Age and Sex-specific survival estimates of Ant-eating Chat (*Myrmecocichla formicivora*)

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Abstract

Studying population dynamics is vital for understanding the short and long-term changes in the structure of populations. Different biological and environmental processes influence these changes. To assess the population structure we need quantitative estimates of survival. Age and sex-specific survival are important components in the dynamics and the structure of birds’ populations. We present the first population dynamics study done on Ant-eating Chat (*Myrmecocichla formicivora*). By means of capture-recapture data of an 8-year dataset (2003-2010), we used program MARK to estimate annual adult survival rates. We also constructed different models to compute age and sex specific survival estimates. In addition, we carried out the first DNA-based sex identification test using P2&P8 primers. First year survival (0.28 ±0.10), was much lower than adult survival (0.74 ±0.12). Annual adult survival estimates varied from (0.338±0.37) to (0.90±0.17). Males showed higher survival (0.60±0.03) than females (0.52±0.03). Nonetheless, this difference was statistically insignificant. Our study gives the first highlights on the population dynamics of the Ant-eating Chat.
Introduction

Population dynamics is the branch of ecology that examines the variation of the distribution of a population in time and space (Begon et al. 1990). It investigates the variations in size and structure of populations, and how the different biological, and environmental processes influence these variations. Examples of biological (demographic) processes are birth rate, mortality, immigration, and emigration (Goodman 1987, Lande 1993). As for the environmental (stochastic) processes, weather conditions, natural catastrophes, and predation can be examples (Ewens et al. 1987, Lande 1993). These are the main components driving populations’ fluctuations from one year to another. Since population size fluctuations are determined by the number of individuals that survive, are recruited, or dispersed, there is therefore a strong reason to study the estimates for all of these components in order to understand the underlying mechanisms of the population turnover. Studying these mechanisms is vital to answer many ecological, evolutionary and conservation questions. From an evolutionary perspective, we can investigate how the evolution of behaviors such as cooperative breeding, i.e. delayed dispersal; affect the age structure of a population. For conservation, if we are interested to know whether a population is expected to grow or decline in the coming years, we can carry out a population viability analysis using quantitative methods to predict the likely future status of a population of conservation concern (Morris et al. 2002).

Estimates of the demographic rates can be calculated statistically if we follow the fate of the individuals through time and space. However, sometimes it is hard to follow marked individuals because they are not always seen in the study site, as they can simply be missed during our observations. This can be due to many factors; Shyer individuals are harder to spot than others for example. Some marked individuals can be hard to identify due to our uncertainty about the ring colors, since the colors can be worn out. Also, females can sometimes be easier to catch than males since they are incubating the eggs. Consequently, this may lead to missing out important information about the fate of the individuals, whether they are alive but not seen or they are dead. Many statistical methods have been developed to overcome this problem (Lebreton et al. 1992).
**Age and Sex-specific survival**

Age and sex specific survival are essential components in the dynamics and genetic structure of bird populations (Oppel et al. 2010). Adult and juvenile survival estimates are known to differ in many avian taxa (Martin 1995). It is important to understand how the basic demographic rates vary with age to provide us with more understanding of the population dynamics of the species (Sæther et al. 2000). For example, population declines are poorly understood. The declines can be due to many reasons; a decline in the prey availability, high predation or more susceptibility to variation in some environmental conditions. To be able to assess the changes, especially to young and inexperienced birds, we will need more information about survival rates of different age classes. Therefore, survival estimates for juvenile and adults are urgently needed to understand the population dynamics mechanism. Survival also differs between sexes in many bird species. In a study done by Payesvsky et al. (1997) they claimed that overall male survival was higher than that of females in 70% of the cases. Such difference is considered to be related to sex-specific investment in reproduction and parental behavior (Robertson et al. 2006) since the female experience more risks being on the eggs for instance.

