UNIVERSITY OF CANBERRA | INSTITUTE FOR APPLIED ECOLOGY

ANALYSIS REPORT

Assessment of hybridization and introgression in Bellinger River turtles, and assessment of parentage of hatchlings destined for release in the recovery program of *Myuchelys georgesi*.

Report prepared for Biodiversity and Conservation, NSW Department of Planning, Industry and Environment, Level 8, 22-24 Moonee St, Coffs Harbour NSW 2450.

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EXECUTIVE SUMMARY

In this report, the frequency of hybridization, and for the first time introgression, between *Myuchelys georgesi* and *Emydura macquarii*, is examined with the addition of new specimens from the 2019 and 2020 surveys, and in particular, the addition of new specimens from the Kalang River. Also, at the request of Taronga Zoo, a parentage analysis is undertaken to confirm or refute the paternal identity of hatchlings generated by the Zoo's breeding program and destined for release to the wild.

Nine F1 specimens, six backcrosses to *Emydura*, and two backcrosses to *Myuchelys* were identified in the Bellinger drainage. Note that specimen BRST_TZ18 is again identified as an F1 hybrid, which confirms as correct the decision to remove it from the insurance breeding colony.

All the *Emydura* captured in the Kalang River in the recent survey were assigned to pure *Emydura* in the NewHybrids analysis. This is a surprising result, given that past surveys by others have found that *Myuchelys georgesi* or hybrids/backcrosses assigned to *Myuchelys georgesi* dominated the catch. It suggests that the *Emydura* in the Kalang River have increased their population size in similar fashion and to similar degree as in the Bellinger River. The capture of only one *Myuchelys* in the Kalang River in the recent surveys also poses the question as to whether the Kalang populations were decimated by the virus. Further surveys are recommended when the waters in the Kalang are clear enough for snorkelling.

The first specimen of *Emydura macquarii* to be genetically sampled in the Bellinger was collected by Peter King in 1990, at a time when the species was rare in the Bellinger River. This specimen clearly originated from the Bellinger Coast, near Coffs Harbour.

Our problematic specimen, JOSH, is among those showing some level of introgression, in conflict with previous assessments that had JOSH as pure *Myuchelys*, as an F1 hybrid and as a backcross. Resequencing this specimen is necessary.

The frequency of hybridization and introgression amongst the specimens of *Myuchelys* caught in the Bellinger River is 13% (9 F1, 7 backcrosses, 36 introgressed), excluding those the 3 specimens initially identified as *Emydura macquarii* and showing deep introgression. A similar analysis of the *Emydura* identified these further 3 specimens (AA048171, BRST_5293, AA036241), as showing evidence of introgression of *Myuchelys* alleles.

Having identified F1 specimens, backcrosses and specimens subject to introgression, we are now in a position, for the first time, to be able to assess the relative heterozygosity of the different populations and species, expected heterozygosity being a measure of genetic diversity. *Myuchelys georgesi* have exceptionally low genetic diversity in comparison with *Emydura macquarii* from the Hastings, Macleay and Bellinger Coast drainages (Figure 4). This suggests that they have gone through a protracted bottleneck at some point in their past, or that the effective population sizes in their very restricted natural distribution (ca 70 km of river) has not been sufficient to prevent erosion of genetic diversity over time. This low genetic diversity may have been a contributing factor to the almost universal loss of adult specimens from the population during the virus epidemic in 2015 (the Irish Potato effect).

The parentage analysis of hatchlings produced by Taronga Zoo yielded results that were broadly consistent with expectation, with biological explanations likely to resolve those inconsistencies that did occur (Michael Mcfadden, pers. comm.).

It is hoped that these analyses will better inform discussions on whether and how to manage the hybridization and introgression between the endemic *Myuchelys georgesi* and the introduced *Emydura macquarii*, and whether and how to manage the rapidly increasing numbers of *Emydura macquarii* and the consequential impact on the endemic already decimated by a devastating viral epidemic.

BACKGROUND

This is an interim report to provide information in support of the monitoring and recovery of the Bellinger River turtle *Myuchelys georgesi*. *Myuchelys georgesi* is a relictual lineage of Australian freshwater turtle now restricted to the Bellinger River drainage basin, including the Kalang River which joins the Bellinger River via a marine estuary. Locally abundant, the species was considered of little concern from a conservation perspective until, in February 2015, the adult population was almost entirely extirpated from the Bellinger River by a novel virus (Zhang et al., 2018). It has since been classified as Critically Endangered at both State and National levels, listed as one of the top 25 species at risk of extinction globally (Stanford et al., 2018), and the subject of intensive monitoring by the NSW authorities (Chessman et al., 2020). An insurance colony of 18 specimens has been established at Taronga Zoo.

To complicate matters, a turtle once absent from the Bellinger River, *Emydura macquarii*, has been introduced, primarily from rivers draining the Bellinger Coast, but also from the Macleay/Hastings (Georges et al., 2011). Known from only a single specimen collected by

Peter King of UNE in 1990, despite extensive surveys by John Cann and Arthur Georges before and immediately after that finding (see Cann 1998 for a history of its discovery), the species has progressively increased in numbers to become the dominant freshwater turtle in the drainage. *Emydura macquarii* is also known from the Kalang River, but the source of these specimens is unknown.

