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Islands of water in a sea of dry land: hydrological regime predicts genetic diversity and dispersal in a widespread fish from Australia's arid zone, the golden perch (*Macquaria ambigua*)

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Abstract

Rivers provide an excellent system to study interactions between patterns of biodiversity structure and ecological processes. In these environments, gene flow is restricted by the spatial hierarchy and temporal variation of connectivity within the drainage network. In the Australian arid zone, this variability is high and rivers often exist as isolated waterholes connected during unpredictable floods. These conditions cause boom/bust cycles in the population dynamics of taxa, but their influence on spatial genetic diversity is largely unknown. We used a landscape genetics approach to assess the effect of hydrological variability on gene flow, spatial population structure and genetic diversity in an Australian freshwater fish, *Macquaria ambigua*. Our analysis is based on microsatellite data of 590 samples from 26 locations across the species range. Despite temporal isolation of populations, the species showed surprisingly high rates of dispersal, with population genetic structure only evident among major drainage basins. Within drainages, hydrological variability was a strong predictor of genetic diversity, being positively correlated with spring-time flow volume. We propose that increases in flow volume during spring stimulate recruitment booms and dispersal, boosting population size and genetic diversity. Although it is uncertain how the hydrological regime in arid Australia may change under future climate scenarios, management strategies for arid-zone fishes should mitigate barriers to dispersal and alterations to the natural flow regime to maintain connectivity and the species' evolutionary potential. This study contributes to our understanding of the influence of spatial and temporal heterogeneity on population and landscape processes.

Keywords: climate change, conservation genetics, landscape ecology, landscape genetics, Murray-Darling Basin, phylogeography

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Introduction

Understanding the structure, scale and function of landscapes is a research priority in biology, especially within the current context of rapid global environmental change. The discipline of landscape ecology provides ways to study spatial patterns of biodiversity and their effect on ecosystem processes (Turner 1989). However, ecological systems are spatially heterogeneous and exhibit considerable complexity and spatial and temporal variability—this problem of pattern and scale has been considered one of the central problems in ecology (Levin 1992). Testing hypotheses about biodiversity structure over the landscape can potentially identify processes, phenomena and scales at which spatial heterogeneity is important or at which its contribution could instead be ignored (Turner 1989). This framework is not

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restricted to terrestrial ecosystems. In fact, riverine networks can be viewed as spatially variable landscapes with a hierarchical nature. They are composed of a dynamic assortment of spatial elements and ecological processes arranged hierarchically, providing an excellent system to study interactions between patterns of biodiversity structure and ecological processes in the context of spatial heterogeneity (Ward *et al.* 2002). A practical way of documenting spatial patterns of biodiversity in difficult to observe aquatic species is by quantifying gene flow and elucidating population structure. Clarifying the genetic architecture of riverine species can therefore facilitate our understanding of the connection between spatial heterogeneity and environmental processes. Landscape genetics can help identify these processes. Landscape genetics is an area of study integrating population genetics, environmental data and spatial statistics (Manel *et al.* 2003; Storfer *et al.* 2007; Holderegger & Wagner 2008). This approach is valuable for exploring the interaction between contemporary population and environmental processes and understanding factors that maintain regional levels of biodiversity (Manel *et al.* 2003; Diniz-Filho *et al.* 2008).

In riverine freshwater fishes, gene flow is spatially restricted by the hierarchical nature of the drainage network and can be further constrained by temporal changes in hydrological connectivity across the network. This is particularly true in the Australian arid zone where the hydrological regime of freshwater environments is among the most variable in the world (Puckridge *et al.* 1998). For the majority of the time, freshwater systems in the Australian arid zone exist as a series of remnant waterholes, often isolated by hundreds of kilometres, like 'islands of water in a sea of dry land'. 'Islands' can be considered as any patch of suitable habitat that is surrounded by unsuitable habitat, and movement among these 'islands' is influenced by several factors, including their spatial and temporal isolation (MacArthur & Wilson 1967). In our study system, 'islands' or waterholes are only connected during flow events, the frequency and magnitude of which are governed by the dominant climatic systems of the continent (Williams 1970; Kotwicki & Isdale 1991; Kotwicki & Allen 1998). The tropical monsoon is active in the northern part of Australia during summer, resulting in rainfall that is highly variable across seasons. In contrast, the southern temperate zones experience more consistent rainfall throughout the year, but tend towards a Mediterranean winter rainfall pattern in the south (Linacre & Hobbs 1977). In addition to seasonal variation, climatic conditions also fluctuate over interannual timescales with extensive drought and flood periods being driven by El Niño Southern Oscillation (ENSO) (Kotwicki & Isdale 1991). The outcome of this combination of conditions is a highly variable hydrological regime encompassing the timing and extent of major runoff events and persistence of connectivity in freshwater systems.