**Capture-recapture data**

Capture-recapture data has been widely used to compute survival estimates, and hence give an overall view of the structural dynamics of a population. Many statistical programs have been developed to deal with this kind of data. These programs deal with data from marked animals and use it to provide survival and recapture parameter estimates when they are re-encountered in a later event. They compute the estimates of model parameters using maximum likelihood estimation (MLE). MLE can accomplish that through some specific statistical methods. Using some observations with unknown mean and variance, MLE can consider these unknowns as parameters and compute parametric values that make the observed results the most probable (given the model). In this study we used program MARK to compute annual survival estimates for adults (age-specific survival), check difference between juvenile and adult survival, and to investigate sex-specific difference in survival estimates of Ant-Eating Chat (AEC) “Myrmecocichla formicivora” using an 8-year (7 seasons) dataset.
The study species (Ant-eating Chat)

Ant-Eating Chat (AEC) is a common bird species of the grassland and open savannah of the southern part of Africa. It is endemic to this region. There is an associative relationship between Ant-eating Chats and Aardvarks, *Orycteropus afer*. Aardvarks locate their food by smell, and they can excavate ants and termites nests underground with their strong claws. The Ant-eating Chats benefit from that through easy access to the food source (termites) exposed by the Aardvark digging (Taylor *et al.* 2001). They can also benefit from the Aardvark burrows where they excavate tunnels in the roof of these burrows to roost and breed in. These tunnels make it hard for most of the mammalian predators, such as the mongoose (*Galerella sanguinea*), to reach the AEC. However, the Cape cobra (*Naja naja*) and few egg eating snakes, which are major predators, can still find their way through. The length of the tunnels varies from 30cm to 150cm. From our experience, we found that short tunnels rarely contained any roosting or nesting chats. Tunnels excavation and nest building (soil dug with bill and shoveled out with feet) take 8-10 days effort from both sexes (Herholdt 1988). The female lays 2-7, usually 3-4 eggs (Maclean 1993). Solely, the female incubates for 14-15 days (Jonathan Barnaby, personal communication). Fledging takes place after 15-18 days. The Ant-eating Chat is known to be a facultative cooperative breeding bird where mature individuals tend to stay with the breeding parents to raise the subsequent brood (Earle & Herholdt 1988). AEC can occur solitarily, in pairs or in bigger family groups.

Sex identification

In general it can be difficult to sex birds. Generally, nestlings rarely show sexual dimorphism. Also, over 50% of the world’s bird species show identical adult (male-female) morphology (Griffiths *et al.* 1998). Sex differentiation in Ant-eating Chat is thought to be fairly easy, based on morphology. Males are known to show a characteristic white patch on the lesser coverts carpal joint (Hockey *et al.* 2005), which can even be seen in nestlings once they grow feathers (4-5 days). Nonetheless, this is a point of debate between South African bird researches about whether or not the white patch is universal in males. Therefore, in this study we carried out a DNA-based sex identification test to confirm this information.
Methods

Study area
The fieldwork for this year was conducted in the period from 6th October 2010 to 22nd December 2010 in Benfontein Nature Reserve (28°49'58.17"S, 24°49'43.73"E) located 10 km south east of Kimberley, in the Northern Cape province, South Africa (Figure 1). The study site covers an area of 8 km², and consists of a Karoo type scrub area, savannah grassland and a bare/eroded ground. Kimberley receives an average of 419mm of rain per year, with driest climate between July and September.

![Figure 1. The study site boundary and reference territories](image)

Survey
Since the AEC roosts in tunnels dug in Aardvark burrows, we had to first carry out an initial survey to locate all the Aardvark burrows in the site. To do that, we divided the study site into smaller areas on the map using Garmin Map Source software (Version 6.16.3), and then transferred the assigned area on daily basis to the GPS. With the assistance of the handhold GPS (Garmin 60Csx), we walked in the assigned area in
parallel lines within a visual range of the line before, to make sure we did not miss any Aardvark burrows. A distance of 10-15 m between each line was found suitable. Once we found an AEC tunnel in a burrow, we marked its location on the GPS, measured its length with a measuring tape, checked if it had a nest or not (using a small square shaped mirror attached to a long aluminum wire), if we found a nest, we checked for the incubating female, eggs or chicks and counted them. Finally we recorded the condition of the tunnel *i.e.* if it was derelict, newly dug, had other animals in it. All the GPS points were then transferred to a laptop computer and were stored as a Garmin map database file.

