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Final Report

Estimation of the population size and distribution of the southern cassowary, *Casuarius casuarius*, in the Wet Tropics Region of Australia

David Westcott, Suzanne Metcalfe, Dean Jones, Matt Bradford, Adam McKeown and Andrew Ford

Australian Government
Department of the Environment

Reef & Rainforest RESEARCH CENTRE

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December 2014

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Acronyms Used In This Report

WTR..... Wet Tropics Region

WTWHA..... Wet Tropics World Heritage Area

Abbreviations Used In This Report

ha..... hectares

MM..... mis-matches

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Introduction

The southern cassowary, *Casuarius casuarius,* has long been the focus of conservation concern in Australia. Early reports indicated that cassowaries were wary but abundant in both coastal and upland forests (White 1912) but by the 1940's concerns were being voiced about the impacts of habitat loss and hunting on their prospects (White 1946). Those concerns have only grown since those times and today cassowary conservation is a high profile issue in the Wet Tropics Region (WTR), Australia and around the world.

There are good reasons to be concerned about the cassowary. It is a large bodied (up to 74 kg, (Westcott and Reid 2002)) species that occurs at low densities, is dependent on closed tropical forests that are of limited extent in Australia, and is one of Australia's few specialist frugivorous species (Dennis and Westcott 2006; Westcott *et al.* 2008). In tropical species these are all characteristics associated with increased vulnerability to the effects of anthropogenic modification of landscapes (Owens and Bennett 2000; Sodhi *et al.* 2010; Strahl and Grajal 1991). This is of concern for these species themselves but is of concern also because of the loss of the ecological role they play in tropical forests as seed dispersers. Loss of seed dispersers results in a reduction in the quantity and quality of seed dispersal with negative consequences for recruitment leading to modifications of plant traits and of community structure (da Silva and Tabarelli 2000; Galetti *et al.* 2006; Mokany *et al.* 2014; Velho *et al.* 2012; Wotton and Kelly 2011). Thus the conservation of a species like the cassowary, which provides a unique dispersal service to a range of plant species which have limited alternative dispersal options (Bradford and Westcott 2010; 2011; Westcott *et al.* 2008), is important for the maintenance of our tropical forests.

Previous Cassowary Monitoring

Despite the early concerns about the status and trend of the cassowary no effort was made to attempt to monitor the species prior to the late 1980s. In 1986, however, Cyclone Winifred caused significant damage to coastal rainforests and prompted the Queensland National Parks and Wildlife Service and the Australian National Parks and Wildlife Service to issue a contract for a single year of monitoring in the Wet Tropics Region. The resulting study by Francis Crome and Les Moore was the first focused study of any cassowary species and resulted in a key report and journal paper which have formed the base-line for our thinking about cassowary conservation in Australia (Crome and Moore 1988; Crome and Moore 1990).

Based on field surveys, a review of the literature and responses to a public survey, Crome and Moore (1990) reported a cassowary population of 1,500-4,000 individuals. They reported on the distribution of the species across the WTR and expressed concern that the trend was still a decline in areas of agricultural and urban development. In the late 1990s, in a series of reports based on extrapolation from surveys at a number of focal sites, Moore and Moore (2001) revised these estimates to a number of less than 1,500 birds, and subsequent to this Moore (2007) reported that the species occurred at densities as little as one third those previously reported. These reports added to a perception that, in Australia at least, the cassowary was in imminent danger of extinction. The long intervening period since Crome and Moore (1990)'s region-wide surveys however made it difficult to assess whether these concerns were justified.

In this report we present the results of a region-wide re-census of the Wet Tropics Region cassowary population conducted between 2012-2014. Our objectives were to i) develop and implement a non-invasive DNA-based mark-recapture monitoring program for the cassowary in an attempt to avoid some of the issues associated with previous methods, ii) to describe the species distribution across the WTR, iii) identify key regions for the cassowary population, and if possible, iv) to conduct a back comparison with the work of Crome and Moore (1990) in an attempt to estimate the population trend of the species. The work undertaken represents Specfic Objectives of the Cassowary Recovery Plan (Latch 2007).

Methodology

Field surveys

Region-wide Surveys

Surveys were conducted on 157 transects distributed across the Wet Tropics Region (Figures 1, 2, 3). Transects were established on existing tracks and trails and their number increased as access to forest areas increased over the study and post Cyclone Yasi. Transect lengths varied from 800 m to 40 km with a mean of 6 km (±7.26 S.D.). Surveys of transects were conducted by walking transects shorter than c. 10 km while transects longer than 10 km were generally vehicle tracks and were traversed by slowly either by bicycle or car. Where a car was used 1 or more passengers scanned the track and its sides while the vehicle moved at <5 kph.

As transects were traversed, the following forms of cassowary sign were searched for; i) sightings of birds, ii) feathers, iii), footprints and iv) dung. When any sign was detected it was recorded along with a GPS fix and the date and time. Additional information for sightings included the number, sex and age of the birds seen. When feathers and dungs were encountered the samples were collected in Ziploc bags, double bagged to prevent cross-contamination, and labelled with GPS location, date, time, size of dung, freshness of dung (dry, damp, wet) and given a unique sample ID. Dung samples were returned to the laboratory on the day of collection and stored in refrigerators until processing, usually within two weeks of collection.

Focal Site Surveys

Ultimately the rate at which fresh dungs were encountered in the region-wide surveys was too low to allow the use of the faecal DNA methods in a mark-recapture analysis as was originally intended. Consequently, in order to translate the recorded incidence of sign into a population estimate we focused our effort on 'focal' sites. These sites were revisited on a roughly weekly basis over a period of 6-7 weeks.

These focal site collections were used in several ways. First, the intensive sampling at focal sites gave us a sufficiently rich data set to conduct capture-mark-recapture analyses in order to estimate population densities at those sites. Second, distributing the choice focal sites across locations with variable dung encounter rates and the altitudinal range of the species it became possible to use them as reference sites for the estimation of the relationship between dung encounter rates and cassowary density for population estimation at the scale of the WTR. Third, by repeatedly visiting a site it was possible to estimate the detectability of cassowaries on transects, i.e. the probability that cassowaries would be detected when in fact present.

At each of the focal sites a set of transects were established on existing tracks, trails and pads. The total length and geometry of these trails was determined by the availability of tracks at a site and sites were chosen on the basis that available tracks gave good coverage of a core area. On average there was 10 km (± 4.2 S.D.) of trails at a focal site. In each survey session the trails were walked and sign recorded and dung collected according to the protocols used in the region-wide surveys.