The introduction or presence of *Emydura macquarii* in the Bellinger River drainage, and its rapid increase in numbers, presents two challenges for the endemic *Myuchelys georgesi*. The first is the possibility of hybridization and introgression with *Emydura macquarii*, which is known to occur (Georges et al. 2018), but for which the trajectory is unknown. Rampant hybridization and introgression could result in genetic swamping of the endemic, and its ultimate extinction. Restricted hybridization and introgression could inject novel genes to the genome of *Myuchelys georgesi*, and the dynamics of this interaction and/or the presence of barrier genes, could enable the species to maintain its distinct identity. At this stage, we do not know which scenario will play out.

The second challenge for *Myuchelys georgesi* presented by the introduction or presence of *Emydura macquarii* in the Bellinger River drainage, and its rapid increase in numbers, is competition for resources in the oligotrophic Bellinger River (Allanson and Georges, 1999; Spencer et al., 2014). *Emydura macquarii* may displace *Myuchelys georgesi* in all but the relatively pristine upper reaches of the drainage, via competitive exclusion, or compromise the ability of *Myuchelys georgesi* to rebound from the devastating viral epidemic. Either way, the risk of extinction of this highly restricted endemic is greatly increased.

In this report, I examine the frequency of hybridization, and for the first time introgression, between *Myuchelys georgesi* and *Emydura macquarii*, with the addition of new specimens from the 2019 and 2020 surveys and in particular, the addition of specimens from the Kalang River. The source population for the first *Emydura macquarii* introduced to the river (the Peter King 1990 specimen) is identified. Finally, at the request of Taronga Zoo, I undertake a parentage analysis to confirm or refute the paternal identity of hatchlings generated by the Zoo's breeding program and destined for release to the wild.

OBJECTIVES

- To evaluate the identity of specimens recently caught from the Kalang River, with special focus on potential hybridization with *Emydura macquarii*.

- To determine the paternity of hatchlings bred by Taronga Zoo, destined for release to the Bellinger River.
- To examine additional specimens of *Myuchelys georgesi* in the Bellinger River for evidence of hybridization and introgression with *Emydura macquarii*.

RESULTS

Preliminary manipulations

The following DArT services were combined for this analysis, comprising genotypes for 1043 specimens scored for 64,937 loci.

> DFwt14-1541 (n=6), DFwt15-1805 (94), DFwt15-2009 (88), DFwt16-2130 (90), DFwt16-2147 (7), DFwt16-2467 (94), DFwt17-2722 (20), DFwt17-3257 (38), DFwt18-3294 (89), DFwt18-3591 (38), DFwt18-3758 (113), DFwt19-4487 (232), DFwt19-4809 (41), DFwt20-5182 (93).

The raw data are stored in R binary file DFwt120-5182_raw.Rdata, which can be retrieved using function readRDS.

Table 1. Numbers of specimens of *Emydura macquarii* (n = 411) and *Myuchelys georgesi* (n = 534) captured and genotyped by location and month of capture. Note that the original specimen of *Emydura macquarii* captured by Peter King in 1990 is included, as are 5 specimens of *Myuchelys georgesi* caught in 1986. Abbreviations: Mg, *Myuchelys georgesi*, Em, *Emydura macquarii*; Bel, Bellinger River; Kal, Kalang River; Coast, Bellinger Coast (Coffs Harbour); Hasting, Hasting River; Macleay, Macleay River.

Emydura macquarii					
Em-Bel_Apr07	Em-Bel_Apr18	Em-Bel_Apr19	Em-Bel_Dec16	Em-Bel_Dec18	Em-Bel_Jan09
49	11	13	11	2	4
Em-Bel_Mar16	Em-Bel_Nov15	Em-Bel_Nov16	Em-Bel_Nov18	Em-Bel_Nov19	Em-Bel_Oct90
89	28	66	93	56	1
Em-Coast	Em-Hastings	Em-Kal_Apr07	Em-Kal_Mar07	Em-Kal_Mar16	Em-Kal_Oct19
44	6	6	1	11	28
Em-Macleay					
15					
Myuchelys georgesi					
Mg-Bel_Apr07	Mg-Bel_Apr15	Mg-Bel_Apr16	Mg-Bel_Apr18	Mg-Bel_Apr19	Mg-Bel_Aug86
208	1	1	5	1	6
Mg-Bel_Dec16	Mg-Bel_Dec19	Mg-Bel_Mar16	Mg-Bel_Nov15	Mg-Bel_Nov16	Mg-Bel_Nov18
12	1	59	39	40	25
Mg-Bel_Nov19	Mg-Kal_Apr07	Mg-Kal_Apr15	Mg-Kal_Oct19		
8	3	1	1		

Specimen identities were recoded to comply with the Wildlife Tissue Database held by the University of Canberra. In most cases, these identities concur with those used by the NSW Department of Planning, Industry and Environment.

Specimens whose identity was uncertain, or where their species designation was contradicted by the New Hybrids assignment were conservatively removed from the dataset:

BRST_10832.23_A, BRST_425315, BRST_325315, BRST_324315, BRST_424315, BRST_MG33_10834.33, BRST_10831.2, BRST_10834.56, BRST_10834.82, AA048095_A, BRST_5253, BRST_10834.66

A further 68 records were duplicated genotypes, and all but one copy of each duplicate were removed from the dataset.