This hydrological regime has a considerable effect on the dynamics of freshwater communities, causing fluctuations in population size and limiting the potential for dispersal (boom and bust cycles, Kingsford *et al.* 1999; Huey *et al.* 2008; Balcombe & Arthington 2009). However, how this regime affects spatial patterns of gene flow and population structure is largely unknown. The majority of genetic studies have focused on evolutionary timescales (Hughes & Hillyer 2003; Byrne *et al.* 2008), and the few studies that considered contemporary processes were restricted to small geographical regions (e.g. Huey *et al.* 2006; Hughes & Hillyer 2006). This study uses golden perch (*Macquaria ambigua*), which is an ideal candidate for exploring the relationship between contemporary population genetic processes and hydrological variability across a broad area of the Australian arid and temperate zones.

Macquaria ambigua is naturally distributed across a large area of the Australian arid zone, including the Murray-Darling (MDB), Lake Eyre (LEB), and Bulloo-Bancannia (BULL) basins, and the coastal Fitzroy (FITZ) basin (Allen *et al.* 2002) (Fig. 1). The species has also been translocated into various coastal basins and stocked into impoundments and rivers across the MDB to support its recreational fishery. In the MDB, three stocking zones exist, but stocking among catchments within these zones does occur. However, a mtDNA phylogeographical study strongly indicates that MDB populations have been historically characterized by low levels of genetic variation and structure (Faulks *et al.* 2010; details below). Therefore, stocking activities are not expected to have significantly impacted the natural genetic architecture of these populations. *Macquaria ambigua* is a relatively common (Wager & Unmack 2000; Pusey *et al.* 2004; Davies *et al.* 2008), generalist species that lives in a broad range of environments (Crook 2004) and possesses good dispersal abilities during all life history stages (Reynolds 1983). During winter and early spring, *M. ambigua* matures in preparation for the reproductive season (Mackay 1973; Rowland 1996) with an increase in water temperature during spring, initiating the upstream migration of fish in preparation for spawning (Rowland 1996; Roberts *et al.* 2008). Some of these dispersal events have been very extensive (>2000 km) and often coincide with high flow conditions (Reynolds 1983; Harris & Gehrke 1994). Spawning occurs in aggregations, where eggs are released, fertilized and drift downstream (Lake 1967). The upstream migration of adults during the reproductive season (spring and summer) is thought to compensate for this

