Fragmentation by major dams and implications for the future viability of platypus populations

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Abstract

Populations of the evolutionarily unique platypus (*Ornithorhynchus anatinus*) have experienced major declines and local extinctions from a range of historical and recent interacting threats, including fur trade, land clearing, water resource developments (dams and extractions), invasive species, fire, pollution, and urbanisation. Although spending most of their time in the water, platypuses can move over land. Nevertheless, significant uncertainties remain whether major dams across the platypus' distribution pose barriers to movement, limiting gene flow and dispersal, which are essential evolutionary and ecological processes. Here we examined disruption of gene flow between platypus groups below and above major dams (wall height > 10 m). Platypuses were surveyed above and below five major dams, matched to four adjacent rivers without major dams. Genetic differentiation (F_{ST}) across dams was 4- to 20-fold higher than along similar stretches of adjacent undammed rivers; F_{ST} across dams was similar to differentiation between adjacent river systems. This indicates that major dams represent significant barriers for platypus movements. Furthermore, F_{ST} between populations was significantly correlated with the year in which the dam was built, increasing by 0.011 every generation (7.9 years), reflecting the effects of these barriers on platypus genetics. This study provides evidence of gene flow restriction, which jeopardises the long-term viability of platypus populations when populations are fragmented by major dams. Alternatives to large dam construction, such as building of by-pass structures and translocation between upstream and downstream populations, should be considered in water conservation and management planning.

Introduction

The semi-aquatic platypus (*Ornithorhynchus anatinus*), along with echidnas, belong to the order Monotremata, the most species-scarce (n = 5) and most basal branch of the mammalian group, which diverged from marsupials and eutherians 187 Mya (Zhou et al., 2021). Platypuses have a unique combination of features, including oviparity, venomous spurs in males, electroreception used to locate freshwater macroinvertebrates, biofluorescent pelage, and multiple sex chromosomes (five pairs instead of one; Veyrunes et al., 2008; Bino et al., 2019; Anich et al., 2021). The uniqueness and rarity of platypus features (*sensu* Pavoine et al., 2005) and the longest evolutionary history in mammals (97.6 million years; Isaac et al., 2007) make it arguably the most irreplaceable mammal existing today.

There is increasing evidence of larger numbers of platypuses in historical times and ongoing declines and extinctions of local populations (Grant & Fanning, 2007; Bino et al., 2019; Hawke et al., 2019; Bino *et al.*, 2020). Declines are likely driven by multiple and synergistic threats, including river regulation, habitat modification, climate change, pollution, by-catch mortality, and predation by invasive species (Grant & Fanning, 2007; Bino et al., 2019; Hawke et al., 2019; Bino et al., 2020). Continued declines due to current and future climate change are predicted as a result of increased frequency and severity of droughts (Bino et al., 2019; Bino et al., 2021; Hawke, Bino, & Kingsford, 2021a), as well as elevated temperature conditions which could lead to the loss of more than 30% of suitable habitat by 2070 (Klamt et al., 2011; Hawke, Bino, & Kingsford, 2021a).

The platypus is currently listed as 'Near Threatened' by the International Union for Conservation of Nature (IUCN; Woinarski & Burbidge, 2016), 'Endangered' in South Australia (*National Parks and Wildlife Act 1972*), 'Vulnerable' in Victoria (Victoria Government Gazette, 2021). Past threats include hunting for fur, while present threats include extensive habitat degradation by agriculture and urbanisation, regulation of water flows, by-catch mortality in fishing gear, diseases, and predation by invasive foxes and dogs (Grant & Temple-Smith, 2003; Bino et al., 2019; Hawke, Bino, & Kingsford, 2021a).

Moreover, all these threats have possibly been intensified by the construction of major dams that have immediate and long-term effects, being one of the more serious threats for platypus conservation, given their likely broad impact on habitat (Grant & Temple-Smith, 2003; Bino et al., 2019; Hawke, Bino, & Kingsford, 2021a). Major dams are widespread across much of the platypus distribution, where as many as 77% (383 out of 495) of the Australian major dams (wall height > 10 m; ancold.org.au) coincide within the regions where platypuses occur (Fig. 1a; see also Bino et al., 2020). Immediate adverse effects of major dams extend over large areas both upstream and downstream. Water impoundments behind major dams form wind-exposed, deep, and standing (lentic) ecosystems. Below major dams, altered natural flow regimes can significantly impact platypus abundances and demographics (Hawke, Bino, & Kingsford, 2021b). Conditions below and above major dams represent poor foraging and burrowing habitat for platypuses, given lower productivity of macroinvertebrate prey species (Grant & Llewellyn, 1991; Bethge et al., 2003; Grant, 2004; Grant & Fanning, 2007; Marchant & Grant, 2015).

Long-term effects of major dams include reduction in the ability of platypuses to move between potential habitat areas. This fragmentation has twofold effects; first, it restricts the ability to recolonise available habitat or migrate to areas with more suitable conditions (Baguette et al., 2013). Secondly, and importantly, fragmentation also simultaneously reduces both local population size and gene flow, each of which is expected to lead to increased inbreeding and reduction of the genetic variation necessary for adaptation to changes including threats (Frankham et al., 2017). One adverse consequence of small population size is lower survival and lower reproduction output due either to inbreeding depression or to catastrophic stochastic events. Another adverse consequence is reduced variation between individuals, necessary for adaptation to changes such as the threats listed above (Frankham, 2015). These genetic changes may be prevented by immigration because gene flow replenishes the gene pool of populations, but of course, this will only happen if the small population is not a fragmented isolate (Garant et al., 2007; Tigano & Friesen, 2016).

