31 July2016
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Dr Mark Eldridge
Chairperson
NSW Scientific Committee

Further Information for the Nomination to the NSW SCIENTIFIC COMMITTEE, the "Koala population in the Port Stephens LGA" as an "endangered population" under the *Threatened Species Conservation Act 1995.*

Dear Mark,

The following correspondence (dated the 26 November 2016) was received from the NSW Scientific Committee requesting further information regarding the above nomination.

"While the committee noted there was evidence of decline, the population does not appear to be disjunct or genetically, morphologically or ecologically distinct. Nor does it appear that the population has significant conservation value that would distinguish it from other coastal populations in NSW.

"In order to assist with the assessment the committee would appreciate additional information demonstrating how the population meets the following criteria:

□ it is disjunct or near the limit of its geographic range;

□ it is genetically, morphologically or ecologically distinct;

□ it is otherwise of significant conservation value

"It should be noted that the committee may reject a nomination if the information is inadequate and does not satisfy the Committee's request."

Information pursuant to this request is outlined below. It details some new developments in our understanding of Koala population genetics and provides an update on the Koala care data for last year.

□ it is disjunct or near the limit of its geographic range;

The original nomination made the assertion that the Port Stephens Koala population is not disjunct or near the limit of the species' range. This can now be qualified following further analysis of the extent and barriers to populations in the lower Hunter (Figure 1).

This nomination is concerned with the two sub- or local populations in the Port Stephens LGA, #1 (Tomaree) and #2 (Tilligerry). These two neighbouring populations while closely located now seem to be divided principally by the tidal Tilligerry Creek, with few records of

animals in parts of the LGA which connect the two peninsulars. Records of Koalas over the last 12 years indicate that there may be a local population associated with the western section of the LGA (#3) between the towns of Morpeth and Clarence Town and possibly ranging up to the Dungog. Historically, these three populations would have formed one meta-population, though given levels of habitat fragmentation and the paucity of recent records, this inland sub-population may not be in genetic contact with the two coastal populations. Data from this inland population is not strong enough to indicate trends and so consideration of data from this locality has not been further considered in this nomination, though would be included within the definition of the Endangered Population.

