

Revised estimation of abundance for breeding stock C3 of humpback whales, assessed through photographic and genotypic mark-recapture data from Antongil Bay, Madagascar, 2000-2006.

SALVATORE CERCHIO^{1,2,3}, PETE ERSTS^{1,5}, CRISTINA POMILLA^{1,2,4}, JACQUELINE LOO^{1,8}, YVETTE RAZAFINDRAKOTO^{1,6}, MATT LESLIE^{1,2}, NORBERT ANDRIANRIVelo^{1,6}, GIANNA MINDON^{7,8}, JENNIFER DUSHANE^{1,9}, ANITA MURRAY^{1,9}, TIM COLLINS^{1,7} AND HOWARD ROSENBAUM^{1,2}

¹WCS and AMNH Cetacean Conservation and Research Program, c/o Marine Conservation, Wildlife Conservation Society, 2300 Southern Blvd., Bronx, NY 10460-1099, USA.

²Center for Biodiversity and Conservation and Conservation Genetics Program, American Museum of Natural History, 79th Street and Central Park West, New York, NY 10024, USA.

³Evolutionary Biology Program and Bioacoustics Research Program, Cornell Laboratory of Ornithology 159 Sapsucker Woods Rd. Ithaca, NY 14850, USA.

⁴Department of Biology, New York University, 1009 Silver Center, 100 Washington Square East, New York, NY 10003-6688, USA.

⁵Center for Biodiversity and Conservation, American Museum of Natural History, 79th Street and Central Park West, New York, NY 10024, USA.

⁶Madagascar Country Program, Wildlife Conservation Society, B.P. 8500, Antananarivo, Madagascar.

⁷Environment Society of Oman P.O. Box. 3955, P.C. 112, Ruwi Sultanate of Oman

⁸University of Malaysia Sarawak, Piasau 70, Miri 98009, Sarawak

⁹Columbia University, Department of Conservation Biology, New York, NY, USA.

INTRODUCTION

Humpback whales (*Megaptera novaeangliae*) in the southern hemisphere are distributed in circumpolar high latitudes during the austral summer and migrate to discrete or semi-discrete low latitude breeding areas in the austral winter. Population structure and status in the breeding areas is currently the focus of ongoing research (Pomilla 2005, Pomilla et al. 2006, Rosenbaum et al. 2006) as is the relationship of specific breeding regions and feeding areas. The International Whaling Commission (IWC) currently designates seven breeding stocks (populations) labeled A through G, ranging from the western South Atlantic eastward to the eastern South Pacific. The breeding population that winters in the western Indian Ocean is considered Breeding Stock C, and is distributed primarily from the eastern coastal waters of South Africa to Kenya, off the islands of the Mozambique Channel, and the coastal waters of Madagascar.

Best et al. (1998) proposed three potential subpopulations and migratory corridors of humpback whales in the western Indian Ocean, based upon historical whaling records, land based observations of migrations, and shipboard surveys. IWC delineation of Breeding Stock C is consequently divided into three sub-regions: C1, wintering off the east coast of South Africa to Mozambique; C2, a group that potentially migrates up the Mozambique Channel to winter grounds in the Comoros Islands; and C3, wintering in the coastal waters of Madagascar (Best et al. 1997, 1998, Rosenbaum et al. 1997). The C3 sub-region has been investigated primarily in the semi-protected waters of Antongil Bay in the northeast of Madagascar (Rosenbaum et al. 1997; Rosenbaum 2003). Here we present an analysis and assessment of population abundance for the Madagascar breeding assemblage of humpback whales using individual identification photographs and microsatellite multi-locus genotypes. We discuss potential biases affecting these estimates, particularly related to migratory behavior and timing of individual whales, as well as their geographic or population level significance.

METHODS

Data used in this study were collected on the breeding area of Antongil Bay, Madagascar (Fig. 1) during the austral winters of 2000 through 2006. Antongil Bay, in the northeastern corner of Madagascar, is a shallow, semi-protected bay that extends approximately 60km northward from the mouth of the bay and is on average approximately 30km in width. Humpback whales can be observed in Antongil generally from June to October with the highest concentrations occurring in July through early September (Rosenbaum et al. 1997). Behaviors widely accepted to indicate breeding activity are regularly observed in Antongil Bay, as are females with young calves, and thus the bay is considered a breeding area for the western Indian Ocean population (Rosenbaum et al. 1997). The degree to which the bay represents an endpoint “destination” for migratory whales with high residency, versus a “stopover” point with relatively transient residency is as yet undetermined.

Individual identification photographs and skin samples for genetic identifications used in this analysis were collected from 2000 to 2006. Effort was relatively consistent each year from July to September (Table 1) with the exception of 2002, which was an anomalously short season due to political upheaval in Madagascar. Standard procedures were used for identification photography using primarily Nikon D1 digital cameras. Photographs were collected of both sides of the dorsal fin as well as the ventral tail flukes whenever possible, however recapture analysis of only tail flukes are reported here. Skin samples were collected using biopsy dart procedure (Lambertsen 1987) or, when available, as sloughed skin, and stored in 95% EtOH until processed.

Photographic comparison procedure. Whenever possible a single photograph was chosen to represent the flukes of an individual for a single day. Photographs were first compared within each year to establish within-year sample size of individuals and within-year recaptures. Between-year comparisons were then conducted starting with the first two years and sequentially comparing each subsequent year to the reconciled catalogue of all previous years. All photographs used in the comparison were rated for quality in three separate categories: *photographic*, which included focus, exposure, contrast and pixilation of digital images; *orientation*, which included angle of the flukes in the horizontal and vertical planes, amount of the flukes above water, and obstruction by splash; and *distinctiveness*, which was an intrinsic characteristic of the fluke involving the uniqueness of the pattern and degree of scarring (although this was inevitably influenced by photographic and orientation quality). Quality was rated on a five-level scale: excellent, good, fair, poor, and not useable. Flukes were also rated on the proportion of the fluke that was showing above the water plane as whole, left fluke only, right fluke only, trailing edge or leading edge. By defined protocol the latter four categories (essentially partial flukes) could only receive a fair, poor or not useable quality rating in orientation. Flukes of all qualities were compared and used for assessing recapture rates of individuals within season and temporal characteristics of individual captures. In mark-recapture statistical procedures for the estimation of population abundance, we used only flukes with quality of fair or better in photographic and orientation categories. Photographs of only the right or left fluke were also eliminated from the sample since they cannot be compared to each other.

Genotypic comparison procedure. The genetic capture-recapture approach is based on the resolution of unique genetic profiles to permit unambiguous identification of individuals (Palsbøll et al. 1997). Total genomic DNA was extracted from the epidermal layer of biopsies or sloughed skin, using standard Phenol/Chloroform extraction method or using DNAeasy tissue kit (Qiagen). The samples were genotyped using 10 cetacean microsatellite markers selected from literature (Pomilla 2005). A detailed description of molecular methodology, quality control protocols and statistical analyses of genetic variation can be found in Pomilla & Rosenbaum (2006).

