -80 km in length (see Map 1) along dirt roads at a speed of between 20-30 km/ hr whilst looking for the characteristic bright eye shine of cats. Two people, Jason Herrick and Alex Sliwa, would stand on the open back of the vehicle operating two spotlights (1 million candle power Lightforce®) and additional people assisting in the search.

(B) Catching via searching and chasing: Once black-footed cats were located using their eye-shine with the spotlights, their species identity was swiftly confirmed with 10x42 binoculars. If positively identified, they were pursued quickly by vehicle for a short distance, of between 100-600m until the cat squatted low on the ground in front of the stopped vehicle. One or two people with fish landing nets then got off the vehicle and netted the cats. On other occasions the cats would find a den system (dug by aardvark, ground squirrel, springhare) and were either captured by exposing them after digging or were lost to the capture team by escaping deep into the den system. All accessible cats were subsequently anesthetized with an intramuscular injection of medetomidine, midazolam, and butorphanol and covered with a blanket to shield them from lights and sounds. After taking them back to the research house, they were examined, sampled, and measured. The anesthetic drugs were antagonized with intramuscular atipamezole and naltrexone and the cats were placed in a small plastic crate for recovery. All black-footed cats were released back into a den, close to their capture locations. A blanket was used to cover the den entrance, keeping them inside until they were fit to leave on their own account.

(C) Digging: One male cat, radio-collared during the November 2006 trip (see report 2006) was followed via telemetry to his den and dug out of an extensive aardvark den system. This method has been used previously at length by Sliwa (2004, 2006). The operation took 3.5 hours, because the den chamber lay 2.2 m underground (Fig. 7). The cat was injected via a syringe on a small pole stick and subsequently extracted by hand.

(D) **Live-trapping – not employed during this trip**

In contrast to the past three years, we did not conduct any trapping on Benfontein Farm this year as the previous two years were unsuccessful for the target species despite an intensive and large-scale effort. Trapping has been successful for non-target small carnivores that share black-footed cat habitat and prey base and may be resumed in the future.

The capture via vehicle was conducted and manned by:

Dr. Alexander Sliwa, Curator, Wuppertal Zoo, Germany / currently Kölner Zoo (sliwa@zoo-koeln.de)
Ms. Beryl Wilson, Ethologist, McGregor Museum, South Africa (Berylwa@museumsnc-co.za)
Dr. Nadine Lamberski, Veterinary Clinical Operations Manager, San Diego Wild Animal Park, USA (nlamberski@sandiegozoo.org)
Dr. Jason Herrick, Gamete Biologist, CREW, Cincinnati Zoo and Botanical Garden, USA (Jason.Herrick@cincinnatizoo.org)
Renate Stock, private sponsor, Offenbach, Germany

Results:

Spot-lamp searching: In total, there were 8 black-footed cat sightings during 9 nights. The number of sightings varied between 0-2 per night. Black-footed cats were seen during six out of 9 nights (67%), with three nights driven without any black-footed cat sightings. One of those nights was due the preceding exhaustive activity involving the digging out of the one radio-collared cat, where after we did not seriously search for additional cats.

All areas that were part of the previous ecological study of Sliwa from 1992-1998, and during previous capture trips were searched during at least three nights, with some being covered on all 9 nights.

During these night drives we consistently observed other carnivores including aardwolves (*Proteles cristatus*), two families of black-backed jackals (*Canis mesomelas*), and several small groups of bat-eared foxes (*Otocyon megalotis*) every night. During one or two drives caracal (*Caracal caracal*), Cape fox (*Vulpes cana*), striped polecat (*Ictonyx striatus*), and small-spotted genet (*Genetta genetta*) were also seen. Some other nocturnal mammals we recorded were: aardvark (*Orycteropus afer*) and porcupine (*Hystrix africaeaustralis*). On only several occasions, a spotted eagle owl (*Bubo africanus*) was seen.

Catching via searching and chasing: Out of the above 8 sightings we caught five different black-footed cats via chasing on (Maps 1 & 2). One of the cats (Cat 1 07) we caught once again after 3 nights, 2.4 km away from the first capture location (Map. 1). We also chased a large male Cat 7-07, who managed to loose us repeatedly in long grass and a likely female Cat 7-07 escaped in to a very extensive springhare den system where we could not dig her out.

The first capture was an independent kitten (Cat 1 07), about 5 months old when compared to data from hand-raised black-footed cats, judging from its milk dentition, the upper canines were just being replaced by the permanent ones, his small size and low body mass (Mass = 0.92 kg, Table 1) (Leyhausen & Tonkin 1966, Armstrong 1975, Olbricht & Sliwa 1995). The next black-footed cat we caught was an adult male that we already caught last year in November (Cat 3 06), identified by his microchip, 5.6 km away. We radio-collared him and named him “Okko”. The third cat (Cat 3 07) we caught only 1.1 km to the south was a sub-adult male. We judged him to be about 7 months old from his mass (1.44 kg) and the permanent dentition, except for the lower molars. He had white un-chipped teeth and was in good condition, but certainly not territorial. On 13 May we caught the adult female we named “Gogo” and radio-collared her. She had used nipples and her teeth were clean and unchipped, however with a bit of plaque on the carnassials. We estimate her to be older than 2 years. On 14 May we dug out the male “Panga” (Fig. 7) and repeated biological sampling.