**Catching & banding**

We started the catching process by uploading a chosen area to survey on the GPS with all the tunnels to be checked for roosting chats. To catch the chats, we first had to build a special catcher, which is a plastic container open from one side (to be put on the tunnel entrance) and the other side has a plastic bag attached to it to trap the bird. To drive the birds out the tunnel we used a fake snake, which forced the birds out, straight to the plastic bag attached to the entrance. We did all the catching at night from 20:00 to approx 24.00-1.00. Using our dim head torches light we navigated to the different burrows, quietly and quickly checked the tunnels (since the birds showed a high sensitivity to the outside noise and they attempted to escape if they felt something approaching their roosting tunnel). Once we had a bird in hand, we banded it with one metal ring and a unique combination of color rings. We measured the beak, head, tarsus, and wing lengths. We also took 2 blood samples from each bird we caught for DNA analysis later on in the lab.

**Sexing**

DNA-based sex identification tests offer a solution for sex identification in birds. However, finding a suitable sex-linked marker is not easy (Griffiths et al. 1998). In birds, W sex chromosome occurs in the females only (ZW) while the males have only the Z chromosome (ZZ). Nonetheless, this chromosome is small and contains a high amount of junk DNA (Stefos & Arrighi 1971). “An improved basis for a DNA sexing technique is to use a gene, and the first and only avian W chromosome gene that has been discovered is chromo-helicase-DNA binding gene CHD” (Ellegren 1996, Griffiths *et al.* 1998). It is available on all avian sex chromosomes, with the exception
of ratites (ostriches, Struthioniformes) (Griffiths&Tiwari 1996). PCR primers amplify homologous parts of the CHD-W and the related gene CHD-Z. Since CHD-Z is available in both sexes, it should be amplified anyway, and that indicates that the PCR did work. We used P2 and P8 as our primers for the PCR reaction, and as described by Griffiths et al. (1998), these primers anneal to conserved exonic regions and amplify across an intron in both genes. The PCR was carried out in a total volume of 10μl. The final reaction conditions were: 100μl buffer without MgCl2, 53μl dNTP’s, 40μl MgCl2, 5μl of the enzyme (Biotaq), 53μl of each primer. The PCR cycle conditions were: an initial denaturation step at 94°C for 1 mins 30 s, followed by 30 cycles of 48°C for 45 s, 72 C for 45 s and 94°C for 30 s and then a final run of 48°C for 1 min and 72°C for 5 min. We carried out the test on 80 different samples. We then used MegaBace for the genotyping and MegaBace Fragment profile (Version 1.2) to score the alleles.