The average probability of different random individuals in the population sharing the same genotype by chance (Probability of Identity, PI) was estimated to evaluate the reliability of the genetic tagging based

on the number of loci used. Duplicate samples were detected from genotype identity using the Microsoft Excel add-in GENALEX package version 5.1 (Peakall and Smouse, 2001). Additionally, for all samples with matching genotypes that represented putative recaptures between years, genotype probability (GP) was generated separately for the specific genotype. The genotype probability estimates the probability of a random match to a given specific genotype in the given population. PI and GP were estimated using the Microsoft Excel add-in mentioned above.

Abundance estimation procedures. Abundance estimates were generated using several combinations of sample years and estimation models. All models used assumed a closed population; due to sparse recaptures our data was not appropriate for application of open models, attempts to use such resulting in nonsensical results. Pair-wise estimates were generated using the Chapman's modified Petersen model (Begon 1979, Hammond 1986) and each consecutive pair of sample years. The Program MARK (White & Burnham 1999) was used to generate estimates from multiple years of data applying closed capture likelihood models. Program MARK allows the construction of models that relax the assumption of equal probability of capture in several manners. For each dataset, several models were run, including the null model (M_0), variation of capture probability with sampling occasion (time, M_t), individual (heterogeneity, M_h), and time in combination with individual (M_{th}). Selection of the most parsimonious model was done within MARK using information theory and comparison of Akaike's Information Criteria (AIC) values (Burnham & Anderson 2002). Models that vary probability of capture and recapture periods (behavior, M_b) were not included as these models uniformly provided nonsensical results and were a poor fit to the data. MARK models heterogeneity as a mixture of capture probabilities for each individual, and estimates the probability of capture, p_x , for each mixture and the mixture proportion, pi_x . In this analysis the number of mixtures was limited to two, in order to minimize the number of parameters in the model; very often the heterogeneity model produced nonsensical estimates for one or more of the parameters, irrespective of its rank in the AIC assessment.

RESULTS

Photographic recaptures. Within-year sample size of captured individuals varied for tail flukes photographs (all qualities better than "not useable") from a low of 24 in 2002 to a high of 184 in 2001. The distribution of photographic identifications varied by date across each season (Fig. 2). Periods of few or no collected identifications resulted primarily from poor weather, however was also influenced by variation in the density of whales. The sample of identifications is particularly small in 2002 due to a limited season of 20 days during which photographs were collected on 12 days. Season duration in all other years ranged from 52 to 66 days with photograph effort ranging from 28 to 37 days (Table 1). Within-year recapture rate ranged from 6% to 18% of individuals captured on more than one day. Recaptured individuals had short "residency" intervals between first and last capture with a mean ranging from 3 to 8 days and median values of 2 days for all years except 2002, the anomalous sampling year.

A total of 33 individuals were captured in multiple years, accounting for 44 pair-wise recapture events when using data of all qualities. Between 2000 and 2004 (2005 and 2006 data yet to be examined), yearly timing of first capture day for recaptured individuals displayed remarkable consistency, with the majority of recaptured individuals being seen on similar dates in different years (Table 2, Fig. 3). In 21 cases, 16 (76%) were recaptured within 10 Julian days of the date of their initial year's capture, 13 (62%) within 5 days, and 7 (33%) within 2 days. To assess the probability of these data in a random distribution, a simple permutation routine was written. For each year, the first date of capture for each individual was randomly permuted among individuals captured that year, and the mean difference in Julian days between recapture dates was calculated for all pair-wise events. The use of the actual data structure (i.e., capture dates) controls for variation and inconsistencies in data collection effort between years. After 10,000 iterations a random distribution was constructed of both the actual individual pair-wise differences in Julian day of capture (Fig. 3), and the mean difference in Julian days across the 21 recapture events.

The random mean was 23.26 Julian days (s.d. 15.98). The observed mean of 6.76 Julian days (s.d. 6.64) had a $p < 0.0001$ in the randomly generated distribution (e.g., in 10,000 iterations, there were no runs that had a mean equal to or less than the observed mean).

After eliminating poor quality and partial flukes for use in capture-recapture parameter estimation, the sample of individuals was reduced to a low of 16 individuals in 2002, and between 89 and 159 individuals for the remaining years (Table 3). Recaptures between pairs of years were sparse, ranging from 0 to a maximum of 4. In 2006 a total of 9 of 158 individuals (5.7%) had been seen in any previous year. Of 812 individuals identified across the five years, 28 (3.4%) were captured in more than one year, with one individual captured in a maximum of four different years, and three individuals captured in three different years. Limiting recapture data by quality resulted in the elimination of four recaptures of poor quality or partial flukes.

Genetic recaptures. A total of 1126 biopsies collected between 2000 and 2006 in Antongil Bay were analyzed. Yearly sample size ranged from 35 to 208 during 2000-2006 (Table 4). Based on genotype identity ($PI=5.6 \times 10^{-12}$), the samples were assigned to 922 unique individuals. Thirty-nine individuals (4.2%) were encountered in multiple years for a total of 47 recaptures between pairs of years. For individual pairs of samples with matching genotypes, GP ranged from 7.0×10^{-17} to 2×10^{-9} , therefore there is strong support for the assumption that the samples came from the same individuals. The yearly sample sizes of individuals ranged from 28 to 185 with a resample rate of 11.1% to 20.8% (Table 4) and the number of recaptures between each pair of years ranged from 0 to 6 (Table 5). Cross checking of genotypic recaptures against tail flukes photographs revealed three missed photographic matches, or false negatives in the photographic dataset; these were corrected for computation of the capture-recapture estimates using the photographic data.

Abundance estimates. Considering first estimates derived from flukes photographs, pair-wise Chapman's estimates from consecutive years ranged from 539 (CV=0.39) for years 2002-2003, to 6434 (CV=0.49) for years 2003-2004 (Table 6a). Both estimates involving 2002 were anomalously small, and considered unreliable due to the small sample size of 2002 photographs, as well as biased due to timing characteristics of individual recaptures (see discussion below). There was no apparent increasing trend in the magnitude of the point estimates, with the estimates for years 2005 and 2006 being smaller than those for 2003 and 2004 (the highest as noted above). CV's were generally large, close to 0.4 or higher, and thus confidence intervals were large and broadly overlapping.

Estimates using genotypic recaptures from 2000-2006 were predominantly larger than estimates using flukes identification photographs from the same years, presumably due to larger sample sizes without commensurate increase in recaptures (Table 6b). Pair-wise Chapman's estimates using consecutive years yielded estimates ranging from 770 (CV=0.30) for years 2002-2003, to 10167 (CV=0.49) for years 2003-2004. As with photographs, these estimates were inconsistent with no apparent trend and high CV's (Table 6b).