For platypuses, major dams are predicted to be a barrier for dispersal (Kolomyjec, 2010; Furlan et al., 2013), with potential long-term ramifications for gene flow, genetic variation, and adaptation to threats as described above. However, both the restriction of dispersal and the genetic consequences remain largely unquantified. Population viability analyses suggest significant impacts by major dams, particularly in synergy with lower habitat quality and droughts, which are projected to increase (Bino et al., 2020). However, the extent to which major dams restrict platypus dispersal remains unclear because landscape connectivity varies due to both the species' life history and landscape features (Baguette et al., 2013). Platypuses are known to climb around dams up to 10m high (Jenolan Karst Conservation Reserve Newsletter, https://sway.office.com/qI6BOrvW8CO5vS8i?ref=email), although their ability to find their way around higher structures is currently unknown. Their ability to swim across the large deep-water impoundments above the dam is unclear.

Therefore, our research uses genetic methods to focus on the connectivity of platypus populations above and below major dams. Genetic-based methods used to infer patterns of dispersal and gene flow (Balkenhol et al., 2015) commonly examine the positive relationship between the amount of genetic differentiation between populations or individuals and the geographic distance separating them (Ramachandran et al., 2005). The presence of a dispersal barrier could be inferred by testing whether populations or individuals, separated by potential barriers, are more genetically differentiated than populations or individuals in landscapes lacking such barriers but separated by a similar distance. Genetic differentiation can increase due to dispersal barriers within one to 15 generations during simulations (Landguth et al., 2010), but is unlikely to arise if population size is large (> 50 individuals) or if the species lifespan is long (> 22 years; Hoffman et al., 2017).

To determine whether major dams have reduced dispersal and gene flow between platypus groups, we analysed genetic data from platypuses sampled in nine rivers; five rivers were regulated by major dams, and four were unregulated (Fig. 1). If major dams adversely affected gene flow between platypus groups, we predicted the following: a) individuals and groups separated by a major dam in a river should be more differentiated than in an unregulated river, and; b) genetic differentiation across major dams should correlate with the time since the dam was built.

Methods

Study areas and fieldwork

Samples from platypuses were collected from nine different rivers (five regulated by major dams and four unregulated) across four regions in south-east Australia (see Fig. 1 and Table 1), also described in Hawke et al., (2021b) Kolomyjec et al. (2008, 2009). River flows upstream of major dams were minimally regulated, contrasting with heavily regulated downstream flows. Throughout their range, the platypus comprises four major geographically defined genetic clusters: North Queensland, central Queensland, New South Wales and Tasmania (Martin et al., 2018). The samples used in this study belong to the New South Wales cluster.

Table 1
The four study systems and the major dams. See Fig. 1 for details of geography. The letters c, d, e and f refer to panels in Fig. 1.

Region	River/Creek	Dam name	Year of completion	Dam height (m)	Dam volume (GL)
Upper Murray Rivers[c]	Ovens	-	-	-	-
	Mitta-Mitta	Dartmouth	1979	180	3,856
Snowy Rivers[d]	Snowy	Jindabyne	1967	72	688
	Thredbo	-	-	-	-
	Eucumbene	Eucumbene	1958	116	4,798
Central NSW Rivers[e]	Wingecarribee	-	-	-	-
	Nepean	Nepean	1935	85	68
Border Rivers[f]	Tenterfield	-	-	-	-
	Severn	Pindari*	1969	85	312

GL - Gigalitres.

* Pindari Dam. The height of the dam wall was doubled from 45m to 85m in 1995.

Platypuses were captured across 81 sites (Fig. 1). In this study, we used two different molecular markers: single nucleotide polymorphism (SNPs) for all samples except Central NSW, and microsatellites for Central NSW (Kolomyjec et al., 2008; Kolomyjec et al., 2009). Sampling for microsatellites in Central NSW is described in Kolomyjec *et al.* (2008, 2009). For SNPs at all other sites, we aimed to cover a minimum of 40 km of each unregulated river and 20 km of river above and below major dams on regulated rivers. The procedure of trapping and sampling platypuses, including details of anaesthesia, used in this study have been described elsewhere (Bino et al., 2018; Hawke, Bino, & Kingsford, 2021b). Briefly, platypuses were captured using fyke nets or unweighted mesh (gill) nets and implanted with a Passive Integrated Transponder (PIT) tag (Trovan) to identify recaptured individuals. Platypuses were then weighed, measured, sexed, aged, and blood collected (~2 ml) and stored in Qiagen RNAprotect® animal blood tubes (Qiagen, Hilden, Germany). For the SNP sampling, our proxy of abundance for each river was the following metric: unique number of captures/number of sampling nights x length of the river surveyed (see Hawke, Bino, & Kingsford, 2021b).