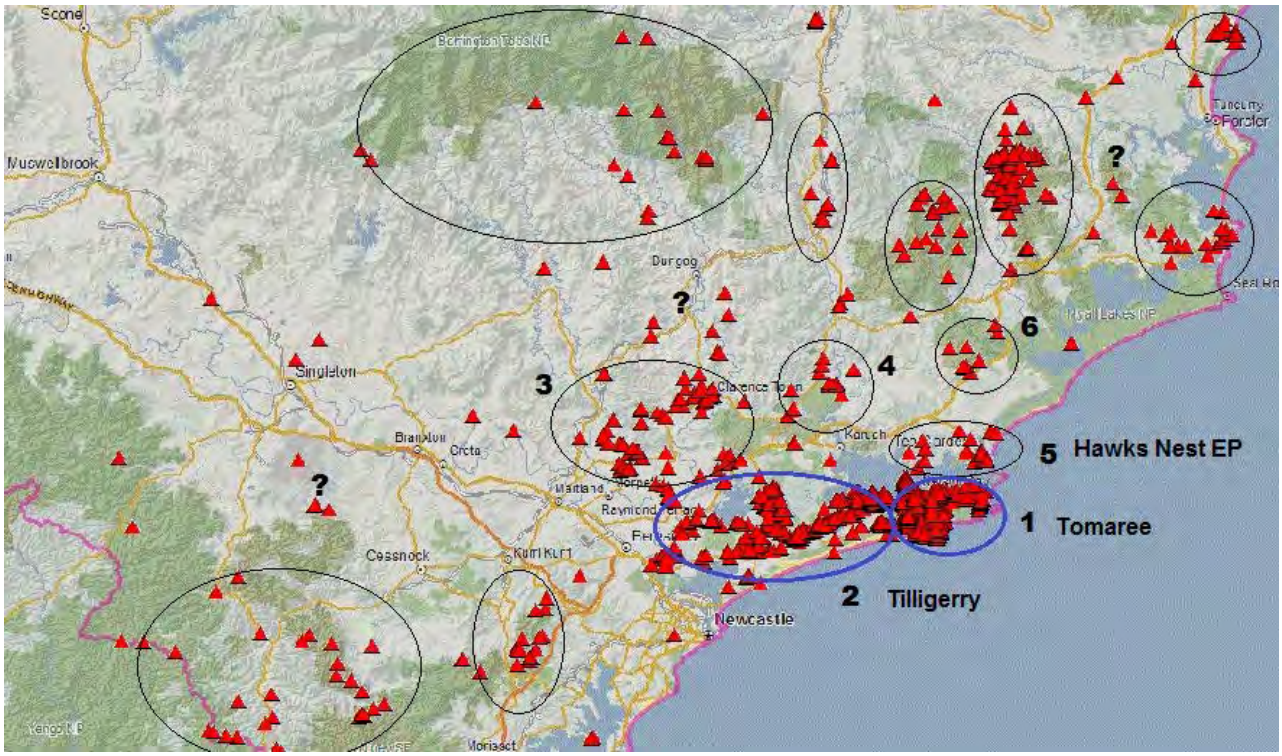

Figure 1. Distribution of populations in the lower Hunter region. All locations in the last 12 years are indicated (Source: BioNet 31/07/2016)

None of the other populations indicated in Figure 1 are in direct contact with the Port Stephens koalas. The Karuah River forms a significant barrier to movement of koalas into the Great Lakes LGA (#4 Karuah, #5 Hawks Nest Endangered Population and #6 Myall Lakes). To the south, the Hunter River seems to be a significant barrier to koala dispersal, particularly since large areas of the Hunter valley have been cleared of native vegetation. This is reflected in an absence of records of koalas in the central part of the Hunter valley, with closest populations south of the Hunter associated with the inland areas of Lake Macquarie and Yengo National Park.

Given the extent of habitat removal from the more fertile parts of the lower Hunter and the natural barriers of the Hunter and Karuah Rivers, it would be reasonable to say that the Port Stephens populations are disjunct from neighbouring meta-populations.

□ it is genetically, morphologically or ecologically distinct;

Morphological and geographical variation in the koala is recognised, generally based on skull morphology and general phenotype (size and fur colour). Koalas have tentatively in the past been grouped into three 'sub-species', loosely corresponding to the state political borders along the east coast of Australia (Natural Resource Management Ministerial Council (2009). Genetic studies carried out at the time did not detect sufficient levels of differentiation for a sub-species classification (Houlden et al.1999). This has led to the widely held view that koalas have reduced genetic diversity across their range (Lee et al. 2010; Melzer et al. 2000; Phillips 2000), generally based on limited studies using neutral markers. However, this has been challenged by a number of more recent studies which highlight the genetic diversity both within and between populations (Fowler et al. 2000; Lau et al. 2014). In particular, the results a recent genomic study, which included samples from five of the sites used in previous studies, indicate that in fact koalas may have equivalent genetic diversity to other stable outbred wild taxa (Kjeldsen et al. 2016).

Two grants from the Australian Research Council (ARC)/Linkage Projects to a cross institution team has provided the first genome-wide SNP marker panel in the koala, using a reduced representation genome sequencing method known as double digest restriction-associated sequencing. 33,019 loci were identified in the koala with a filtered panel of 3060 high-utility SNP markers, including 95 sex-linked markers. These were "…*used to provide key insights into population variability and genomic variation in 171 koalas from eight populations across their geographic range*". Broad-scale genetic differentiation between geographically separated populations was assessed and revealed significant differentiation between all populations (FST range=0.01–0.28), with the largest divergence observed between the three geographically distant subgroups in Queensland, New South Wales and Victoria (average FST range=0.17–0.23). Assignment tests correctly assigned 100 % of individuals to their source population, with only 15.85 % of individuals being assigned to a second, geographically close population.