The poorly represented 2002 sample year was removed for multiple sample closed model estimation. For all but one dataset, the AIC selection procedure recommended either Model M_0 or M_i , and in all cases these abundance estimates were very similar, within 100 (see Appendix I). Using the span of 7 years from 2000-2006 generated the highest estimates (Table 7a); these are highly likely to be positively biased, possibly severely so, due to violations of the assumption of population closure due to births and deaths over the extended period. Using 4 years of data from 2003-2006 produced a smaller estimate (7715, CV=0.24) from photo-IDs, but the genotypic estimate was comparable with that generated with 2000-2006 data; again the larger genotypic estimates are a result of larger sample sizes without an increase in recaptures. Since these models assume closure, and therefore no change in population size, the estimates

should be considered for the final year, 2006, although over a such a long span of years are likely to be positively biased.

Breaking the sample down into two non-overlapping, temporally consecutive spans of years, the same models were run for 2000-2003 and 2004-2006 data (Table 7b). This resulted in estimates for sample years 2003 and 2006, respectively, each using 3 years of data, which should consequently be less biased by closure assumption violation than the estimates above from longer expanses of years. The 2006 estimate from 2004-2006 photographic data was the only one in which the AIC procedure recommended M_h as the most parsimonious model (Appendix I); however the results were nonsensical with a abundance estimate of 64050, and a mixture proportion of $p_i = 0.0006$, so model M_0 was used. Consequently, almost all runs with M_h resulted in similarly low estimates of p_i , thus calling into question the appropriateness of this model with our data. The 2006 estimates of both data types are smaller than the 4 year estimates derived from 2003-2006 (Table 7b), suggesting that closure violations may be inflating the 4 year closed population estimate. Conversely, the 4 year estimate may reflect better sampling of a temporally heterogeneous population. The 2003 estimates are similar between the two data types, 5612 for photos and 5807 for genotypes, whereas the 2006 genotype estimate, 8348, is substantially larger than that for the photos, 6737 (Table 7b). There is a clear increasing trend in both data types that allows the estimation of a preliminary annual rate of increase. The ROI value of 0.063 estimated from the Photo-ID data is reasonable, whereas the ROI value of 0.136 for the genetic data is likely improbable. The improbable ROI for genetic data and the points mentioned previously brings into question the validity of the higher estimates generated by the genotypic data, and demands further scrutiny.

DISCUSSION

There are several considerations to keep in mind when evaluating these abundance estimates. The primary concerns effecting accuracy are heterogeneity of capture probability introduced by the consistent timing of capture of individuals, the small sample size relative to population size (low probability of capture), and the potential for positive bias due to using closed capture models. In addition the photographic and genetic datasets gave very different estimates for some sets of years, which must be evaluated. We must also consider what region these estimates represent, or rather, the geographic bounds of the population that is likely to be frequenting Antongil Bay.

Heterogeneity, Closure and Reliability of Estimates

An important aspect of this dataset is the strong consistency observed in Julian date recaptures of individuals between years. This has significant ramifications for sampling design on the resultant estimate bias. On one extreme, sampling during the same short period each season would introduce heterogeneity and negative bias, since those animals that have a tendency to arrive during the period of sampling would have a much higher probability of capture than others. Conversely, sampling during short periods at different non-overlapping times of the season could result in significant positive bias due to sampling essentially different sub-populations. It is therefore clearly important to sample throughout the season and during the same periods each year, or otherwise there is a high risk of sampling different portions of the population moving through the sampling site at different times. This clearly has important implications for both mark-recapture as well as genetic studies looking at population structure across broad regions.

In our study, this issue calls into question the use of yearly samples that are not completely concurrent, such as the highly abbreviated 2002 photographic sample. The anomalous estimates that are derived from these samples when using them in pair-wise Chapman's estimate corroborate this conclusion, as does the observation that when the 2002 photographic data is removed, the respective estimates increase. To further explore this effect, we generated a set of estimates using photographic data that was constrained to the brief period of sampling in 2002 between Julian days 230 and 255 using the Chapman's Modified

Petersen (Table 8). Estimates were in all cases lower than those for the same models and years when using all data, indicating significant heterogeneity when restricting the data to the truncated sample period. In the most extreme case, the pair-wise estimate for 2000-2001 was nearly tenfold smaller. For this reason we believe the most reliable estimates are those that exclude the 2002 sampling year.

Due to the relatively small yearly sample sizes for the apparent size of this population (i.e., low capture probabilities), the observed number of recaptures were small and resulted in poor precision. This is particularly true with the pair-wise Chapman's estimates, with CV's ranging from ca 0.30 to 0.49, rendering them of questionable value. Even the best precision achieved through multiple year estimates, ranging from a CV of 0.17 to 0.34, requires caution when applying these estimates to population assessment and management decisions.

Using multiple year data increases precision as seen in the improved CV's. Using multiple years will also have the effect of "smoothing" out variation in composition of individuals from year to year. This may act to counteract the heterogeneity introduced by the observed periodicity of recaptured individuals. By including more years of data, there may be a greater representation of the entire population; this is a potential explanation for the estimates increasing with when adding additional years (e.g., the 2004-2006 estimates compared to the larger 2003-2006 estimates). However, the use of multiple years of data in closed models can introduce a potentially significant positive bias due to violation of the assumption of closure, a result of recruitment and death (Hammond 1986). Therefore the estimates generated by 2003-2006 data models, may represent overestimates due to closure violations relative to the 2004-2005 data estimates. The largest estimates were generated by longest term data set, 2000-2006, and thus is highly suspect for potential violation of closure.

Photographic vs. Genetic data

It is difficult to explain the discrepancy between the photographic and genotypic estimates, particularly that the discrepancy flips direction after 2003. For the earlier pair-wise estimates, 2000-2001 and 2001-2003, the genetic estimates are smaller than the photographic estimates, and this was similarly noted with data from 1996 to 1999 (Cerchio et al. 2006) not presented here. Conversely after 2003, starting with 2003-2004 the genetic estimates are substantially larger than the photographic estimates. Similarly in the MARK models, the runs using 2004-2006 genetic data are substantially larger than those using photographic data, whereas the 2000-2003 estimates are very similar. This is clearly due to an increase in the sample size of the genetic data with no commensurate increase in recaptures (e.g., for 2003-2004 data both data types have 2 recaptures, and for 2004-2005 data, there are 4 photographic recaptures whereas that are actually fewer, 2, genetic recaptures, despite the larger genetic samples). There are three potential explanations for the observed discrepancies:

1. Within the genotypic sample there are errors in genotyping which result in "false negatives" or the same individual sampled twice but assigned different genotypes. Genotyping error is a typical characteristic of large samples of individuals typed for many microsatellite loci, and it is important to assess error and resultant effect on parameter estimation (Waits and Leberg 2000). If false negatives occurred between years the result would be that some recaptures are missed and m is under represented. In a dataset such as this with a small probability of capture and sparse recaptures, missing even 1 or 2 recaptures will have substantial positive bias on the abundance estimate. If it occurred within a year, it would result in the inflation of the sample size for the year, also resulting in a positive bias on abundance estimation. Waits and Leberg (2000) used simulations to assess potential magnitude of bias, and concluded that a positive bias could be >200% when using 7-10 loci with an average error of 0.05/locus. This drops substantially (e.g., <20%) when error is decreased to 0.01 or 0.005/locus. As our genetic data from 2002 to 2006 was generated very recently, we have not yet been able to properly estimate error in the entire dataset, and thus we can not assess this effect for the current report. That the direction of the discrepancy between genetic and photographic data flips after 2003 suggests that the 2002-2006 analysis

requires further scrutiny. This will include error checking of the genotypic data and cross-checking with photographic data associated with the biopsies of recaptured individuals, in order to accurately estimate genotype error and potentially model the effect for our specific sample of loci and individuals. An initial error check was completed for SC60, but raised the questions above. Following the *Implementation Review* of BCB bowheads as it related to stock structure (Annex F, JCRM 2008), a thorough error check is needed for the genotypic data before they should be reliably used for stock structure and abundance estimates in completing a Breeding Stock C Assessment.