After this we made a trip to Loxton in the southwestern part of the Northern Cape Province to search for suitable habitat to study and also capture black-footed cats, unfortunately without success.

Upon our return to Benfontein we searched for two more nights for cats. We caught the last cat (Cat 6 07), a subadult female we named “Maya” after radio-collaring her. She was in good condition, with unused nipples and black nose, which is indicative of young cats. She was monitored by Beryl Wilson and Jan Kamler for the subsequent month and was sadly found dead on 22 June. Judging from the spacing of the two canine punctures that pierced her eye and skull, and the condition of her body, she was killed by a female caracal the day before.

We also managed to capture a black female domestic cat on the western side of Benfontein, while hunting in the veldt. She was sampled and released for comparisons in her health status to the black-footed cats sharing the space where she was caught (Maps 1&2).

We also spotted and then attempted to capture an African wildcat, which managed to escape.

Behavioural observations:

On 12May 2007, the night following the radio-collaring of the adult male cat "Okko" (Cat 2 07), we witnessed a remarkable encounter with a black-backed jackal. At 22h00 we located the black-footed cat in short grass (S 28 53.441/ E 24 46.651) and followed him for about 200m. An adult jackal discovered and cornered him in between some small bushes (30 cm tall). The jackal circled him 6 times in a radius of ~ 3 m while trying to get into the back of the cat, which squatted amongst the small bushes, whenever the jackal harassed him too much. On three occasions the cat ventured up to 2 m from his hiding spot to attack the jackal from behind. The cat swatted the jackal on the legs and at least once in the face. After a while the jackal employed a distraction technique, walking a couple of meters away, and when the cat came out from the bushes to swipe at him, the jackal turned quickly and attempted to grab the cat. The jackal was seriously testing / harassing the cat, to assess the cat's quality. ***Hypothesis*:** adult male cats hold their ground, but females and sub-adult cats or kittens would be under pressure or even in mortal danger when encountered by black-backed jackals.

Locating the radio-collared cats:

Subsequent to their respective capture we attempted to get a location fix of each cat in its den during daylight each day and then another fix during the course of the nights. Due to the short duration of the field trip we were only able to collect a limited number of fixes, and thus arrive at relatively small estimated ranges (Table 1). Further work continued by Beryl Wilson and Jan Kamler following the trip, and these will give a clearer picture of the ranges by the advent of the next trip.

Discussion and Conclusions:

Valuable data on censusing and catching has been collected again on this trip in the area, which was intensively studied between 1992 -1998. A record number of black-footed cats were seen and/or captured, with 6 different black-footed cats captured 7 times during a mere 9 nights. The rate of spotting frequency was improved over the previous field trips (see progress reports 2005 & 2006 – available from the authors). On average, one can expect that experienced observers, using the described spotting method, would be able to spot a black-footed cat almost every night, the latest every second night, in an area with a good black-footed cat population.

There were at least eight different black-footed cats sighted and most of them captured. The one large male (Cat 8 07) was probably experienced to escape the capture team using the pursuit technique since he may have been pursued already during the previous two field trips. He was a very fast runner and managed to evade the spotlights on numerous occasions possibly making him impossible to catch using this method.

As in the previous years we recorded 2 families of black-backed jackals with their offspring. The observations of a caracal, probably female, were repeated from last years' observations. We can assume that at least one caracal is resident in the northern half of Benfontein. During Sliwa's study, both of these medium-sized predators were very rarely seen. The behavioural observation of the black-backed jackal harassing the adult male black-footed cat and also the death of the sub-adult radio-collared female "Maya" through the killing by a caracal has confirmed previous hypotheses of potential predators of the black-footed cat (Olbricht & Sliwa, 1997). With high densities of these two medium sized carnivores black-footed cats may be negatively affected in their densities and may alter their behaviour, a hypothesis to be tested in new future comparative study sites:

The approximate aging of the two male black-footed cats (Cat 1 07 & Cat 3 07) is interesting. Assuming their age to be 5 and 7 months according to their dental condition (Olbricht & Sliwa 1995) they would have been born sometime in October and December 2006. This would be spring / summer which, is in accordance with previously observed mating activity in August on Benfontein (Sliwa, pers. obs.). The younger cat would have been sired only later, during October. This may have been due to last years especially late rains promoting some late breeding. Also, in 2006 the winter was fairly mild so the older cat may in fact have been from a second litter, the first having being lost early on.

Altogether the trip was very successful, with the capture rate improved and even exceeding the capture success obtained during the previous field trips. During this trip we decided to radio-collar any captured cat that was large enough (> 1 kg) in order to get repeated biological samples during future trips and allowing for the comparison of home ranges to the sizes estimated by Sliwa (2004). Beryl Wilson and Jan Kamler will be able to collect more location fixes, at least once a week for each of the three remaining radio-collared black-footed cats.

We hope to return to Benfontein for further capturing and sampling of wild black-footed cats in March 2008. On our next visit we will also attempt to work further afield in the Highveld close to Johannesburg and in the Kalahari.

Summary of Health Assessment of Black-footed Cats

Anesthesia:
A total of 6 black-footed cats were captured and subsequently anesthetized using a combination of medetomidine, midazolam, ketamine, and butorphanol administered via intramuscular injection. All cats required supplementation following initial induction to facilitate maintenance of anesthesia. There were no significant anesthetic complications. Drugs were antagonized with naltrexone and atipamezole. Cats were placed in a small plastic recovery crate for 30-60 minutes before being released into a den near the site of capture. Recaptures (both within a few days and in subsequent years) confirm the safety of the anesthetic protocol used.