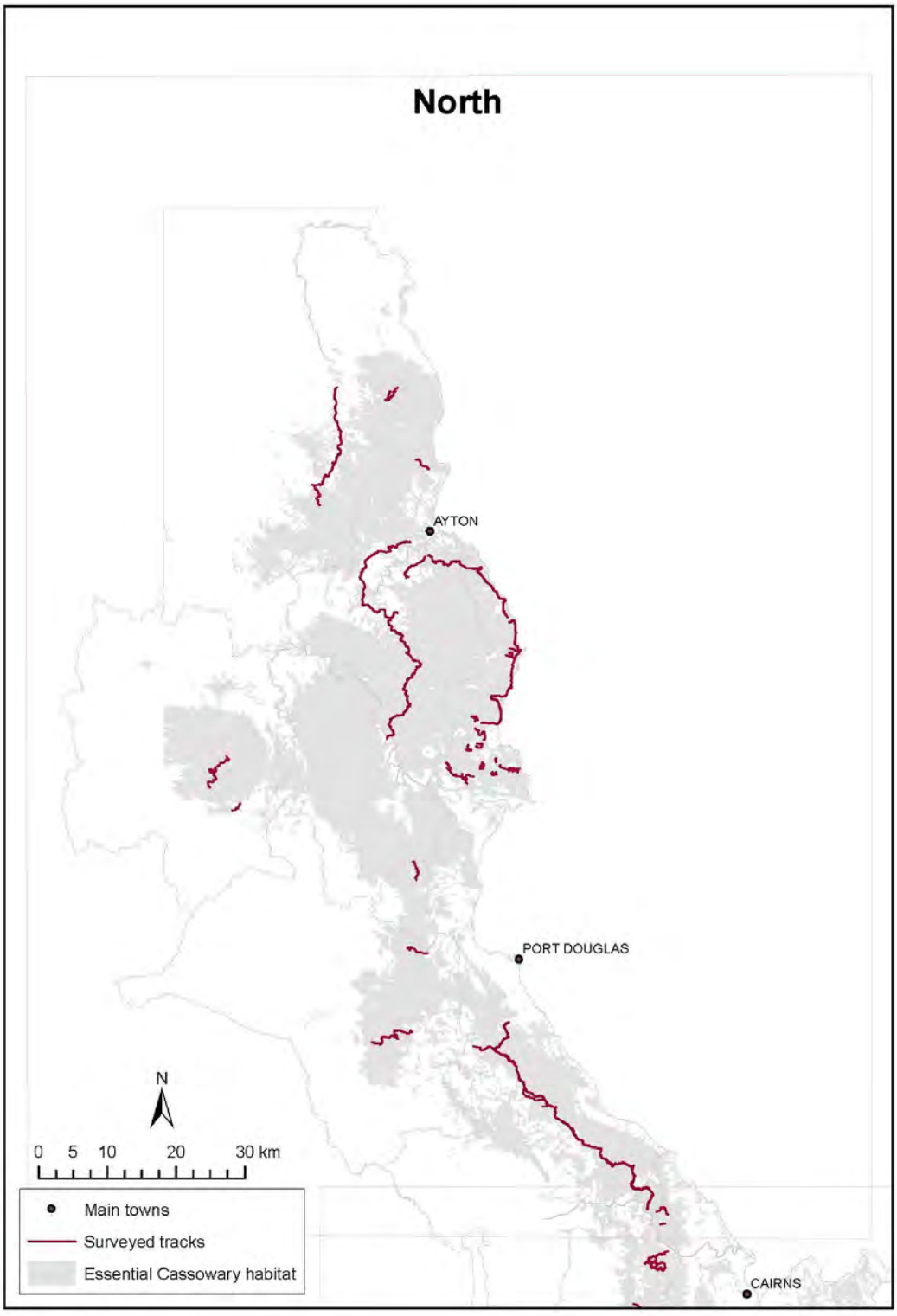


Figure 1. Map of the northern Wet Tropics Region showing the distribution of essential cassowary habitat (Kutt et al. 2004) and the distribution of survey transects

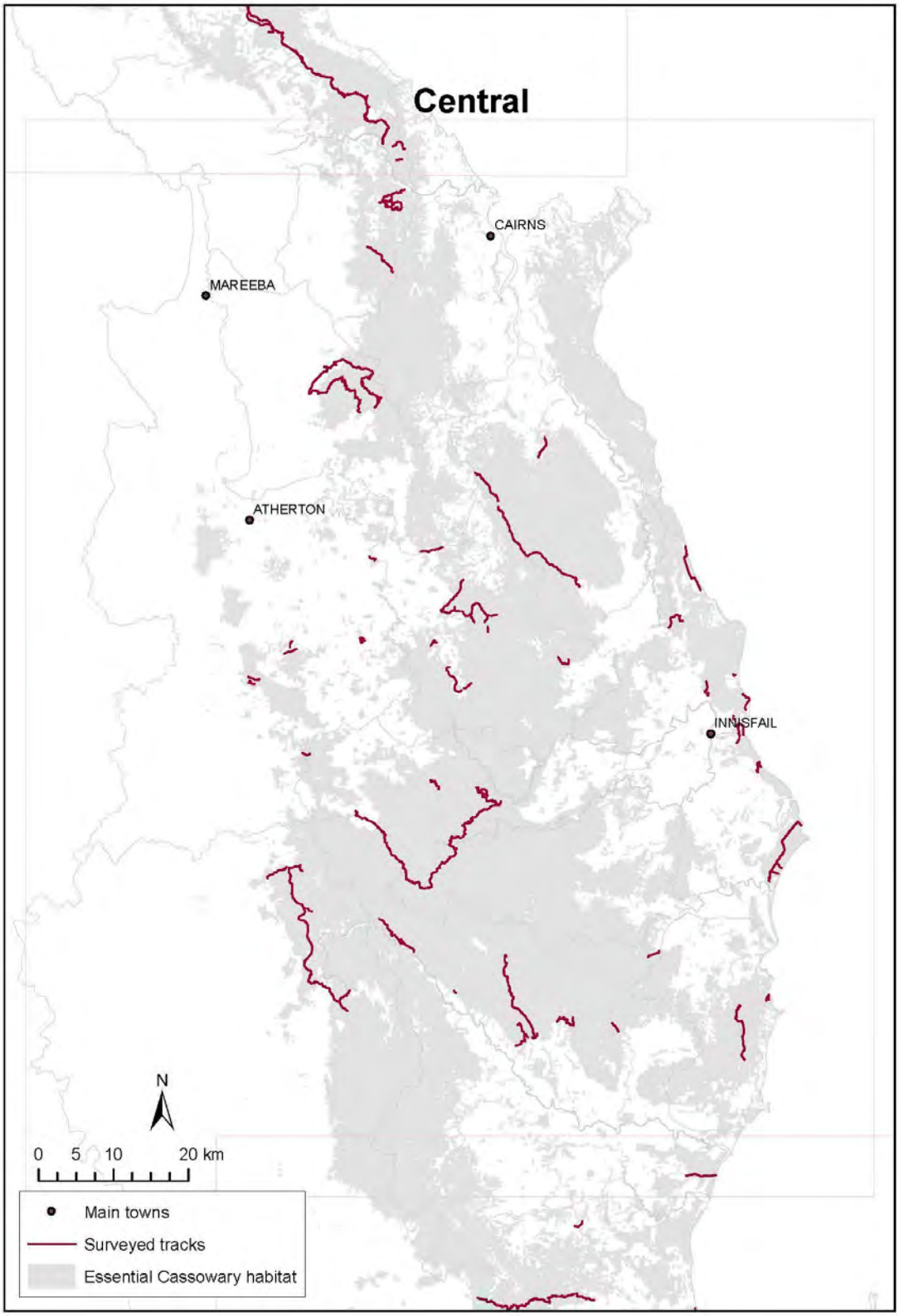


Figure 2. Map of the central Wet Tropics Region showing the distribution of essential cassowary habitat (Kutt et al. 2004) and the distribution of survey transects