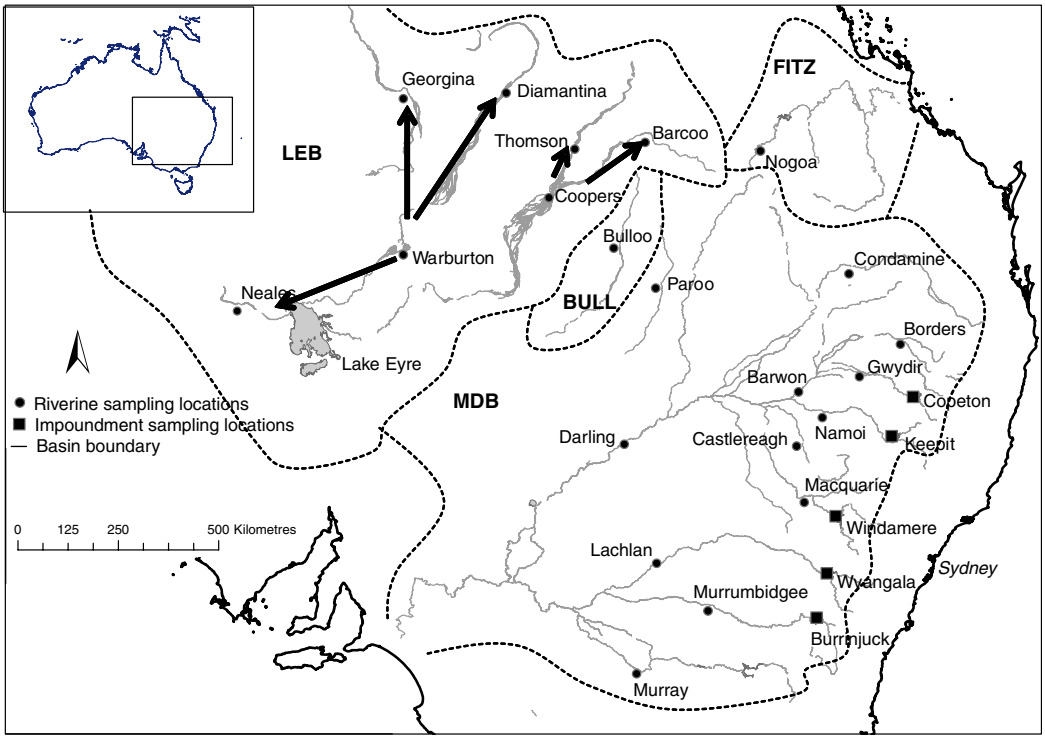

Fig. 1 Map of the drainage network and sampling location for *Macquaria ambigua* in this study with an inset showing the location of the study area in eastern Australia. Dots indicate riverine sites and squares impoundments. Grey dotted lines represent basin boundaries with names abbreviated as follows: BULL, Bulloo; FITZ, Fitzroy; LEB, Lake Eyre Basin; MDB, Murray-Darling Basin. Arrows indicate direction of gene flow as calculated in BAYESASS+ (all ~0.3 migrants per generation).

downstream drift of pelagic eggs and larvae, particularly during high-flow events (Mackay 1973; Rowland 1996). *Macquaria ambigua* has often been observed spawning in response to flood pulses (Balcombe *et al.* 2007; Roberts *et al.* 2008), but there is increasing evidence that the unpredictable conditions in Australian freshwater environments require opportunistic reproductive strategies, including spawning during low/no-flow events (King *et al.* 2003; Mallen-Cooper & Stuart 2003; Ebner *et al.* 2009).

We have recently used coalescent-based phylogeographical methods to assess the influence of past climatic conditions on the evolutionary history of *M. ambigua* (Faulks *et al.* 2010). In addition to the findings mentioned previously, we demonstrated that populations of *M. ambigua* underwent expansions in both range and population size during moister climatic phases of the Pleistocene, with subsequent isolation of drainage basins as conditions became increasingly arid. Here, we investigate the relative importance of climate and hydrology in shaping contemporary gene flow within and between river drainages and over the entire species range. Our general aim is to use a landscape genetics approach to clarify how this common and widespread freshwater fish disperses throughout a vast area of the Australian arid zone. In particular, we aimed to test the effect of hydrological variability on rates of gene flow, spatial population structure and genetic diversity. We hypothesized that regions with greater hydrological variability would lead to unpredictable and infrequent connectivity of populations, resulting in greater population structure. In addition, we predicted that the boom and bust cycles experienced by freshwater organisms would result in a series of population bottlenecks, reducing genetic diversity in the most variable environments. We anticipate that this study will increase our understanding of the influence of spatial and temporal heterogeneity on population and landscape processes of aquatic species.

Methods

Sample collection and DNA extraction

A total of 590 samples were obtained from across the species range in eastern Australia (Fig. 1). Twenty-six locations were sampled, including samples from each of the 21 major catchments within basins and five major

impoundments in the MDB. Samples were collected using nondestructive methods such as electrofishing and netting. A piece of caudal fin tissue approximately 5 mm^2 was taken from each fish, placed in 100% ethanol and stored at −20 °C in the laboratory. Total DNA was extracted from the tissue using a modified salting out method. We modified the method of Sunnucks & Hales (1996) by doubling the volume of TNES buffer and proteinase K in the initial digestion and doubling the volume of saturated salt solution used to precipitate proteins.