2. There is “trap response effect” in the genotypic captures, introduced by some portion of individuals in the population successfully avoiding biopsy after being biopsied once (specifically in response to being biopsied). This would result in different capture and recapture probabilities, and thus systematically reduce the number of recaptures (particularly relative to photographic data) introducing a positive bias into abundance estimation for the genetic data.

3. There is a negative bias in the photographic estimates introduced by a source of heterogeneity to which the genetic sampling is not subject. A potential such source could be fluking up behavior; if there is an individually specific and consistent tendency to either always or rarely lift the flukes out of the water (resulting in heterogeneity of capture probability), then flukes photograph identification would preferentially capture high rate “flukers”, and negatively bias the estimates. Importantly, individual fluke-up probability would have to be consistent for all encounters; if an individual’s probability to fluke-up varied with occasion (particularly randomly), then there should be no such bias. Note that for both explanations (2) and (3) we would expect that this effect be consistent across years, and thus the observation that the discrepancy between photographic and genetic estimates flips direction after 2003 argues against these effects as contributing substantial bias.

RECOMMENDATIONS

Given these caveats, for the purposes of the comprehensive assessment we recommend the following. The large genetic estimates should be approached with caution until explanation (1), regarding genotyping error can be assessed. We thus do not recommend the use of these estimates. We suggest use of three estimates for current abundance in 2006, bracketing a lower-bound, mid range or “best” estimate, and an upper-bound. As a lower bound estimate, we recommend the photographic Chapman’s 2005-2006 estimate of 4610, CV=0.39, because this estimate should be the least effected by closure assumption violations and is most conservative. We recognize that this estimate is potentially negatively biased due to representing only 2 years of data. As a mid-range, or “best” estimate, we recommend the Closed Capture M_t estimate from 2004-2006 photographic data of 6737, CV=0.31, because this model incorporates more data and therefore is likely more representative of the population, while keeping closure violation at a minimum. As an upper bound estimate, we choose the 4-year estimate of 2003-2007 photographic data of 7715, CV=0.24, with the understanding that with four years of data in a closed model there could be substantial positive bias.

Geographic implications

Lastly, we must consider the question of exactly what group of whales we are estimating the abundance for. These samples were collected in one restricted area, Antongil Bay, within the C3 breeding sub-region off Madagascar. There are several levels in which we need to evaluate what this sample represents: Antongil Bay relative to sub-region C3; sub-region C3 relative to all of region C; and region C relative to other breeding regions, particularly B and D.

We have little data from other areas around Madagascar, therefore attributing these estimates to C3 in general makes an assumption regarding mixing of animals around Madagascar. There are observations and reports of concentrations of humpback whales further south on the east coast (e.g., Ile St. Marie, Ft. Dauphin) as well as along the west coast from Toliara north to Nosy Be (Best et al. 1997, Cerchio,

Razafindrakoto and Rosenbaum unpub. data, Findlay pers. comm.). Further research is required to determine how these whales mix, particularly those off the west relative to the east coasts. Given the general mobility of humpbacks whales in other regions (e.g. among the Hawaiian Islands, Cerchio et al 1998), the short apparent residency time in Antongil Bay (Table 2) and data indicating much greater distance movements between C3 and C2 (Ersts et al. 2006) as well as C3 and C1 (Pomilla 2005, Pomilla et al 2006), we find it reasonable to assume this estimate at least represents the C3 sub-region specifically.

Regarding the relationship of the three currently designated sub-regions within region C, there is emerging evidence suggesting differential exchange. Genetic analyses have indicated significant differentiation between C3 and C1 for both mtDNA (Rosenbaum et al. 2006) and nDNA microsatellites (Pomilla 2005, Pomilla et al. 2006). However, the same analyses indicated no differentiation between C3 and C2. Recapture of individuals (from both photographic and genetic data) indicate exchange of individuals, potentially significant, between C3 and C2 (Ersts et al. 2006). Further work and larger samples from C2 are required to test whether there is random exchange and panmixis between individuals from C2 and C3. However, our current understanding suggests the possibility, if not likelihood, that these sub-regions are contiguous and our estimate may reflect abundance for both C2 and C3. Regarding C3 and C1, although population genetic analyses reveal significant differentiation, it is not as great as between C3 and equivalently sampled sub-regions in region B or D (Pomilla 2005, Rosenbaum et al. 2006; Pomilla et al. 2006, Rosenbaum et al. 2006). Furthermore we have reported a genetic recapture between C3, Antongil Bay, and C1, East South Africa (Pomilla 2005, Pomilla et al. 2006), and report at this meeting two photographic recaptures also between Antongil Bay and East South Africa (Cerchio et al. 2008 SC60SH33). These localities in C1 are thought to be predominately a migration corridor in the southern range of C1, and thus might represent a stream of animals migrating to breeding areas in northern C1 as well as C2/C3. The results of the recent photographic comparison between (southern) C1 and C3 (Cerchio et al. 2008) suggest that these groups are not randomly associating; however, there is clearly some exchange and the photographic sample from C1 was too temporally and geographically inconsistent, and recaptures too sparse to draw definitive conclusions regarding degree of exchange. There is very little data from Mozambique and other areas of known breeding aggregations in northern C1, so it is as yet impossible to comment on the relationship between these breeding assemblages. Therefore, we conclude that there is likely sufficient exchange between these sub-regions to caution against the simple addition of independent estimates from C3 and C1 to arrive at a region-wide estimate for C. Further characterization of migratory movements and exchange among the sub-regions of C are necessary before we can satisfactorily assess the impact of exchange rates and patterns on estimation of abundance. Given the genetic and observational data combined and the uncertainty regarding population structure in this region, it is ultimately difficult to provide a precise characterization of this estimate as representing C3 with some 'known' degree of mixing between C1 and C2. Based on the evidence available for SC60, we recommend revisiting the models for stock structure that were initially drafted at the SH humpback whale workshop in 2006 (Rep 5, JCRM 2007)

Finally, the relationship between regions B, C and D needs to be more clearly defined to assess the degree of exchange between regions. Our current evidence regarding regions B and C indicate significant differentiation at a level greater than between sub-regions within either region (Pomilla 2005, Pomilla et al. 2006, Rosenbaum et al. 2006). However, there is also a documented movement between Gabon, B1, and Madagascar, C3, detected with genotypic recapture and confirmed with dorsal fin photographs (Pomilla and Rosenbaum 2005). Movements of whales from breeding grounds (and sub-regions) mediated through adjacent feeding grounds has also been recently detected (Loo et al. 2008). Moreover, recent acoustic analysis indicates that whales from these two regions sing very similar songs sharing all major phrase types (Razafindrakoto, Cerchio, Collins and Rosenbaum, unpubl. data) indicating a cultural exchange that requires a non-trivial exchange of individuals. Little is known about the relationship of Breeding Stocks C and D beyond significant genetic differentiation at mtDNA (Rosenbaum et al. 2006),

however there is recent evidence of shared song content and therefore acoustic cultural exchange, although minor in comparison to the B-C similarities (Murray 2007).