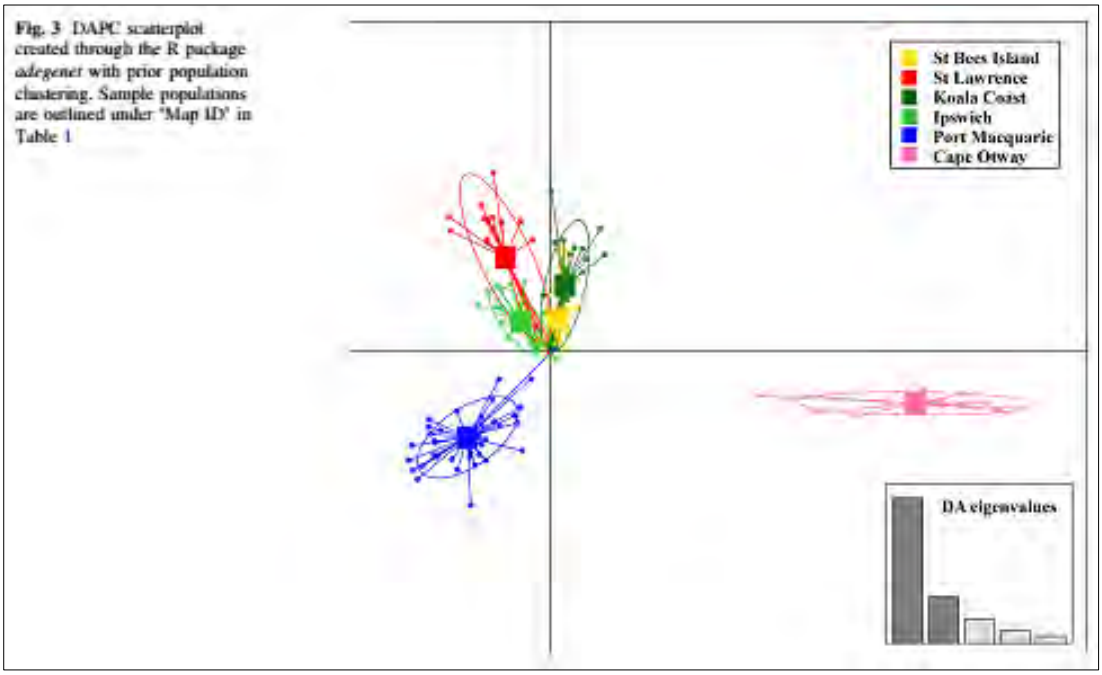

Fig. 3 DAPC scatterplot created through the R package *adegenet* with prior population clustering. Sample populations are outlined under 'Map ID' in Table 1

The study found that despite being geographically further apart, the Port Macquarie population revealed less differentiation from the population in Ipswich, QLD (FST0.11) than from Campbelltown, NSW (FST0.13). Broad-scale genetic structuring using NETVIEW

(which does not include the Campbelltown population in the analysis) revealed three major genetic clusters across the sampled populations, with the QLD populations in one large group, Port Macquarie clustering out on its own, and Cape Otway clustering apart from Port Macquarie (as shown in the DAPC scatterplot above). A similar trend was observed in the MHC diversity study (Lau et al.2014).

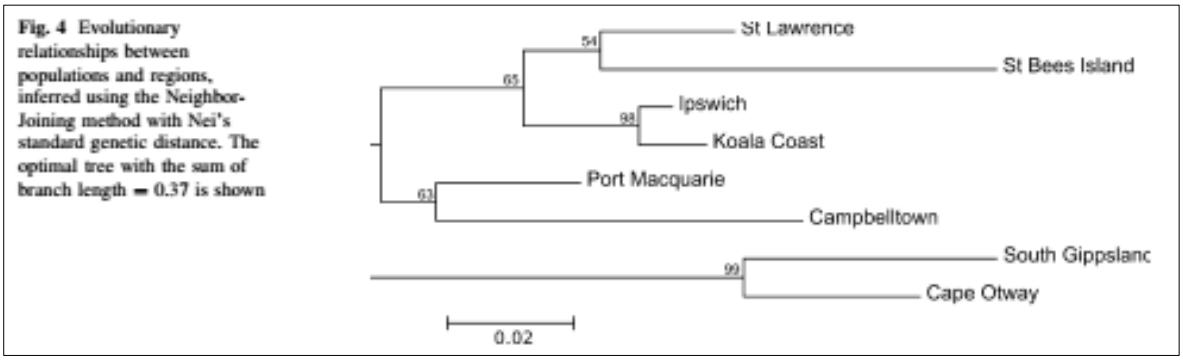

Fig. 4 Evolutionary relationships between populations and regions, inferred using the Neighbor-Joining method with Nei's standard genetic distance. The optimal tree with the sum of branch length = 0.37 is shown

The NJ tree (Fig.4 above) shows three genetic clusters, but also reveals that both the NSW populations cluster more closely with populations in QLD, rather than their Victorian counterparts. This is supported by smaller FST values between these groups.

