Chapter 3

Individual Identification and population estimation of tigers
in the Bandipur Tiger Reserve, Karnataka state, India
Introduction:

Abundance (population size) is a state variable of primary interest to scientists and conservationists because of its decisive influence on ecological and behavioral attributes, and, thus the potential viability of any animal population (Williams et al., 2002). However, abundance is a particularly difficult parameter to estimate for low density, wide-ranging, elusive, carnivore species, most of which are threatened (Schipper et al., 2008). For example, wild tigers (*Panthera tigris*) have suffered a massive range contraction of ~93% in historical times, with a global population size less than 3000-4000 animals surviving in fragmented local populations (Sanderson et al., 2006; Ranganathan et al., 2008). However, it is not practical to employ invasive physical tagging methods to derive tiger density estimates because of logistical difficulties, high costs and small numbers of captures possible (Karanth and Nichols, 2002). Consequently, non-invasive, photographic capture-recapture methods were initially developed by Karanth (1995) and thereafter refined (Karanth and Nichols, 1998; Karanth and Nichols, 2002) for estimating tiger numbers. Since then photographic capture-recapture sampling has been successfully employed on tigers (Karanth et al., 2004; Wegge et al., 2004; Kawanishi and Sunquist, 2004; Simchareon et al., 2007) and other elusive felids like leopards (Henschel and Ray, 2003), jaguars (Silver et al., 2004) and ocelots (Trolle and Kery, 2003). The photographic capture-recapture method takes advantages of the fact that in all these species, individuals are identifiable from natural markings. However, they do not work for species in which individuals are not uniquely identifiable. Other potential disadvantages of photographic capture methods include: requirement of large number of camera traps, scarcity of skilled personnel, vulnerability of cameras to theft, vandalism, animal
damage or adverse weather, and, potentially low animal densities or wariness resulting in small number of detections.

In this context, non-invasive genetic typing of DNA extracted from animal hair or faeces has emerged as an alternative option for capture-recapture sampling of populations of such rare, endangered or cryptic species (Waits, 2004). Genetic identification methods have often been simply used to count the minimum number of individuals or as part of pilot studies (e.g. Sloane et al., 2000; Creel et al., 2003; Fickel et al., 2006; Bhagavatula and Singh, 2006). However, since not all individuals in the population may be detected such estimates are of limited utility (Williams et al., 2002). Several different approaches have been used to estimate animal abundance from genetic data, in the face of such imperfect detections. Principally, these include rarefaction curve from unique genotypes (Kohn et al., 1999; Eggert et al., 2003; Wilson et al., 2003; Frantz et al., 2003, Frantz et al., 2004), genetic mark-recapture method (Mowat and Paetkau, 2002; Piggot et al., 2002; Boersen et al., 2003; Solberg et al., 2006) with jackknife estimators (Flagstad et al., 2004), Lincoln-Peterson estimator (Triant et al., 2004), closed capture model (Rudnick et al., 2008), open population model (Prugh et al., 2005) and relatively recent CAPWIRE method (Zhan et al., 2006; Peuchmaille and Petit, 2007). Different estimation methods have been evaluated from both theoretical (McKelvey and Schwartz, 2004; Lukacs and Burnham, 2005) and empirical (Petit and Valiere, 2006, Piggott et al., 2006, Dreher et al., 2007;) perspectives.

The above methods based on ‘genetic captures’ require data from multiple hypervariable loci for unambiguous individual identification (Taberlet and Luikart, 1999; Mills et al., 2000; Lukacs and Burnham, 2005; Waits and Paetkau, 2005; Broquet et al., 2006). However, getting reliable genotypes at sufficient loci for
unambiguous individual identification poses a challenge. As an example, amplifying all ten loci from a degraded genetic sample is much harder than amplifying five loci. This requirement of increased genetic information lowers the number of identifiable individuals counted from the survey sample, thereby reducing numbers of ‘individual captures’ available for capture-recapture analysis, resulting in trade-offs between accuracy of individual identification and statistical needs of robust capture-recapture estimation.

This study addressed key methodological issues related to field survey design, laboratory protocols and capture-recapture analyses relevant to genetic capture-recapture sampling of tigers based on faecal DNA.

Main objectives of this study were: (1) develop and validate a genetic identification protocol for tigers using known distinct individuals (2) employ field survey protocols explicitly designed for robust capture-recapture analyses under various plausible models (Williams et al., 2002; Karanth et al., 2004; Karanth et al., 2006) (3) evaluate fecal DNA-based tiger abundance estimates in comparison to standard photographic capture-recapture methods, and, (4) explore methodological trade-offs between attaining greater certainty in individual identifications versus achieving higher capture rates. In addition to estimation of tiger numbers, the results from this study have wider relevance for non-invasive, faecal DNA-based population assessments in many scarce, elusive, wide-ranging animal species.

Methods

Sample collection:

This study was carried out in the 880 km² Bandipur Tiger Reserve (76°12’-76°46’ E and 11°37’-11°57’ N) in Karnataka state, India. This landscape supports high densities of ungulate prey species (~35.2 animals/km²), and consequently, a
large tiger population (Karanth et al., 2004). For sampling, the study area (671 km²
total area) was partitioned into three blocks (Karanth and Nichols, 2002: design 4,
page 133), each containing six spatially well-distributed search routes (Figure 1).
These routes were surveyed on foot to collect ‘fresh’ tiger faeces (as classified by
Andheria, 2006). These surveys followed dirt roads and trails, which were used as
regular travel routes by tigers (Karanth and Sunquist, 2000). Within each block, two
trained trackers covered each route on successive days. The entire study area was
surveyed over six successive days by three teams, which covered a distance of 235.4
km. On the 8th day the survey was repeated, and the tiger faeces, deposited after the
previous effort were collected. In terms of capture-recapture sampling data structure
(Otis et al., 1978; Williams et al., 2002), tiger scats collected during days 1-6 were
assigned to the first ‘sampling occasion’, on days 8-14 to the second sampling
occasion, and so on, to attain a total of six sampling occasions. The survey yielded a
total of 63 fresh faecal samples.