Laboratory work

For SNPs (single nucleotide polymorphisms), Genomic DNA was extracted from whole blood using a Qiagen DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany). DNA quality and concentration were visualised using agarose gel electrophoresis and quantified fluorimetrically with a Qubit 2.0 (Thermo Fisher Scientific). Samples were genotyped using DArTseq™ (DArT Pty Ltd, Canberra, ACT, Australia). DArT's procedure uses a combination of genome complexity reduction methods using restriction enzymes, implicit fragment size selection and next-generation sequencing to produce thousands of SNPs randomly distributed throughout the genome (Kilian et al., 2012). Read sequences were processed using proprietary DArT analytical pipelines (Kilian et al., 2012) and mapped to the representative platypus genome (mOrnAna1.p.v1, GenBank assembly accession: GCA_004115215.2; total sequence length of 1.8 Gbps, 305 scaffolds with an N50 of 83 Mbp). Refer to Georges et al. (2018) for details of DArT sequencing, genotyping and filtering processes. DArT's genotyping has various advantages such as limiting the potential for ascertainment bias (Steane et al., 2011), providing metadata for each locus with various quality and BLAST alignment measures, including the proportion of replicates for which the marker score is consistent (RepAvg) and the average of the polymorphism information content of the reference and SNP allele (AvgPIC).

For microsatellites, DNA was extracted from toe-web biopsies (2 x 2 mm specimens stored in 70% ethanol) using a proteinase K/salt precipitation method (Sunnucks & Hales, 1996). Twelve published microsatellite sites were amplified and scored according to standard techniques (Kolomyjec et al., 2008; Kolomyjec et al., 2009).

SNP filtering

The criterion for SNP filtering used to analyse variation between populations (*e.g.*, F_{ST}) can bias estimates of variation within populations (e.g., heterozygosity; Schmidt et al., 2020). Therefore, we used different filtering settings for each type of analysis

(Table 2).

Table 2
Filters and their thresholds used for SNPs to remove sites for the analyses based on variation between groups and variation within groups.

Filter	Variation between groups	Variation within groups
Reproducibility (RepAvg)	> 100%	> 100%
Retain only one SNP per read	Used	Used
Departure from Hardy-Weinberg proportions	< 0.05	< 0.05
Mapped to chromosome	Used	Used
BLAST alignment E-value	< 1e-20	< 1e-20
Missing data by site	> 90%	> 100%
Missing data by individual	> 90%	> 100%
Minor allele count (MAC)	> 3	Not used
Linkage disequilibrium (r^2)	< 0.2	Not used
Remove sites located within coding regions	Used	Not used
Remove sites located within sex chromosomes	Not used	Used
Monomorphic sites	Used	Not used
Total SNPs after filtering	2,252	4,790

We first describe the filters applied to the entire SNP dataset, then those applied for analyses of variation between groups, and finally the filters applied to analyses of variation within groups. The filtering was performed using the R (R Core Team, 2021) package *dartR* (Gruber et al., 2018).

Filters applied to the entire SNP dataset. Because filtering for Hardy-Weinberg proportions requires the delimitation of populations or groups, we assigned individuals *a priori* into groups, based on the river that individuals were sampled and whether they were sampled below or above major dams. To reduce genotyping errors that might have arisen during library preparation and SNP calling (O'Leary et al., 2018), we discarded sites that had a reproducibility of less than 100% (RepAvg). We also discarded sites if the read had more than one SNP; or showed a significant departure from Hardy-Weinberg proportions within any one group after Bonferroni correction with a p-value of less than 0.05; or was not mapped to a chromosome and had a BLAST alignment E-value of less than $1X10^{-20}$.

Filters applied to analyses of genetic variation between groups in the SNP dataset. We discarded sites with more than 10% of missing data and then discarded individuals with more than 10% of missing data. By discarding sites based on minor allele frequency (i.e., proportion, MAF), there is the potential to alter subsequent analyses (Linck & Battey, 2019). Therefore, we discarded sites with a minor allele count (MAC) of less than three. Because physical linkage between sites can affect analyses of genetic structure (Abdellaoui et al., 2013), we removed one of two sites if they were in linkage disequilibrium (LD). For this, we used a threshold of the LD statistic r^2 (Hill & Robertson, 1968) of > 0.2 and the clumping algorithm using the R package *bigsnpr* (Privé et al., 2018). Then the SNP with lower polymorphic information content (AvgPIC) was discarded. Selectively neutral sites are better suited to infer population dynamics, such as dispersal, than sites under selection because they allow separation from potential confounding factors arising from natural selection (Holderegger & Wagner, 2008). Consequently, we removed sites located within coding regions using the GFF (General Feature Format; ref_mOrnAna1.p.v1_top_level.gff3) file retrieved from *Ensembl* (www.ensembl.org; Yates et al., 2020). Despite 3rd position SNPs usually being silent (*i.e.*, synonymous mutations), we also filtered out these SNPs for two reasons. Firstly, recent research suggests that synonymous mutations also affect fitness (Lebeuf-Taylor et al., 2019). Secondly, natural selection can affect not only genetic variation with direct consequences on fitness but can also affect

adjacent neutral genetic variation due to genetic linkage (Smith & Haigh, 1974). We also discarded sites monomorphic within each group.