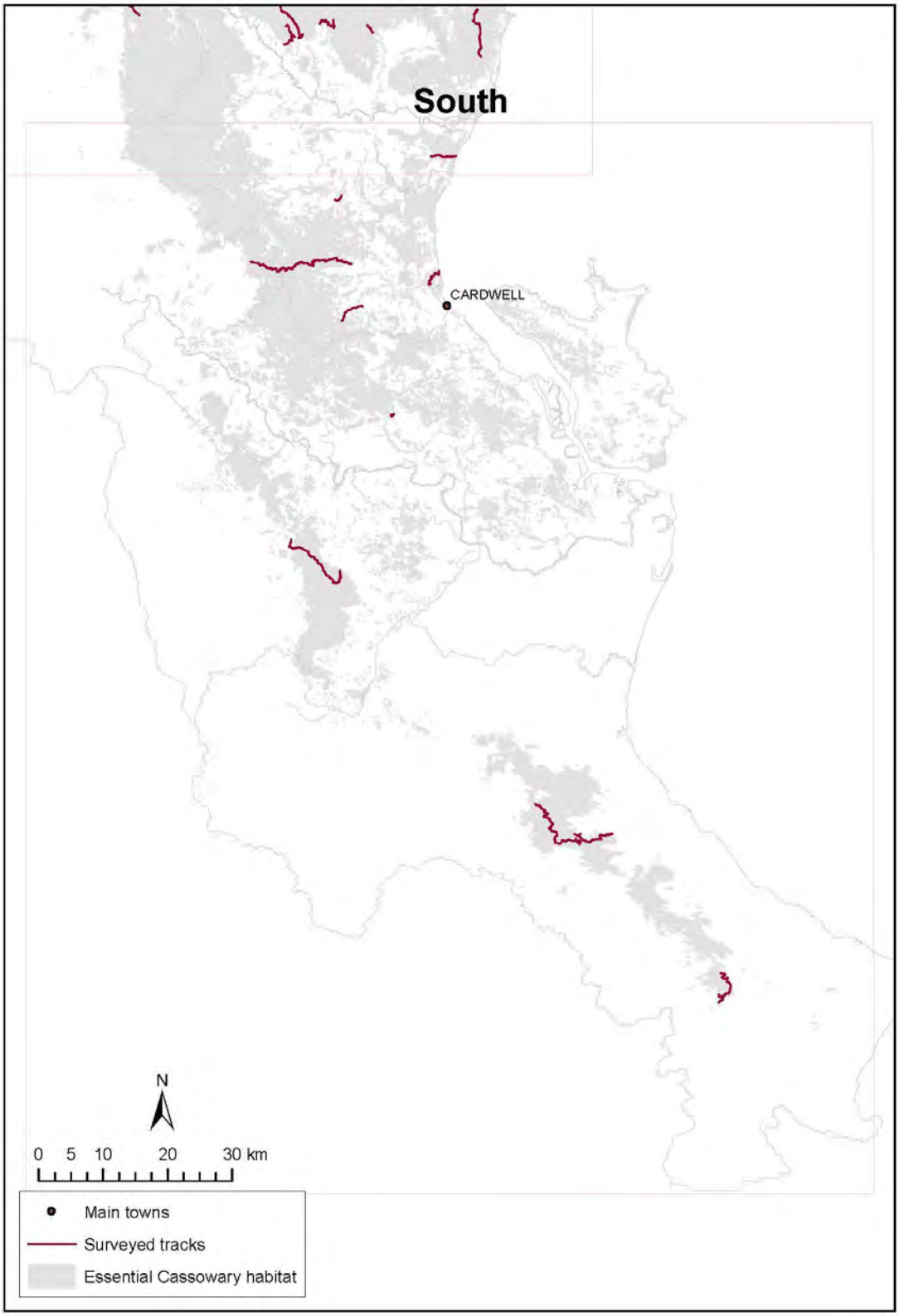


Figure 3. Map of the southern Wet Tropics Region showing the distribution of essential cassowary habitat (Kutt et al. 2004) and the distribution of survey transects.

Genetic Analyses

All fresh dungs samples were bagged at the point of collection and immediately refrigerated until laboratory analysis began.

DNA Extraction using 2CTAB/PCI Buffer

Four replicates samples, 50-100 mg in size, were scraped from different sections of the dung and placed in labeled eppendorf tubes. The samples were vigorously mixed in 1300 µl of extraction buffer, containing a mixture of hexadecyltrimethylammonium bromide (CTAB), 1 M Tris-HCL, 5 M NaCl, and 0.5M EDTA, and incubated overnight at 60 °C. The samples were centrifuged at 13,200 rpm for 5-10 min to carefully separate the supernatant from dung debris. To the supernatant, 110 µl of lysis buffer was added, containing a mixture of CTAB and 0.5 M NaCl and incubated for 2 hours at 60 °C. To this, 15 µl of Proteinase K was added and incubated for a further 1 hour, followed by 4 µl RNase A (100 mg/ml) stock and an incubation period of 1 hour at 37°C. Following this 350 µl of phenol: chloroform (1:1) mixture was added, the tubes gently inverted by hand and centrifuged at 13,200 rpm for 5 min. The resulting upper layer was then removed to a new tube. To this, 350 µl of chloroform: isoamyl alcohol (20:1) mixture was added, the tubes gently inverted by hand and centrifuged at 13,200 rpm for 5 min. The upper layer was again removed to a new tube and DNA precipitated with the addition of 1000 µl cold 75% isopropanol and allowed to stand overnight in a -20 °C freezer. The tubes were centrifuged at 14,000 rpm for 20 min, the supernatant removed and the DNA pellet washed twice with 80 µl of cold 70% ethanol and a spin step at 13,000 rpm x 10 minutes. The air dried DNA pellet was resuspended in 70 µl of AE buffer.

DNA Amplification

Genomic DNA was quantified using a Nanodrop using 2 µl of DNA. Wavelength readings were recorded at 260 nm and 280 nm. A 260/280 nm ratio between 1.8 – 2.0 nm was considered for downstream steps. Polymerase chain reaction (PCR) amplifications were performed in 10 µl reaction volumes, using 10-50 ng genomic DNA, 5 pmole of fluorescent-labelled forward primer, 5 pmole of unlabelled reverse primer, and 2x Multiplex PCR Master Mix buffer (Qiagen). PCR amplification of genomic DNA was conducted with 12 microsatellite markers (**cass 3.1, cass 5.2.3, cass 7.1, cass 3.2.4, cass 2.2.2, cass 6.2, cass 1.1.3, cass 5.2.1, emu 63**, cau 11, list 007, **cau 64**). We used fluorescent labels PET, NED, VIC and FAM, from Life Technologies . As a negative control, DNA template was replaced with distilled water. PCR steps consisted of an initial hotstart at 95 ˚C for 15 min, followed by 7 cycles of 55 ˚C x 10 sec, 72 ˚C x 30 sec, 94 ˚C x 30 sec, 55 ˚C x 10 sec, 72 ˚C x 30 sec in denaturation steps, then 32 cycles of 94 ˚C x 20 sec, 52 ˚C x 10 sec, 72 ˚C x 30 sec in amplification steps and a final extension step of 72 ˚C for10 minutes.

Fragment analyses capillary reactions were conducted on the Applied Biosystems 3730 DNA Analyzer using LIZ500 as the size standard. This was outsourced at the Ramaciotti Centre for Gene Function Analysis located at the University of New South Wales, Sydney. Fragment sizes were measured using GeneMapper Software (Life Technologies).

Sex determination

Sex determination is still underway with the sexing primers W1 and K7. Current PCR protocol using DNA extracted from the 2CTAB/PCI buffer appears to have been inhibited by residual phenol products and has produced background primer dimer and weak DNA bands. This has

necessitated a reanalysis of samples with sufficient remaining DNA. A number of different amplification steps are being tested, including several DNA column cleanup methods.

Scoring

For each microsatellite marker the frequency distribution of fragment sizes across the samples was determined. To minimize the impact of allelic dropout and false alleles we used a consensus of the sub-samples taken from each dung to define the score at each allele. Where consensus was not possible, e.g. due to equal numbers scoring for different alleles, the unique genotypes were included in the analysis as separate samples. In all such cases these sub-samples ultimately clustered together and so could be assigned to a single sample.

Genotypes were compared in GenALEx 6.5 (Peakall and Smouse 2012) to identify those that differed at fewer than four alleles: each of these genotypes were rechecked for accuracy. We considered samples that differed at three or fewer alleles but matched in size and location to be the same individual, a conclusion that will be further assessed when the sexing results are available. Including erroneous genotypes as captures in the CMR analysis would positively biased any estimates of population size (Creel *et al.* 2003).

