Assault from all sides: hybridization and introgression threaten the already critically endangered *Myuchelys georgesi* (Chelonia: Chelidae)

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ABSTRACT: Hybridization and introgression between species is remarkably common, even between distantly related taxa. This suggests that the frequency of hybridization between species has been greatly constrained, not by evolutionary divergence in isolation, but by lack of opportunity. This constraint is being relaxed by human-mediated dispersal. Here, we document a case where such dispersal of a widespread species of freshwater turtle (*Emydura macquarii*) into the highly restricted range of a critically endangered endemic turtle (*Myuchelys georgesi*) has provided opportunity for the two to hybridize. This has raised concerns about the potential impact of hybridization on the endemic species, and its continued persistence in the face of challenges brought about by habitat alteration, increased competition, disease and genetic pollution. This study serves to highlight the risks associated with human-mediated dispersal, which can bring into contact species that would otherwise never or only rarely have met, and thus provide opportunities for hybridization and introgression between even distantly related species, with uncertain consequences for already threatened species.

KEY WORDS: Human-induced dispersal · Genetic pollution · Genetic swamping · Single nucleotide polymorphism

INTRODUCTION

Rare species typified by low abundances are often considered the most vulnerable to changing circumstances that drive them to the brink of extinction (Davies et al. 2000, Lawler et al. 2003). Another class of rarity (sensu Rabinowitz 1981) applies to species that are locally abundant but very restricted in distribution. For these species, local extinction means global extinction. They are particularly vulnerable to geographically restricted processes, such as local alteration of land use, local establishment of invasive predators or competitors, pollution events, pathogen outbreaks or the vicissitudes of natural events. Lo-cally abundant but geographically restricted species can be recognized under criteria used to classify species as Vulnerable or Endangered (IUCN 2015). Such species rarely attract attention until they are already impacted by one or more threats, by which time options for effective mitigation may have become limited.

One neglected factor potentially impacting localized endemics is hybridization and introgression. Hybridization is remarkably common in nature. One estimate has at least 25% of plant species and 10% of animal species, mostly recently diverged species, involved in hybridization and potential introgression with other species (Mallet 2005). Reptiles were not

included in this estimate, but they too include many instances of natural hybridization. Natural hybridization among well-established species has been reported for snakes (LeClere et al. 2012), lizards (Jančúchová-Lásková et al. 2015), crocodiles (Milián-García et al. 2015) and turtles (Seminoff et al. 2003). In a study of hawksbill sea turtles *Eretmochelys imbricata* nesting in the Bahia State in Brazil, 42% of genotyped adults morphologically identified as hawksbill sea turtles carried mtDNA lineages of the loggerhead turtle *Caretta caretta* (Lara-Ruiz et al. 2006, Vilaça et al. 2012), indicating past, and potentially contemporary, hybridization. Hybrids have also been reported between *C. caretta* and the olive ridley turtle *Lepidochelys olivacea*, between *E. imbricata* and *L. olivacea*, and between the green sea turtle *Chelonia mydas* and *C. caretta* (Karl et al. 1995). Hybridization was considered to provide the best explanation for a species tree that strongly conflicted with the mtDNA phylogeny and traditional taxonomy for taxa within the genus *Trachemys* (Parham et al. 2013). Among Australian freshwater turtles, *Chelodina burrungandjii* and *Chelodina oblonga* (formerly *rugosa*) hybridize at their contact zone in northern Australia, *C. oblonga* and *Chelodina canni* hybridize in the rivers that drain into the Australian Gulf of Carpentaria, and *C. canni* and *Chelodina longicollis* hybridize in the lowland swamps of the Styx River of eastern Australia (Georges et al. 2002). While there is a negative correlation between instances of hybridization and level of genetic divergence between species (Mallet 2005), the above examples of intergeneric hybridization in marine turtles and between divergent lineages of *Chelodina* (subgenera *Chelodina* and *Macrochelodina*) suggest that partial or complete reproductive compatibility can be maintained over millions of years of independent evolution. Thus, the frequency of hybridization between species has been greatly constrained, not by evolutionary divergence in isolation, but by lack of opportunity.