**Program Mark**

This software deals with ‘marked individuals’. In this study, we used MARK 5.1 developed by Gary White (White & Burnham 1999, Cooch & White 2009). For marked individuals, the number encountered in the next sampling occasion is a function of 2 probabilities: probability of survival and probability that if the individuals survived, they are seen (encounter probability). An individual can be alive but we miss it in an observation. So, the proportion of individuals encountered alive on a second occasion, ‘return rate’= survival probability (Phi) x encounter probability (P). Program Mark computes the model parameters estimates by using the maximum likelihood tool which fixes the problem of considering an unseen individual(s) dead when it can still be alive. I sorted out all encounter histories for each individual in the data to match Program Mark format (i.e. when the individual was marked, and if it was re-observed or not (It takes 1 if it was seen and 0 if it wasn’t). The sampling occasion starts in July and ends in June the following year with constant capturing effort during the breeding season from October to December. We worked on a dataset of 8 capture-recapture occasions from 2003-2010 to estimate annual survival probabilities. I constructed various models to examine the effect of age, sex and year. I used Akaike’s information criterion (AIC;Akaike 1973, Leberton et al. 1992, Burnham and Anderson 2002) to compare between models. The AIC aims
to reach the balance between precision and parsimony between different models. The model with the lowest AIC value will have the highest likelihood; it will therefore be considered as the best model. If ∆AIC is ≤ 2, both models are considered to have the same support (Cooch & White 2009). An initial step to test for the goodness of fit (GOF) of the global model (most parameterized) is necessary before selecting the model. Variance inflation factor (Ĉ) should be calculated and corrected for, in case of any lack of fit. I used program Release (Version 3.0) and bootstrap GOF to compute Ĉ from a simulation of 100 replicates, and then I used it to correct for over dispersion. After correction, program MARK gives Quasi-Akaike (QAICc) estimates instead of Akaike index criterion (AIC). First, I constructed various models that considered only 2 age classes (juveniles “1 year old” vs. adult “2+ survival) using all the data from the birds banded as juveniles (males=234, females=248) and then I constructed models to test for age-specific survival for every year. For the sex-specific survival we used data from birds that were banded as adults (males=171, females=198), where I separated the sexes into 2 groups and we ran models with and without sex difference.

Results

Age-specific survival

The AECs were ringed as both juveniles and adults. We considered the birds as juveniles up to one year old. Anything beyond that was considered as an adult. After running the different models in MARK that investigates survival in both juveniles and adults, we found that the global model (Phia2t/t)p(t) (Phi: the survival estimate, P: the recapture estimate, while a2 means 2 age classes and t indicates time dependence) for the age specific survival estimate showed some over dispersion (Ĉ=2.17). This was considered a minor over dispersion. Therefore, we corrected for the Ĉ value and we based our model selection on Quasi-Akaike criterion (QAICc) instead of the Akaike criterion (AIC) to evaluate the relative plausibility of each model. The model with 2 age classes (Phi a2) was the most supported model based on QAICc value (Table 1) and had much higher support (w_i=94%) than all other models including the 7-age class model. Juveniles seemed to have low survival in their first year with survival estimate of (0.28 ±0.10), while adults seemed to survive much better with estimate of (0.74 ±0.12) (Figure 2).
Table 1. Model selection results to assess age difference in survival. (Phi) is the survival probability and (p) is the re-encounter probability. a2 means 2 age classes, while (.) indicates time independence and (t) indicates time dependence.

<table>
<thead>
<tr>
<th>Model</th>
<th>QAICc</th>
<th>ΔQAICc</th>
<th>Wi</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phi(a2./.)p(.)</td>
<td>304.241</td>
<td>0</td>
<td>0.618</td>
<td>3</td>
</tr>
<tr>
<td>Phi(a2./.)p(t)</td>
<td>306.685</td>
<td>2.441</td>
<td>0.182</td>
<td>9</td>
</tr>
<tr>
<td>Phi(a2./t)p(.)</td>
<td>306.986</td>
<td>2.744</td>
<td>0.156</td>
<td>8</td>
</tr>
</tbody>
</table>

Figure 2. Mean, ±SE of juvenile and adult Ant-eating Chat. Mean juvenile survival estimates (0.28±0.10) and mean adult survival estimate was (0.74±0.12)