Survey Analysis

Crome and Moore (1990) comparison

We replicated the Wet Tropics-wide score comparison presented by Crome and Moore (1988) and Crome and Moore (1990) as best as possible given the information about their analysis method that was available. This included conducting transect surveys and soliciting information on cassowaries from locals (first year only). With the passage of time and cyclones over the region it was not possible to repeat survey many of the tracks used by Crome and Moore, even had these all been identifiable. However, our surveys covered the entire region as did theirs and used similar methods making visual comparison possible.

Estimating the Population Size at Focal Sites

To estimate population size at focal sites the relative capture rates of individuals was analysed using Capwire (Miller *et al.* 2005) to give an estimate of the true number of birds using the transect. Capwire uses a maximum likelihood estimation approach to capture-mark-recapture problems. It assumes that individuals are correctly identified and that capture does not influence the probability of recapture, a safe assumption for dung. Overall Capwire works well when population sizes are small and the method has high precision and accuracy when capture rates are heterogenous. Furthermore, Capwire allows multiple captures within a sampling session, an important consideration when using faecal DNA sampling as individuals can be captured with replacement.

The resulting analyses provided estimates of size of the population using a focal site, along with the 95% CI range of this estimate. The density of cassowaries at each focal site was then estimated by adding a 500 m buffer around the transects at each site to estimate the search area and dividing the number of individuals by the resultant area. A buffer of 500 m was chosen as it corresponds to the radius of the average of previously published estimates of cassowary home range size, c. 80 ha (Bentrupperbäumer 1998; Campbell *et al.* 2012) and of our own unpublished data.

Based on the results of our focal site surveys and Capwire analyses we determined the relationship between the rates of dung and total sign encounter at a site and the number of individuals and the density of cassowaries estimated for each site. This relationship was then used to assign a population density that corresponded to the dung encounter rates recorded on each transect.

Simple Area Extrapolation Model

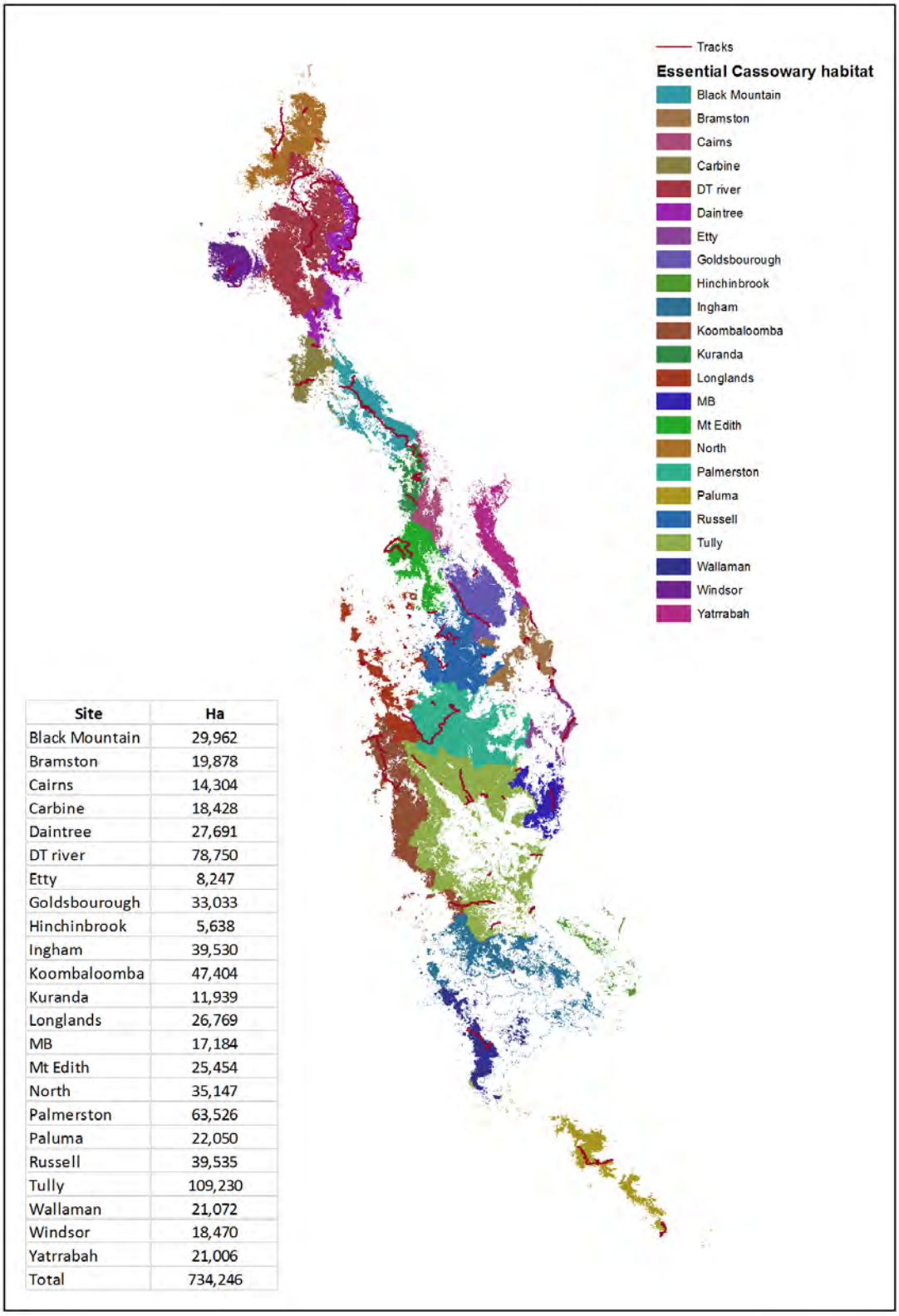
Our simplest estimate of total cassowary population size was derived as an extrapolation of the densities observed at the focal sites across the WTR. Using GIS we identified the areal extent of Essential cassowary habitat as identified by Kutt *et al.* (2004) for the Wet Tropics Region. We then multiplied this by the mean density of cassowaries across the focal sites. Three densities were used: i) estimated density, ii) low 95% CI of estimated density, iii) high 95% CI of estimated density. Precision was estimated as the 95% CI range divided by the estimated density and expressed as a percentage.

Altitudinal Extrapolation Model

Our second estimate is an estimate based on dung encounter rates recorded at different altitudes, reflecting the commonly held belief that cassowary densities decrease with increasing altitude. We determined the areal extent of Essential habitat at each in each of three altitudinal ranges, 0-450 m, 450-950 m and >950 m. We then determined the density of cassowaries at our focal sites and averaged the densities across sites in each of the altitudinal bands. Three densities were used: i) estimated density, ii) low 95% CI of estimated density, iii) high 95% CI of estimated density. These average densities were then used to estimate the population and its 95% CI range. Again, precision was estimated as the 95% CI range divided by the estimated density and expressed as a percentage.

Sub-Regional Population Estimation

Our final approach to estimating the population was to derive estimates for sub-regions based on the dung encounter rates recorded in each of the sub-regions. Sub-regions were defined on the basis of drainages with some modifications to avoid 'splitting' areas that would otherwise logically be included in the same area (**Figure 4**). For each sub-region we derived an average dung encounter rate based on the results from all transects in that sub-region. This measure was used to estimate an average cassowary density based on the relationship between dung encounter rates and cassowary density (Figure 6**Figure 6**). This density was then multiplied by the area of essential habitat in the sub-region to give its population estimate. The 95% CI range for this estimate was based on the standard deviation of the mean dung encounter score of all regions. Precision was estimated as the 95% CI range divided by the estimated density and expressed as a percentage.