This constraint is being relaxed by human-mediated dispersal, which, in freshwater turtles, is driven by a trade in turtles for pets, meat and medicinal products. Introduced species are now considered to be the second most important cause of global biodiversity loss after direct habitat destruction (McNeeley et al. 2001, Baskin 2002). A direct consequence of species intro-

duction can be hybridization when the introduced species comes into contact with native species (Arnold 2004). There are now numerous instances of hybridization in the wild, even between very divergent taxa (Buskirk et al. 2005), occurring because of the inadvertent release of exotic species well outside their natural range (Fong & Chen 2010). This is generally considered to be a deleterious process, potentially disrupting local adaptation and threatening the genetic identity of the species occupying its native range, a process referred to as genetic pollution (Fong & Chen 2010, Rhodin et al. 2017, p. 20).

Here, we show that hybridization and introgression threaten an abundant but highly geographically restricted freshwater turtle, *Myuchelys georgesi*, which is endemic to the Bellinger River drainage basin, a small coastal oligotrophic river system (including the Kalang River) in northern New South Wales, Australia (Fig. 1). This drainage once flowed through a mosaic of rainforest and wet sclerophyll forest, and had a rocky substrate with open beds of coarse gravel ideally suited to stream-dwelling *M. georgesi*, but not to other short-necked species of riverine freshwater turtle. Extensive land clearing for dairy and agriculture has opened up the riparian and adjacent forest canopy and increased rates of erosion and sedimentation (Laronne et al. 1994, Telfer & Cohen 2010), and led to the establishment of exposed sand banks suited to the nesting of a wider range of turtle species. One species taking advantage of these changes is *Emydura macquarii*, a polytypic species widespread in eastern and southeastern Australia (Georges & Thomson 2010). The Bellinger River form of this species was, for a time, regarded as a distinct taxon endemic to the Bellinger River (Cogger et al.
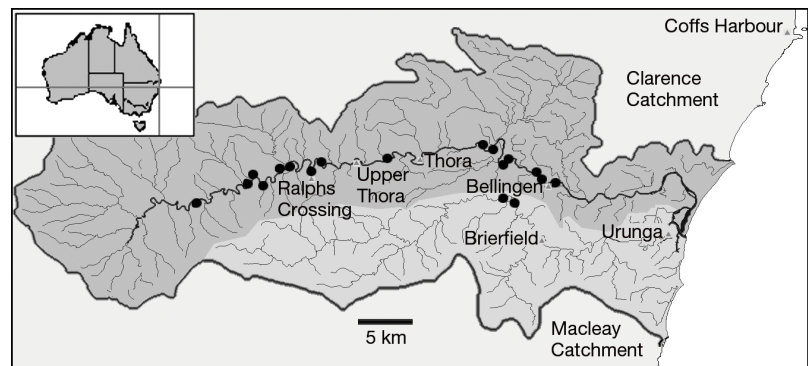

Fig. 1. Bellinger catchment (at the junction of the crosshairs in the inset) showing the capture localities of the Bellinger *Emydura*. The Kalang River is essentially a tributary of the Bellinger River, and is shown on the map as running through Urunga and Brierfield (lighter shading). The Bellinger and Kalang Rivers comprise the entire natural range of the now Critically Endangered *Myuchelys georgesi*

1993, Cann 1998), which led to it being listed on the state and federal schedules of endangered species. Closer examination using mtDNA markers (Georges et al. 2011) revealed this second 'endemic' to be an unremarkable population of *E. macquarii* and, further, that it was a recent invader of the Bellinger River, probably introduced in the 1980s (see Cann 1998 for a history of its discovery) and on several occasions since. The conservation requirements of the true endemic, *M. georgesi*, were overlooked.

The introduced *E. macquarii* has steadily increased in abundance since the early 1990s, when it was first reported, and now poses a serious threat to the endemic *M. georgesi* through potential competition for scarce shared resources in an oligotrophic system (Chessman 1986, Allanson & Georges 1999, Spencer et al. 2014). Furthermore, in 2015, *M. georgesi* suffered from a catastrophic dieback involving a virus not previously recorded from the catchment, or indeed from turtles (Jakob-Hoff et al. 2017, Zhang et al. 2018). Adult *M. georgesi* were effectively extirpated from their entire range, while the exotic *E. macquarii* were unaffected. The Australian government moved *M. georgesi* from unlisted to critically endangered, and the NSW Office of Environment and Heritage undertook surveys to determine the magnitude and extent of the decline, and to urgently establish an assurance breeding colony. The species is now listed as one of the 25 most endangered freshwater turtles globally (Stanford et al. 2018).