Filters applied to analyses of genetic variation within groups in the SNP dataset. When sites with missing data are not removed, observed (*Ho*) and expected heterozygosity (*He*) estimates diverge (Schmidt et al., 2020); accordingly, we removed sites with missing data. *He* is generally lower for SNPs with rare alleles than for SNPs with common alleles (Schmidt et al., 2020). Therefore we did not filter out sites using minor allele count (MAC). Because filtering out sites based on linkage disequilibrium results in a decrease of rare and monomorphic alleles and excess of the common alleles and therefore biases estimates of *He* (Dementieva et al., 2021), we did not remove sites that were in linkage disequilibrium. Estimates of *He* should reflect as much as possible genome-wide patterns (Miller et al., 2014). Therefore we kept sites located outside and inside coding regions. We removed sites in sex chromosomes for two reasons. Firstly, *He* in sex chromosomes differs from *He* in autosomes because sex chromosomes occur in different proportions in males and females (Schaffner, 2004). Secondly, the platypus has five pairs of sex chromosomes (Veyrunes et al., 2008), which will bias the overall estimation of *He*. Including monomorphic sites to estimate *He* reduces the bias from sample size (Schmidt et al., 2020); consequently, we retained all monomorphic sites. Finally, relatedness analyses using the R package *related* (Pew et al., 2015) were performed to identify any recaptures not identified or mislabelling in the field or the laboratory.

For SNPs, a total of 295 platypuses were captured and blood sampled across four river regions in southeast Australia (Extended Data Table 2). DNA extraction and DArT^{TM} sequencing were successful in 218 blood samples from individuals. Two samples, each collected in a different river (V30 in Ovens and V32 in Mitta Mitta), showed contrasting genetic patterns relative to samples collected in the same river (Extended Data Figure 1). Relatedness analyses performed in the R package *related* (Pew et al., 2015) revealed these two samples had closer relatives in the opposite river (Extended Data Table 1). Additionally, the locations of these two samples were separated by 46 Km, steep mountainous terrain, and a river system. Under these conditions, we considered that dispersal events were unlikely and concluded that samples were mislabelled and therefore assigned them to the presumed correct river and site. Relatedness analyses also identified two pairs of samples in which each pair was collected from the same individual (*i.e.*, recaptures; samples T3-T5 and T28-T42; Extended Data Table 1). Consequently, we removed one sample from each pair. In the unlikely event that these were pairs of identical twins, it would still be appropriate to remove one of each pair.

For SNPs, sequencing provider DArT™ (Canberra) successfully genotyped 17,631 single nucleotide polymorphism (SNP) sites. After stringent filtering, our dataset for analysing genetic variation between populations comprised 2,252 SNPs genotyped in 214 platypus samples (108 females, 106 males). After filtering, our SNP dataset for analysing genetic variation within populations comprised 4,790 SNPs genotyped in 214 platypus samples (108 females, 106 males).

Data analyses

Genetic variation within groups

To measure genetic variation within rivers, we calculated observed heterozygosity (*Ho*), expected heterozygosity (*He*) and allelic richness using the R package *Hierfstat* (Goudet, 2005). After identifying that the data did not conform to a normal distribution, using a Shapiro-Wilk test of normality (R function *shapiro.test*), we tested whether *He* was significantly different between groups using a non-parametric Mann-Whitney U test (R function *wilcox.test* with option paired = FALSE). Additionally, we calculated the inbreeding coefficient (F_{IS}) of each river group using *Hierfstat*.

Investigating whether major dams affect connectivity between platypus groups

We used multiple approaches to investigate whether major dams affect gene flow between platypus groups. Firstly, to test whether groups separated by major dams are more genetically different than otherwise, we divided the sampling sites of each pair of rivers into comparable upstream and downstream groups. For regulated rivers (Nepean, Severn and Mitta-Mitta), the dam, ignoring the reservoir, was used as reference point for the division. For unregulated rivers (Wingecarribee, Tenterfield and Ovens), the division point was chosen at a comparable position to the dam in the paired regulated river. We then calculated the genetic differentiation using Weir & Cockerham's F_{ST} estimator (1984) between the two groups within each river. We tested the significance of the difference of F_{ST} values between dammed and unregulated rivers using a Mann-Whitney U test (R function *wilcox.test* with option paired =

FALSE). Additionally, we used Mutual Information (Sherwin et al., 2017) and Jost's D (Jost, 2008) two measures that assess between-group differentiation independently of within-group variation.

Secondly, to test whether the number of platypus generations since the building of the dams can predict the genetic differentiation of SNPs and microsatellites between populations (F_{ST}), we used univariate linear regression models (R function *lm*). We considered one platypus generation to be 7.9 years based on Pacifici et al. (2013), who used information on age at first reproduction and reproductive life span to estimate generation length in platypus.

Thirdly, to visualise the spatial distribution of genetic variation of the sampled individuals, we performed principal component analysis (PCA) using the R package *dartR* (Gruber et al., 2018) using our two datasets of SNP's and microsatellites. PCA is a statistical method that summarises the variance in the data and projects the top principal components onto a series of orthogonal axes (McVean, 2009). PCA is a method that does not rely on any genetic model or principle, but spatial patterns revealed by PCA are mathematically equivalent to coalescent analysis, so that they are representative of evolutionary processes such as genetic structure, gene flow and founder effects (McVean, 2009).