The survey was specifically designed to meet two key requirements of
standard capture-recapture analyses: full spatial coverage of the area in each sampling
occasion to ensure exposure of all individual tigers to potential ‘capture’, and, to
ensure a short survey duration of 42 days, which reasonably met the assumption of
demographic closure (Karanth and Nichols, 1998).

Validation and development of laboratory protocols

Blood samples were collected from 14 captive tigers from three different zoos
in Southern India. These animals included close relatives such as siblings, and were
used for standardization of the final set of loci used for individual identification.
Additionally, faeces were collected from 13 of these tigers (housed separately) to test
the effects of different sources of DNA (i.e. blood or fresh faeces) on the
amplification process and associated error estimates. All these faeces were collected fresh (less than 12 hours) from the zoos.

To test the efficiency of the microsatellite loci from faecal samples of wild tigers, 10 fresh samples were collected from the Bandipur-Nagarahole landscape. These samples were possibly deposited the same night, and found sufficiently far apart within the landscape, allowing us to assume reasonably that they came from different individuals within the same genetic population. These scats, further mentioned as ‘field test samples’, were used to standardize protocols but not in the abundance estimation. These field scat samples were carefully collected to avoid contamination and preserved in absolute alcohol for DNA extraction. All the samples were stored in ethanol as opposed to other storage mediums (for example, silica gel) following recommendations in Murphy et al., 2002.

**DNA Extraction and Species Identification:**

DNA was extracted in duplicate from all faecal samples using commercially available QIAamp DNA Stool mini kit (QIAGEN Inc.) following the manufacturer’s instructions with slight modifications. Around 180–200 mg of sample from the outer parts of the faeces was used for each extraction. The first overnight incubation was conducted at room temperature with 1 ml ASL buffer, followed by a second incubation with 700 µl ASL buffer at 65°C for 60 minutes. 4 µg of carrier RNA (Poly-A from NEB) was added to increase DNA yield from faecal samples as recommended by Kishore et al., 2006. DNA Elution was performed with 120 µl of Tris-EDTA buffer (pH 7.8). All extractions (in sets of 11 samples) included an extraction control to monitor contamination. DNA was extracted in a separate, pre-PCR laboratory space (no prior extractions from mammalian samples had been conducted in this space). Blood DNA was extracted using QIAamp DNA Tissue Kit
following manufacturer’s instructions. All the faecal samples collected from wild (both Bandipur and field test samples) were analyzed for species identification using species-specific primers developed earlier (Mukherjee et al., 2007). This was necessary as there is a chance of misidentifying faecal samples of similar sized sympatric carnivores in the field (Davison et al., 2002), in this case leopards. This additional step ensured that all further analyses are conducted only on tiger faecal samples.

Microsatellite primer selection:

33 microsatellite loci, characterized for either domestic cat (Menotti-Raymond et al., 1999) or different tiger subspecies including P. t. sumatrae (Williamson et al., 2002), P. t. amoyensis (Zhang et al., 2005) and P. t. tigris (Bhagavatula and Singh, 2006) were selected for individual identification of tigers in for this study. These loci were selected based on their polymorphic information content and expected heterozygosity. The details of these are provided in Table 1.

PCR standardization and genotyping:

All PCR standardizations were initially conducted using the blood DNA samples. For all standardizations amplification was carried out in 10 µl reaction volumes containing 5 µl Qiagen multiplex PCR buffer mix (QIAGEN Inc.), 0.2 µM labeled forward primer (Applied Biosystems), 0.2 µM unlabeled reverse primer, 4 µM BSA and 1 µl of the DNA extract. For faecal DNA, 3 µl of the extract was used in the reaction mixture. The temperature regime included an initial denaturation (94 °C for 15 min); 45 cycles of denaturation (94 °C for 30 sec), annealing (T_a for 45 sec) and extension (72 °C for 45 sec); followed by a final extension (72 °C for 30 min) in an Eppendorf thermocycler. Post-temperature standardization primers with same annealing temperatures were optimized for multiplex reactions from the blood and
faecal DNA samples. For multiplex reactions, the PCR conditions were identical to normal reactions. The number of PCR cycles was optimized as 30 and 45 cycles for blood and faecal DNA samples, respectively. PCR negatives were incorporated in all reaction setups to monitor contamination. The PCR products were visualized in 2% agarose gels. 1 µl of the amplified product was added into 12 µl of formamide (Applied Biosystems) and 0.5 µl of ROX 500 size standard (Applied Biosystems) and then run into an automated sequencer ABI3100XL (Applied Biosystems). Microsatellite alleles were scored with GENEMAPPER version 4.0 (Applied Biosystems).

To obtain reliable genotypes the whole genotyping procedure was repeated thrice from blood DNA samples. For all the faecal DNA samples, the genotyping process was performed four times. Average amplification success (over individuals) for a given locus was calculated as the percent positive PCR (Broquet et al., 2006). In other words, amplification success was calculated as the number of times a particular locus showed amplification for all samples in all the trials. This definition of amplification success allows us to distinguish it from sample quality. Microsatellite loci that revealed positive amplification were tested on 13 faecal DNA samples from the captive individuals. The results from blood and scat genotyping were compared to evaluate the suitability of these particular microsatellites. Further, the average allelic dropout (across individuals) and the average number of false alleles (or genotyping success) as the number of dropouts or the number of false alleles over the total number of amplifications respectively were compared from both kinds of samples. Program GIMLET (Valiere, 2002) was used to calculate the $P_{ID(obs)}$ and the $P_{ID(sibs)}$ based on the captive sample genotypes (blood and scat genotypes were the same). Finally a set of 10 loci was selected for further analysis based on their amplification
success in the fecal DNA samples. These 10 microsatellite loci were combined into six PCR reactions (Table 2).