Resultant monomorphic loci were removed. The raw dataset (unfiltered) comprised 963 specimens scored for 64,783 loci, and is available as DFwt120-5182_raw.Rdata. This file can be read with the readRDS function in R.

The raw data are broken down on the basis of the river of capture (Bellinger, Kalang, Macleay, Hastings, Bellinger Coast – Coffs Harbour), and the month of capture (Table 1).

Hatchlings from the breeding program were grouped into Mg-Hat_2017 (n = 7), Mg-Hat_2018 (22), Mg-Hat_May18, and Mg-Hat_2019 (16). The captive specimens held by Taronga Zoo were grouped into Mg-Taronga_Apr15 (n=17), and an additional 11 animals scheduled for release were grouped under Mg-Tmt_Sep19.

Population Structure

A standard series of filters were applied. All but one SNP within a single sequence tag were removed because of linkage considerations. Loci with a repeatability (across technical replicates run for 30% of specimens) less than 0.99 averaged across loci were removed. Loci that were called for less than 95% of specimens were similarly removed, and specimens with a call rate of less than 60% were removed.

BRST_10829.5[Mg-Bel_Mar16], AA036821[Em-Bel_Apr07]

This left 958 specimens scored for 19,142 loci, assigned to 47 groupings. A Principal Components Analysis applied to the *Emydura maquarii* in the Bellinger drainage showed considerable structure (Figure 1), presumably reflecting their multiple origins (Georges et al., 2011).

The first specimen of *Emydura macquarii* to be genetically sampled in the Bellinger was collected by Peter King in 1990, at a time when the species was rare in the Bellinger River. This specimen clearly originated from the Bellinger Coast, near Coffs Harbour (Figure 2).

Figure 1. Summary of the genetic structure of the *Emydura macquarii* genotyped in this study as represented by PCA ordination. Note that the Bellinger River specimens show close affinity to specimens from the Bellinger Coast, whereas those in the Kalang River have greater affinity to specimens from the Hastings/Macleay. The cluster of individuals at the bottom of the figure is curious. They may be putative introgressed individuals, though introgressed individuals would be expected to lie along the axis connecting *Myuchelys* with *Emydura*. Those animals require further scrutiny.

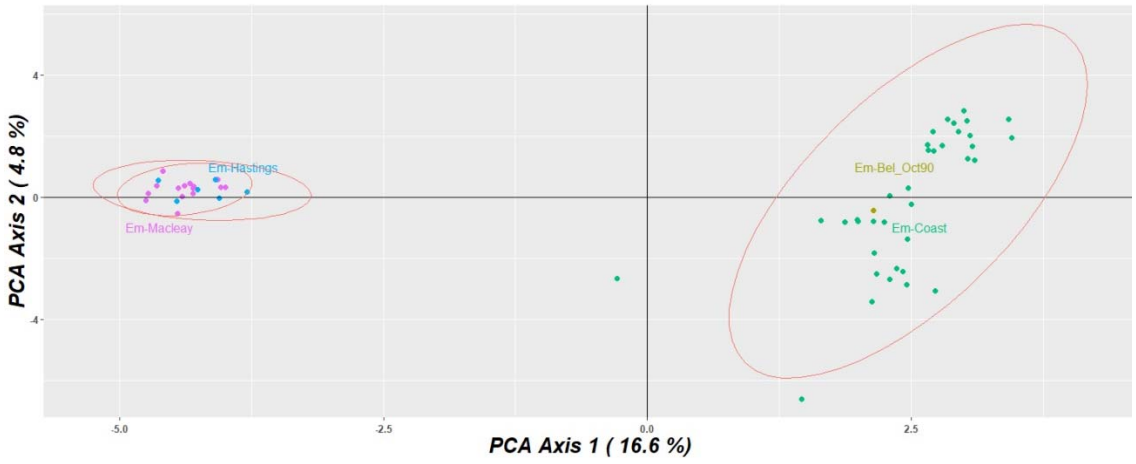

Figure 2. PCA plot of specimens from the Hastings, Macleay and Bellinger Coast to show clearly that the specimen collected by Peter King in 1990 from the Bellinger River had is provenance in the Bellinger Coast, Clarence drainage basin.

Hybridzation

The incidence of hybridization was assessed using NewHybrids (Anderson and Thompson, 2002). This software takes user identified parental species, in this case *Myuchelys georgesi* from 1986 and *Emydura macquarii* from the Hastings, Macleay and Bellinger Coast rivers,

and generates by simulation likelihood bins for each parental species, F1 hybrids, F2 crosses, and backcrosses between F1 and one or the other parental species. Specimens are assigned to these likelihood bins based on their individual likelihood of belonging to each bin. These likelihood bins are refined with each addition of new data, which can lead to a firming up of the posterior probabilities, and on occasion shifting an specimen from one classification to another (F2 to F1 for example). The likelihoods are rescaled to deliver the posterior probabilities of bin membership shown in the Table 2. Prior to the analysis, errors in the SNP calls were minimized by more stringent filtering than normal – only SNPs that had 100% repeatability were used, and the SNP read depth was required to exceed 10x (normally 5x).