Physical exam findings:
In general, all cats were in fair to good body and haircoat condition. Some had fleas and ticks in the ear pinna. One juvenile female had evidence of healing bite wounds involving the ears and base of the tail.

Sample collection:
Following physical examination, the following biological samples were collected: blood, pharyngeal swabs, rectal swabs, skin, fat, hair, feces, ectoparasites, and urine. The majority of samples were collected for diagnostic purposes (general health determination and infectious disease surveillance); however, some samples were collected for genetic analysis and banking for future studies. Hair, blood, and skin samples were deposited in the Wildlife Biological Resource Center bank in Pretoria.

Scientific studies in progress:
- Fat biopsies are being evaluated for the presence of amyloid (P. Zimmermann, Wuppertal Zoo)
- Skin samples have been used to generate cell lines for future genetic studies (O. Ryder, Zoological Society of San Diego)
- Serologic prevalence of infectious diseases in black-footed cats and small carnivores (N. Lamberski, San Diego Wild Animal Park)
- Ectoaparasite identification (University of Pretoria, Onderstepoort)

Significant findings to date (2004-2007):
- 32 samples were submitted for rabies serology and only one from a black-backed jackal pup (collected in 2005) was positive. This result likely reflects maternal antibody. Additional samples will be sent to the CDC in Atlanta, Georgia for additional lyssa virus testing.
- A few black-footed cats had evidence of exposure to Feline Calicivirus, Canine Distemper virus, and West Nile Virus.
- One of two domestic cats sampled (in 2005 and 2007) were positive for Feline Immunodeficiency Virus, Feline Calicivirus, and Feline Parvovirus.
- One of four Cape fox had serologic evidence of Canine Distemper Virus and three of four were exposed to West Nile Virus.
- Both domestic dogs sampled were exposed to Canine Distemper Virus and Canine Parvovirus.

- 27 of 29 yellow mongoose sampled had serologic evidence of exposure to Canine Distemper Virus. These data require additional investigation to determine if mongoose are reservoirs for Canine Distemper Virus, if mongoose sera reacts nonspecifically with the reagents used, or if the results are due to cross-reactivity with exposure to a related virus.

Summary of Reproductive Assessment of Black-footed Cats

Reproductive Exam and Semen Collection:
During the 4 trips (3-4 weeks per year during 2004, 2005, 2006, and 2007) to date there were 10 successful captures of 8 different male cats. Five of the males were sexually mature at the time of capture and the remaining 3 males were pre- or peripubertal, estimated to be 5-7 months old based on body size and dentition. Once the cat was stable under anesthesia, the penis was examined by manually extruding it from the prepuce and assessed for normal morphology and presence of penile spines. Extrusion of the penis was not possible for the 2 pre-pubertal males captured during the 2007 trip. Testicular dimensions (width and length of each testicle) were determined with calipers and used to calculate testicular volume.

For electroejaculation, a lubricated probe (domestic cat probe - 1.0 cm diameter) was gently inserted into the rectum with the electrodes directed ventrally and a warmed, sterile collection cup was placed over the end of the penis. An electroejaculator was used to deliver 3 series of electrical stimulations beginning at 2 volts and progressing to 5 volts (Series 1 and 2, 30 stimulations each at 2, 3, and 4 volts; Series 3, 30 stimulations at 3, 4, and 5 volts) with 10 stimulations at each voltage and 3-5 minute rests between series. The entire procedure lasted less than 30 min.

Sperm Cryopreservation:
The combined, diluted sample was centrifuged for 10 min at 300x g. The supernatant was centrifuged again at 1100x g for 10 min and the bottom 20 µl was added to the original pellet. The sperm was diluted to ~25 x 10^6 motile sperm/ml with TEST EY medium containing 4% glycerol. Aliquots (~30 µl) of the sample were loaded into 0.25 cc straws. Straws were then heat-sealed, placed in a beaker of room temperature water (350-400 ml), and cooled to 5 °C for 3.5 h in the refrigerator. Cooled straws were frozen over liquid nitrogen vapor in two steps (1 min, 7.5 cm above surface and 1 min, 2.5 cm above surface) before plunging straws into the liquid nitrogen. Straws are in storage at the Wildlife Biological Resource Centre (wBRC) in Pretoria.

Discussion:
Semen containing viable spermatozoa was collected from all adult males during both study seasons (Sept.-Nov. in 2004, 2005, and 2006; May in 2007; Table 2). However, ejaculate quality was reduced in samples collected in May 2007 from 2 males that had been sampled six months previously (Nov. 2006). Testicular volume (1.48 ± 0.2 cm^3 vs. 2.0 ± 0.1 cm^3), semen volume (0.096 ± 0.027 ml vs. 0.178 ± 0.037 ml), sperm concentration (83.1 ± 32.3 x 10^6 sperm/ml vs. 148.0 ± 86.0 x 10^6 sperm/ml), and sperm number (8.9 ± 5.4 x 10^6 vs. 23.2 ± 9.9 x 10^6) were all reduced in samples collected in May compared to samples collected from the same males in Nov. The proportion of normal spermatozoa was reduced in samples collected in May, but this was only observed in one of the 2 males ("Okko", #6895205) sampled during both seasons (53% vs. 10%). In contrast, "Panga" (#6897006) exhibited 38% normal spermatozoa in both samples. Since only 2 samples were collected in early winter (May), it is difficult to determine if these differences represent seasonal changes in reproductive function. In captive cats in the U.S., including 6 samples collected during Nov.-Mar. and 10 samples collected during May-Sept., there was no evidence of seasonal changes in any reproductive parameter except a slight decline in mean fecal testosterone concentrations (Table 3). Since ambient temperature and food availability would not fluctuate as much for captive cats as it would for those in the wild, it is possible that seasonal changes are dependent on environmental conditions. Interestingly, the anesthesia regimen used in May 2007 for the wild cats included midazolam, in addition to ketamine and medtomidine which were used for all collections. Midazolam, in combination with ketamine and acepromazine, was also used during 2 collections from captive cats resulting in the lowest numbers of sperm with the worst motility of any