Kjeldsen et al. (2016) suggest it "…*appears that across Australia only two large genetic groups may be present, although a denser sampling strategy should be observed in future studies to confirm this. The largest variation between groups was observed between two of the most geographically distant mainland populations assessed in this study (St Lawrence, QLD and South Gippsland, VIC) and genetic distances between populations were generally high. However, FST values of 0.3–0.4 and a greater variation between sub-species groups would have been needed to provide support to any sub-species classification (Frankham et al. 2002), and this was not observed in this dataset*."

So, while it is clear that a general isolation by distance effect is being observed in koala populations, what is not clear is the relationship between the different genetic groups in New South Wales and whether there is a transition zone between northern and southern genetic units. The data presented in this study clearly suggests that the Port Macquarie population is more closely related to the QLD populations than it is to the Campbelltown one, though Kjeldsen et al (2016) complicate the matter by suggesting that the two NSW populations may have a common evolutionary source.

Further sub-sampling of populations within New South Wales is clearly needed to complete an understanding of the relationships between these groups. While the Port Stephens population was not sampled in this study, it is a population of genetic interest lying between the Port Macquarie and the Campbelltown units. Samples are currently being gathered from Port Stephens (Prof. H. Raadsma, UoS, pers. comm.) who is of a view that the Port Stephens population may lie at the southern extremity of the 'northern' genetic group.

This does make some sense if the significant barrier of the Hunter River and lack of records of the koala between the Port Stephens and the Yengo and Wollemi area are considered. Given this new information, I believe the Committee should take into account the potential genetic importance of the Port Stephens population.

There is also the documented trend among koala populations to show increased genetic isolation through the growing magnitude of barriers. Dudaniec et al. (2013) found that gene flow resistance was assisted by man-made barriers at a local scale;

"…foliage projective cover (FPC) facilitates high gene flow (i.e. low resistance) until it falls below approximately 30%. Out of six additional land-cover variables, only highways and freeways further explained genetic distance after accounting for the effect of FPC".

Increasing levels of habitat fragmentation in the Port Stephens area also leading to reduced contact between the two sub-populations on Tomaree and Tilligerry peninsulars and possibly to the west of the LGA. An update on the status of the koalas in the LGA is provided below, though it is apparent that Koalas have all but disappeared from many parts of the LGA and that trends of decline indicated in the initial nomination have continued in 2015 and 2016.

From an ecological point of view, while Koala populations tend to have unique ecological characteristics, such as local food tree and habitat preferences, the ecological preferences of the Port Stephens Koalas is broadly not that distinctive from other coastal populations on the north coast where food tree preferences, particularly a preference for Swamp Mahogany, Forest Red Gum and Grey Gum are shared by other populations in the Great Lakes, Port Macquarie and Iluka areas, though differs in two respects:

- species composition of this swamp ecological community is not the same in these different locations.
- the important north coast food tree Tallowwood *E. microcorys* is virtually absent in the Port Stephens LGA.

References

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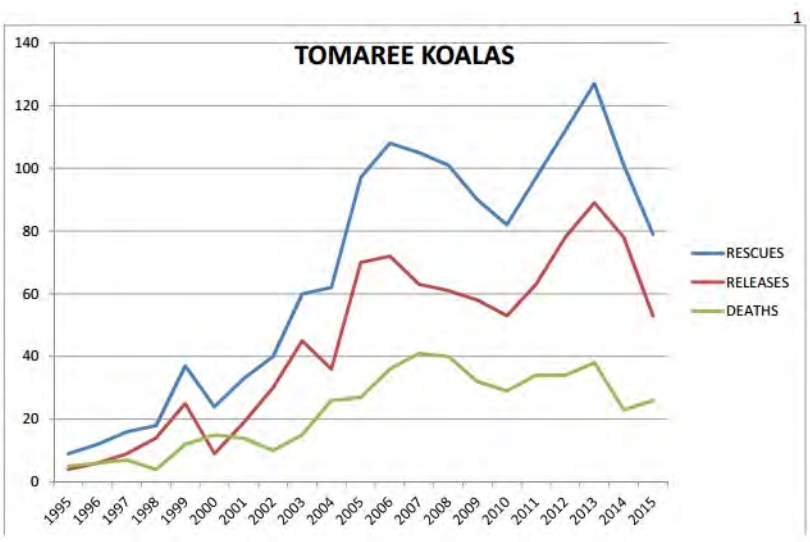
Update on call-out figures from HKPS (2015-16).