Nine F1 specimens, six backcrosses to *Emydura*, and two backcrosses to *Myuchelys* were identified. Note that specimen BRST_TZ18 is again identified as an F1 hybrid, which confirms as correct the decision to remove it from the insurance breeding colony.

It is notable that all the *Emydura* captured in the Kalang River in the recent survey to target that drainage, assigned to pure *Emydura* in the NewHybrids analysis. This is a surprising result, given that anecdotal reports have *Myuchelys georgesi* or hybrids/backcrosses assigned to *Myuchelys georgesi* dominating the catch. John Cann in particular should be contacted for records of capture in the Kalang prior to 2007.

Specimens not involved in hybridization were typically assigned by New Hybrids to the species to which they were identified on capture. Those few that were not were removed from the dataset during the initial cleaning process. The hybrid specimens were reassigned to populations as shown in Table 2, and the new dataset saved as DFwt120-5182_hybrids_defined.Rdata.

Introgression

New Hybrids has limits to how deep it can define introgression – beyond backcrosses between the F1 and the parentals, the likelihood bins overlap to the point of ambiguity. To examine introgression at a finer scale, I used a novel technique. This technique rests upon the observation that specimens of one species with an injection of alleles from a second species will appear closely related, on a background of general relatedness. In Figure 3A, the F1 hybrids and associated backcrosses form a tight cluster of "related" specimens, that separates out from the background relatedness among *Myuchelys* specimens from the Bellinger River. Within that cluster lie a number of other specimens (Figure 3B), and they can be interpreted as having some degree of introgression. Note that the specimens in the insurance colony held

by Taronga Zoo are scattered among the specimens showing average relatedness, and so do not include any specimens subject to introgression. Note also that our problematic specimen, JOSH, is among those showing some level of introgression, in conflict with previous

Table 2. Summary of the results of the New Hybrids analysis. This analysis uses parental populations (Mg-Bel_Oct86 and Em_Hastings, Macleay, Coast) to establish by simulation, a series of likelihood bins corresponding to the parental genotypes, F1 crosses, F2 crosses, and back crosses between F1 and each of the parentals. Specimens are then assigned to the bin with the highest likelihood. The likelihoods are rescaled to deliver the posterior probabilities of bin membership shown in the table. Specimens that were clearly misclassified are omitted. Note that specimens that arose from deeper introgression will be assigned to one or the other parental populations.

id	pop	Emydura	Myuchelys	F1	F2	F1xEmydura	F1xMyuchelys	Counts	Species	Source
Multiple	Em-Bel	1	0	0	0	0	0	382	Emydura	Bellinger
Multiple	Em-Outside	1	0	0	0	0	0	56	Emydura	Macleay, Hastings, Coffs
Multiple	Em-Kal	1	0	0	0	0	0	45	Emydura	Kalang
UC_0669	Em-Bel_Oct90	1	0	0	0	0	0	1		Bellinger
AA048061	F1-Kal_Apr07	0	0	1	0	0	0	1	F1	Kalang
AA048159	F1-Kal_Apr07	0	0	1	0	0	0	1	F1	Kalang
AA048175	F1-Kal_Apr07	0	0	1	0	0	0	1	F1	Kalang
BRST_TZ18	F1-Kal_Apr15	0	0	1	0	0	0	1	F1	Kalang
BRST_10758.13	F1-Bel_Mar16	0	0	1	0	0	0	1	F1	Bellinger
BRST_5008	F1-Bel_Dec16	0	0	1	0	0	0	1	F1	Bellinger
BRST_6115	F1-Bel_Nov19	0	0	1	0	0	0	1	F1	Bellinger
BRST_4968	F1-Bel_Nov16	0	0	1	0	0	0	1	F1	Bellinger
BRST_4979_A	F1-Bel_Nov16	0	0	1	0	0	0	1	F1	Bellinger
AA36801	F1xEm-Kal_Mar07	0	0	0	0	1	0	1	F1xEmydura	Kalang
AA036018	F1xEm-Bel_Apr07	0	0	0	0	1	0	1	F1xEmydura	Bellinger
AA048054	F1xEm-Bel_Apr07	0	0	0	0	1	0	1	F1xEmydura	Bellinger
BRST_11008.1_C	F1xEm-Kal_Mar16	0	0	0	0	1	0	1	F1xEmydura	Kalang
BRST_10834.47	F1xEm-Bel_Mar16	0	0	0	0	1	0	1	F1xEmydura	Bellinger
BRST_5220	Em-Bel_Apr18	0.09932	0	0	0	0.90068	0	1	F1xEmydura	Bellinger
AA036212	F1xMg-Bel_Apr07	0	0	0	0	0	1	1	F1xMyuchelys	Bellinger
AA36128_A	F1xMg-Bel_Apr07	0	0	0	0	0	1	1	F1xMyuchelys	Bellinger
Multiple	Mg-Tmt	0	1	0	0	0	0	36	Myuchelys	transmittered
Multiple	Mg-Hat	0	1	0	0	0	0	22	Myuchelys	Taronga hatchlings
Multiple	Mg-Taronga	0	1	0	0	0	0	17	Myuchelys	Taronga adults
BRST_JOSH_10831.1	Mg-Bel_Nov15	0	1	0	0	0	0	1	Myuchelys	Josh
BRST_6057	Mg-Kal_Oct19	0	1	0	0	0	0	1	Myuchelys	Kalang
BRST_11087.6	Mg-Bel_Mar16	0	0.98074	0	0	0	0.01926	1	Introgressed?	Bellinger
Multiple	Mg-Bel	0	1	0	0	0	0	378	Myuchelys	Bellinger

assessments that had JOSH as pure *Myuchelys*, as an F1 hybrid and as a backcross. Resequencing this specimen is necessary.