samples collected throughout the study. Such small sample sizes preclude drawing any conclusions, but the effects of anesthesia on semen quality may warrant further investigation.

Overall, the reproductive traits of the adult males captured on Benfontein were very similar to data obtained captive males in the U.S. (Tables 2 and 3). Although the sample size of wild males is small, it is encouraging that ejaculate characteristics in the captive males are so similar to their wild counterparts. This would suggest that the reproductive health of the captive males has not been adversely affected by captivity.

During the study period, the functionality of frozen-thawed, black-footed cat spermatozoa was evaluated using samples collected from captive males and cryopreserved using the same techniques as those used for samples from wild males. Thawed samples were coincubated with domestic cat oocytes to determine if the spermatozoa was still competent to complete fertilization after the cryopreservation process. Frozen-thawed spermatozoa from black-footed cats was shown to be equally as effective as fresh spermatozoa from domestic cats for producing embryos through in vitro fertilization (Fig. 8). These results suggest that samples collected and cryopreserved in South Africa can be used to produce embryos using in vitro fertilization. Although production of kittens from embryos produced in vitro has yet to be accomplished, this technology may useful for infusing the genetics of wild populations into captive populations without necessitating the removal of cats from their native range.

Acknowledgements: We thank DeBeers Consolidated Mines for permission to work on Benfontein and the use of the research house for accommodation and lab facilities. We are grateful for the use of the campground. Ecology Division of De Beers who gave us permission for the sampling, and supported us in employing the pursuit method, while live-trapping failed to procure black-footed cats. We thank Edwin and Gisela Sliwa for the use of their Land Rover, especially since the vehicle was provided free of charge. Funds for fieldwork came from the *In Situ* Conservation Fund of the Cincinnati Zoo and Botanical Garden, San Diego Wild Animal Park and Mrs. Renate Stock's donation through Wuppertal Zoo. The International Society of Endangered Cats (ISEC) Canada Branch sponsored the purchase of 8 ATS® radio-collars. We also want to thank our respective employers for letting us carry out this field work.

References:

Armstrong, J. 1975. Hand rearing black-footed cats (*Felis nigripes*) at the National Zoo Park, Washington D,C. . Int. Zoo. Yb. 15: 245-249.

Leyhausen, P & Tonkin, B. 1966. Breeding the black-footed cat. . International Zoo Yearbook. 6: 178-182

Olbricht, G. & Sliwa, A. 1995. Comparative development of juvenile black-footed cats at Wuppertal Zoo and elsewhere. International Studbook for the Black-footed cat (*Felis nigripes*). Wuppertal 1995: 8-20.