During surveys, a few animals from the Bellinger drainage showed an admixture of characters between the 2 species (Cann 1998: p. 115, top plate), and have been regarded as potential hybrids (Georges et al. 2011), though hybrids and introgressed individuals could not be reliably identified by superficial morphological examination. We addressed the question of potential hybridization and the associated challenges for management by generating a panel of single nucleotide polymorphisms (SNPs) to genotype animals for which we had existing tissue samples, including individuals suspected of being hybrids. We also genotyped the animals held in the assurance breeding colony established by the NSW Office of Environment and Heritage in collaboration with Taronga Zoo and the Western Sydney University. Our study provides an example of where next-generation DNA technologies have progressed to a point where they can be used to respond to management needs on a time frame commensurate with the need to make urgent decisions on the conservation of endangered species. Our demonstration of hybridization between *E. macquarii* and *M. georgesi* in the wild introduces unwelcome uncertainty into the future of the phylogenetically distinctive and critically endangered *Myuchelys georgesi*.

MATERIALS AND METHODS

Specimen collection

A total of 536 individuals were available for examination. These included 68 *Emydura macquarii* from the Bellinger River, 14 from the Kalang River, which links to the Bellinger River via a common estuary, 6 and 10 from the Hastings and MacLeay Rivers to the south, respectively, and 25 from the vicinity of the township of Coffs Harbour (Clarence drainage) to the north, a suspected source of the Bellinger River *E. macquarii* (Georges et al. 2011). The 396 *Myuchelys georgesi* individuals consisted of 4 individuals collected in 1985 (Georges & Adams 1996) and 392 animals collected during surveys for and by the NSW Office of Environment and Heritage (Georges et al. 2011). Four of these were from the Kalang River. Tissue samples were typically collected from these individuals by removing a portion of the trailing webbing of the clawless toe on the rear foot, though in some cases blood was taken from the readily accessible jugular vein. Neither technique is thought to have long-term impact on the turtles (Bjorndal et al. 2010). The samples were preserved and stored in 75% ethanol, and held in storage at −20°C at the University of Canberra in the UC Wildlife Tissue Collection before being subsampled for this study. An additional 18 heparinized blood samples were taken from *M. georgesi* originally held as an assurance colony at the University of Western Sydney, now held at Taronga Zoo (specimens TZ01 to TZ18). They were shipped to Canberra by courier and subsampled for analysis (samples AA70200 to AA70217, respectively).

DNA extraction and sequencing

Sequencing for SNP genotyping was done using DArTseq™ (Diversity Arrays Technology Pty Ltd, www.diversityarrays.com), which uses a combination of complexity reduction using restriction enzymes, fragment size selection and next-generation sequencing, as described in detail by Kilian et al. (2012). The technique is similar to double-digest restriction associated DNA sequencing (ddRAD) (Peterson et al. 2012) but has the advantages of accepting lower

quantities of DNA, having greater tolerance of lower quality DNA and having higher call rates (Sansaloni et al. 2011). Five commonly used combinations of restriction enzyme (HpaII, NSpI, MseI, PstI and SphI; data not presented) were evaluated for the chelid turtle *E. macquarii* in an earlier study (Georges et al. in press), and on that basis the restriction enzyme combination of PstI (recognition sequence 5′-CTG CA|G-3′) and SphI (5′-GCA TG|C-3′) was selected for the double digestion in both *E. macquarii* and *M. georgesi*. The PstI-compatible adapter included the Illumina flow cell attachment sequence, a sequencing primer sequence, a barcode region of variable length (see Elshire et al. 2011) and the PstI-compatible overhang sequence. The reverse adapter contained a flow cell attachment sequence and an SphI-compatible overhang sequence. Only fragments generated by the PstI–SphI double digest were effectively amplified in 30 rounds of PCR. After PCR, equimolar amounts of amplification products from each sample of the 96-well microtiter plate were bulked and applied to c-Bot (Illumina) bridge PCR for sequencing on the Illumina Hiseq2500. The sequencing (single read) was run for 77 cycles.