Data Validation:

In this study, a modified multiple tube approach monitored by the quality index approval was employed to ensure good data quality. All the faecal samples from the study area that showed positive amplification for at least 3 loci after first genotyping were selected and the whole process was repeated thrice, independently. After scoring the alleles with GENEMAPPER version 4.0 (Applied Biosystems) the genotypes from four different amplifications were compared and the quality index value was assigned as per Miquel et al. (2006). In other words, samples that revealed the same genotype in all four repetitions had a quality index of one, compared to samples that yielded three same genotypes out of the four amplifications (quality index = 0.75).

Individual identification:

The selected ten-microsatellite panel was used to create genotype profile for all the samples collected in the field. All these profiles were then compared using the identity analysis module in program CERVUS (Marshall et al., 1998) to identify samples with identical genotypes for the specified number of loci. Identical genotypic profiles (based on five or more loci) were used to identify multiple instances of the same individual. As samples with incomplete genotype profiles were included in analyses (using an approach similar to that described by Frantz et al., 2003), if individuals had identical genotypes for at least five or more common loci they were considered as recaptures. Such analyses allowed discerning both the number of unique individuals as well as the number of recaptured individuals from multilocus genotypes. While using this approach there was a possibility that the incomplete
genotypes might belong to a new individual, this method of grouping them with matching samples ensured conservative population estimation (Frantz et al., 2003) by minimizing non-existing individuals through erroneous multilocus genotype.

Similarly unique and recaptured individuals based on seven (genotypes identical for at least seven loci) and three loci (genotypes identical for at least three loci) were identified for population estimation.

*Analysis of 'Tiger Capture' Data:*

In this study, an explicit survey design (Karanth and Nichols 2002; Andheria 2006) for sampling has been combined with standard closed model capture-recapture analyses (Otis et al., 1978; Williams et al., 2002; Amstrup et al., 2005).

Systematic and rigorous validation and development of genetic identifications has lead to a high degree of certainty in individual identification of tigers even from potential siblings, which avoided additional nuisance parameters that account for such uncertainties in capture-recapture models (Lukacs and Burnham, 2005).

Each genetic capture of an individual tiger was assigned to the appropriate sampling occasion to generate individual capture histories in the standard X matrix format (Otis et al., 1978). These capture histories were analyzed using program CAPTURE (Rexstad and Burnham, 1993; White et al., 1982). Earlier work (Karanth and Nichols, 2002; Karanth et al., 2004) showed that closed capture–recapture model $M_h$, which accounts for heterogeneity of capture probabilities among individuals, is often most useful for tiger populations. Because of its’ stability and better convergence properties during iterative computations, and, based on reasonable results in the past with tiger data (Karanth and Nichols, 2002), the $M_h$-Jackknife estimator (Burnham and Overton, 1978) implemented in program CAPTURE was preferred over the $M_h$ models implemented in program MARK (White and Burnham,
1999) wherein heterogeneity is handled using the method of finite mixtures (Pledger, 2000). Additionally, analysis was performed using the simpler $M_0$ (null) model to enable comparisons, although this model is not robust to violation of assumptions of invariant capture probabilities.

Finally, these faecal DNA-based tiger abundance estimates were compared to abundances derived from a 48-day photographic capture–recapture survey of tigers conducted 15 weeks earlier in the same area. The photographic capture–recapture survey involved 118 trap locations set along some of the same scat sampling routes (Figure 2). It expended 1246 camera trap nights of effort across 12 sampling occasions. The survey resulted in 35 photographic ‘captures’ of 29 distinct individuals (Karanth et al., 2008, unpublished report), from the same tiger population that has been sampled subsequently for genetic samples. The methods used in the photographic capture–recapture survey are described in detail elsewhere (Karanth and Nichols, 1998, 2002; Karanth et al., 2004, 2004).

**Results**

*Species identification from the field samples:*

Genetic screening showed that of the 73 samples collected from wild (63 from Bandipur and 10 field test samples) 68 were found to be from tigers. All the 10 field test samples were from tigers while five samples from Bandipur were identified to belong to leopards. The survey from Bandipur hence yielded a total of 58 tiger faeces.

*Amplifying microsatellites from DNA samples of Indian tigers:*

While reporting the results following terms have been used; “captive samples” (for blood and faecal samples from zoo tigers), “field test samples” (described in methods section) and “Bandipur samples” for scat samples obtained from the field survey.
A total of 33 microsatellite primers were tested for the captive tiger blood DNA samples, of which 30 primers showed amplification (Table 1). The microsatellite loci varied from extremely polymorphic (locus Tiger 6, nine alleles; loci FCA164 and FCA279, $H_o = 1$) to being monomorphic (locus FCA170, one allele; loci 6HDZ056, Tiger24 and FCA170, $H_o = 0$). Results for captive tiger faecal DNA samples revealed that amplification success was higher for loci with shorter amplicon size. Out of these 30 loci, 19 loci showed 100% amplification success from these faecal samples (Table 1). The remaining loci showed amplification success range from 17-92% for the captive tiger faecal samples. Further observation revealed that loci with larger amplicons (generally < 200 bp) produce low amplification success (in this case, highest of 65%, Table 1). Since none of the amplifiable loci showed allelic dropout, it can be inferred that amplification success depends only on amplicon size of the locus, at least for captive faecal DNA samples (in which DNA quality is presumed high, because of their freshness).