The frequency of hybridization and introgression amongst the specimens of *Myuchelys* caught in the Bellinger River is 13% (9 F1, 7 backcrosses, 36 introgressed), excluding those initially identified as *Emydura macquarii* and showing deep introgression.

A similar analysis of the *Emydura* identified a further 3 specimens (AA048171, BRST_5293, AA036241), initially identified as *Emydura macquarii*, that showed evidence of introgression of *Myuchelys* alleles.

The detection of 36 putative introgressed individuals presents us with a challenge. How do we explain their presence, requiring 3 generations (parental, to F1, to backcross, to introgressed), when *Emydura* has arguably been in the Bellinger River since only 1990. The turtles have barely had time to generate any introgression. This question requires further examination that goes beyond a visual assessment.

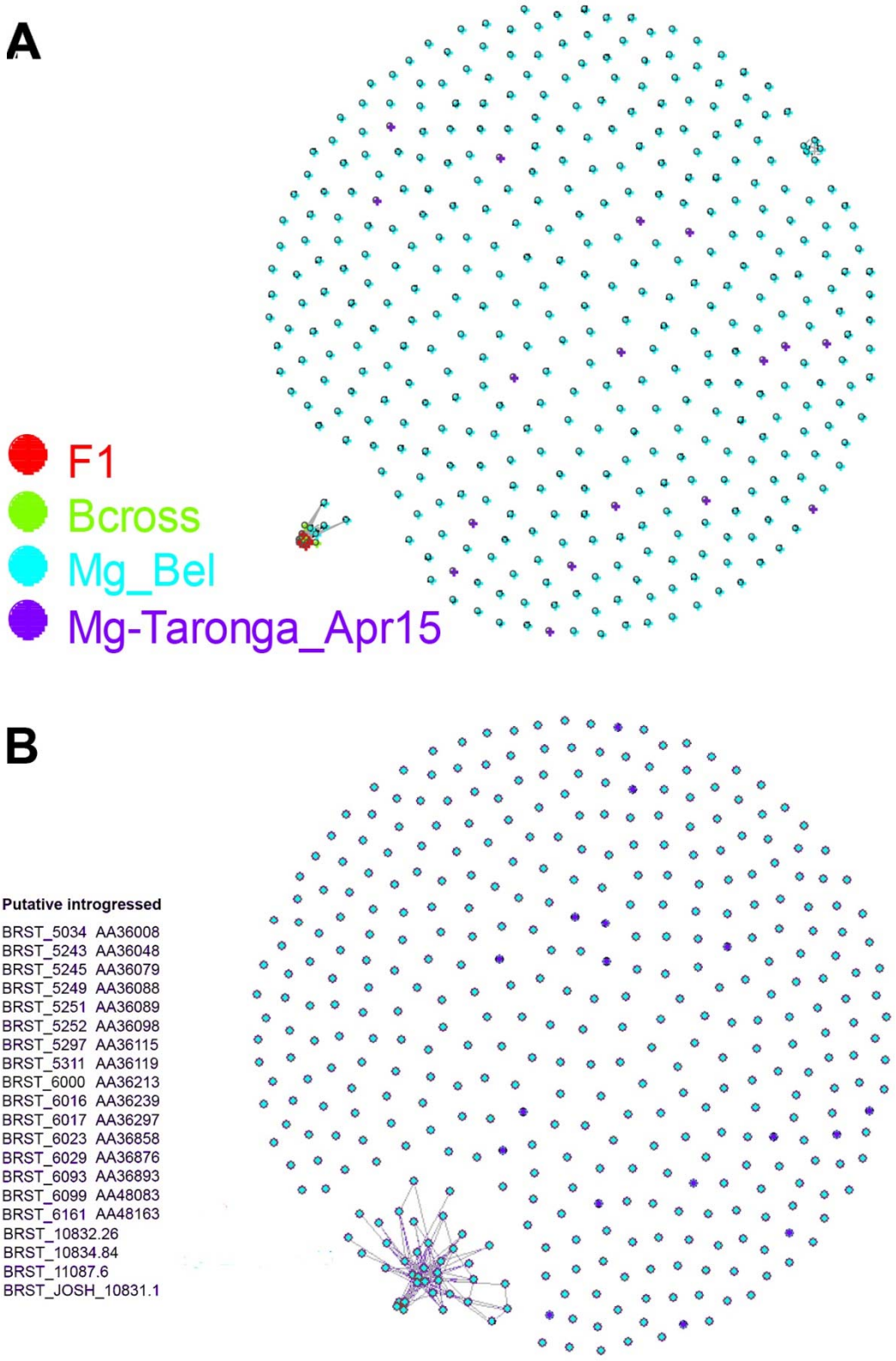

Figure 3. A network graph of relatedness among specimens. Because hybrids, backcrosses and introgressed specimens share alleles from the second species, they appear more related to each other than the average 'background' relatedness of the G matrix. Graph A shows the network plot with the F1 and backcrossed specimens included, to establish the identity of the cluster. The second plot (B) is without the F1 and backcrossed specimens included, to identify putative introgressed specimens (listed).