Site	Ha
Black Mountain	29,962
Bramston	19,878
Cairns	14,304
Carbine	18,428
Daintree	27,691
DT river	78,750
Etty	8,247
Goldsbourough	33,033
Hinchinbrook	5,638
Ingham	39,530
Koombaloomba	47,404
Kuranda	11,939
Longlands	26,769
MB	17,184
Mt Edith	25,454
North	35,147
Palmerston	63,526
Paluma	22,050
Russell	39,535
Tully	109,230
Wallaman	21,072
Windsor	18,470
Yatrrabah	21,006
Total	734,246

Figure 4. Map of the Wet Tropics showing each of the sub-regions and the extent of essential cassowary habitat in each or them.

Results

Surveys

In the region-wide surveys a total of 1444 instances of cassowary sign were encountered over 1886 km of surveys across 156 transects. This sign comprised 1231 dungs (216 fresh, 1015 too old for extraction), 163 sets of tracks, 16 feathers and 34 sightings of one or more birds. Sign was encountered on 49% of transects with dungs encountered on 43%. Fresh dung was obtained on 30% of transects and multiple fresh dungs on just 15% of transects. This low encounter rate of multiple fresh dungs from individual transects effectively precluded the use of mark-recapture analyses based on data from the transects alone and required estimation methods based on the data obtained on the focal sites.

In the focal site surveys a total of 296 records of sign were encountered during 170 surveys. Of these 259 records were dung, 34 were tracks and 3 were sightings of one or more birds. No sign was encountered in 82 focal site surveys indicating a detection probability of 0.52. Dung was encountered at all focal sites.

Faecal DNA Analysis

Four hundred and thirty five sub-samples were successfully extracted from 134 dung samples, an average of 3.2 sub-samples per dung. The level of observed genetic diversity as indicated by the microsatellite genotyping was not high. The average number of alleles over the 12 loci was 4.5 (range 1-7) and the average observed heterozygosity was just 0.18 (range 0-0.6). While the average number of alleles per loci was higher than in our pilot work (4.5 versus 3.2), observed heterozygosity was much lower (0.18 versus 0.46). Nine of the 12 loci did not conform to Hardy-Weinberg expectations with lower than expected heterozygosities. Allelic drop out was estimated at 1.5% and samples for which it was not possible to score all alleles were not used in the analysis.

A cut-off point of less than 4 or more mis-matches (MM) between samples was used to discriminate between individuals at a site. This cut-off was chosen on the basis that sub-samples from the same dung differed by an average of 0.7 MM (±0.9 S.D.), while samples from different sites differed by a mean of 4.7 MM (±1.4 S.D.). These results are in accord with our previous comparison of dungs from known individuals and those from different individuals which indicated that a 4MM cut-off provided an appropriate balance between type 1 and type 2 errors with 96% of within dung comparisons having ≤3MM.

Crome and Moore (1990) comparison

A comparison of our sign and reporting results with those of Crome and Moore (1988) are shown in Figure 5 where the sign encounter scores are mapped on a catchment basis. Scores for sign appear to have decreased overall but the decreases are most noticeable in the Herbert catchment and Paluma Range in the south and in the Cairns area. These results indicate that there have been changes in the distribution of the cassowary, though the species is still found throughout the region. Over the three years of our study significant variation in the score of individual catchments is noticeable.

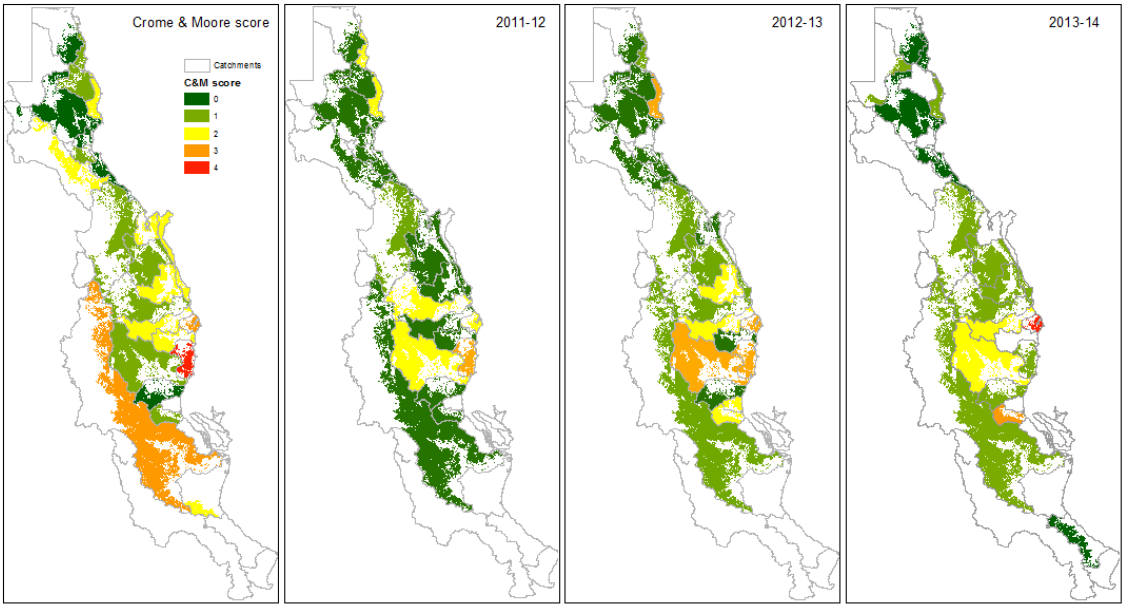


Figure 5. Comparison of the sub-regional scores derived by Crome and Moore (1988) compared with the scores derived from this study for each year of the study.

Population estimates for intensively sampled sites

The number of individuals detected at the intensively sampled sites and the corresponding estimate and confidence range estimated with Capwire are shown in **Table 1**. At all sites where more than one individual was identified, sample sizes were sufficient to give an estimate to within 10% of N (Miller *et al.* 2005). There were only minor changes in the estimated population size from the number of individuals identified with the estimate at the lower bounds of the CI interval (**Table 1**).

There was a significant relationship between dung encounter rate (dungs/km) and the estimate density of cassowaries across the sites (r_p=0.84, p<0.005; Figure 6). There was also a significant relationship between the total sign encounter rate (total sign/km) and the estimated density of cassowaries across the sites although this was slightly weaker than that of dung encounter rate (r_p=0.78, p=0.01).

Simple Area Extrapolation Model

Assuming an average home range size of 80 ha our simple area extrapolation model gave a population estimate of 4,053 with a 95% confidence range of 3,836-4,752, or a precision of 23%. Assuming a population structure of 95% sub-adult and adult birds, 5% juveniles this would correspond to an adult population of 3,836 adults and a juvenile population of 217 birds.

We assessed the effect of error in the assumption of an 80 ha home range by recalculating the population estimates for an average home range of 50 ha and 100 ha. These correspond to the smaller and the larger ends of the range of estimates of individual cassowary home range sizes.

These estimates resulted in population estimates of 5,028 (4,747 – 5,898) for a 50 ha home range, which corresponds to a 24% increase in the population estimate, and for 100 ha of 3,312 (3,134 – 3,883) or an 18% decrease in the population estimate.

Table 1. Extraction success and sample sizes for the faecal DNA analyses along with the number of individuals detected and the number of individuals estimated to be using the focal site surveys.