Results

Genetic variation within groups

Mean SNP genetic variation across all rivers (expected heterozygosity) was *He* = 0.141. *He* was significantly different between all groups within one river system (except for Severn above the dam/Severn below the dam; p-value > 0.05; Table 3). *He* was also significantly different between regions (except for Snowy Rivers/Upper Murray Rivers; p-value > 0.05; Table 3). Border Rivers, located in the north, had the lowest *He* (range: 0.132–0.138), followed by the Snowy Rivers (0.136–0.143) and the Upper Murray Rivers (0.143–0.153), river regions in the south (Fig. 1). Estimates of allelic richness follow the same trend as heterozygosity estimates. Inbreeding estimates (F_{IS}) were close to zero except for the microsatellite dataset (Table 3).

Table 3
Summary genetic statistics across the four rivers regions, the number of samples and a proxy of abundance calculated as (unique number of captures / number of sampling nights) x (length of the river surveyed) based on Hawke et al., (2021b); *Ho* - observed heterozygosity; *He* - expected Hardy-Weinberg heterozygosity; F_{IS} - inbreeding coefficient.

Region	River/Creek	Survey section (km)	Sample size	Proxy of abundance	*Allelic richness*	*Ho*	SE	*He*	SE	F_{IS}	SE
Upper Murray Rivers	Ovens	36	19	27	1.295	0.145	0.004	0.146	0.004	0.005	0.006
	Mitta-Mitta above dam	23	13	19	1.296	0.141	0.005	0.143	0.005	0.011	0.008
	Mitta-Mitta below dam	18	4	4	1.304	0.153	0.007	0.153	0.006	-0.021	0.013
Snowy Rivers	Snowy	26	56	46	1.282	0.141	0.004	0.141	0.004	0.007	0.004
	Thredbo	33	19	37	1.281	0.142	0.004	0.141	0.004	-0.005	0.006
	Eucumbene above dam	18	4	36	1.289	0.145	0.006	0.143	0.006	-0.028	0.012
	Eucumbene below dam	20	20	50	1.267	0.137	0.005	0.136	0.005	-0.004	0.007
Central NSW Rivers	Wingecarribee*	7	42	**	4.113	0.703	0.060	0.731	0.044	0.053	0.047
	Nepean above dam*	0.5	11	**	3.942	0.549	0.063	0.646	0.064	0.142	0.051
	Nepean below dam*	4	7	**	4.706	0.589	0.095	0.608	0.059	0.096	0.107
Border Rivers	Tenterfield	96	39	207	1.492	0.137	0.005	0.138	0.004	0.011	0.006
	Severn above dam	50	23	115	1.453	0.135	0.005	0.134	0.005	-0.008	0.007
	Severn below dam	60	17	83	1.440	0.133	0.005	0.132	0.005	0.000	0.007

* Microsatellite data

SE – standard error

NSW - New South Wales

Note that small sample sizes in Mitta-Mitta below the dam and Eucumbene above the dam (both 4 individuals) are likely to result in unreliable estimates of diversity.

** Comparable estimates are not available due to different survey techniques see Kolomyjec et al. (2009, 2010 and 2014).

Connectivity between platypus groups – effects of major dams

For unregulated and regulated river comparisons, the river with the dam showed higher genetic differentiation: Mitta-Mitta above versus below dam had F_{ST} = 0.021, whereas Ovens above versus below had F_{ST} = 0.001; Nepean below versus above dam had F_{ST} = 0.073, whereas Wingecarribee above versus below had F_{ST} = 0.016; and Severn below versus above dam had F_{ST} = 0.051, whereas Tenterfield above versus below had F_{ST} = 0.009 (Table 4). In each case, the dammed versus undammed F_{ST} values differed by more than two standard errors of the mean; the average F_{ST} for the three dammed rivers (0.048) was about five times higher than the paired undammed rivers (0.0087). The relatively high within-locality variation for microsatellites has the potential to lower F_{ST} for microsatellites relative to SNPs (Meirmans & Hedrick, 2011), however such a trend was not evident – in fact, the opposite trend was seen. Finally, in the more complex Snowy Rivers system (Fig. 1), this simple paired F_{ST} analysis was not easy to interpret, so we relied upon the other analyses presented below. Using Mutual information and Jost's D to assess genetic differentiation with and without major dams gave results that were comparable to those from F_{ST} (Extended Data Tables 3–6).

Table 4
Genetic differentiation (F_{ST}) between rivers in different connectivity scenarios.