In summary, we have generated the most current estimates and extensive evaluation of humpback whale population abundance for the C3 breeding region in the southwestern Indian Ocean. Sample size limitations resulted in relatively low precision, and characteristics of individual behavior may introduce bias. However, relative consistency among a variety of models and sample combinations suggests that this population is likely within 5000-7000 individuals, however the geographic region to which this population estimate applies needs to be carefully considered and better defined.

ACKNOWLEDGEMENTS

We are grateful to the staff of the WCS/AMNH New York Staff and WCS Country Programs of Madagascar, in particular George Amato, Rob DeSalle, Eleanor Sterling, Helen Crowley and Matthew Hatchwell, and all the summer interns, students, volunteers and research assistants who have contributed to field and laboratory work, in particular Carla Freitas and Vanessa Rasoamampianina. Many other individuals were involved and helped throughout the years of this study from the WCS Madagascar Country Program, WCS Marine Program, AMNH Center for Biodiversity and Conservation and AMNH Conservation Genetics Program. Funding for this work was provided from grants and awards to WCS and AMNH.

LITERATURE CITED

- Ayres KL, Overall DJ (2004) api-calc 1.0: a computer program for calculating the average probability of identity allowing for substructure, inbreeding and the presence of close relatives. *Molecular Ecology Notes*, **4**, 315-318.
- Begon M (1979) Investigating animal abundance: capture-recapture for biologists. University Park Press, Baltimore.
- Best PB, Findlay KP, Sekiguchi K, *et al.* (1998) Winter distribution and possible migration routes of humpback whales (*Megaptera novaeangliae*) in the southwest Indian Ocean. *Marine Ecology Progress Series*, **162**, 287-299.
- Best PB, Sekiguchi K, Rakotonirina BP, Rossouw A (1997) The distribution and abundance of humpback whales off southwest Madagascar, August-September. *Report to the International Whaling Commission*, **46**, 323.
- Burnham K, Anderson, D (2002) *Model Selection and Multi-Model Inference* (2nd Ed.) Springer-Verlag. 496 pp.
- Cerchio S, Gabriele C, Norris T, Herman LM (1998) Movements of humpback whales between Kauai and Hawaii: implications for population structure and abundance estimation in the Hawaiian Islands. *Marine Ecology Progress Series* **175**: 13-22.
- Clapham PJ, Palsbøll PJ (1997) Molecular analysis of paternity shows promiscuous mating in female humpback whales. *Proceedings of the Royal Society of London - series B*, **264**, 95-98.
- Ersts P, Pomilla C, Rosenbaum HC, Kiszka J, Vely M (2006) Humpback whales identified in the territorial waters of Mayotte [C2] and matches to eastern Madagascar [C3], p. 5. Paper

- SC/A06/HW12 presented to the IWC, Intersessional Workshop for the Comprehensive Assessment of Southern Hemisphere humpback whales (unpublished).
- Hammond, PS (1986) Estimating the size of naturally marked whale populations using capture-recapture techniques. *Report to the International Whaling Commission, Spec Issue No 8*: 253-282.
- Lambertsen RH (1987) A biopsy system for large whales and its use for cytogenetics. *Journal of Mammalogy*, **68**, 443-445.
- Loo* J., Méndez* M., Pomilla C., Leslie, Best, P.B., Collins, T., Engel, M.H., Ersts, P.J., Findlay, K.P., Bonatto, S., Kotze, P.G.H., Meyer, M., Minton, G., Barendse, J., Thorton, M., Razafindrakoto, Y., Ngouessono, S., Vely, M., Kiszka, J. Olavarría C., Baker S., Aguayo A., Thiele D., Ensor P., and Rosenbaum H. 2008. Update on the evaluation of genetic structure on the feeding grounds and their connectivity to Breeding Regions. SC/60/SH11
- Murray, A. (2007) *A Characterization and Comparison of Humpback Whale (Megaptera novaeangliae) Song from the Southern Indian Ocean*. M.A. thesis. Columbia University, New York.
- Otis DL, Burnham KP, White GC, Anderson R (1978) Statistical inference for capture data closed animal populations. *Wildlife Monographs* **62**: 1-135.
- Palsbøll PJ, Allen J, Bérubé M, *et al.* (1997a) Genetic tagging of humpback whales. *Nature*, **388**, 767-769.
- Pomilla C. (2005) Genetic structure of humpback whale populations on the Southern Hemisphere wintering grounds. PhD Dissertation. New Your University.
- Pomilla C, Rosenbaum HC (2006). Estimates of relatedness in groups of humpback whales on two wintering grounds of the Southern Hemisphere. *Molecular Ecology*, **15**, 2541-2555.
- Pomilla C, Rosenbaum HC (2005) Against the current: an inter-oceanic whale migration event. *Biology Letters*, **1**, 476-479.
- Pomilla C. *et al.* (2006) MtDNA diversity and population structure of humpback whales from their wintering areas in the Indian and South Atlantic Ocean (Breeding regions A, B, C and X), p. 12. Paper SC/A06/HW41 presented to the to the IWC, Intersessional Workshop for the Comprehensive Assessment of Southern Hemisphere humpback whales.
- Rosenbaum HC, Razafindrakoto Y, Ersts P, Ventasca G. (2000) A preliminary population estimate for humpback whales from the Antongil Bay, Madagascar wintering grounds in the southwestern Indian Ocean. Paper SC/52/IA10 presented to the IWC Scientific Committee, (unpublished).
- Rosenbaum HC, Walsh PD, Razafindrakoto Y, Vely M, DeSalle R (1997) First description of a humpback whale breeding ground in Baie d'Antongil, Madagascar. *Conservation Biology* **11**: 312-314.
- Rosenbaum HC, Pomilla C, Leslie M, *et al.* (2006) Population structure and sex biased gene flow of humpback whales from their wintering areas in the Indian and South Atlantic Ocean (Breeding regions A, B, C and X) based on nuclear microsatellite variation. p. 12. Paper SC/A06/HW38. presented to the to the IWC, Intersessional Workshop for the Comprehensive Assessment of Southern Hemisphere humpback whales.