The putative introgressed specimens were reassigned to populations with the prefix ADM (for admixed) and the new dataset saved as DFwt120-5182_with_introgression.Rdata.

Heterozygosity

Having identified F1 specimens, backcrosses and specimens possibly subject to introgression, we are now in a position, for the first time, to be able to assess the relative heterozygosity of the different populations and species, expected heterozygosity being a measure of genetic diversity. *Myuchelys georgesi* have exceptionally low genetic diversity in comparison with *Emydura macquarii* from the Hastings, Macleay and Bellinger Coast drainages (Figure 4). This suggests that they have gone through a protracted bottleneck at some point in their past, or that the effective population sizes in their very restricted natural distribution (ca 70 km of river) has not been sufficient to prevent erosion of genetic diversity over time. This low genetic diversity may have been a contributing factor to the almost universal loss of adult specimens from the population during the virus epidemic in 2015. The Bellinger River had the highest heterozygosity of the *Emydura* populations, possibly a reflection of their multiple origins and insufficient time for allele profiles to come to equilibrium (171 loci, 0.9%, significantly out of HWE).

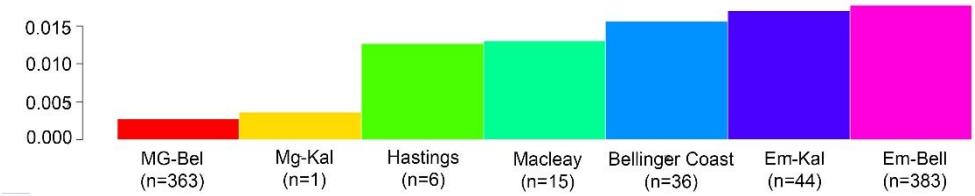

Figure 4. Expected heterozygosities for populations of *Myuchelys georgesi* and *Emydura macquarii*.

We can also again assess how representative the genetic diversity of the specimens in the captive colony is of the genetic diversity of the population from which they were drawn (Figure 5).

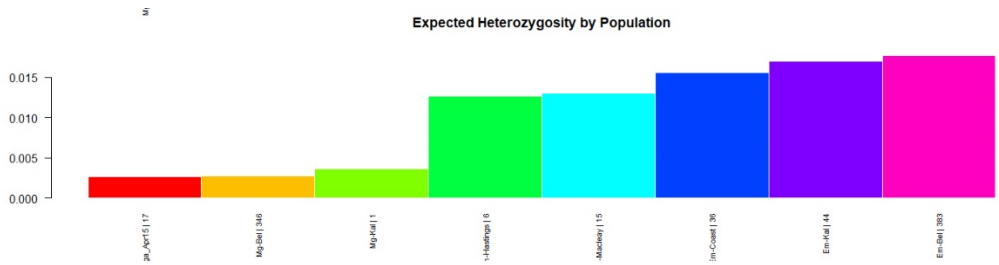

Figure 5. Expected heterozygosities including the 17 animals in the captive colony. Cleary, the genetic diversity of the captives is the same as for the population as a whole.

Parentage Analysis – Taronga Hatchlings

Specimens of the Bellinger River snapping turtle held in the captive colony of Taronga Zoo were genotyped together with a series of hatchlings scheduled for release to the Bellinger River as part of the recovery actions. Before release, the identity of the fathers was requested.

The offspring were genotyped using the same set of SNP markers, and 50 loci that were informative for ascertaining parentage, and that had no allelic dropout, were selected. This subset of data were used in program FRANz (https://www.bioinf.uni-leipzig.de/Software/FRANz, Riester et al., 2009) to assign offspring to fathers with a measure of reliability (Table 2). All but nine offspring could be assigned to a father, and these assignments were typically concordant with the suspected father (Table 3). Those hatchlings whose father was suspected to be either TZ02 or TZ17 were assigned by FRANz a father TZ02 in 8 cases and TZ05 in 3 cases, with 2 cases undetermined. Those hatchlings whose father was suspected to be either TZ14 or TZ15 were assigned TZ14 in 3 cases and TZ15 in 2 cases by FRANz, with 7 cases unresolved.

The assignment of father TZ05 to hatchlings arsing from matings with either father TZ02 or TZ17 is clearly an unsatisfactory result, unless there were some opportunities by fathers to sire the hatchlings that are not yet clear. This requires further review to identify the cause of this mismatch between results and opportunity of the males to mate with the females.