SNP genotyping

Sequences generated from each lane were processed using proprietary DArT analytical pipelines. Approximately 2 000 000 (±7%) sequences per sample were identified and used in marker calling. Identical sequences were collapsed into files referred to as FASTQcoll files and error-corrected using a DArT Pty Ltd proprietary algorithm, which corrects a low-quality base from a singleton tag by consensus. The error-corrected FASTQcoll files were analyzed using DArT Pty Ltd proprietary software (DArTsoft14) to output candidate SNP and silicoDArT markers, which are polymorphic within the set of samples. All unique sequences from the set of FASTQcoll files were identified, and clustered by sequence similarity at a distance threshold of 3 bp (number of different bases occupying a specific position). The sequence clusters were then parsed into SNP markers using a range of metadata parameters derived from the quantity and distribution of each sequence across all samples in the analysis: the average count for each sequence or row sum (sequencing depth), the balance of average counts for each SNP allele, and the call rate (proportion of samples for which the marker is scored). Calling quality was assured by high average read depth per locus (ca. 20×). In addition, approximately one-third of samples were processed twice from DNA to allelic calls as technical replicates, and scoring consistency (repeatability) was used as the main selection criteria for high quality/low error rate markers.

Additional SNP filtering

After receiving the SNP data from DArT Pty Ltd, the SNP data were read into a genlight object as implemented in the R package adegenet (Jombart 2008) to facilitate subsequent processing (Gruber et al. 2018). Stringent initial screening was undertaken on the basis of repeatability. Only loci for which the repeatability was 100% were chosen for subsequent analysis. Further filtering was undertaken on the basis of call rate. An SNP allele at a locus may not be identified during genotyping because of a concurrent mutation at one or both of the restriction enzyme recognition sites. Because of the high read depth, most 'missing data' are of this form. Only loci with a call rate of 98% or higher were included in the analyses. Finally, we filtered out duplicate SNPs from single sequenced fragments, retaining only 1 SNP selected at random. The data remaining after this additional filtering are regarded as highly reliable.

Hybridization and introgression

For qualitative assessments, we applied principal coordinates analysis (PCoA) (Gower 1966) using the centred Euclidean distance metric (functionally equivalent to Rogers' *D*) applied to individuals. The PCoA was implemented in the R package dartR (Gruber et al. 2018) available in the CRAN repository. The results are presented in 1 dimension because there were only 2 major clusters, corresponding to the 2 species, a decision supported by examination of the associated scree plot.

For quantitative assessments, the data were analysed with the software package NewHybrids (Anderson & Thompson 2002) to identify individuals representing F1 and F2 hybrids and backcrosses with one or the other parental species. Strictly, this software identifies F1 hybrids, and then groups gene frequencies into classes representing different levels of hybridization (F2 and backcrosses) based on posterior probabilities, often with some overlap. In the case of the Bellinger turtles, where the introduction of *Emydura macquarii* is relatively recent, the interpretation of the F1, F2 and associated backcrosses is likely to be unambiguous. The power of the analysis

was improved by inclusion of known parental geno-types, in the case of *M. georgesi* by using the samples collected in 1985, and for *E. macquarii* by using samples drawn from drainages adjacent to the Bellinger: Hastings-Macleay to the south, and in the vicinity of the township of Coffs Harbour (Clarence drainage) to the north. The NewHybrids software can handle only 200 loci before experiencing memory issues, a number far less than the number of loci available even after stringent filtering (7247). We selected a subset of 200 loci that were most informative in assessing hybridization, namely loci that showed fixed differences between the parental populations.

RESULTS

A total of 61244 polymorphic SNP loci were scored for the 534 individuals of *Emydura macquarii* and *Myuchelys georgesi*. After stringent filtering on repeatability (absolute repeatability) and call rate (0.98), the number of SNP loci in the data set dropped to 37906 and then 7335, respectively. Two individuals had call rates of less than 80% and were removed from the analysis (leaving n = 532), with the creation of 1 monomorphic locus that was also removed. Some sequence tags have more than 1 SNP, whereby they are included in the data by DArT Pty Ltd as separate entries. All but one of these entries were removed at random to leave 7247 loci which were used in subsequent analyses.