For the 10 field test samples from wild tigers in the region, all these 19 loci were tested. Reliable data from multiple loci is required for individual identification that can be used further for population estimation. Out of the 19 loci tested, all produced reliable data but only 10 of the 19 tested loci showed an amplification success higher than 70%. These ten loci thus were used in this study and results from these 10 loci are presented in Table 2.

Probability of identity curves was calculated based on these ten loci for both captive and field test samples (Figure 3). This graph highlighted the $P_{ID(sibs)}$ for 3, 5 and 7 most informative loci, and revealed that the $P_{ID(sibs)}$ was higher for the captive samples compared to the field test samples, and that it increased to 0.008 and 0.0003 for field test and captive individuals respectively for seven loci.
All fresh Bandipur scats (n= 58) were amplified for the above 10 loci. Amplification success for these samples was high at > 74% and allelic dropout was less than 1.7% (Table 3). For all the ten loci presented in Table 3, the number of alleles observed for the tiger samples from Bandipur survey was higher than that observed for the captive or field test tigers. Genotyping success for the Bandipur tigers ranged from 98-100%.

**Individual identification and data validation:**

Of the 58 fresh tiger scats collected from the study area, only 50 produced genetic data. Further only 38 of the 50 samples produced five or more loci genotypes with quality index greater than 0.75. 12 of these samples produced data for all ten loci, and 11, six, five and four samples produced data for nine, eight, seven and five loci, respectively. Before conducting individual identification, an investigation of how the probability of identity changes as a function of loci was performed for the Bandipur samples. Analysis with GIMLET resulted in a probability of identity for siblings ($P_{ID(sibs)}$) value of 0.0005 for ten loci (Figure 3) for the Bandipur scat samples.

Using prior knowledge that tiger population size was likely to be less than 80 individuals in the study area based on earlier work (Karanth et al., 2008, unpublished report), a conservative cutoff level for $P_{ID(sibs)}$ was set for Bandipur tigers. A $P_{ID(sibs)}$ value of $5 \times 10^{-3}$ was used for individual identification, which corresponded an error rate of 1 in every 200 tigers based on suggestions of earlier researchers (Waits et al., 2001, Miller et al., 2005). Figure 3 revealed that to achieve this degree of certainty, 5 loci were required for the Bandipur samples, suggesting Bandipur tigers were more polymorphic than the ‘captive’ or ‘wild’ field-test tigers.

After grouping the incomplete genotypes with the complete ones, 26 unique genotype profiles were identified from the samples based on five loci criteria. All five
loci were among the eight most informative loci (based on the $P_{ID(sibs)}$ analyses, loci FCA628, FCA205, FCA126, FCA672, FCA232, FCA441, F41 and FCA391) of the complete ten loci panel.

Similarly based on three loci criteria 29 different genotype profiles were identified. The number of individuals increased when three loci was used because more number of samples could be used for this analyses (n= 41) in comparison to the other two cases (n=38 for 5 loci and n= 34 for 7 loci).

*Model choice and tiger abundance estimates*

Because of the short survey duration of 42 days, demographic closure could be assumed (Otis *et al.*, 1978, Karanth and Nichols, 1998), with no births, deaths, immigration or emigration. Results of the closure test (Otis *et al.*, 1978) in program CAPTURE ($z = 1.39, p = 0.92$), as well result of the Stanley and Burnham (1999) test in program CloseTest ($\chi^2 = 3.65, df = 4, p = 0.46$) supported this assumption of demographic closure (Table 4).

Following standard practice (Waits *et al.*, 2001; Miller *et al.*, 2005) in genetic studies and based on the results so far (Tables 1-3), it was evident that with five polymorphic loci individual tigers from Bandipur could be identified with sufficient certainty. The genetic individual identification resulted in ‘captures’ of 26 distinct individual tigers during this study. The capture histories (Otis *et al.*, 1978) of these individuals were analyzed using closed model $M_0$-Jackknife estimator implemented in CAPTURE (Rexstad and Burnham, 1993). In addition, capture histories were also created based on genetic recaptures using 3 and 7 loci to test the effects of the number of loci on population estimation.

Although the overall model comparison results based on discriminant function test scored the $M_0$ null model higher than model $M_0$ (score of 1.0 versus 0.77) it could
be assumed that heterogeneity is likely to exist in tiger populations because of social spacing mechanisms (Karanth and Sunquist, 2000) and differential exposure of tigers to survey routes (Karanth and Nichols, 1998, Borchers and Efford, 2008, Royle et al., 2009), making the results from the heterogeneity model \( M_h \) more reliable. The tiger abundance estimates from both CAPTURE analyses (based on genetic captures using 5 loci) were reasonably close: \( \hat{N} (S\hat{E}[\hat{N}]) = 47 (11.21) \) for the ‘null \( (M_0) \)’ model, and, \( \hat{N} (S\hat{E}[\hat{N}]) = 66 (12.98) \) for the ‘heterogeneity \( (M_h\text{-jackknife}) \)’ model, respectively. Results generated by heterogeneity models under the method of finite mixtures (Pledger, 2000) implemented in program MARK are reported in Table 5, however, all further discussion considered the results generated by the jackknife estimator.