Table 3. Results of paternal prediction using software package FRANz. Input data were SNP loci that showed variability among the parental stock potentially informative in parental assignment. Maternal identity is assumed to be known, as provided by Adam Skidmore. Where an assessment could be made, it was in accordance with expectation except in the cases highlighted in red. A biological explanation should be sought to explain these discrepancies (such as moving individuals among tanks).

Offspring	Loci Typed	Parent 1	Loci Typed	Parent 2	Loci Typed	LOD	Posterior	Common Loci Typed	Mismatches	n_f	n_m	Pair LOD Parent 1	Pair LOD Parent 2	Posterior Parent 1	Posterior Parent 2	Suspected Dad	Suspected Alternate Dad	Predicted Dad (FRANz)	Predicted Mum (FRANz)
B70146	50	BRST_TZ10	50			0.00E+00	1	50	0	12	22	8.56E+00		1		BRST_TZ04	BRST_TZ15		BRST_TZ10
B70148	50	BRST_TZ10	50			0.00E+00	1	50	0	12	22	7.67E+00		1		BRST_TZ04	BRST_TZ15		BRST_TZ10
B80239	50	BRST_TZ10	50	BRST_TZ14	50	6.74E+00	0.9986	50	1	12	22	4.99E+00	5.05E+00	1	0.999	BRST_TZ14	BRST_TZ15	BRST_TZ14	BRST_TZ10
B80240	50	BRST_TZ10	50	BRST_TZ15	50	3.51E+00	0.703	50	1	12	22	3.48E+00	4.65E+00	1	0.703	BRST_TZ14	BRST_TZ15	BRST_TZ15	BRST_TZ10
B80241	50	BRST_TZ10	50	BRST_TZ14	50	3.61E+00	0.8366	50	1	12	22	8.44E+00	2.29E-02	1	0.837	BRST_TZ14	BRST_TZ15	BRST_TZ14	BRST_TZ10
B80242	50	BRST_TZ10	50	BRST_TZ15	50	5.28E+00	0.994	50	0	12	22	6.22E+00	2.74E+00	1	0.994	BRST_TZ14	BRST_TZ15	BRST_TZ15	BRST_TZ10
B80243	50	BRST_TZ10	50	BRST_TZ14	50	6.04E+00	0.9974	50	1	12	22	6.46E+00	3.29E+00	1	0.997	BRST_TZ14	BRST_TZ15	BRST_TZ14	BRST_TZ10
B80245	50	BRST_TZ10	50		50	1.92E+00	0.8494	50	1	12	22	6.98E+00		0.849		BRST_TZ14	BRST_TZ15		BRST_TZ10
B80249	50	BRST_TZ10	50			0.00E+00	1	50	0	12	22	9.17E+00		1		BRST_TZ14	BRST_TZ15		BRST_TZ10
B90118	50	BRST_TZ10	50		50	9.25E+00	0.9941	50	0	12	22	8.40E+00		0.994		BRST_TZ14	BRST_TZ15		BRST_TZ10
B90123	50	BRST_TZ10	50		50	4.55E+00	0.9106	50	2	12	22	9.62E+00		0.911		BRST_TZ14	BRST_TZ15		BRST_TZ10
B90127	50	BRST_TZ10	50		50	4.05E+00	0.5107	50	2	12	22	6.52E+00		0.511		BRST_TZ14	BRST_TZ15		BRST_TZ10
B90130	50	BRST_TZ11	50	BRST_TZ02	50	7.37E+00	0.9992	50	1	12	22	6.24E+00	7.02E+00	1	0.999	BRST_TZ02	BRST_TZ17	BRST_TZ02	BRST_TZ11
B90131	50	BRST_TZ11	50	BRST_TZ05	50	1.41E+01	1	50	1	12	22	3.30E+00	9.48E+00	1	1	BRST_TZ02	BRST_TZ17	BRST_TZ05	BRST_TZ11
B90132	50	BRST_TZ11	50	BRST_TZ02	50	1.07E+00	0.7125	50	2	12	22	8.86E+00	-9.06E-01	1	0.713	BRST_TZ02	BRST_TZ17	BRST_TZ02	BRST_TZ11
B90133	50	BRST_TZ11	50	BRST_TZ05	50	7.69E+00	0.9994	50	2	12	22	7.72E+00	9.36E+00	1	0.999	BRST_TZ02	BRST_TZ17	BRST_TZ05	BRST_TZ11
B90134	50	BRST_TZ11	50	BRST_TZ02	50	8.69E+00	0.9998	50	0	12	22	4.44E+00	5.17E+00	1	1	BRST_TZ02	BRST_TZ17	BRST_TZ02	BRST_TZ11
B90135	50	BRST_TZ11	50	BRST_TZ02	50	9.58E+00	0.9972	50	1	12	22	6.88E+00	5.32E+00	1	0.997	BRST_TZ02	BRST_TZ17	BRST_TZ02	BRST_TZ11
B90136	50	BRST_TZ11	50	BRST_TZ02	50	1.07E+01	1	50	1	12	22	8.33E+00	5.87E+00	1		BRST_TZ02	BRST_TZ17	BRST_TZ02	BRST_TZ11
B90138	50	BRST_TZ11	50			0.00E+00	1	50	1	12	22	3.46E+00		1		BRST_TZ02	BRST_TZ17		BRST_TZ11
B90139	50	BRST_TZ11	50	BRST_TZ02	50	8.84E+00	0.9999	50	1	12	22	7.55E+00	5.39E+00	1	1	BRST_TZ02	BRST_TZ17	BRST_TZ02	BRST_TZ11
B90140	50	BRST_TZ11	50			0.00E+00	1	50	1	12	22	1.61E+00		1		BRST_TZ02	BRST_TZ17		BRST_TZ11
B90141	50	BRST_TZ11	50	BRST_TZ02	50	9.31E+00	0.9998	50	0	12	22	4.95E+00	5.40E+00	1	1	BRST_TZ02	BRST_TZ17	BRST_TZ02	BRST_TZ11
B90142	50	BRST_TZ11	50	BRST_TZ05	50	1.38E+01	0.9998	50	1	12	22	4.45E+00	1.01E+01	1	1	BRST_TZ02	BRST_TZ17	BRST_TZ05	BRST_TZ11
B90143	50	BRST_TZ11	50	BRST_TZ02	50	9.79E+00	0.9999	50	1	12	22	4.38E+00	6.13E+00	1	1	BRST_TZ02	BRST_TZ17	BRST_TZ02	BRST_TZ11