Population	# Dungs Extracted	Total Individuals detected	Estimated population (95% CI)	Mean # dungs/ individual
Mission Beach	21	7	7 (7-9)	3.00
Goldsborough	1	1	1	1.00
Kuranda	26	7	7 (7-8)	3.71
Palmerston	2	2	2	2.00
Robson's Ck	7	1	1	7.00
Paluma	0	0	0	0.00
Gourka Rd	61	18	21 (18-25)	3.39
Coochimbeerum	46	13	13 (13-15)	3.54
Longlands	13	4	4 (4-6)	3.25

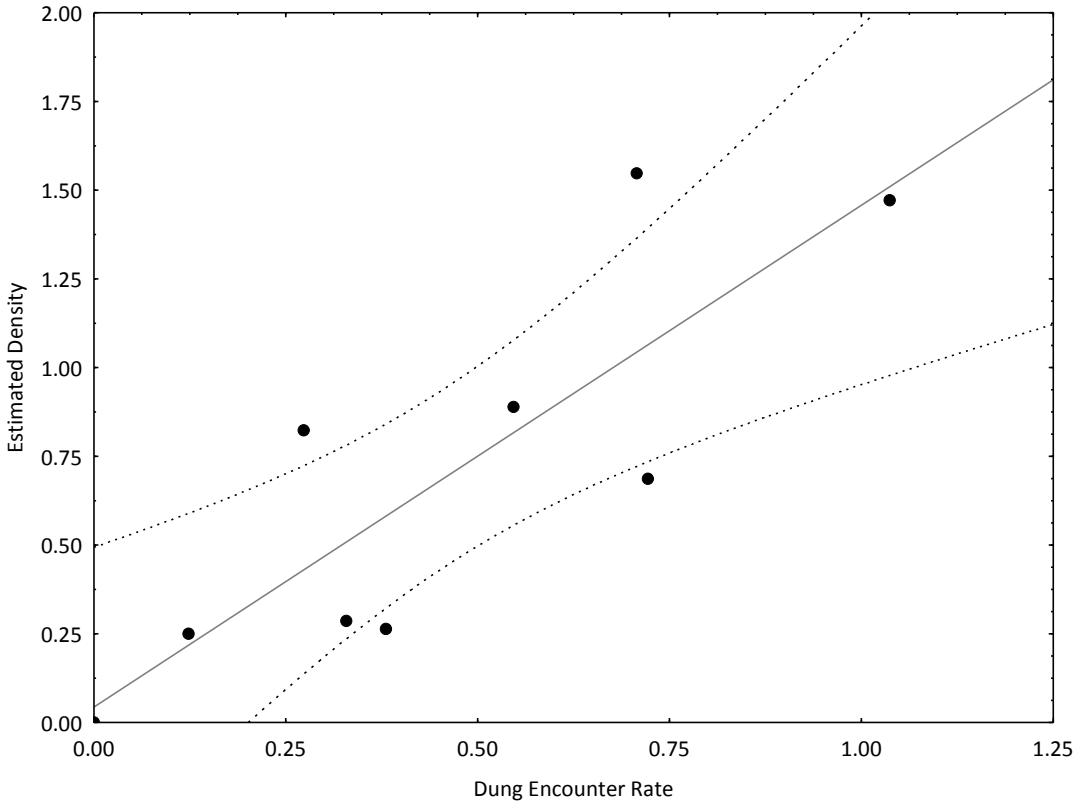


Figure 6. The relationship between the dung encounter rate (dungs/km of transect searched) at a site and the density of cassowaries estimated for the site using faecal DNA and Capwire analysis. The regression line and 95% confidence intervals for the regression are shown.

Altitudinal Area Extrapolation

Our second estimation model is based on the identified relationship between dung encounter rates and the estimated population density at the focal sites (Figure 6**Figure 6**). In this approach we assigned each of the 157 transects walked to an altitudinal range class (0-450m, 450-950m, >950m) and then estimated the mean encounter rate for each of those classes. Dung encounter rates for lowland sites averaged 0.91 dungs per km surveyed (± 1.9 S.D.), while upland and highland sites averaged 0.67 (±1.5 S.D.) and 0.26 (± 0.4 S.D.) respectively. For sign/km these values were 1.66 (±6.7 S.D.), 0.75 (±1.6 S.D.) and 0.26 (±0.44 S.D.) respectively. The average density for each altitudinal class corresponding to this mean encounter rate was then estimated using the regression equation for the relationship between dung encounter rates and estimated density and the resulting densities for each altitudinal class. The resulting estimate for the WTR as a whole is 4,353 (2,656 – 6,272 95% CI range, precision 83%).

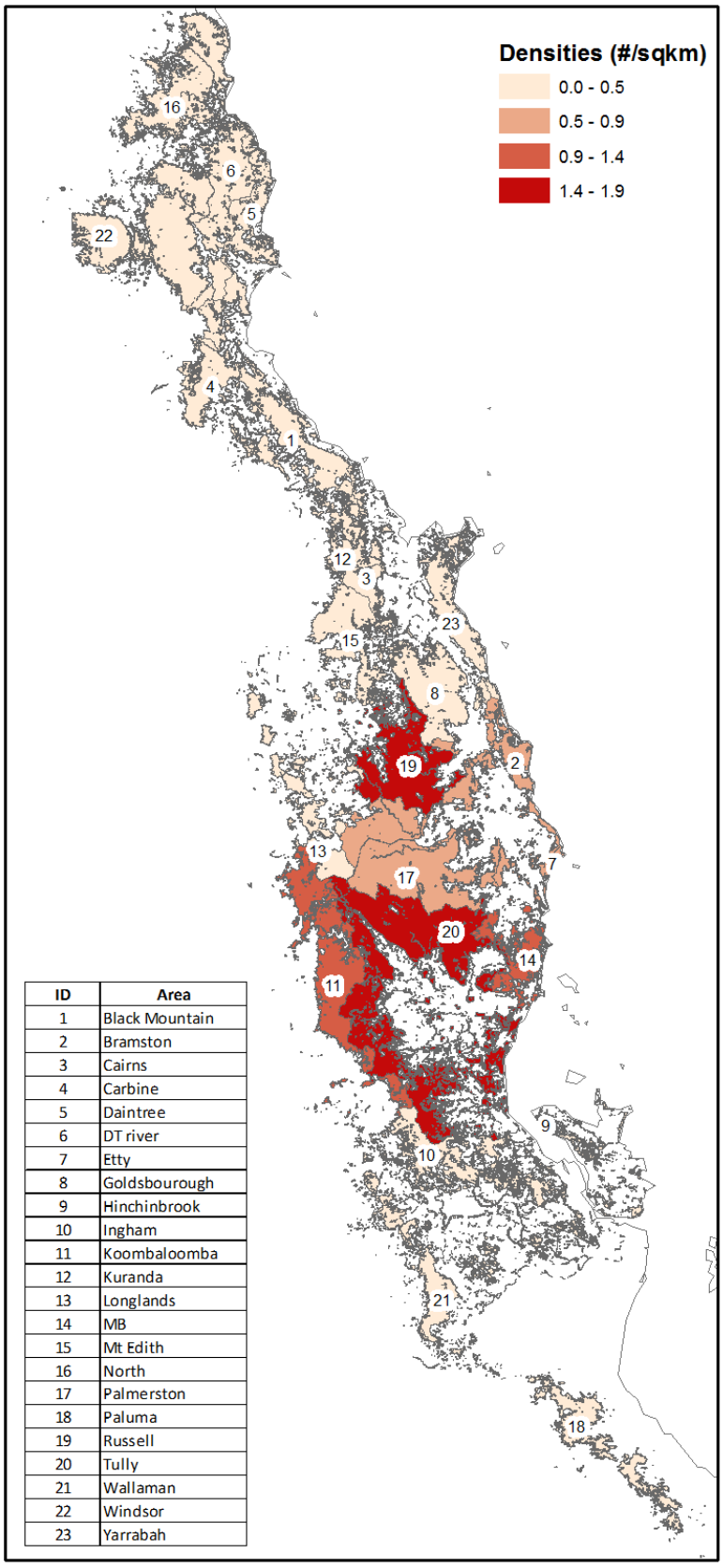
Sub-regional estimation

At the scale of the WTR the sub-regional approach gave a final population estimate of 4,381 (4,059-4,707 95% CI range, precision 15%) cassowaries with the greatest numbers of birds being found in the largest forest blocks, i.e. the Tully, Russell, Koombooloomba and Palmerston sub-regions (Table 2). There was a significant correlation between the area of a sub-region and the dung encounter rate recorded there ($r = 0.4906$, $p = 0.02$) though this correlation explained only 25% of the variation observed. Dung encounter rates in the north were lower than in the south, mean north = 0.16 (± 0.29 S.D.), mean south = 0.43 (± 0.43 S.D.), though there was no significant difference between the two areas ($t=-1.75$, $df=19$, $p=0.09$).