Genetic similarity of individuals in the PCoA established 2 clear groups corresponding to *M. georgesi* and *E. macquarii* (Fig. 2) on axis 1 (97.1% of variation). Axis 2 did not represent significant variation (0.1%). Individuals falling at positions intermediate

between these 2 major groups are putative hybrids or backcrosses between F1 individuals and one or the other parental species, or individuals showing some other level of introgression.

The qualitative analysis confirmed the assignment of 63 *E. macquarii* from the Bellinger River and 14 *E. macquarii* from the Kalang River to the species *Emydura macquarii*, in addition to the 41 parental *E. macquarii* from the Macleay, Hastings, Bellinger and Clarence (Coffs Harbour) drainages (Table 1). Similarly, the analysis confirmed the assignment of 380 *M. georgesi* to *Myuchelys georgesi*, in addition to the 4 nominated parentals collected in 1985. However, in addition, there were individuals with genetic profiles consistent with hybridization and introgression. Seven individuals (2 males and 5 females) had profiles most consistent with F1 hybrids (alleles universally heterozygous when fixed as alternate states in the parentals). Three of these specimens from the Kalang River were initially classified as *M. georgesi* based on superficial morphology, another specimen from the Bellinger River was originally classified as *M. georgesi*, and 2 specimens from the Bellinger River were originally classified as *E. macquarii* (Table 1). One female F1 individual (AA070217) from the Kalang River was among those transferred to the assurance colony, having been identified as *M. georgesi*. Three individuals had genetic profiles consistent with a backcross between an F1 individual and *E. macquarii*, and 3 individuals had genetic profiles consistent with a backcross between an F1 individual and *M. georgesi*. No F2 individuals were detected. All individuals from the Kalang River had genetic profiles consistent with *E. macquarii*, an F1 or a backcross between F1 and *E. macquarii*. There were no individuals in the Kalang River that assigned to pure *M. georgesi*.
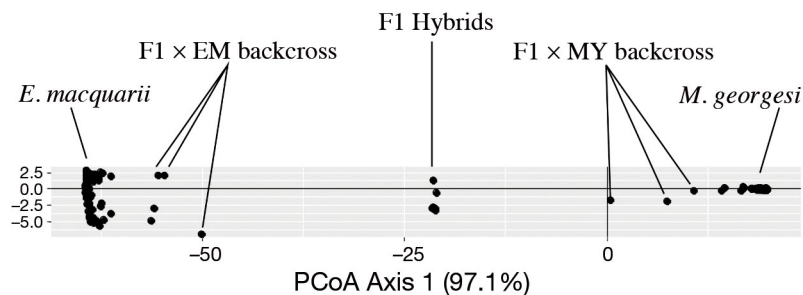

Fig. 2. A plot of genetic similarity among individuals using ordination applied to Euclidean genetic distance. Only axis 1 in the ordination contained substantial structural information (97.1%); variation against the vertical axis is insubstantial (0.1%), shown only to draw points out. Assignments of individuals to classes (parental *Emydura macquarii* [EM] and *Myuchelys georgesi* [MY]); F1 hybrids (F1); backcrosses of F1 hybrids to one or the other parentals, F1 × EM or F1 × MY) were made using NewHybrids (Anderson & Thompson 2002) as implemented in the dartR package (Gruber et al. 2018). No F2 hybrids were observed

DISCUSSION

Myuchelys georgesi is one of several highly divergent species in Australia that are restricted to single drainages, presumably a result of progressive aridification of the Australian continent over the past 15 million years (Byrne et al. 2008). These species also include the Fitzroy river turtle *Rheodytes leukops*, restricted to the Fitzroy-Dawson drainage of eastern Queensland; the Mary River turtle *Elusor macrurus*, restricted to the Mary River also of eastern Queens-

Table 1. Summary of individuals assigned to likelihood classes (parental *Emydura macquarii* [EM] and *Myuchelys georgesi* [MY]); F1 hybrids (F1); backcrosses of F1 hybrids to one or the other parentals, F1×EM or F1×MY) by NewHybrids (Anderson & Thompson 2002). Posterior probabilities of class membership are given in the body of the table. Sample sizes (N) are summed for individuals with the same profile. The F1 hybrid in the Taronga captive colony was from the Kalang River. Gray shading indicates non-zero posterior probabilities of assignment