Waits JL, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Molecular Ecology*, **10**, 249-256.

Waits, JL Leberg, PL (2000) Biases associated with population estimation using molecular tagging. *Animal Conservation*, **3**, 191-199.

White GC, Burnham, KP (1999) Program MARK: Survival estimation from populations of marked animals. *Bird Study* 46 Supplement, 120-138.

Table 1. Within year effort by year in Antongil Bay, Madagascar, for photographic data.

	2000	2001	2002	2003	2004	2005	2006
Yearly Effort							
Start Date	17 July	10 July	22 Aug	11 July	10 July	13 July	16 July
End Date	17 Sept	14 Sept	11 Sept	9 Sept	5 Sept	5 Sept	4 Sept
Duration	62	66	20	60	59	56	52
Sample Days	37	35	12	34	34	28	37

Table 2. Date of first capture for between-year photographic recaptures in Antongil Bay, Madagascar, indicates strong consistency in timing of arrival. Of 21 recapture events between years (comprised of 19 individuals), 7 (33%) were sighted within two days of their first sighting date, 13 (62%) within five days, and a total of 16 (76%) within ten days.

Individual	2000	2001	2002	2003	2004	Difference in Julian Days ¹
TF-MAD-00-008	7/22/2000				7/20/2004	2
TF-MAD-00-019	8/7/2000			8/26/2003		18
TF-MAD-00-031	8/27/2000				8/14/2004	13
TF-MAD-00-041	9/7/2000			9/7/2003		1
TF-MAD-00-081	7/29/2000			7/29/2003		1
TF-MAD-00-095	9/2/2000	9/9/2001	9/4/2002			6,1,5
TF-MAD-00-098	9/8/2000	9/6/2001				3
TF-MAD-01-041		7/27/2001			7/19/2004	7
TF-MAD-01-077		8/19/2001		8/18/2003		1
TF-MAD-01-139		7/18/2001			7/21/2004	4
TF-MAD-01-189		8/7/2001			8/5/2004	1
TF-MAD-01-194		9/6/2001		8/16/2003		21
TF-MAD-02-001			8/28/2002	8/24/2003		4
TF-MAD-02-003			9/2/2002	8/26/2003		7
TF-MAD-02-010			9/4/2002	8/17/2003		18
TF-MAD-02-021			9/6/2002	9/7/2003		1
TF-MAD-03-037				7/28/2003	8/14/2004	18
TF-MAD-03-118				7/12/2003	7/16/2004	5
TF-MAD-03-140				7/19/2003	7/13/2004	5

¹Julian Day is calculated using 1 January as "1"; leap years in 2000 and 2004 with 366 days account for the apparent inconsistencies between differences in calendar dates and Julian days.

Table 3. Yearly sample sizes and recaptures for tail flukes in Antongil Bay, Madagascar.

	2000	2001	2002	2003	2004	2005	2006
Individuals captured	89	159	16	126	151	144	158
	Year of Recapture						
Year of Initial Capture	2000	2001	2002	2003	2004	2005	2006
2000	X	2	1	3	1	0	1
2001		x	1	3	3	3	2
2002			x	3	0	0	0
2003				x	2	1	3
2004					x	4	3
2005						x	4
2006							x

Table 4. Within year sample characteristics by year in Antongil Bay, Madagascar, for genetic data.

	2000	2001	2002	2003	2004	2005	2006	Total
Number samples	142	187	35	208	193	188	173	1126
Number Individuals	114	161	28	185	163	161	153	922
Resample Rate	19.7%	13.9%	20.0%	11.1%	15.5%	14.4%	11.6%	18.1%

Table 5. Yearly sample sizes and recaptures for genotypes in Antongil, Madagascar.

	2000	2001	2002	2003	2004	2005	2006
Individuals captured	114	161	28	185	163	161	153
	Year of Recapture						
Year of Initial Capture	2000	2001	2002	2003	2004	2005	2006
2000	x	4	1	2	2	0	0
2001		x	2	6	2	1	2
2002			x	6	1	1	1
2003				x	2	2	3
2004					x	2	4
2005						x	3
2006							x

Table 6. Abundance estimates using (a) tail flukes photo-IDs and (b) microsatellite genotypes applying Chapman's modified Petersen pair-wise estimates.

Years	(a) Photo-IDs					(b) Genotypes				
	N	SE	CV	LCI	UCI	N	SE	CV	LCI	UCI
2000-2001	4799	2337	0.49	218	9380	3725	1465	0.39	2871	6596
2001-2002	1359	733	0.54	0	2796	1565	734	0.47	1440	3005
2002-2003	539	208	0.39	132	946	770	233	0.30	456	1226
2001-2003	5079	2208	0.43	752	9406	4304	1460	0.34	1441	7166
2003-2004	6434	3148	0.49	264	12603	10167	4996	0.49	9793	19960
2004-2005	4407	1739	0.39	999	7815	8855	4346	0.49	8519	17374
2005-2006	4610	1820	0.39	1042	8178	6236	2719	0.44	5328	11564

Table 7. Abundance estimates using tail flukes photo-IDs and microsatellite genotypes and applying Program MARK likelihood models for capture-recapture. For each dataset several models were run and the most parsimonious model was chosen (shown in table) based upon Akaike's Information Criteria (AIC); M_0 =Null Model; M_t =capture probability varies with sampling occasion. (a) Two estimates for 2006 making the use of the most data, using all years but excluding the poorly sampled year of 2002, and using the final four years of data after 2002. (b) In the last set of models, two non-overlapping datasets were used each having three sample years, providing estimates for the years 2003 and 2006; these estimates were then used to calculate a cursory rate of increase (ROI) for the population.

Dataset	Photo-IDs						Genotypes					
	Mod	N	SE	CV	LCI	UCI	Mod	N	SE	CV	LCI	UCI
(a) 2000-2006, for 2006	M_t	9243	1629	0.18	6604	13081	M_t	10498	1738	0.17	7649	14549
2003-2006. for 2006	M_0	7715	1869	0.24	4885	12399	M_t	10123	2462	0.24	6389	16285
(b) 2000-2003, for 2003	M_t	5612	1925	0.34	2980	10896	M_0	5807	1618	0.28	3452	10008
2004-2006, for 2006	M_0	6737	2067	0.31	3804	12229	M_0	8348	2712	0.32	4558	15650
ROI		0.063						0.136				

Table 8. Abundance estimates using tail flukes, exploring the effect of sampling during a short period. Using the abbreviated 2002 field season as a reference, these abundance estimates were generated using only concurrent sampling, thereby truncating the data to only captures made during the 25-day period of Julian day 230 to 255 for all years. In all cases, these estimates are two to ten-fold smaller than when using all data.