Region	River 1	River 2	F_{ST}	SE	Connectivity scenario
Border Rivers	Tenterfield	Severn above dam	0.059	0.002	Separated by a river system
	Tenterfield	Severn below dam	0.063	0.002	Separated by a river system
	Severn below dam	Severn above dam	0.051	0.002	Separated by dam for 47 years (*Circa* 1969)*
	Tenterfield above	Tenterfield below	0.009	0.001	No dam
Upper Murray Rivers	Ovens	Mitta-Mitta above dam	0.045	0.002	Contiguous river systems
	Ovens	Mitta-Mitta below dam	0.035	0.003	Contiguous river systems
	Mitta-Mitta above dam	Mitta-Mitta below dam	0.021	0.003	Separated by dam for 39 years (*Circa* 1979)
	Ovens above	Ovens below	0.001	0.002	No dam
Snowy Rivers	Snowy	Thredbo	0.024	0.001	Separated by dam for 50 years (*Circa* 1967)
	Snowy	Eucumbene above dam	0.042	0.003	Separated by dam for 59 years (*Circa* 1958)
	Snowy	Eucumbene below dam	0.040	0.001	Separated by dam for 50 years (*Circa* 1967)
	Thredbo	Eucumbene above dam	0.043	0.003	Separated by dam for 59 years (*Circa* 1958)
	Thredbo	Eucumbene below dam	0.030	0.002	Separated by lake for 50 years (*Circa* 1967)
	Eucumbene above dam	Eucumbene below dam	0.053	0.003	Separated by dam for 59 years (*Circa* 1958)
Central NSW Rivers	Wingecarribee**	Nepean above dam	0.060	0.023	Contiguous river systems
	Wingecarribee**	Nepean below dam	0.062	0.013	Contiguous river systems
	Nepean above dam**	Nepean below dam	0.073	0.018	Separated by dam for 74 years (*Circa* 1935)
	Wingecarribee above**	Wingecarribee below	0.016	0.007	No dam

SE - standard error.

* Pindari Dam. The height of the dam wall was doubled from 45m to 85m in 1995.

** Microsatellite data

Over all four river systems, we observed a positive and significant relationship ($R^2 = 0.719$; p-value = 0.008) between F_{ST} and the number of platypus generations since the building of the dam (Fig. 2). We note again that potential bias towards lower F_{ST} values microsatellites than SNPs, mentioned above, was not evident – the oldest dam was in the river system analysed by microsatellites, and this system showed the highest F_{ST} (Fig. 2). The regression is also significant if this system is excluded (data not shown).

PCA analyses of the Upper Murray Rivers (Mitta Mitta and Ovens Rivers) did not show complete separation of samples for different locations, but there was noticeable clustering of platypuses into three groups: all Ovens river (unregulated); below the dam in the Mitta-Mitta River, and above the dam in the Mitta-Mitta River (Fig. 3a). Snowy Rivers (Snowy, Thredbo and Eucumbene Rivers) did not follow the paired experimental design due to geographic constraints. PCA analyses showed that platypuses from the Snowy

River formed a separated cluster to that of the Thredbo and Eucumbene Rivers (Fig. 3b), whereas platypuses from the two latter rivers overlapped somewhat on the PCA plot. Notably, platypuses from the Eucumbene River above the dam were closer to platypuses from Thredbo River than platypuses from the Eucumbene River below the dam. PCA analyses of the central New South Wales Rivers (Nepean and Wingecarribee Rivers) showed that platypuses from below and above the dam in the regulated Nepean River formed separated clusters in contrast to platypuses sampled at the unregulated Wingecarribee River, which forms only one cluster (Fig. 3c). For the Border Rivers (Tenterfield Creek and Severn River), the principal component analysis (PCA) of these rivers indicated three well-separated clusters (Fig. 3d), with platypuses collected below and above the dam in the Severn River, and Tenterfield Creek forming different groups.

Discussion

Dispersal and gene flow are essential for the viability of natural populations, critical for ecological and evolutionary processes such as recolonisation, dispersal to suitable habitats, increased genetic diversity to avoid inbreeding depression and allow adaptation (Garant et al., 2007; Baguette et al., 2013; Tigano & Friesen, 2016). Our analyses suggest that major dams are barriers to platypus dispersal and gene flow, because genetic differentiation increased proportionally with time after the building of a dam and was higher in dammed than undammed rivers.

In relation to whether major dams affect the connectivity between platypus groups, F_{ST} values were higher when there was a dam, and some F_{ST} values between groups separated by a dam were as high as F_{ST} values between groups in different rivers (Table 4). Additionally, we found a significant association between F_{ST} and the number of platypus generations since dam construction (Fig. 2), suggesting that F_{ST} increases at a rate of 0.011 by generation. Even though the Nepean dam, built in 1935, was analysed with a different type of molecular marker (microsatellites, not SNPs), recent research indicates that estimates of F_{ST} using SNPs and microsatellites are comparable (Lemopoulos et al., 2019; Sunde et al., 2020). If anything, we would expect the microsatellites used in this system to have lower F_{ST} due to the effect of their high within-population variation (Meirmans & Hedrick, 2011; Sherwin et al., 2017), but in fact the opposite trend was seen. We noticed that F_{ST} values in the Snowy Rivers were higher between groups separated by the Jindabyne Dam (Eucumbene below dam / Snowy; F_{ST} = 0.04) than between groups divided by the Jindabyne reservoir but not a dam (Eucumbene below dam / Thredbo; F_{ST} = 0.03). This observation suggests that some limited gene flow might have occurred across the Jindabyne reservoir.