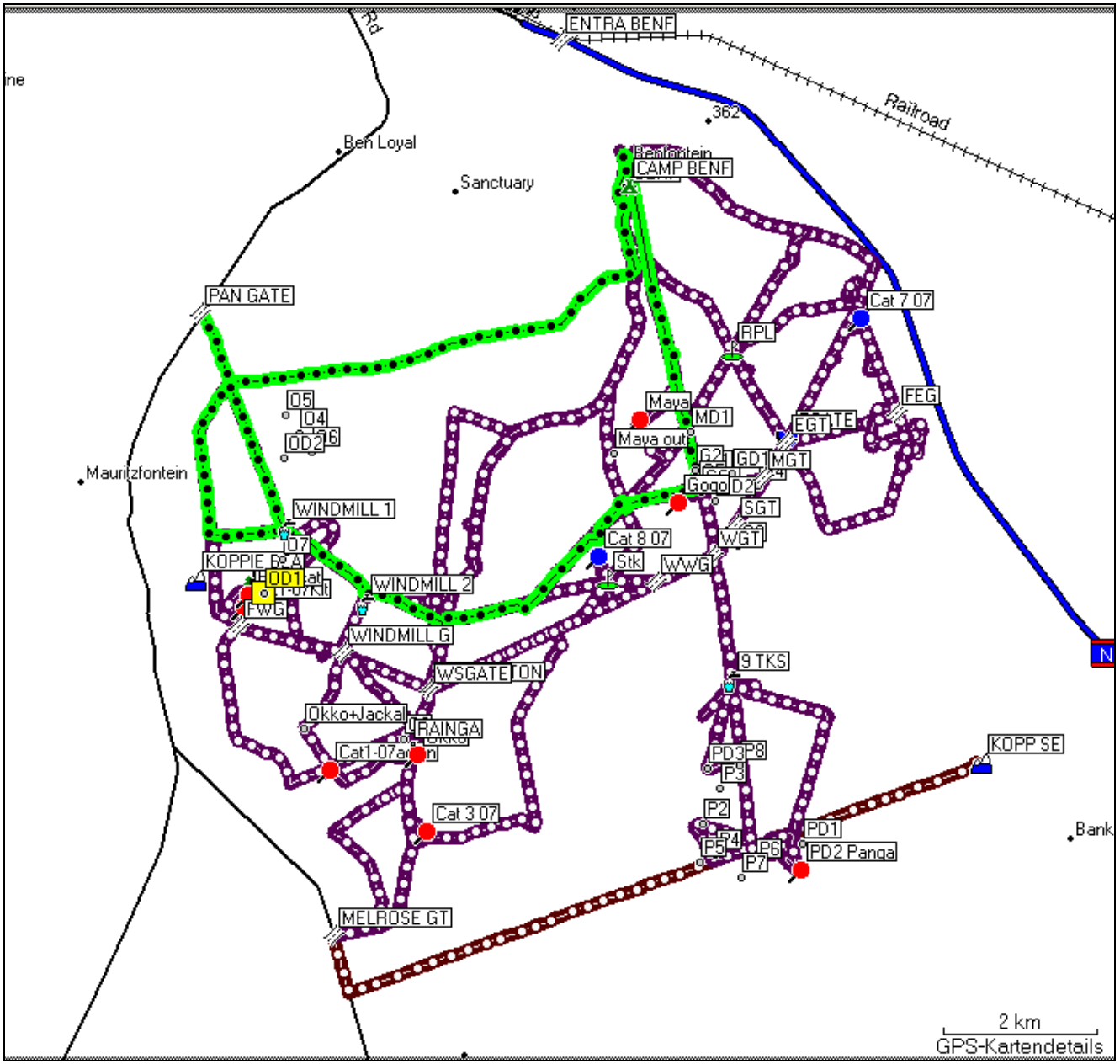
Olbricht, G. & Sliwa, A. 1997. In situ and ex situ observations and management of black-footed cats *Felis nigripes*. International Zoo Yearbook 35: 81-89.

Sliwa, A. 1993. A habitat description and first data on ecology and behaviour of the black-footed cat (*Felis nigripes*) in the Kimberley area, South Africa. International Studbook for the Black-footed cat (*Felis nigripes*). Wuppertal 1993: 8-11.

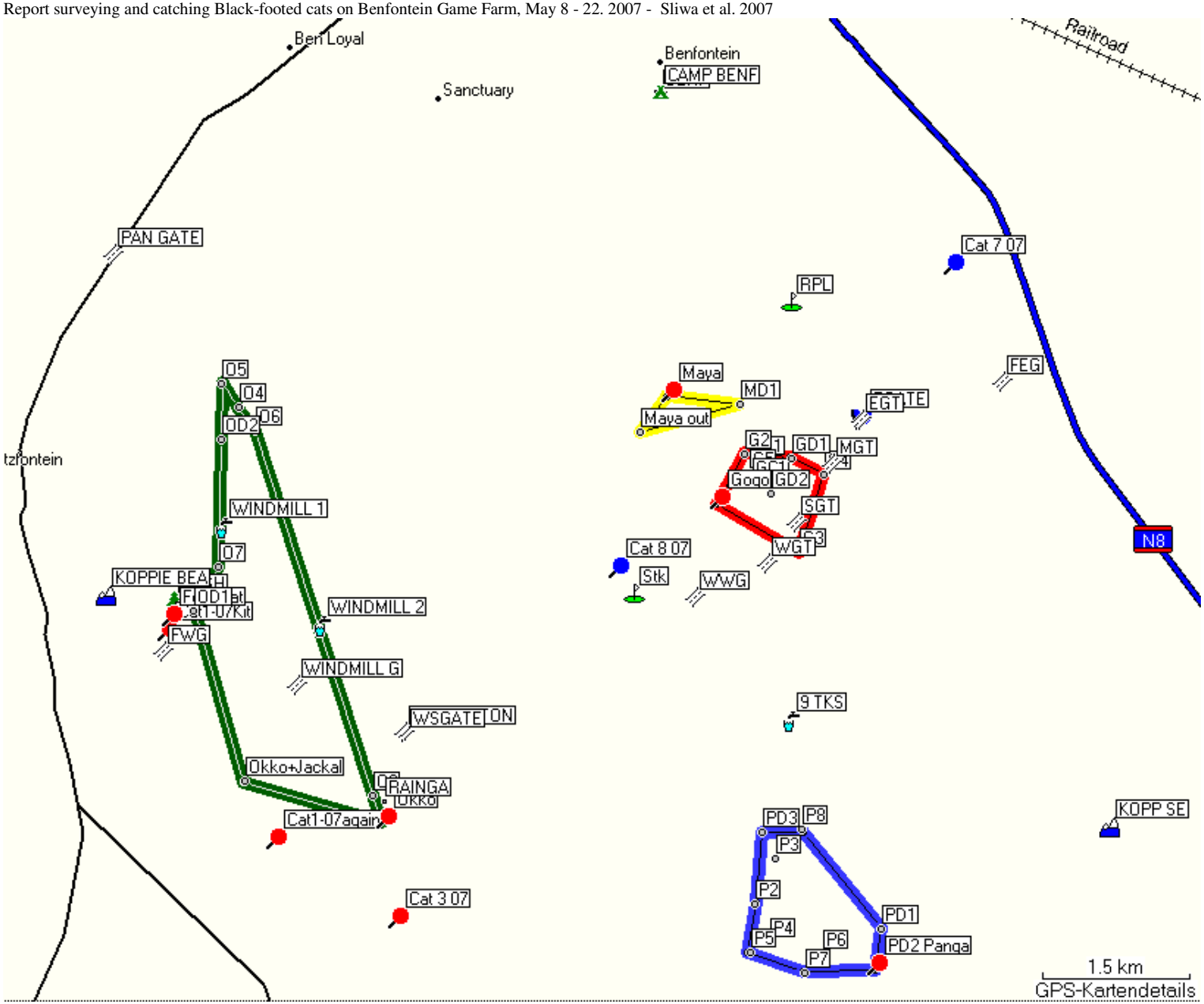
Sliwa, A. 1994. Diet and Feeding Behaviour of the Black-footed Cat (*Felis nigripes* Burchell, 1824) in the Kimberley Region, South Africa. Zoologischer Garten 64 (1): 83-96.