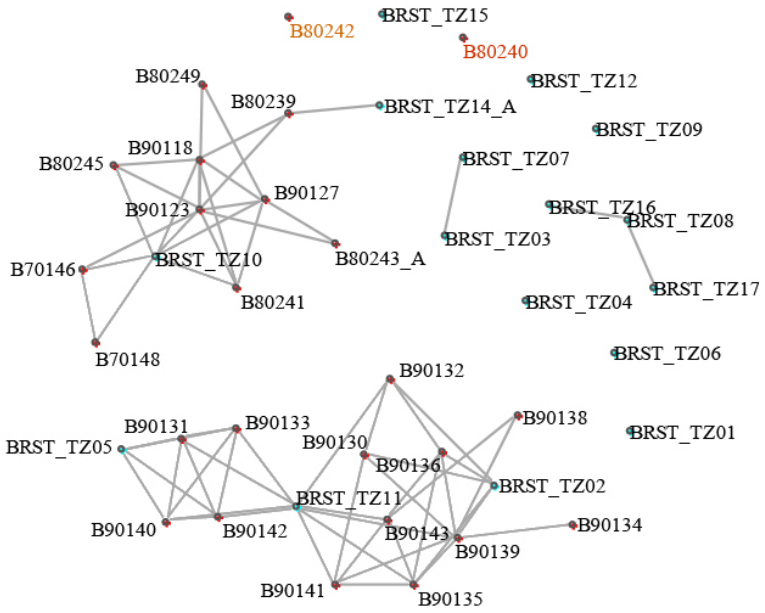

Figure 6. A visual representation of the relatedness among hatchlings against the background average relatedness of adult breeders in the Taronga colony. Note that hatchlings B80240 and B80242 could not be assigned a cluster.

REFERENCES

Riester, Stadler and Klemm (2009). FRANz: Reconstruction of wild multi-generation pedigrees. Bioinformatics. 25:2134-2139.

Allanson M, Georges A (1999). Diet of *Elseya purvisi* and *Elseya georgesi*, a sibling species pair of freshwater turtles from Eastern Australia. Copeia 1999: 473−477

Anderson E.C., Thompson E.A. (2002). A model-based method for identifying species hybrids using multilocus genetic data. Genetics 160: 1217−122

Cann J (1998). *Australian freshwater turtles*. Beaumont Publishing, Singapore.

Chessman, B.C., McGilvray, G., Rumming, S., Jones, H.A., Petrov, K., Fielder, D.P., Spencer, R-J and Georges, A. (2020). On a razor's edge: status and prospects of the critically endangered Bellinger River snapping turtle, Myuchelys georgesi. Aquatic Conservation 30:586-600.

Georges A, Spencer RJ, Welsh M, Shaffer HB, Walsh R, Zhang X (2011). Application of the precautionary principle to taxa of uncertain status: the case of the Bellinger River turtle. Endangered Species Research 14: 127−134.

Luiselli, L., Haitao, S., Singh, S. and Walde, A. (2018). Turtles in Trouble. The World's 25+ Most Endangered Tortoises and Freshwater Turtles -- 2018. Turtle Conservancy, Ojai, California, USA.

Spencer RJ, Georges A, Lim D, Welsh M, Reid AM (2014). The risk of inter-specific competition in Australian short-necked turtles. Ecological Research 29: 767−777.

Stanford, C.B., Rhodin, A.G.J., van Dijk, P.P., Horne, B.D., Blank, T., Goode, E.V., Hudson, R., Mittermeier, R.A., Currylow, A., Eisemberg, C., Frankel, M., Georges, A., Gibbons, P.M., Juvik, J.Ol, Kuchling, G.,

Zhang J, Finlaison DS, Frost MJ, Gestier S, Gu X, Hall J, et al. (2018) Identification of a novel nidovirus as a potential cause of large scale mortalities in the endangered Bellinger River snapping turtle (*Myuchelys georgesi*). PLoS ONE 13(10): e0205209. https://doi.org/10.1371/journal.pone.0205209.