Table 2. Dung encounter rates, estimated densities and estimated populations for each of the sub-regions

Sub-region	Mean dung encounter rate (dungs/km)	Cassowary density (birds/km^2)	Area (ha)	Estimated Population
North	0.13	0.22	35,147	79
Windsor	0.00	0.04	18,470	0
Daintree	0.25	0.39	27,691	109
Daintree River	0.02	0.07	78,750	59
Carbine	0.00	0.04	18,428	8
Black Mountain	0.06	0.12	29,962	37
Kuranda	0.09	0.17	11,939	21
Cairns	0.00	0.04	14,304	6
Mt Edith	0.07	0.14	25,454	36
Goldsbourough	0.06	0.13	33,033	43
Russell	1.07	1.55	39,535	614
Longlands	0.22	0.35	26,769	94
Bramston	0.55	0.81	19,878	162
Palmerston	0.34	0.52	63,526	333
Koombaloomba	0.75	1.11	47,404	527
Etty	0.30	0.47	8,247	39
Mission Beach	0.74	1.09	17,184	187

Tully	1.26	1.82	109,230	1986
Ingham	0.00	0.04	39,530	17
Wallaman	0.00	0.04	21,072	9
Paluma	0.02	0.07	22,050	15



ID	Area
1	Black Mountain
2	Bramston
3	Cairns
4	Carbine
5	Daintree
6	DT river
7	Etty
8	Goldsbourough
9	Hinchinbrook
10	Ingham
11	Koombaloomba
12	Kuranda
13	Longlands
14	MB
15	Mt Edith
16	North
17	Palmerston
18	Paluma
19	Russell
20	Tully
21	Wallaman
22	Windsor
23	Yarrabah

Figure 7. Distribution of cassowary densities across the Wet Tropics Region.

Discussion

Cassowary distribution and population size

Our estimates of the size of the Wet Tropics Region's cassowary population range from 4,053 (3,134 – 3,883 CI range) for the simple area estimate, to 4,353 (2,656 – 6,272 95% CI range) and 4,381 (4,059-4,707 95% CI range) for the sub-regional estimate. The narrowness of this band of estimates is in part a function of the fact that all three of these methods are based on shared assumptions about the relationship between dung encounter rates and abundance and about the size of cassowary home ranges. Even so, these methods make very different assumptions about how these relationships are utilized and the concordance observed suggests that, given these assumptions, the results are relatively robust. While the three different approaches produced relatively similar population estimates, we estimated very different levels of precision for each of them (23, 83, and 15% respectively). These precision values would suggest that the greatest confidence can be had in the sub-regional estimate of 4,381.

The estimates we report here are much higher than is commonly claimed in the media for cassowaries, however, they are in line with most previously published estimates and densities. Crome and Moore (1990) estimated the 1988 population size to be 1,500-4,000 animals. In contrast, based on extrapolation from focal sites, Moore and Moore (2001) suggested that there were fewer than 1,500 cassowaries in the region, though it is unclear how this figure was arrived at. In subsequent work, Moore (2007) used individual identification based on sightings and footprint characteristics to estimate population densities at Mission Beach and (presumably using the same methods) at Daintree. He concluded that the densities he found were far lower than those encountered by Bentrupperbäumer (1998) and Crome and Moore (1988). There are significant issues associated with identifying individuals based on track characteristics with other species and there is little reason to think that similar concerns do not also hold for cassowaries. If we ignore these concerns for the moment, however, we find that Moore (2007)'s estimated densities for Mission Beach and the Daintree (0.49 and 0.45 individuals/km^2 respectively) sit well within the range of our density estimates for the different sub-regions (**Table 2**) and when extrapolated to the WTR using a simple area extrapolation result in estimates that are only 15-25% lower than ours. Overall, our estimates, though higher than the previous estimates, are not greatly different to them and this convergence adds to confidence in our results. We suggests that cassowary population sizes, in the absence of any apparent driver of population change, have probably remained relatively stable since the surveys of Crome and Moore (1990).

Our surveys found evidence of cassowaries in all sub-regions with the exception of Mt Windsor, and we collected dung from all but six regions; Cairns, Paluma, Ingham, Wallaman, Carbine and Mt Windsor. The failure to detect cassowary dung in the three southern sub-regions is possibly a function of low numbers due to the direct and indirect impacts of Cyclone Yasi in these areas and is consistent with reports from locals that cassowaries are less frequently encountered since that event and is surprising, particularly in the case of Wallaman where sign has previously been common. The failure to detect any sign of cassowaries at Mt Windsor, however, probably reflects reality. We do not know of any records or reports of cassowaries at Mt Windsor, despite significant forestry, research and grazing activity there over the last 70 years. It is possible that this is a function of the barrier that the Daintree River valley represents to cassowary movement combined with the relatively small area and perhaps an insufficient year-round fruit resource in the area (Crome and Moore 1988; Crome and Moore 1990).

Comparison of the distribution results of our work with that of Crome and Moore (1988; 1990) suggests that there has been little change in the distribution of cassowaries in the 26 years since their surveys (**Figure 5**) with cassowaries still found throughout the region. There were however significant differences in the sign scores obtained for different regions in the two studies. For example, Crome and Moore (1988; 1990) encountered many cassowaries in the Lamb Range and Lake Tinaroo area and they identified these areas as important for the population. At the

same time they recorded very low numbers of birds in locations such as the Palmerston. In contrast, we estimate low numbers at Lamb Range and Tinaroo and much healthier numbers in the Palmerston area. The variation in sign scores across the three years of our surveys leads us to suggest that while these differences may in part be the result of changes in population at these sites over the intervening period, chance is likely to also play a significant role. With cassowary densities being low, the chance of detecting sign on any given visit to a transect, or even set of visits, also remains low even if the animals are present. This has the important implication that confidence in the score assigned to a transect can only improve with each additional visit and that multiple visits and multiple years of visits are required in order to get a high level of certainty.

Across the three years of surveys conducted in this project we found that the sub-regions with the highest estimated densities were those at low to mid elevation with extensive areas of essential habitat, for example the Tully and Russell sub-regions. Contiguous high elevation, large area sub-regions also scored highly, e.g. Koombooloomba. Ranking slightly below these are the coastal sub-regions that are generally assumed to be the stronghold of the species, and below these an undefined group of sub-regions that span a variety of altitudes and contexts. High density sub-regions were all located in the central section of the WTR between the Herbert River and Atherton Tablelands (Figure 7).

Our estimates of population structure were at 5%. This is a surprisingly low figure given that we conducted surveys towards the end of the breeding season each year. However, given that the second round of surveys were conducted during the wet season and well after the breeding season had finished it is possible that surviving young of the year had grown to a size where their identification by dung size was less reliable.