Sliwa, A. 2004. Home range size and social organisation of black-footed cats (*Felis nigripes*). Mammalian Biology 69 (2): 96-107.

Sliwa, A. 2006. Seasonal and sex-specific prey-composition of Black-footed cats *Felis nigripes*. Acta Theriologica 51 (2): 195-206



Map 1. GPS map of Benfontein Farm with land marks all sightings (blue) and capture locations (red beacons + Names of radio-marked cats) of black-footed cats.



Map 2. GPS map of Benfontein Farm, with Minimum convex polygons encompassing the locations of the 4 radio-collared black-footed cats collected during the field period (different colours), land marks and gates, sightings (blue beacons) and capture locations (red beacons) of black-footed cats.

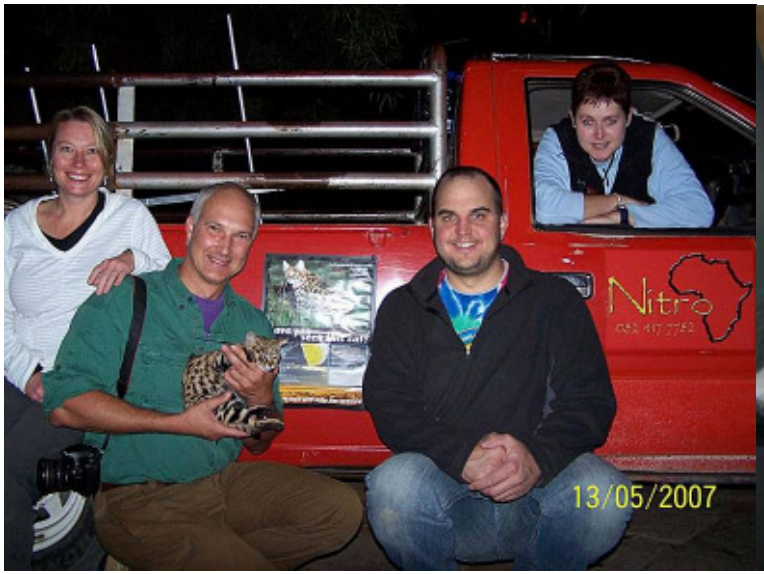


Fig.1. The capture team with vehicle (Photo Selfrelease)

Fig.2. Radio-collars sponsored by ISEC Canada (Photo Alex Sliwa)

Fig. 3 Beryl Wilson with "Maya" (Alex Sliwa)

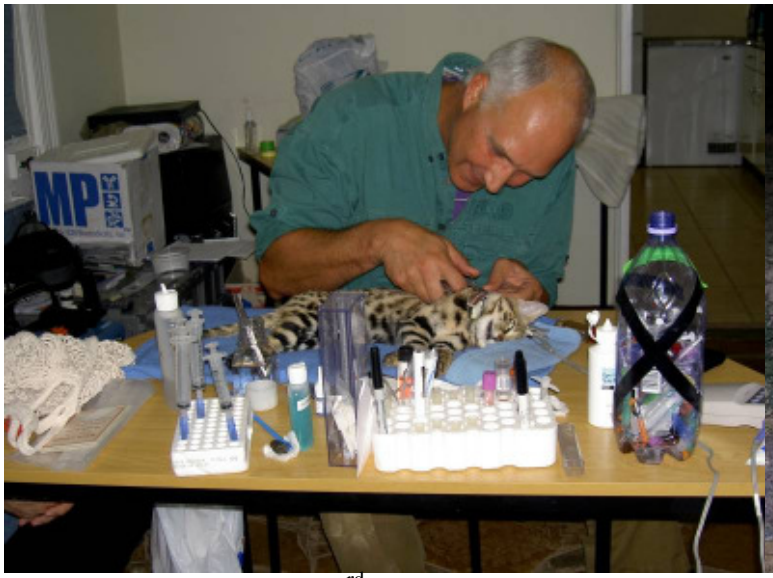


Fig.4. Radio-collaring of 3rd Cat. (Photo Nadine Lamberski)

Fig.5. Releasing Cat (photo Beryl Wilson)

Fig. 6.Capture team with vehicle. (Photo U. Stenkewitz)

Fig.7. Team work digging out “Panga”, radio-collared already November 2006 (Photos Beryl Wilson)

Table. 1: Measurements, range size and remarks on the 6 captured black-footed cats and one black domestic (feral) cat – Benfontein, May 2007.