To validate these DNA-based tiger abundance estimates, the results were compared with estimates derived from a photographic capture-recapture survey (Karanth et al., unpublished data) of the same tiger population but 15 weeks earlier (Table 6). These comparisons revealed that whereas DNA studies (using 5 loci) identified 26 unique individual tigers in the sampled population, the photographic survey had yielded 29 individuals. As with the genetic survey, the photographic capture survey results also supported the assumption of population closure (Table 4). Both models \( M_0 \) and \( M_h\text{-Jackknife} \), in program CAPTURE, had high model selection test scores (0.97 versus 0.94) for the photographic-capture data set. The average tiger abundance estimates from photographic capture-recapture surveys were \( \hat{N} (S\hat{E}[\hat{N}]) = 81 (27.7) \) and \( \hat{N} (S\hat{E}[\hat{N}]) = 66 (13.8) \) tigers, under the \( M_0 \) and \( M_h \) models, respectively (Table 6).
Discussion

Protocol for individual identification:

In comparison to other studies involving tiger individual identification using genetic data (Xu et al., 2005; Bhagavatula and Singh, 2006), a large panel of 30 microsatellite loci was used for initial assessment of variability/microsatellite suitability. This allowed selecting a good combination of loci that resulted in lower probability of identity for siblings. This is particularly relevant given that tigers have relatively low genetic diversity (Luo et al., 2004), making the individual identification more demanding than other species with higher genetic variation. Molecular screening of a large number of loci did not significantly improve our P_{ID} (0.005, with six loci (Bhagavatula and Singh, 2006) versus five loci in this study). However, the amplification success with the selected loci was high (< 85% for the Bandipur samples in this study compared to 60% in Bhagavatula and Singh, 2006) and allelic dropout was lower (between 0.5 and 1.5 %).

Under field conditions the ‘freshness’ and ‘quality’ of tiger faeces could vary greatly among samples. As a result, faeces from captive tigers might yield higher quantity and quality of DNA. Results from this study supported this hypothesis, as the DNA from captive faecal samples amplified more loci (13 versus 10) than the field test DNA samples, and amplification success was also higher on an average (100 versus 90%).

Using distinct captive and wild individuals for investigating P_{ID(sibs)} revealed that wild test samples were less polymorphic than captive individuals, possibly because of high outbreeding in zoo tigers. Polymorphism was highest for the Bandipur samples, possibly due to higher numbers of individuals (25 versus 10 and 13) from the Bandipur survey population.
The quality index measure suggested by Miquel et al. (2006) was initially proposed for pilot studies on genotype data quality/reliability, and generally allows better data quality management within a study and comparability across studies. This was probably the first genetics-based individual identification study for any elusive large carnivore, in which the quality index approach has been used for data validation.

*Modeling and Estimation of Abundance from Tiger Capture Data:*

This study targeted to use standard capture–recapture models (Otis et al., 1978; Williams et al., 2002; Amstrup et al., 2005) in which the influence of factors such as time, trap–response and individual heterogeneity on tiger capture probabilities could be assessed as opposed to the null model which assumes that these factors do not affect capture probabilities. Results revealed that various capture–recapture analytical approaches produced similar estimates even when five loci are used in genetic identifications. However, it could be noted that unlike with physical tagging or photographic captures, in the case of ‘genetic captures’, individual capture probabilities might additionally vary depending both on number of scats produced and the quality of DNA available in the scats collected non-invasively from different individuals (Broquet et al., 2007). As capture–recapture analyses can explicitly deal with potentially complex forms of individual heterogeneity (such as the Jackknife estimator under model M₉ in program CAPTURE), it might be more appropriate for analysis of genetic capture data from tigers. Analyses in this study also demonstrated the robustness (Otis et al., 1978) in the parameter estimates under the M₉-Jackknife model to small variations in recapture rates, when results were compared between photographic and genetic surveys using the same model (Tables 5 and 6).

Since the precision of individual identification is highly dependent on the number of loci used in comparisons (through their effect on P_{ID}), this study also
explored the effects of using different numbers of loci on tiger population estimates (Table 7). As expected, using very few loci (three loci) resulted in underestimates of population size due to low probability of identity for siblings resulting from ‘shadow effect’. Interestingly, using five versus seven loci did not result in very different population estimates, despite use of seven loci reducing the number of identified individuals to 25. These results are thus in contrast to that of Creel et al. (2003), which found that increasing number of loci for individual identification could result in artificially creating new individuals due to genotyping errors. Since the number of ‘recaptures’ is also reduced as the number of loci used for individual identification is increased, the estimated mean population size should not differ greatly, especially under the robust Mₖ-Jackknife model. These results demonstrated that five loci might be sufficient to accurately identify individual tigers in the context of abundance estimation.

Utility of genetic capture-recapture sampling for estimating tiger abundance:

Tiger abundance estimates derived from genetic capture-recapture sampling closely matched abundance estimates generated from photographic capture-recapture sampling, which is a standard method successfully used for estimating abundances in populations of tigers (Karanth and Nichols, 1998; Karanth et al., 2004; Wegge et al., 2004; Kawanishi and Sunquist, 2004; Simchareon et al., 2007) and other elusive carnivores (Henschel and Ray, 2003; Trolle and Kery, 2003; Silver et al., 2004). However, results from this study were in contrast to several other studies, where genetic capture–recapture yielded higher (Zhan et al., 2006; Creel et al., 2003; Fickel and Hohmann, 2006) or lower (Wilson et al., 2003) abundance estimates compared to more traditional survey methods. Further, it is important to point that photographic capture-recapture estimates of tiger abundance reported here have additionally been
validated against independent estimates based on density of principal ungulate prey and food intake rates of tigers (Karanth et al., 2004). Overall, carefully conducted non-invasive genetic-capture sampling leading to high degree of certainty in individual animal identifications appears to be a reliable approach for estimating abundances of tigers and other species. Furthermore, at a metapopulation level, for estimating long-distance dispersal movement, genetic sampling of individual tigers appears to be a potentially very attractive tool, because photographic capture surveys are difficult to conduct at regional spatial scales.

Genetic sampling can be used as a valuable additional tool to estimate tiger abundance in situations such as the Russian Far East, Sundarbans mangrove swamps and rainforests of Southeastern Asia, where densities are low and camera trapping is often impractical due to various environmental and logistical constraints. Even where camera trap methods work well, faecal genetic sampling may provide additional data on age–sex structure and relatedness among individuals, information useful for answering some scientific or management questions.