Population	EM	MY	F1	F2	F1 × EM	F1 × MY	N
Parental *Emydura*	1.0000	0.0000	0.0000	0.0000	0.0000	0.0000	41
Emydura (Bellinger)	1.0000	0.0000	0.0000	0.0000	0.0000	0.0000	63
Emydura (Kalang)	1.0000	0.0000	0.0000	0.0000	0.0000	0.0000	14
Emydura (Bellinger)	0.4931	0.0000	0.0000	0.0000	0.5069	0.0000	1
Emydura (Bellinger)	0.0000	0.0000	1.0000	0.0000	0.0000	0.0000	2
Myuchelys (Bellinger)	0.0000	0.0000	1.0000	0.0000	0.0000	0.0000	1
Myuchelys (Kalang)	0.0000	0.0000	1.0000	0.0000	0.0000	0.0000	3
Myuchelys (Taronga)	0.0000	0.0000	1.0000	0.0000	0.0000	0.0000	1
Emydura (Bellinger)	0.0000	0.0000	0.0000	0.0000	1.0000	0.0000	2
Myuchelys (Kalang)	0.0000	0.0000	0.0000	0.0000	1.0000	0.0000	1
Myuchelys (Bellinger)	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	3
Myuchelys (Bellinger)	0.0000	0.9994	0.0000	0.0000	0.0000	0.0006	1
Myuchelys (Bellinger)	0.0000	1.0000	0.0000	0.0000	0.0000	0.0000	380
Myuchelys (Taronga)	0.0000	1.0000	0.0000	0.0000	0.0000	0.0000	17
Parental *Myuchelys*	0	1.0000	0.0000	0.0000	0.0000	0.0000	4

land; the Manning River turtle *Myuchelys purvisi*, found only in the Barnard-Manning River of eastern New South Wales; the western sawshell turtle *Myuchelys belli*, from the granitic headwater streams of the Murray-Darling drainage; and the western swamp turtle *Pseudemydura umbrina* of southeast Western Australia. These species are vulnerable simply because of their highly restricted distributions, regardless of local abundance (Georges 1993), and many deserve greater attention than they currently receive in conservation planning. *P. umbrina* has long been classified as Critically Endangered, but the case of *M. georgesi* serves to highlight how quickly a species with a restricted distribution (in this case, ca. 60 km of riverine habitat) can move from being secure to critically endangered. Two primary influences appear to be the cause: a rapidly spreading virus causing blindness and poor vigor that virtually extirpated the adult population in a matter of months (Jakob-Hoff et al. 2017, Zhang et al. 2018); and the founding of a population of the widespread generalist species *Emydura macquarii* (sensu Georges & Thomson 2010) in the Bellinger catchment. Its subsequent rapid increase in distribution and abundance within the oligotrophic Bellinger River has raised concerns that it is displacing the more specialized *M. georgesi* through competition for food, as the 2 species have considerable overlap in diet (Chessman 1986, Allanson & Georges 1999, Spencer et al. 2014). To this, we now add a risk that *E. macquarii* and *M. georgesi* are hybridizing, despite

their having diverged in the Lower Cretaceous (Ferreira et al. 2018), with 5 F1 hybrids detected, and evidence of backcrossing to the parental types. The F1 individuals are presumably fertile, as indicated by the presence of backcrosses, and the absence of F2 individuals is assumed to be the result of their low frequency in the population and sampling intensity. *E. macquarii* has genotypic sex determination, as do all chelid turtles studied so far (Bull et al. 1985, Georges 1988, Thompson 1988, Georges & McInnes 1998), and chromosome number 2n = 50 (Martinez et al. 2008). The karyotype of *M. georgesi* has not yet been described and so chromosomal homology across the 2 species has not been studied in detail.