Date	09.05.07	11.05.07	12.05.07	13.05.07	14.05.07	24.05.07	11.05.07
Name (also on Map)		**Okko**		**Gogo**	**Panga**	**Maya ***	**Black**
No. captured	Cat 1 07	Cat 2 07	Cat 3 07	Cat 4 07	Cat 5 07	Cat 6 07	Feral Cat
Sex	M	M	M	F	M	F	F
Age	indep. Kitten	adult	subadult	adult	adult	subadult	adult
Microchip #.	TRV 00-068A 119F	TRV 00-0689-5205	TVN 00-068A-E822	TVN 00-0689-561F	TRV 00-0689-7006	TVN 00-0689-7D88	
Mass (kg)	0,92	2,00	1,44	1,50	2,00	1,20	
Ear (cm)	4,60	4,90	5,0	5,2	5,50	5,1	
Shoulder (cm)	21,00	26,00	25	22	26,00	23,00	
Total Length (cm)	52,50	59,00	57,5	58	62,00	55	
Hind foot (cm)	8,46	9,40	9,18	-	9,80	9,10	
Front foot (cm)	1,90	2,00	1,95	1,80	2,20	1,90	
Tail (cm)	15,50	17,00	17,00	18,00	18,50	17,00	
Neck (cm)	8,00	14,00	12,5	12	14,00	11,00	
Canine UR (cm)	0,63	1,01	0,85	0,86	1,02	0,80	
Canine LR (cm)	0,48	0,91	0,84	0,70	0,88	0,66	
Canine UL (cm)	0,62	1,04	0,83	0,83	1,04	0,79	
Canine LL (cm)	0,55	0,91	0,78	0,73	0,86	0,62	
Testes RL (cm)	Not descended	1,5	1,2	/	1,6	/	
Testes RW (cm)	Not descended	0,9	0,7	/	1,0	/	
Testes LL (cm)	Not descended	1,5	1,2	/	1,6	/	
Testes LW (cm)	Not descended	0,9	0,7	/	1,0	/	
Number of fixes	-	8	-	8	10	3	
Range size (100%)	-	3.8 km²	-	0,7 km²	1.4 ²	0.1 km²	

REMARKS

Cat1-07 Kitten of about 5 months (milk teeth, but permanent canines coming out),otherwise good condition. Caught two times, on 9th and 12th May

Okko Cat 2-07 Adult male, recapture from November 2006 (Cat 3 06, good condition, some plaque, all canines un-chipped, **radio-collared)**

Cat 3-07 Subadult of about 7 months (permanent dentition, apart from lower molar (milk)), white teeth, good condition

Gogo Cat 4 07 Adult female, good condition, used nipples, teeth clean, un-chipped canines, some plaque on carnassials, **radio-collared**

Panga Cat 5 07 Adult male, good condition, un-chipped canines, **left radio –collar on.**

Maya Cat 6 07) subadult female, permanent teeth, white, unused nipples, blackish nose pad, good condition, many ticks in ears, fleas¸ **radio-collared – she was found dead a month later – probably killed by female caracal (measuring canine puncture distances.**

Domestic Cat Black female cat, caught 21:51 on 11.05.07; sampled and released

Table 2: Reproductive traits (mean ± S.E.M.) in wild male black-footed cats on the Benfontein Farm.

	Spring (Sept.-Nov.) **5 ejaculates, 5 males**	**Autumn/Winter** (May) **2 ejaculates, 2 males**
Total Testicular Volume (cm^3)	1.92 ± 0.24	1.48 ± 0.2
Total Semen Volume (µl)	131.0 ± 30.7	96.0 ± 27.0
Sperm Concentration ($x10^6$ sperm/ml)	174.8 ± 36.1	83.1 ± 32.4
Total Sperm Count ($x10^6$ sperm)	21.0 ± 5.2	8.9 ± 5.4
Motility (%)	80.0 ± 3.5	80.0 ± 10.0
Forward Progression (0 to 5)	3.6 ± 0.2	3.0 ± 0.5
Morphologically Normal Sperm (%)	45.2 ± 2.9	24.0 ± 14.0
Intact Acrosomes (%)	90.0 ± 2.7	88.8 ± 5.3

Table 3: Reproductive traits (mean ± S.E.M.) of captive male black-footed cats in the U.S.