	N	CV	95%CI
2000-2001	549	0.46	+/- 498
2001-2002	296	0.53	+/- 307
2002-2003	350	0.53	+/- 365
2003-2004	1754	0.69	+/- 2374

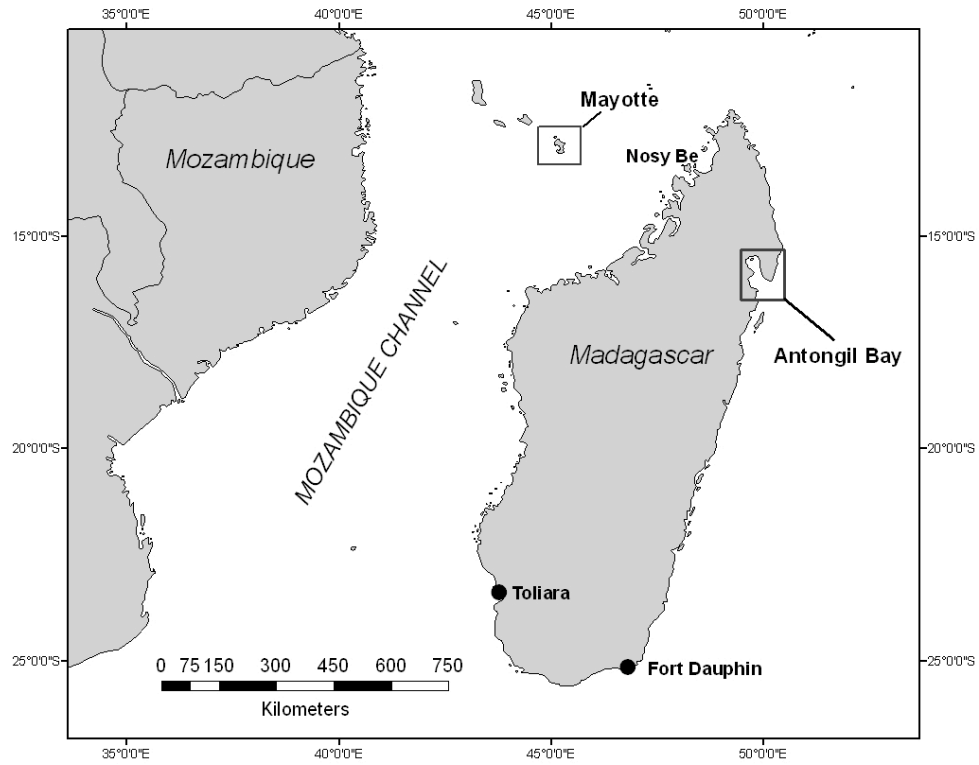


Figure 1. Location of study site, Antongil Bay, Madagascar.

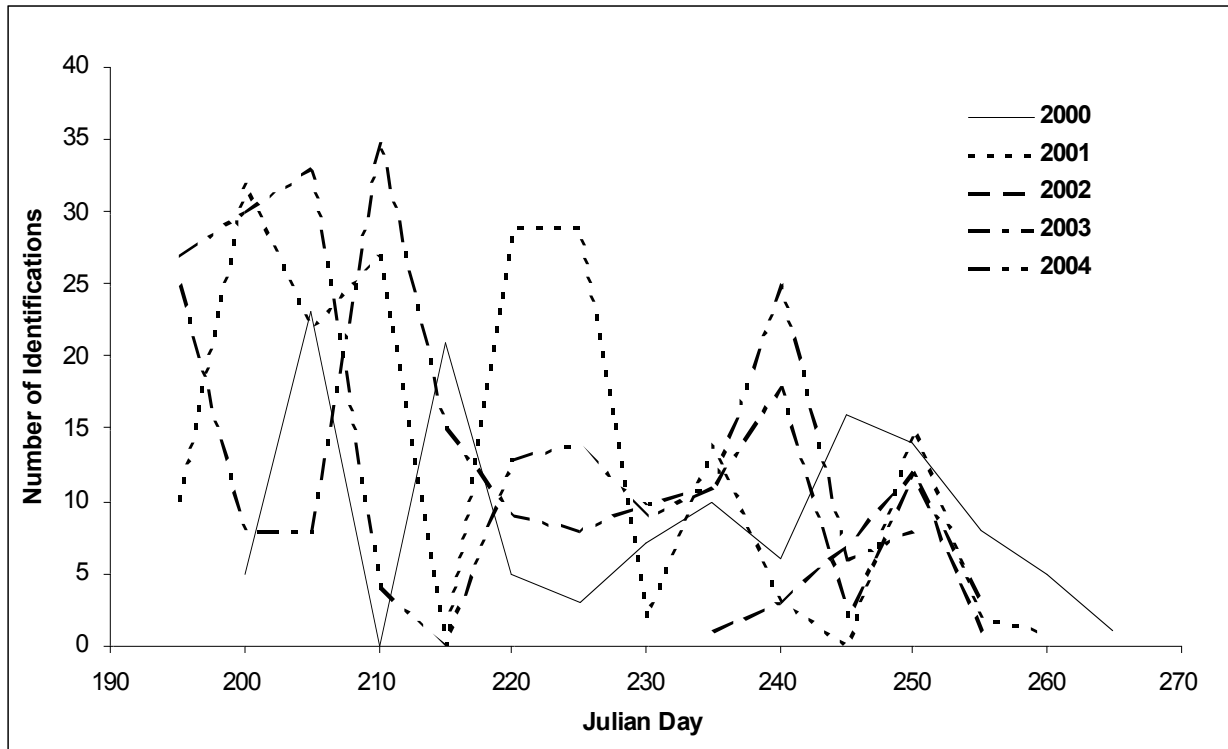


Figure 2. Sampling profile in Antongil Bay, Madagascar, expressed as number of photographic identifications collected in 5-day blocks for 2000 through 2004. Number of IDs collected varied throughout each year primarily due to weather constraints as well as density of whales. The 2002 season (red) was strongly abbreviated due to political upheaval in Madagascar early in the year.