In the analysis of year-by-year survival using data only from the birds banded as juveniles, adult survival estimates varied from (0.54 ±0.193) to (0.93 ±0.40) and some of the values were estimated to be 1.0 and 0.0, which is biologically unaccepted. The reason of such estimates is mainly due to the poor power due to lack of data. Therefore, to estimate annual survival estimates for adults we included data from both birds banded as juveniles and birds banded as adults. We used a 7-year model representing the 7 fieldwork seasons, excluding the first year (i.e. 2003/2004) survival estimate since it included birds banded as juveniles (i.e. Juvenile survival). Estimates are shown in Table 2 and Figure 3.
Table 2. Annual survival estimates (i.e. season) for adults with standard errors, upper and lower confidence intervals.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>SE</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) 04/05</td>
<td>0.7237647</td>
<td>0.0941755</td>
<td>0.5099904</td>
<td>0.8683513</td>
</tr>
<tr>
<td>2) 05/06</td>
<td>0.5864141</td>
<td>0.0988023</td>
<td>0.3895275</td>
<td>0.759076</td>
</tr>
<tr>
<td>3) 06/07</td>
<td>0.9099936</td>
<td>0.1714468</td>
<td>0.1431734</td>
<td>0.998368</td>
</tr>
<tr>
<td>4) 07/08</td>
<td>0.4438448</td>
<td>0.1345634</td>
<td>0.215172</td>
<td>0.6990718</td>
</tr>
<tr>
<td>5) 08/09</td>
<td>0.8392956</td>
<td>0.3735933</td>
<td>0.0224028</td>
<td>0.9991605</td>
</tr>
<tr>
<td>6) 09/10</td>
<td>0.3381828</td>
<td>0.3735933</td>
<td>0.0244846</td>
<td>0.9123055</td>
</tr>
</tbody>
</table>

Figure 3. Annual survival estimates (i.e. season specific) and their SE of adult Ant-eating Chat from season (June 2004/July 2005) to the last study season in 2010. Average survival for adults (0.67±0.033)

Sex-specific survival

Using program Release to test for GOF, the general model showed a minor under dispersion that we ignored and we continued model selection using the Akaike criterion (AIC). There was a sex difference in survival according to AIC model selection. Males showed a mean survival estimate of (0.645±0.06) and females mean survival estimate was (0.464±0.06). Nevertheless, using a paired T-test this difference
was statistically insignificant (P-value= 0.365). I constructed two different models with and without sex effect. The first model, I assumed full time dependence with no sex effect and the other one, full time dependent but including the sex effect. According to the model AICc values, the model with no sex effect was favored to the model with sex effect (Table 3).

### Table 3. The models used in Program MARK to investigate effect of sex on the survival of Ant-eating Chat

<table>
<thead>
<tr>
<th>Model</th>
<th>AICc</th>
<th>∆AICc</th>
<th>Wi</th>
<th>K</th>
<th>Deviance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phi(t)p(t) no sex effect</td>
<td>720.362</td>
<td>0</td>
<td>0.869</td>
<td>13</td>
<td>142.557</td>
</tr>
<tr>
<td>Phi(t)p(t) - sex effect</td>
<td>728.099</td>
<td>7.736</td>
<td>0.018</td>
<td>20</td>
<td>135.121</td>
</tr>
</tbody>
</table>

### Sexing

From the 80 samples we tested, we found a 98% percent match between the observations and the DNA-based test. All the 41 tested male samples matched our observations of the (white patched) males. Whereas, we had only one sample that did not match our observations, as it was observed as a female but it appeared on the fragment profiler to have only one peak at the CHD-Z gene. However, we believe that this sample was particularly noisy and perhaps the peak at the CHD-W gene was covered by the noise. An example of how the alleles peaks look like when they are being scored is shown in Figure 4 and 5. Figure 4 shows a single male peak, and figure 5 shows a female with 2 strong peaks.

![Figure 4](image.png)

**Figure 4.** A fragment profile for a male Ant-eating Chat, showing a single peak that indicates the presence of only the CHD-Z gene.
Figure 5. A fragment profile for a female Ant-eating Chat, showing 2 peaks that indicate the presence of both CHD-Z and CHD-W genes.