Erosion of genetic variability in geographically restricted endemics increases vulnerability (Lande & Barrowclough 1987, Újvári et al. 2002). Low genetic diversity may indeed have contributed to the efficacy of the virus in killing *M. georgesi* while leaving the introduced *E. maquarii* and naturally resident *Chelodina longicollis* unaffected. In this context, some level of hybridization and horizontal transfer of alleles is not necessarily cause for concern. Injection of novel alleles may rescue genetic variability in the endemic species, increase its adaptive potential, and increase its resilience to future change in response to disease and environmental change (Becker et al. 2013, Hedrick 2013, Stelkens et al. 2014, Pfennig et al. 2016, Meier et al. 2017, Phillips et al. 2018). An example of genetic rescue is provided by the Florida panther *Puma concolor coryi*, in which 8 individuals from

another subspecies in Texas (*P. c. stanleyana*) were introduced to Florida (Johnson et al. 2010). Panther numbers in Florida have since increased threefold, genetic heterozygosity has doubled, survival and fitness indices have improved, and inbreeding correlates have declined significantly (Johnson et al. 2010). Similar success was achieved in restoration of an inbred population of the adder *Vipera berus* (Madsen et al. 1999). Recruitment of new alleles via hybridization occurs regularly without assistance from humans, and genetic variation arising from natural hybridization has conservation value. Thus, natural introgression between sympatric or parapatric sister species could be considered an *in situ* conservation strategy, particularly where the pure species are at risk of extinction (Becker et al. 2013). However, as *E. macquarii* has come to dominate the freshwater turtle fauna of the Bellinger River, the risk is that the genetics of the endemic species will be compromised by swamping (sensu Todesco et al. 2016) through increasing levels of hybridization and introgression. Indeed, in the Kalang River, all specimens of *M. georgesi* were either F1 hybrids or showed some level of introgression. We believe that the situation in the Kalang River provides a window to the future of the population of *M. georgesi* in the Bellinger River and supports the contention that the process is one of genetic swamping.

This presents a serious conundrum for managers and the community more broadly. Do we intervene to manage the steadily increasing population of *E. macquarii*, or do we let nature run its course? *E. macquarii* is not endemic to the Bellinger River drainage but is widespread in adjacent river systems (the Macleay River to the south; Clarence River to the north) and throughout eastern Australia. Translocation of specimens of *E. macquarii* from the Bellinger River back to their native range is not an option because of the risk of disease spread, the known virus being of particular concern. Euthanasia may need to be considered as a management option. Such an option has been adopted in the management of native populations of the endangered European white-headed duck *Oxyura leucocephala* in Spain and the United Kingdom, where it is threatened by hybridization with the more genetically diverse ruddy duck *Oxyura jamaicensis* introduced from North America (Muñoz-Fuentes et al. 2007). Management of Australian native vertebrates through lethal techniques has proven controversial and led to community backlash in many instances (Warburton & Norton 2009). *E. macquarii* may now also fill a role in the ecosystem that *M. georgesi* was filling before the disease outbreak. Hence, the question of inter-

vention is not solely based on risks of hybridization or competition between *E. macquarii* and *M. georgesi*. Management must balance the impacts that a steadily increasing population of *E. macquarii* will have on *M. georgesi* with broader ecological, community considerations and disease risk.

The prospect of hybrids in the natural population of *M. georgesi* is also clearly a concern when establishing a captive assurance colony. Until now, it was not possible to address this issue adequately using available mitochondrial sequences alone. We were able to identify an F1 hybrid among the animals taken from the wild to establish the assurance colony before the animals were brought together for breeding. That animal was removed so that we now can be certain that any head-started individuals returned to the river are genetically pure *M. georgesi* and not inadvertently contaminated with alleles from *E. macquarii*.

In summary, we contend that genetic swamping by the increasing population of *E. macquarii* is an additional major risk to the already critically endangered *M. georgesi*. This should inform management decisions on the likely efficacy of release of captive-bred *M. georgesi* into the wild and on the priority given to management of the impact of the introduced *E. macquarii*. As these 2 species are distantly related (Georges & Adams 1992, Georges et al. 1999, Fielder 2013, Ferreira et al. 2018), this study also serves to reinforce perceptions of the risks associated with human-mediated dispersal more generally. Such human-mediated dispersal can bring into contact species that would otherwise never or only rarely have met, and so provide opportunity for hybridization and introgression between even distantly related species, with uncertain consequences for already threatened species.

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