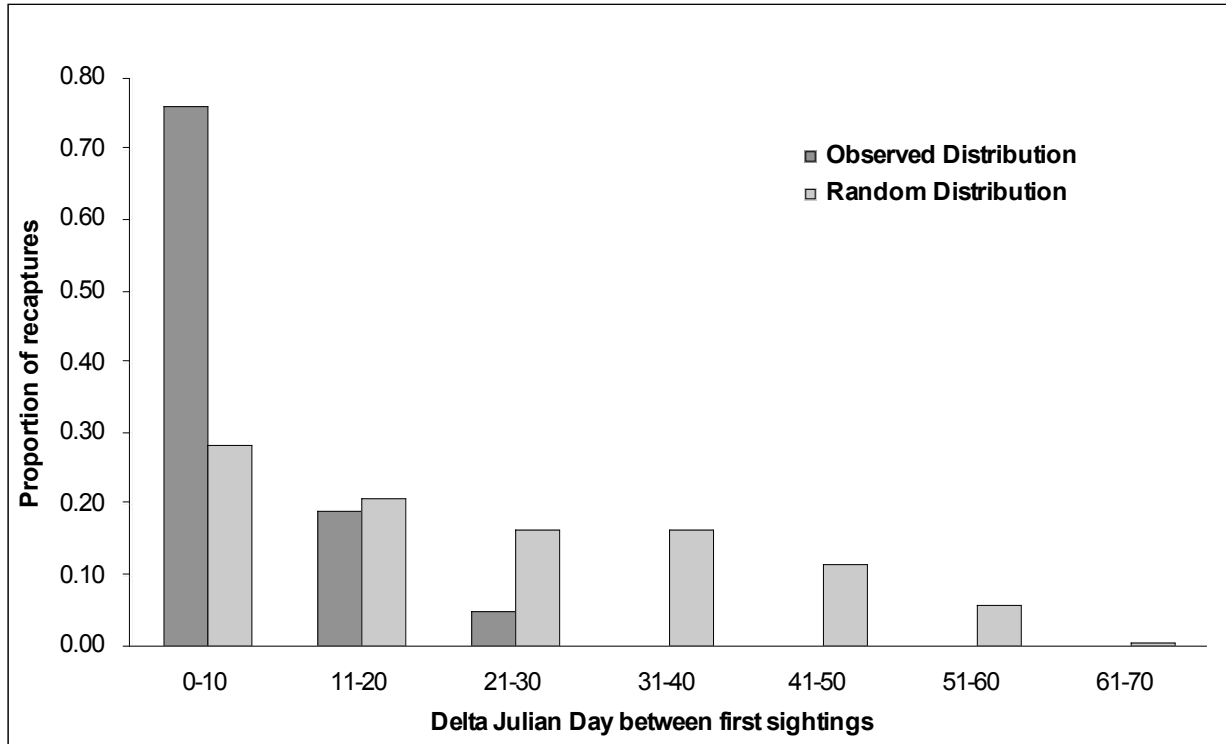


Figure 3. Timing of between-year recaptures in Antongil Bay, Madagascar, indicating highly significant consistency in capture date between years (10,000 permutations, $p < 0.0001$). Random distribution was generated by randomly permuting the capture date of all captured individuals within each year and recalculating the difference in Julian day of capture for each recaptured individual.

APPENDIX I

Photographic Data – Model outputs from Program MARK including AIC results and parameter estimates for abundance (N).

Mad 2000-2006, no 2002

	N	SE	LCI	UCI
Model Mt	9243	1629	6604	13081
Model Mo	9300	1640	6644	13163
Model Mh2	13080	4302	7102	24722
Model Mth2	9243	1629	6604	13081

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Num. Par	Deviance
{Mt}	-5902.1	0.0	0.7301	1.0000	7	47.413
{Mth2}	-5900.1	2.0	0.2677	0.3666	8	47.413
{Mh2}	-5890.5	11.6	0.0022	0.0030	4	65.025
{Mo}	-5883.5	18.6	0.0001	0.0001	2	76.064

Mad 2003-2006

	N	SE	LCI	UCI
Model Mo	7715	1869	4885	12399
Model Mt	7697	1864	4873	12369
Model Mh2	25697	252426	933	1701191
Model Mth2	9795	3328	5216	18877

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Num. Par	Deviance
{Mo}	-4271.7	0.0	0.5640	1.0000	2	18.033
{Mt}	-4270.0	1.7	0.2448	0.4340	5	13.680
{Mh2}	-4269.4	2.3	0.1764	0.3128	4	16.344
{Mth2}	-4264.4	7.3	0.0148	0.0263	10	9.219

Mad 2000-2003, no 2002

	N	SE	LCI	UCI
Model Mt	5612	1925	2980	10896
Model Mo	5763	1979	3057	11193
Model Mh2	5763	1979	3057	11193
Model Mth2	3670	3421	986	17970

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Num. Par	Deviance
{Mt}	-2720.0	0.0	0.9341	1.0000	4	8.466
{Mth2}	-2714.6	5.3	0.0655	0.0702	7	7.713
{Mo}	-2703.5	16.5	0.0003	0.0003	2	28.957
{Mh2}	-2701.5	18.5	0.0001	0.0001	3	28.957

Mad 2004-2006

	N	SE	LCI	UCI
Model Mo	6737	2067	3804	12229
Model Mt	6733	2066	3802	12222
Model Mh2	64050	0	64050	64050
Model Mth2	11284	7814	3494	38962

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Num. Par	Deviance
{Mh2}	-3431.5	0.0	0.6535	1.0000	3	8.871
{Mo}	-3429.9	1.6	0.2882	0.4409	2	12.518
{Mt}	-3426.5	5.1	0.0515	0.0787	4	11.942
{Mth2}	-3422.4	9.1	0.0069	0.0105	8	7.890

Genotypic Data– Model outputs from Program MARK including AIC results and parameter estimates for abundance (N).

Mad 2000-2006, no 2002

	N	SE	LCI	UCI
Model Mt	10498	1738	7649	14549
Model Mo	10536	1745	7676	14603
Model Mh2	10536	1745	7676	14603
Model Mth2	9751	1716	6974	13798

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Num. Par	Deviance
{Mt}	-6900.4	0.0	0.7886	1.0000	7	30.472
{Mth2}	-6897.6	2.8	0.1961	0.2486	14	19.199
{Mo}	-6891.9	8.5	0.0113	0.0143	2	48.988
{Mh2}	-6889.9	10.5	0.0041	0.0053	3	48.988

Mad 2003-2006

	N	SE	LCI	UCI
Model Mt	10123	2462	6389	16285
Model Mo	10140	2466	6399	16313
Model Mh2	10140	2466	6399	16313
Model Mth2	13879	5112	7017	28131

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Num. Par	Deviance
{Mo}	-5110.0	0.0	0.6068	1.0000	2	13.858
{Mh2}	-5108.0	2.0	0.2227	0.3670	3	13.858
{Mt}	-5107.3	2.6	0.1637	0.2699	5	10.459
{Mth2}	-5101.0	9.0	0.0068	0.0112	9	8.780

Mad 2000-2003 no 2002

	N	SE	LCI	UCI
Model Mo	5807	1618	3452	10008
Model Mt	5701	1587	3392	9822
Model Mh2	5807	1618	3452	10010
Model Mth2	4655	3930	1338	20320

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Num. Par	Deviance
{Mt}	-3488.9	0.0	0.9656	1.0000	4	9.316
{Mth2}	-3482.1	6.7	0.0333	0.0344	8	7.975
{Mo}	-3474.8	14.0	0.0009	0.0009	2	27.374
{Mh2}	-3472.8	16.0	0.0003	0.0003	3	27.374

Mad 2004-2006

	N	SE	LCI	UCI
Model Mo	8348	2712	4558	15650
Model Mt	8345	2711	4556	15644
Model Mh2	8347	2712	4558	15649
Model Mth2	8077	4289	3186	21770

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Num. Par	Deviance
{Mo}	-3697.6	0.0	0.6499	1.0000	2	9.212
{Mh2}	-3695.6	2.0	0.2381	0.3663	3	9.212
{Mt}	-3694.0	3.7	0.1043	0.1605	4	8.851
{Mth2}	-3688.8	8.9	0.0077	0.0119	7	8.003