However, with faecal DNA capture methods there is some potential for a particular age–sex class being virtually undetectable on account of behavioral reasons. For example, if transient individual tigers very rarely defecate on regular travel routes to avoid detections by territorial residents, scat sampling may underestimate that segment of the population. However, various studies have employed trained dogs to increase detections rates of carnivore scats (Wasser et al., 2004; Nagata et al., 2005; Kerley and Salkina, 2006), thus offering an avenue to overcome such biases.

This study has used conventional capture–recapture models for analyses, particularly relying on the $M_k$-Jackknife estimator that performed well in earlier studies of tigers (Karanth et al., 2004). However, recent developments in spatial
capture–recapture models (Royle and Young, 2008; Borchers and Efford, 2008; Royle et al., 2009) could potentially offer a superior analytical framework for data analysis eventually when they are explicitly designed for scat detection surveys. Therefore, designing scat sampling surveys explicitly to meet capture–recapture modeling assumptions, particularly via the construction of sampling occasions that generate additional information on sampling effort is advantageous (Williams et al., 2002; Amstrup et al., 2005; Royle et al., 2009) over ad hoc surveys employed in many genetic sampling studies (Bhagavatula and Singh, 2006; Fickel and Hohmann, 2006). Greater integration with standard capture–recapture sampling approaches will allow non-invasive genetic methods to become an even more important tool in the future for research on tigers and other endangered species.

In the broader context of animal abundance estimation, genetic capture sampling provides an useful alternative wherever photographic sampling or conventional tagging studies cannot be employed for reasons such as lack of distinguishing natural marks on study species, logistical difficulties of physical captures, low-animal density, lack of skilled survey personnel or camera equipment, potential for camera theft/damage and sometimes even survey cost considerations. Genetic capture method may also be easier to apply in broader surveys that target multiple species, as opposed to camera-trap or physical tagging surveys, which are typically tailored specifically for one or two species at a time. The problem of evasive animal response to conventional traps or cameras by some elusive species can also be overcome with faecal DNA surveys.

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**Figure 1:** Study area showing the 18 sampling routes in Bandipur national park used for collecting tiger faecal samples

![Study Area with Sampling Routes](image1)

**Figure 2:** Study area showing the camera trap locations in Bandipur national park for the photographic capture-recapture survey

![Study Area with Camera Traps](image2)
Figure 3: A plot showing the effect of number of microsatellites on the probability of identity assuming all individuals are siblings $P_{ID(sibs)}$ for a set of captive (n=14), field test (n=10) and Bandipur samples (n=25). In this plot the number of loci are taken in the order of decreasing information contents. In other word, the locus having the highest power to discriminate among the individuals is the first one and the least one is in the end. The order of the loci (1-10) is as follows: FCA628, FCA205, FCA441, FCA672, FCA232, F41, FCA391, FCA453 and F115.
Table 1: Genetic variability at 30 microsatellites for 14 captive individual tigers (blood samples)