Tomaree Peninsular

The alarming downward trend in overall numbers of Koalas continued in 2015 with very low numbers so far in 2016 (up to May) with a small rise in the number deaths in 2015 over 2014.

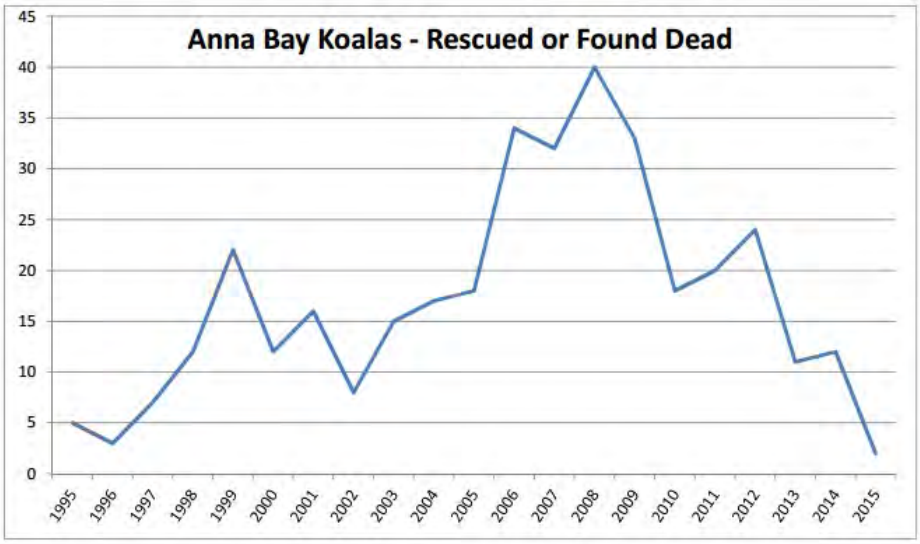
Year	Rescues	Release	Deaths
	1		1
1995	9	4	5
1996	12	6	6
1997	16	9	7
1998	18	14	4
1999	37	25	12
2000	24	9	15
2001	33	19	14
2002	40	30	10
2003	60	45	15
2004	62	36	26
2005	97	70	27
2006	108	72	36
2007	105	63	41
2008	101	61	40
2009	90	58	32
2010	82	53	29
2011	97	63	34
2012	112	78	34
2013	127	89	38
2014	101	78	23
2015	79	53	26
2016	20	14	5



By location the figures are presented below show the total number of call-outs decreased from 101 in 2014 to 79 in 2015. Koalas have not been seen in Bobs Farm or Fingal Bay for some years and the numbers from Anna Bay have dropped alarmingly in recent years (2nd below).