Trait	**Winter** (Nov.-Mar.) **6 ejaculates, 3 males**	**Summer** (May-Sept.) **10 ejaculates, 5 males**	**Overall** **18 ejaculates*, 5 males**
Testicular Volume (cm^3)	1.80 ± 0.17[a]	1.81 ± 0.08[a]	1.77 ± 0.07
Fecal Testosterone (ng/g feces)	***3283.9 ± 84.4[a]***	***2905.3 ± 81.4[b]***	3106.9 ± 53.4
Ejaculate Volume (µl)	262.2 ± 15.9[a]	219.8 ± 11.2[a]	246.5 ± 11.8
pH	9.0 ± 0.02[a]	8.72 ± 0.09[a]	8.77 ± 0.06
Sperm Concentration ($x10^6$/ml)	164.0 ± 55.9[a]	134.7 ± 21.2[a]	130.4 ± 23.6
Total Sperm ($x10^6$)	39.7 ± 11.5[a]	29.5 ± 4.8[a]	29.9 ± 5.1
Motility(%)	85.0 ± 1.8[a]	84.5 ± 1.7[a]	82.5 ± 1.9
Forward Progression (1-5)	3.8 ± 0.1[a]	3.8 ± 0.1[a]	3.6 ± 0.1
Sperm Motility Index	80.0 ± 1.1[a]	80.3 ± 1.6[a]	77.6 ± 2.0
Normal Sperm (%)	47.9 ± 6.6[a]	46.9 ± 3.8[a]	46.8 ± 3.0
Abnormal Sperm (%)	52.1 ± 6.6[a]	53.1 ± 3.8[a]	53.3 ± 3.0
Bent Midpiece w/Droplet	2.7 ± 0.5[a]	7.3 ± 2.3[a]	5.4 ± 1.4
Bent Midpiece	6.4 ± 1.5[a]	5.0 ± 1.3[a]	5.4 ± 0.9
Proximal Droplet	3.8 ± 1.1[a]	7.2 ± 1.8[a]	5.7 ± 1.1
Distal Droplet	11.6 ± 7.6[a]	7.2 ± 1.3[a]	8.6 ± 2.5
Bent Tail	24.3 ± 2.8[a]	24.1 ± 4.3[a]	25.4 ± 2.6
Intact Acrosome (%)	94.5 ± 1.4[a]	89.2 ± 3.1[a]	90.5 ± 1.9

* Two ejaculates collected in the winter were excluded from the seasonal comparison. These samples were the only samples collected using an alternate anesthetic regimen, and contained the lowest number of sperm with the worst motility of any samples collected throughout the study.

[a,b] Different superscripts indicate a significant ($P<0.05$) difference between seasons.

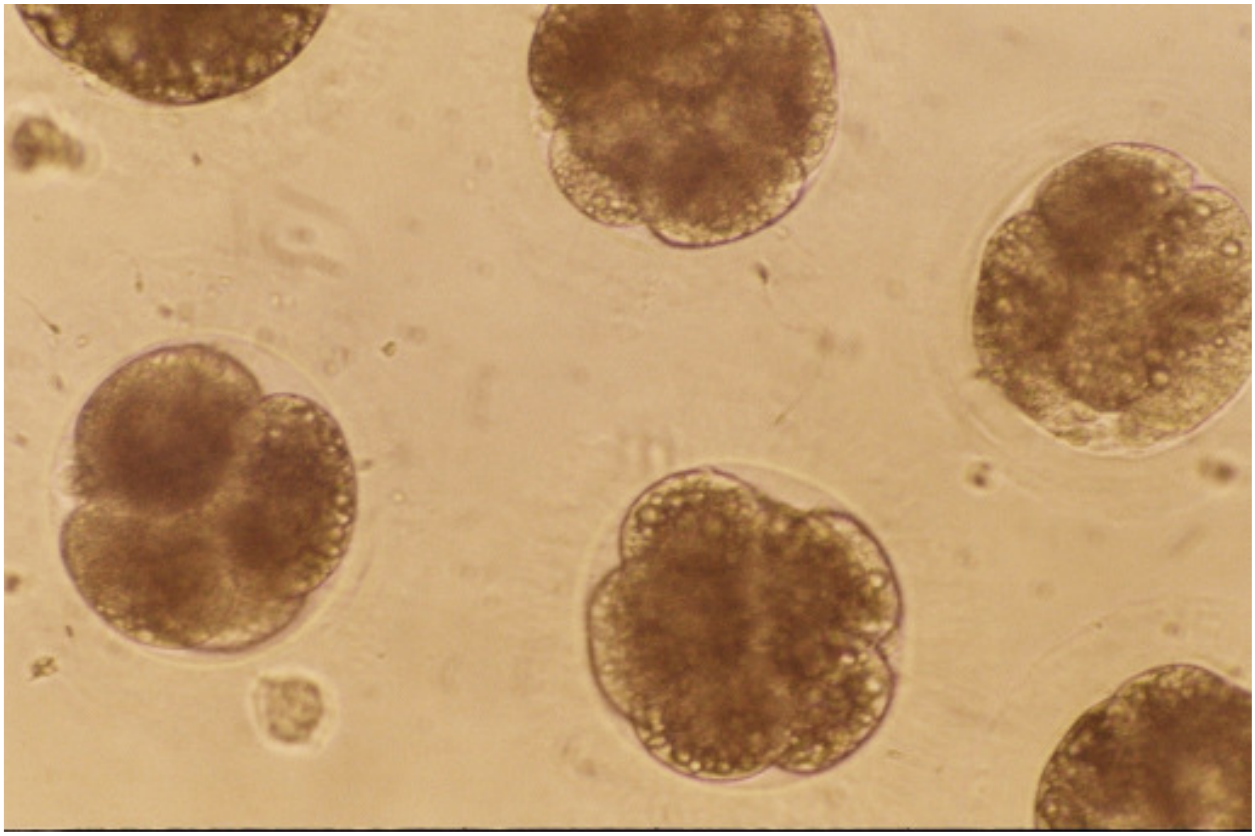


Figure 8: Embryos (~48 h post-insemination) produced from domestic cat oocytes and frozen-thawed spermatozoa from a male black-footed cat at the Cincinnati Zoo and Botanical Garden.