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele size range (bp)</th>
<th>Blood amplification success (For faeces)</th>
<th>T_a</th>
<th>Allelic dropout</th>
<th>False alleles</th>
<th>H_e</th>
<th>H_s</th>
<th>No. of alleles</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIGER6</td>
<td>221-243</td>
<td>100 (40)</td>
<td>59</td>
<td>0</td>
<td>0</td>
<td>0.82</td>
<td>0.79</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>6HDZ170</td>
<td>214-226</td>
<td>100 (52)</td>
<td>55</td>
<td>0</td>
<td>0</td>
<td>0.78</td>
<td>0.5</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>FCA391</td>
<td>197-217</td>
<td>100 (100)</td>
<td>56</td>
<td>0</td>
<td>0</td>
<td>0.77</td>
<td>0.71</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>FCA672</td>
<td>94-112</td>
<td>100 (100)</td>
<td>56</td>
<td>0</td>
<td>0</td>
<td>0.73</td>
<td>0.93</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>F42</td>
<td>206-230</td>
<td>100 (32)</td>
<td>56</td>
<td>0</td>
<td>0</td>
<td>0.73</td>
<td>0.5</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>FCA205</td>
<td>102-116</td>
<td>100 (100)</td>
<td>56</td>
<td>0</td>
<td>0</td>
<td>0.69</td>
<td>0.93</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>F115</td>
<td>188-204</td>
<td>100 (100)</td>
<td>56</td>
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<td>0</td>
<td>0.69</td>
<td>0.79</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>6HDZ2007</td>
<td>227-241</td>
<td>100 (65)</td>
<td>60</td>
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<td>0</td>
<td>0.68</td>
<td>0.36</td>
<td>4</td>
<td>2</td>
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<td>FCA441</td>
<td>146-174</td>
<td>100 (100)</td>
<td>51</td>
<td>0</td>
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<td>0.64</td>
<td>0.64</td>
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<td>3</td>
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<td>TIGER22</td>
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<td>0.64</td>
<td>4</td>
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<tr>
<td>FCA126</td>
<td>126-152</td>
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<td>56</td>
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<td>0.61</td>
<td>0.71</td>
<td>4</td>
<td>3</td>
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<tr>
<td>FCA232</td>
<td>100-108</td>
<td>100 (100)</td>
<td>56</td>
<td>0</td>
<td>0</td>
<td>0.61</td>
<td>0.93</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>F41</td>
<td>194-202</td>
<td>100 (100)</td>
<td>56</td>
<td>0</td>
<td>0</td>
<td>0.58</td>
<td>0.43</td>
<td>3</td>
<td>3</td>
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<tr>
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<td>56</td>
<td>0</td>
<td>0</td>
<td>0.59</td>
<td>0.79</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>FCA506</td>
<td>206-214</td>
<td>100 (42)</td>
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<td>0</td>
<td>0</td>
<td>0.57</td>
<td>0.5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>FCA230</td>
<td>101-107</td>
<td>100 (100)</td>
<td>52</td>
<td>0</td>
<td>0</td>
<td>0.57</td>
<td>0.64</td>
<td>3</td>
<td>3</td>
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<tr>
<td>TIGER23</td>
<td>262-314</td>
<td>100 (17)</td>
<td>55</td>
<td>0</td>
<td>0</td>
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<td>0.21</td>
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<td>1</td>
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<tr>
<td>FCA628</td>
<td>090-096</td>
<td>100 (100)</td>
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<td>0</td>
<td>0.52</td>
<td>0.57</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>FCA453</td>
<td>156-176</td>
<td>100 (100)</td>
<td>59</td>
<td>0</td>
<td>0</td>
<td>0.49</td>
<td>0.29</td>
<td>4</td>
<td>3</td>
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<tr>
<td>FCA164</td>
<td>74-84</td>
<td>100 (100)</td>
<td>59</td>
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<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>FCA279</td>
<td>98-100</td>
<td>100 (100)</td>
<td>52</td>
<td>0</td>
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<td>0.5</td>
<td>1</td>
<td>2</td>
<td>3</td>
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<tr>
<td>FCA001</td>
<td>136-140</td>
<td>100 (100)</td>
<td>54</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td>0.5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>FCA304</td>
<td>120-138</td>
<td>100 (100)</td>
<td>56</td>
<td>0</td>
<td>0</td>
<td>0.46</td>
<td>0.14</td>
<td>4</td>
<td>3</td>
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<tr>
<td>FAC052</td>
<td>108-114</td>
<td>100 (100)</td>
<td>55</td>
<td>0</td>
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<td>0.46</td>
<td>0.71</td>
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<td>3</td>
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<td>E21B</td>
<td>159-167</td>
<td>100 (78)</td>
<td>55</td>
<td>0</td>
<td>0</td>
<td>0.35</td>
<td>0.36</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>6HDZ056</td>
<td>174-176</td>
<td>100 (67)</td>
<td>56</td>
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<td>0</td>
<td>0.34</td>
<td>0</td>
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<td>2</td>
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<tr>
<td>TIGER24</td>
<td>176-178</td>
<td>100 (90)</td>
<td>51</td>
<td>0</td>
<td>0</td>
<td>0.24</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>FCA069</td>
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<td>0.21</td>
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<td>FCA090</td>
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<td>100 (92)</td>
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<td>0</td>
<td>0.14</td>
<td>0.14</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>FCA170</td>
<td>76</td>
<td>100 (77)</td>
<td>55</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

*Loci amplified from faecal samples of captive individuals

1Zhang et al. 2005
2Williamson et al. 2002
3Menotti-Raymond et al. 1999
4Bhagvatula and Singh, 2006
**Table 2:** Genetic variability at 10 microsatellites for ten field test samples (Faecal samples) of wild tigers from Bandipur National Park and Nagarahole National Park

<table>
<thead>
<tr>
<th>Locus</th>
<th>Product size range (bp)</th>
<th>Amplification success (%)</th>
<th>Allelic dropout (%)</th>
<th>False alleles</th>
<th>He</th>
<th>Ho</th>
<th>No. of alleles</th>
<th>Multiplex sets for PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCA453</td>
<td>192-200</td>
<td>71</td>
<td>0</td>
<td>0</td>
<td>0.62</td>
<td>0.14</td>
<td>3</td>
<td>Singleplex</td>
</tr>
<tr>
<td>FCA391</td>
<td>205-217</td>
<td>71</td>
<td>0.035</td>
<td>0</td>
<td>0.54</td>
<td>0.29</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>FCA628</td>
<td>86-108</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0.64</td>
<td>0.71</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>FCA205</td>
<td>100-108</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0.78</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>FCA126</td>
<td>140-146</td>
<td>86</td>
<td>0</td>
<td>0</td>
<td>0.64</td>
<td>0.86</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>F41</td>
<td>112-124</td>
<td>100</td>
<td>0.035</td>
<td>0</td>
<td>0.36</td>
<td>0.29</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>FCA232</td>
<td>98-106</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0.44</td>
<td>0.43</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>FCA441</td>
<td>146-158</td>
<td>71</td>
<td>0</td>
<td>0</td>
<td>0.18</td>
<td>0.14</td>
<td>2</td>
<td>Set 3</td>
</tr>
<tr>
<td>FCA672</td>
<td>100-102</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0.46</td>
<td>0.71</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>F115</td>
<td>192-196</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0.49</td>
<td>0.29</td>
<td>2</td>
<td>Set 4</td>
</tr>
</tbody>
</table>