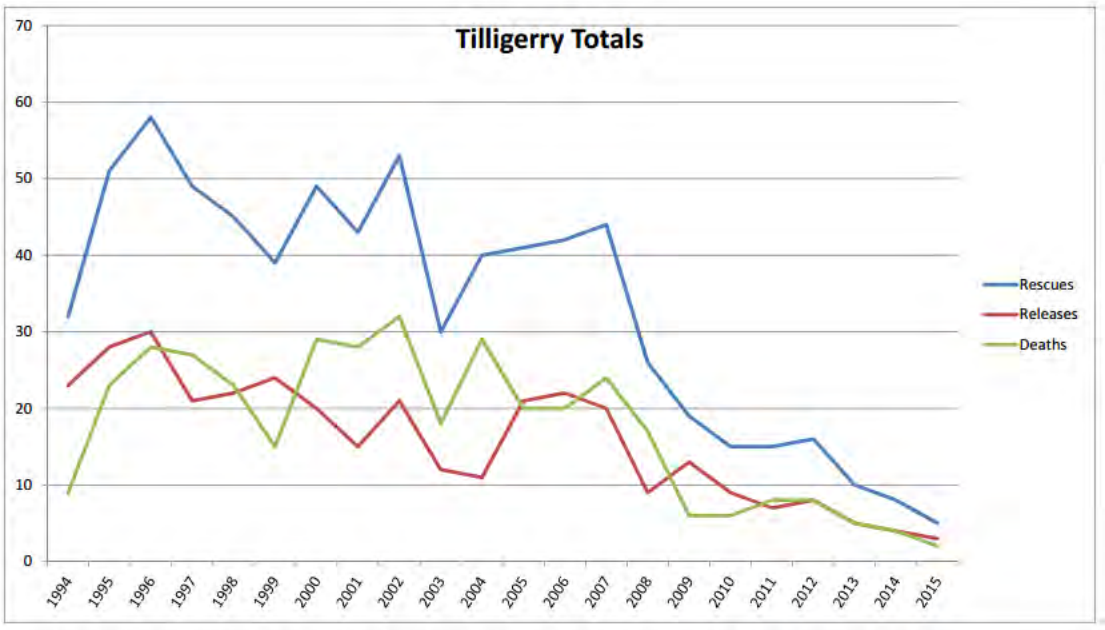
Year	*Total*	*Anna Bay*	*Boat Harbour*	*Bob's Farm*	*Fingal Bay*	*Nelson Bay*	*One Mile*	*Salamander Bay*	*Shoal Bay*	*Soldier's Pt*	*Taylor's Beach*
2009	90	33	3	4	1	9	18	13	1		4
2010	82	18	5	4	2	7	17	17	3	6	3
2011	97	20	7	4	2	5	15	30		4	4
2012	112	24	10	2	5	4	11	24	2	6	7
2013	127	11	1	1	1	8	18	46	3	16	8
2014	101	12	1			5	8	30	3	21	9
2015	79	2	2	7		4	4	26	2	15	8
2016	20	3	1			3	2	6	1	2	1
Total	**1431**	**364**	**71**	**30**	**20**	**84**	**240**	**334**	**24**	**83**	**100**

Year	LR or FD
1995	5
1996	3
1997	7
1998	12
1999	22
2000	12
2001	16
2002	8
2003	15
2004	17
2005	18
2006	34
2007	32
2008	40
2009	33
2010	18
2011	20
2012	24
2013	11
2014	12
2015	2
2016	3



Tilligerry Peninsular

The Tilligerry Peninsular has seen some alarming decline in the last few years with only 2 call-outs in 2015, down from 12 the year before.



The statistics by location show koalas seem to have virtually disappeared from all locations. The HKPS consider it to be absent from the Raymond Terrace, Medowie and Tomago areas where they were once common 20 years ago (Murray Black pers. comm.).

Year	*Total*	*Lemon Tree Passage*	*Mallabula*	*Tanilba Bay*	*Oyster Cove*	*Salt Ash*
1994	27			13		14
1995	45	6	3	18	2	16
1996	52	19	2	17	1	13
1997	50	18	7	11		14
1998	43	13	6	7	1	16
1999	38	15	2	7		14
2000	47	16	8	6		17
2001	40	11	5	3		21
2002	49	15	4	12	1	17
2003	29	11	4	2		12
2004	38	13	5	5	1	14
2005	33	7	5	13	1	7
2006	39	8	2	12	3	14
2007	37	16	6	7		8
2008	25	10	6	3		6
2009	19	5	8	3		3
2010	13	2	2	2		7
2011	15	6	1	6		2
2012	16	8	1	4		3
2013	9	5		2		2
2014	1	1				
Total	**665**	**205**	**77**	**153**	**10**	**220**

It is not known how many koalas are left on the Tilligerry Peninsular, though numbers are critically low and we seem to be heading for the imminent collapse of this sub-population. On the Tomaree Peninsular given levels of recaptures, total population size may only be 25% larger than the total number of call-outs (~ 100 animals) – (estimate only).