Discussion

Our study offers the first annual survival probability estimates for the Ant-eating Chat (AEC). The results showed a significant age-specific difference in survival, and it also showed a possible sex-specific difference. As expected, adult survival was significantly higher than the juvenile survival. Juvenile mortality is known to be higher in many birds’ species since they are inexperienced in avoiding predators, and have less experience in foraging, and thus they are more susceptible to predation and starvation. In our study there was a 46% difference between juvenile and adult survival, which ties in with many other studies. For instance, in a study done by Oppel et al. (2010), they found a 20-30% lower survival estimate in hatch year King Eiders compared to adults. Also, Piersma et al. (2005), found a 13.6% lower juvenile survival estimates in Eurasian golden plovers compared to adults. In our study population, juveniles’ high mortality is mainly due to predation. Cape cobra (Naja nevja) is common in the study area and is thought to be a main (observed) nest predator, while the Common egg eater (Dasypeltis scabra) is thought to be a common egg predator. There are also other mammalian predators like the Striped mouse (Rhabdomys pumilio). However, the latter can hardly get access to the nest due to the nepotistic mobbing behavior from the members of the family group, which is a good example of how anti-predator theory works in AEC. Other factors can also affect nestlings’ mortality, such as parasitic mites (Ornithonyssus bursa). It was
observed in previous years that parents deserted the nestlings due to infestation by the parasitic mites (Jonathan Barnaby, personal communication) (Hockey et al. 2005). We could not provide statistically significant estimates for age-specific survival for each age since we had a lack of data in the birds of known age (i.e. birds banded as juveniles). Our annual survival probability estimates on the other hand showed a significant variation from one year to another, and this could be explained by the variation in environmental factors, such as climate. Although the Ant-eating Chat is adapted to live in the open savannah grassland that is characterized by the seasonal precipitation. In some years, the rainfall level varies drastically from too low (i.e. drought) to very high (i.e. flooding). This variation causes a fluctuation in the availability of the food sources of the AEC. For instance, in the 2008/2009 season, there was an unusual drought in the study area, which could have very likely affected both adult and juvenile survival. This year, fieldwork was carried out in dry conditions, and we had very few breeding attempts compared to previous years. Some AEC groups started breeding early in the season but those groups had failed nests, probably because of shortage of food sources, which might have implied some competition between the different chat groups. However, in some years we experienced flooding in the study that damaged many nests. The low survival estimates in parameters 5&7 (Figure 2) are maybe attributed to the harsh environmental variations in these years. A study done by Altweg et al. 2009 showed a significant variation in survival estimates of Blue cranes (Anthropoides paradiseus) with rainfall levels in South Africa. Since the AEC has been previously little studied, we have limited data about predation; seasonality and environmental variations that we could account for in program Mark.

There was a difference between male and female adult survival estimates in our study population. Although not statistically significant, this difference can be attributed to the costly reproductive burden on the females. Females solely incubate the eggs in AECs, which can lead to a higher susceptibility to predation, as well as less foraging time than males. So, in poor conditions, females will suffer more. Our results tie in with a study done by Sagar et al. 2006, where they found a higher survival rate for males in Bellbirds (Anthornis melanura).
The relatively good average survival estimate in adult Ant-eating chat reflects the fact that the birds have a significantly high chance of survival if they managed to survive their first year. Being a cooperative breeder can be a factor that enhances their survival estimates given the indirect, nepotistic benefits of living in big family groups and being philopatric. Not many species can afford to delay dispersal and favor being philopatric unless the benefits are rewarding (Covas et al. 2007). Perhaps, more studies can be done to investigate the effect of kinship on survival of the Ant-eating Chat since it is crucial for cooperative breeding birds. Also, more capture recapture data should be collected from birds of known age so it can give more accurate estimates on age-specific survival for each age.

Finally, the DNA-based sex identification test was the first to be carried out on our study species. The 98% percent matching results indicate a highly significant support to the characteristic white patch on the males of Ant-eating Chat. The results also show that primers P2 and P8 worked well for sex identification in our study species.
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**List of abbreviations**

AEC: Ant-eating Chat
QAIC: Quasi-Akaike information criterion
AIC: Akaike information criterion
MLE: Maximum likelihood estimation.
CHD: Chromo Helicase DNA-binding gene