**Table 3:** Genetic variability at 10 microsatellites for 50 wild samples (Faeces) from Bandipur National Park

<table>
<thead>
<tr>
<th>Locus</th>
<th>Product size range (bp)</th>
<th>Amplification success (%)</th>
<th>Allelic dropout (%)</th>
<th>False alleles</th>
<th>He</th>
<th>Ho</th>
<th>No. of alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCA453</td>
<td>180-200</td>
<td>74</td>
<td>0</td>
<td>0</td>
<td>0.74</td>
<td>0.19</td>
<td>5</td>
</tr>
<tr>
<td>FCA391</td>
<td>189-217</td>
<td>78</td>
<td>0.017</td>
<td>0</td>
<td>0.68</td>
<td>0.4</td>
<td>7</td>
</tr>
<tr>
<td>FCA628</td>
<td>86-110</td>
<td>96</td>
<td>0.007</td>
<td>0</td>
<td>0.88</td>
<td>0.7</td>
<td>12</td>
</tr>
<tr>
<td>FCA205</td>
<td>94-112</td>
<td>100</td>
<td>0.013</td>
<td>0</td>
<td>0.85</td>
<td>0.94</td>
<td>9</td>
</tr>
<tr>
<td>FCA126</td>
<td>132-154</td>
<td>96</td>
<td>0.015</td>
<td>0</td>
<td>0.79</td>
<td>0.91</td>
<td>11</td>
</tr>
<tr>
<td>F41</td>
<td>112-144</td>
<td>85</td>
<td>0</td>
<td>0</td>
<td>0.74</td>
<td>0.4</td>
<td>8</td>
</tr>
<tr>
<td>FCA232</td>
<td>96-124</td>
<td>96</td>
<td>0</td>
<td>0</td>
<td>0.77</td>
<td>0.6</td>
<td>11</td>
</tr>
<tr>
<td>FCA441</td>
<td>138-166</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0.78</td>
<td>0.53</td>
<td>8</td>
</tr>
<tr>
<td>FCA672</td>
<td>082-104</td>
<td>96</td>
<td>0.007</td>
<td>0</td>
<td>0.76</td>
<td>0.81</td>
<td>9</td>
</tr>
<tr>
<td>F115</td>
<td>192-212</td>
<td>85</td>
<td>0.008</td>
<td>0</td>
<td>0.66</td>
<td>0.45</td>
<td>6</td>
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</tbody>
</table>

**Table 4:** Results of the Otis et al. (1978) and Stanley and Burnham (1999) tests for demographic closure

<table>
<thead>
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<th>Dataset</th>
<th>Program CAPTURE</th>
<th>Program CloseTest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Z-statistic</td>
<td>P-value</td>
</tr>
<tr>
<td>Genetic captures: 3 loci</td>
<td>1.19</td>
<td>0.88</td>
</tr>
<tr>
<td>Genetic captures: 5 loci</td>
<td>1.39</td>
<td>0.92</td>
</tr>
<tr>
<td>Genetic captures: 7 loci</td>
<td>0.97</td>
<td>0.83</td>
</tr>
<tr>
<td>Photographic captures</td>
<td>-1.22</td>
<td>0.11</td>
</tr>
</tbody>
</table>

1 Based on Otis et al. (1978)

2 Based on Stanley and Burnham (1999)
Table 5: Abundance estimation of tigers in Bandipur National Park by capture–recapture sampling using DNA collected from faeces using 5 microsatellite loci for individual identification. Models from programs CAPTURE and MARK.

<table>
<thead>
<tr>
<th>Individuals caught $M_{t+1}$</th>
<th>Total captures $n.$</th>
<th>CAPTURE models</th>
<th>MARK models</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parameter</td>
<td>$M_0$</td>
<td>$M_0$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$M_h$-Jackknife</td>
<td>$M_h$-Jackknife</td>
</tr>
<tr>
<td>26</td>
<td>Average per sample capture probability $\hat{p}$</td>
<td>0.13</td>
<td>0.09</td>
</tr>
<tr>
<td>29</td>
<td>Total abundance, $\hat{N}(S\hat{E}[\hat{N}])$</td>
<td>47 (11.21)</td>
<td>66 (12.98)</td>
</tr>
</tbody>
</table>

Table 6: Abundance estimation of tigers in Bandipur National Park by photographic capture–recapture sampling. Models from programs CAPTURE and MARK.

<table>
<thead>
<tr>
<th>Individuals caught $M_{t+1}$</th>
<th>Total captures $n.$</th>
<th>CAPTURE models</th>
<th>MARK models</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parameter</td>
<td>$M_0$</td>
<td>$M_0$</td>
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<tr>
<td></td>
<td></td>
<td>$M_h$-Jackknife</td>
<td>$M_h$-Jackknife</td>
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<tr>
<td>29</td>
<td>Average per sample capture probability $\hat{p}$</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>26</td>
<td>Total abundance, $\hat{N}(S\hat{E}[\hat{N}])$</td>
<td>81 (27.7)</td>
<td>66 (13.81)</td>
</tr>
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</table>
Table 7: Abundance estimation of tigers in Bandipur National Park by capture-recapture sampling using DNA collected from faeces using 3 and 7 microsatellite loci for individual identification. Models from programs CAPTURE and MARK.

<table>
<thead>
<tr>
<th>No. of loci</th>
<th>Individuals caught $M_{i,i}$</th>
<th>Total captures $n.$</th>
<th>Parameter</th>
<th>$M_0$</th>
<th>$M_{h}$ - Jackknife</th>
<th>$M_0$</th>
<th>$M_{h}$-2 point mixture</th>
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</thead>
<tbody>
<tr>
<td>3</td>
<td>29</td>
<td>40</td>
<td>Average per sample capture probability $\hat{p}$</td>
<td>0.13</td>
<td>0.11</td>
<td>0.13</td>
<td>0.46_{Group 1} 0.09_{Group 2}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total abundance, $\hat{N}(\hat{S}\hat{E}[\hat{N}])$</td>
<td>49 (10.49)</td>
<td>62 (11.76)</td>
<td>49.44 (10.65)</td>
<td>61.93 (24.03)</td>
</tr>
<tr>
<td>7</td>
<td>25</td>
<td>31</td>
<td>Average per sample capture probability $\hat{p}$</td>
<td>0.09</td>
<td>0.08</td>
<td>0.090</td>
<td>0.55_{Group 1} 0.09_{Group 2}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total abundance, $\hat{N}(\hat{S}\hat{E}[\hat{N}])$</td>
<td>57 (18.36)</td>
<td>66 (13.04)</td>
<td>57 (18.74)</td>
<td>57 (18.74)</td>
</tr>
</tbody>
</table>