Overall, our results are consistent with the notion that major dams and their associated waterbodies may be considerable barriers for platypuses. Despite platypuses being able to move substantial distances (*e.g.*, male juveniles can move > 40 km; Serena & Williams, 2013; Hawke, Bino, Kingsford, et al., 2021a; Hawke, Bino, Kingsford, et al., 2021b), the effect of major dams on genetic differentiation was considerable. Contrastingly, major dams did not increase genetic differentiation in the blackfish (*Gadopsis marmoratus*), a non-migratory and low-mobility freshwater fish species in eastern Australia, possibly because blackfish population sizes were not small enough to create this effect during the time since dams were built in the blackfish range (Coleman et al., 2018).

Major dams represent dispersal barriers for most freshwater species (Søndergaard & Jeppesen, 2007; Nislow et al., 2011), requiring mitigation strategies to offset negative demographic impacts. For instance, human-mediated relocation of individuals between populations has been implemented successfully to limit the effects of population isolation and small population size (Hoffmann et al., 2020). A common rule of thumb in conservation suggests that one dispersing individual per generation would minimise the effects of population isolation (Mills & Allendorf, 1996). Another strategy to improve connectivity between populations, despite some limitations and caveats, is the construction of dam passages that increase dispersal of freshwater species, including platypuses (Broadhurst et al., 2013; Brown et al., 2013; Silva et al., 2018), although there are adverse consequences of connectivity, such as disease risks (Sainsbury & Vaughan-Higgins, 2012). Efforts to maintain flow regimes, riparian revegetation and urban stormwater management are an essential part in the conservation of the platypus (Coleman et al., 2022).

We have found that platypus population connectivity is adversely affected by major dams, and it is known that reduced connectivity can lead to the adverse long-term conservation outcomes described above (Garant et al., 2007; Baguette et al., 2013; Tigano & Friesen, 2016; Frankham, 2015 Frankham et al. 2017). Therefore there will be a need for the management of platypus to consider ways such as those just described to minimise detrimental effects of river regulation on the platypus (and other species). Some of the long-term effects of major dams might be reduced by rare natural dispersal events between rivers (Kolomyjec et al., 2014), but

our results indicate that this has not been enough to offset the divisive effect of the major dams, so more active management is required. Firstly, new dams within the platypus distribution need to be avoided, for example, by pumping from the river into an off-stream storage without the necessity for a dam on the river itself, as is done in both the Manning and Hastings Rivers. Secondly, for existing major dams, it might be possible to devise platypus-specific versions of methods that have been used to ameliorate dam effects in other species, such as human-mediated relocation of individuals or dam passages that increase dispersal.

Conclusion

We compared regulated rivers, with major dams, to adjacent unregulated rivers with no major dams and identified that major dams were barriers to movement of platypuses within a river system, reflected in genetic variation. Major dams restricted dispersal and gene flow between groups and therefore increased the possibility of inbreeding depression, loss of adaptive genetic variation, failure to recolonise areas where local extinctions have occurred and failure to disperse to areas with more suitable conditions. These are all expected to lower the long-term viability of the platypus (Bino et al., 2020). Our analyses reinforce the growing evidence on the negative impacts of major dams on platypus populations. These studies are relevant to inform the decision-making process of conservation managers and could be used in viability analysis and decision analysis (Drechsler & Burgman, 2004) to develop strategies that ensure the long-term persistence of the unique platypus.

Declarations

AUTHORS CONTRIBUTIONS

Bill Sherwin, Gilad Bino, Richard Kingsford, Jaime Gongora and others conceived the project and acquired the research funds; Harvinder Sidhu and Bill Sherwin supervised Luis Mijangos; Jenna Day, Kimberly Noel Dias and Jaime Gongora performed DNA extraction; Gilad Bino, Tahneal Hawke and Tom Grant carried out fieldwork and collected samples; Stephen Kolomyjec performed the microsatellite analyses; Luis Mijangos analysed the data; Luis Mijangos wrote the manuscript with support from Bill Sherwin. Tom Grant and Stephen Kolomyjec first pondered on the possible effect major dams may be having on the genetics of the platypus, carried out the initial research and encouraged others to further investigate this aspect of platypus conservation. All authors discussed the results and contributed to the final manuscript.