Methodological Considerations

This project was originally conceived of as a mark-capture-recapture study based on surveys conducted on transects across the region. The successful application of this approach relied on the collection of multiple dungs on a majority of transects, an expectation that seemed reasonable based on our previous experience at Wooroonooran, Speerwah and Mission Beach. However, we ultimately encountered fresh dung on only 30% of transects and multiple fresh dungs on just 15% of transects. We initially interpreted these low encounter rates as an effect of Cyclone Yasi which passed over the region just prior to our study, however, as time passed it became clear that low dung encounter rates were to be expected. This effectively precluded the use of mark-recapture methods for estimating density and necessitated a modification of our approach.

The method we ultimately settled upon combines the region-wide surveys with more intensive short-term sampling at focal sites. The region-wide surveys provided data on dung encounter rates while intensive sampling at focal sites provided the means of translating these dung encounter rates into estimates of population density. This method was made possible by the strong and positive relationship between dung encounter rates and the number of individuals detected by the faecal DNA results (Figure 6). While this relationship was not unexpected, previous work based on sightings of individuals, had suggested that it would not be strong enough to use in this manner (Westcott 1999). Fortunately, when faecal DNA fingerprinting was used to identify local population sizes, the relationship becomes highly significant and explains the majority of the variation observed (Figure 6). This made estimation of density from dung encounter rates possible and provides a basis for assessments of cassowary populations in future monitoring. With appropriate resourcing and access this means an effective census of cassowary populations can be achieved over a period of a couple of months of field work and the method can be applied in all parts of the species range. In Australia this opens up the possibility of conducting a census of the population on Cape York for which there is currently little information on cassowary distribution and no data on abundance.

A critical component of this approach is the performance of the individual identification through DNA fingerprinting. In our pilot work we encountered reasonable levels of diversity and heterozygosity that made individual identification relatively simple. In this project, while the levels of diversity were higher than in our pilot work, the levels of heterozygosity encountered were much lower, less than half the previous figure. Though the heterozygosity proved sufficient for individual discrimination, further investment in the development of additional and more variable markers should be a priority to ensure that future censuses are cheaper and can be conducted more efficiently.

Our estimation of density from dung encounter rates is sensitive to assumptions about the average size of a cassowary home range. Unfortunately, this is a difficult assumption to test without adequate telemetry data from across the region or more extensive surveys at the focal sites. Our estimate of 80 ha was the average of the home ranges reported in Bentrupperbäumer (1998) and Campbell *et al.* (2012) and those obtained in our own telemetry work at Wooroonooran (Westcott, unpubl. data). This represents a sample of less than 20 birds with most observed for relatively short periods of time and whether it adequately reflects cassowary home range size is uncertain. To assess the effect of this assumption on our estimates we varied the assumed home range size and recalculated population size using the simple extrapolation method. We found that a reduction in the average home range size to 50 ha, i.e. a 38% decrease and one of the smaller home range estimates reported for non-urban cassowaries (Bentrupperbäumer 1998; Campbell *et al.* 2012), would result in a 24% increase in the estimated population. A 25% increase in the assumed average home range size, to 100 ha and at the larger end of the home range sizes, would result in an 18% decrease in the population estimate. While the changes in the population estimate associated with these differences in average home range size are significant, they are not sufficiently large to change how we might approach management of cassowaries. In other words, the effect of the error is likely to be quantitative rather than qualitative and is unlikely to modify the status of the species or the urgency of management action.

Implications

Our work suggest that the cassowary population of the Wet Tropics Region is comprised of approximately 4400 birds with a minimum of 5% of these being young of the year. While this is a larger population than is commonly reported in the media, we see in it no cause for complacency about the species' status. A population of just 4400 is not large and places a species is at greater risk from chance events and genetic effects than would otherwise be the case (Frankham *et al.* 2014; IUCN 2010; Rosenfeld 2014). In addition, the WTR cassowary population is distributed across a complex landscape and, particularly on the western and eastern margins, across highly fragmented landscapes. While cassowaries are capable of crossing the gaps between habitat islands, their ability to do so is being increasingly eroded by anthropogenic changes and activities in the intervening habitat. When movement between areas of habitat is limited there is reduced opportunity for mixing and less chance of 'rescue' effects should these be required. These two characteristics of a species, small population size and fragmented range, are among the factors given high priority in increasing a species' threat status under classification systems such as the IUCN's Red List criteria (IUCN 2010). This should be sufficient cause for concern alone, however, under projections for the distribution of cassowary habitat under future climates (Mokany et al. 2014; Mokany et al, in press) it appears inevitable that essential cassowary habitat will decrease in areal extent and increase in the degree to which it is fragmented. Add to this the predictions of more intense cyclones and the outlook is not encouraging (Hilbert *et al.* 2014).

The inference to make from this is that we can expect that the already low cassowary population will decrease in size and become increasingly fragmented in coming decades. Add to this the fact that because of the species' low population density and cryptic habits it is very

likely that there would be a long time lag between declines in the wild and detection and the implementation of any form of management response and the status of the species becomes even more tenuous. For these reasons we see no reason to change the species' conservation listing or to consider that we can take our focus off its status and trend.

Based on this assessment we recommend that future investment in cassowary management should focus on four tasks which fall under the categories of habitat protection and monitoring. Ultimately, the size of the cassowary population is a function of the extent of cassowary habitat, thus increasing the availability and quality of that habitat will be fundamental to securing the species' future in the wild. Secondly, improving the connectedness of cassowary habitat will not only increase the availability of habitat but will facilitate the movement of birds, and genes, through the landscape. There are currently a range of private and agency programs operating to facilitate the purchase and rehabilitation of habitat and these programs should be supported.

The third area is the establishment of a regular monitoring program. We can only be confident about the management of cassowaries if we actually know the status and trend of the population. This requires investment in the establishment of an on-going cassowary monitoring program. The current program has demonstrated that an effective monitoring program can be established relatively cheaply at the scale of the species range. With some further refinement such a program can provide regular updates on the species' trends as well as describing key life-history parameters necessary for predictive monitoring of the species' population dynamics. We recommend that i) monitoring at focal sites be continued and expanded in order to describe life history and population parameters to underpin population monitoring and that ii) region-wide monitoring be conducted at regular intervals of not more than five years to ensure up-to-date data on distribution and abundance are available.

Finally, to date the focus of cassowary conservation has been on the WTR. In contrast, Cape York, where potentially as much as 4,885 km^2 of habitat is found (Latch 2007), has received virtually no attention. Recent surveys in the northern section of McIlwraith Range on Cape York suggest that the habitat is more fragmented than that of the WTR and that it most likely supports a lower density of birds. However, without a specific focus on this important population this is just speculation. Increasing momentum for development and improved access to many parts of the Cape have raised concerns about the future of cassowaries in the region, particularly amongst Traditional Owners. There opportunity to work with Traditional Owners to establish a monitoring program on the Cape must be explored.

Conclusion

Our work has demonstrated the utility of faecal DNA-based capture-mark-recapture methods for cassowary monitoring and we suggest that this approach be further refined and adopted as the standard for cassowary monitoring at both local and regional scales. Our estimate of 4,381 cassowaries in the Wet Tropics Region indicates that this is a species that is still of conservation concern and which must remain a focus of management. Monitoring must become a central component of our approach to cassowary management and we recommend that the current approach of a bout of monitoring every 25 years be abandoned in favour of a regular and more frequent program. Because of the potential of faecal DNA monitoring for cost effective elucidation of key population parameters and processes at both local and regional scales we recommend that a monitoring program be maintained as an on-going activity at local scales with regional monitoring conducted at a maximum interval of five years. To date there has been a focus on cassowaries in the Wet Tropics Region and it is important that attention is now given to the species on Cape York. We recommend that a program of faecal DNA-based monitoring be established with Traditional Owners on Cape York immediately.

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