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Figures

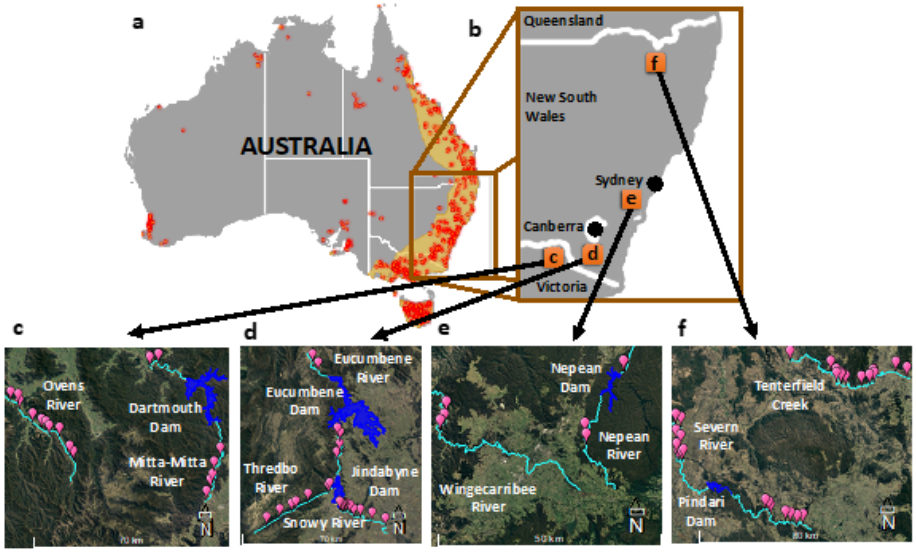

Figure 1

a) Distribution of major dams (> 10 m high; red points) within and outside the IUCN platypus distribution (yellow shade; Woinarski & Burbidge, 2016), and the focus regions for this study (brown inset). b) Location of rivers in south-east Australia where platypuses were sampled (orange squares) in rivers that were regulated (with major dams) and unregulated (no major dams). c) Upper Murray Rivers: Ovens (unregulated) and Mitta-Mitta Rivers (regulated, upstream sections are in the south, confluence with Ovens is out of the frame, in northwest). d) Snowy Rivers (do not follow the paired experimental design, due to geographic constraints; see methods): Eucumbene (regulated), Thredbo (unregulated), and Snowy River (regulated, Snowy flows downstream to the southeast). e) Central NSW Rivers: Wingecarribee River (unregulated) and Nepean River (regulated, downstream sections are in the north, there is no confluence with Wingecarribee). f) Border Rivers: Tenterfield Creek (unregulated) and Severn River (regulated, upstream sections are to the east, confluence with Tenterfield is out of the frame, in northwest). Pink balloons represent the 81 sampling sites; rivers are coloured in light blue, and reservoirs behind major dams are in dark blue.

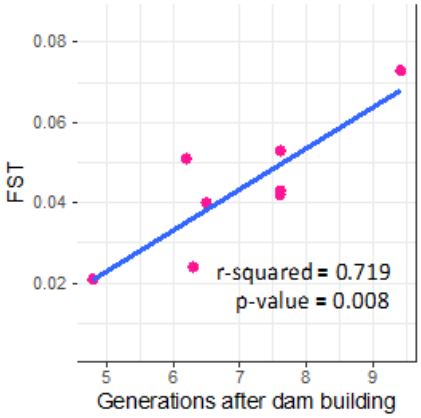

Figure 2

Relationship between genetic differentiation (F_{ST}) between platypus groups separated by major dams and the number of platypus generations (7.9 years; Pacifici et al., 2013) since the building of the dam. Genetic differentiation increased at a rate of 0.011 per generation.

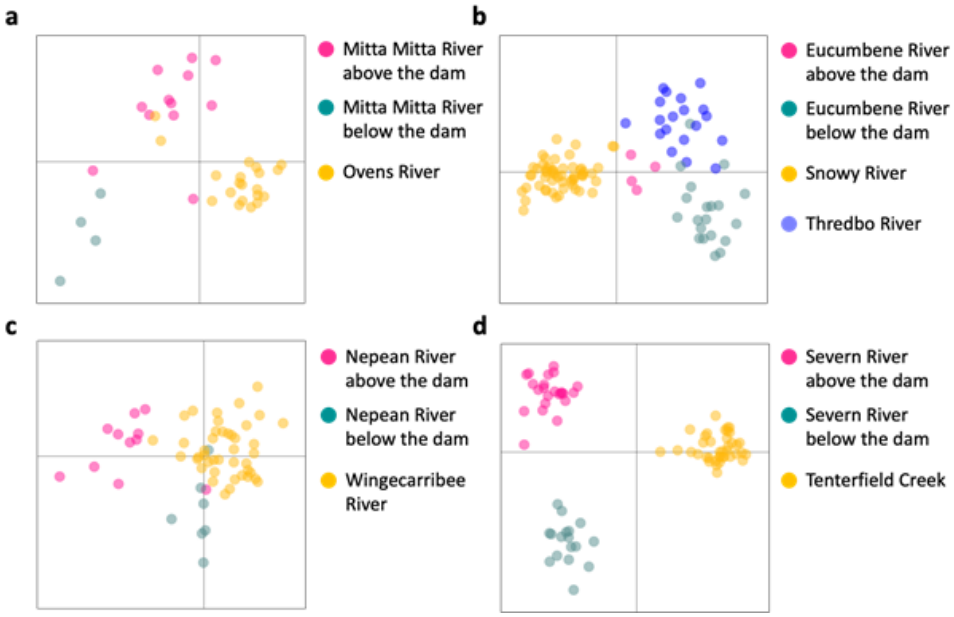

Figure 3

Plot of the first two principal components (PCs) of principal coordinates analysis (points represent platypus individuals). a) Upper Murray Rivers: unregulated (no dam) Ovens and regulated (dam) Mitta-Mitta Rivers. b) Snowy Rivers: regulated (dam) Snowy, unregulated (no dam) Thredbo and regulated (dam) Eucumbene Rivers. These rivers do not follow the paired experimental design due to geographic constraints. c) Central NSW Rivers: regulated (dam) Nepean and unregulated (no dam) Wingecarribee Rivers. d) Border Rivers: unregulated (no dam) Tenterfield Creek and regulated (dam) Severn River.

Supplementary Files

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