Microsatellite genotyping

Eight microsatellite DNA loci were amplified using PCR primers originally developed for Australian bass (*Macquaria novemaculeata*) [AB009 (Schwartz *et al.* 2005)] and Murray Cod (*Macculochella peelii*) [Mpe3.B11, Mpe2.E01, Mpe1.F01, Mpe2.F07, Mpe3.G04, Mpe3.G12, Mpe1.H04 (Rourke *et al.* 2007)]. The forward primer for each primer pair was labelled with an M13 tag (Schuelke 2000). Fluorescent dyes were also labelled with the M13 tag. Loci were amplified and fluorescent dyes incorporated in either single (AB009 VIC, Mpe3.B11 NED, Mpe2.E01 NED, Mpe3.G12 VIC) or multiplex (Mpe3.G04 and Mpe1.H04 PET, Mpe1.F01 and Mpe2.F07 FAM) reactions. PCR consisted of 1 μL template DNA, 2.5 mmol $MgCl_2$, 60 mM KCl, 12 mM Tris–HCl pH 9.0, 0.12% Triton-X, 0.4 mM each dNTP, 0.78 pmol M13 forward primer, 3.8 pmol reverse primer, 3.8 pmol M13 fluorescent dye, 0.5 μg bovine serum albumin, 0.25 U Taq DNA polymerase (Promega) and dH_2O to a final volume of 10 μL. PCR conditions were as described in Schwartz *et al.* 2005. PCR products for the eight loci (3–4 μL) for each sample were pooled, and 1 μL of this product was mixed with 0.1 μL LIZ500 ladder and 9.9 μL HiDi. Products were then screened on an ABI PRISM 377 with GENESCAN software (Applied Biosystems) at the Macquarie University DNA Analysis Facility. Genotypes were visually inspected and scored using GENEMAPPER (Applied Biosystems). In cases of poor amplification, re-runs were conducted with a single locus per reaction and without pooling. Genotypes were checked for scoring errors caused by null alleles, stutter and large allele dropout using MICROCHECKER 2.2.3 (Van Oosterhout *et al.* 2004).

Data analysis

Genetic diversity. Fisher's exact test of linkage disequilibrium between all pairs of loci and conformance to Hardy–Weinberg equilibrium in each population across all loci were tested using GENEPOP 3.4 (Raymond & Rousset 1995). Significance levels were Bonferroni corrected to address the risk of increased Type 1 error associated with multiple tests (Rice 1989) and B-Y corrected to balance the risks of Type 1 and Type 2 errors (Narum 2006). The expected (H_E) and observed (H_O) heterozygosities were calculated in ARLEQUIN 3.1 (Excoffier *et al.* 2006) and allelic richness (A_R) per population in FSTAT 2.9.3.2 (Goudet 2002).

Population structure. Population differentiation was evaluated using global and pairwise F_{ST} tests and levels of population subdivision were assessed using the hierarchical partitioning of molecular variance (AMOVA). Two levels were investigated: among all basins and among locations in the MDB and LEB. The BULL and FITZ were not analysed separately because of the lack of replication within these basins. Weir and Cockerhams F_{ST} values (Weir & Cockerham 1984) and AMOVA were conducted in ARLEQUIN 3.1. We used F_{ST} rather than R_{ST} as a result of our relatively small number of loci (<20) and recently diverged populations (Gaggiotti *et al.* 1999; Faulks *et al.* 2010).

Preliminary data analyses using both STRUCTURE (nonspatial) (Pritchard *et al.* 2000) and GENELAND (spatial) (Guillot *et al.* 2005) to estimate population structure obtained similar results. GENELAND was chosen for final analyses, as the incorporation of spatial information provides a more realistic representation of the study system optimizing the ability to determine population structure (*sensu* Dionne *et al.* 2008; Latch *et al.* 2008). Three analyses were conducted: all populations, populations from the MDB and populations from the LEB. This approach was used, as the analysis of different hierarchical levels has been shown to help resolve finer scale genetic structure (Evanno *et al.* 2005). This program uses a Markov chain Monte Carlo (MCMC) approach to identify genetic spatial discontinuities. Five replicate runs were performed each with 1×10^6 MCMC iterations, the Dirichlet model of allele frequencies, no uncertainty in the spatial coordinates, maximum rate of Poisson process 100 and the maximum number of nuclei in the Poisson-Voronoi tessellation set to 200. Plots of MCMC runs were assessed for mixing. The most probable number of population clusters (K) was the same for each run. The final output displays maps of posterior probabilities of locations belonging to a particular genetic cluster or population (K).

Gene flow. Estimates of recent gene flow among populations within the MDB and LEB were assessed in BAYESASS+ 1.3 (Wilson & Rannala 2003). The program was run for 3×10^6 iterations including a burn-in of 1×10^6 iterations. Delta values for allele frequencies, inbreeding coefficients and migration rate were set at 0.55, 0.55, 0.1 in the LEB and 0.35, 0.45, 0.05 in the MDB. These values

achieved the recommended acceptance rates of changes (40–60%) (Wilson & Rannala 2003). Convergence was assessed by plotting the cumulative log likelihoods of the iterations. Estimates were considered 'real' if they were consistent in at least 7 of 10 replicate runs and the run with the best acceptance rates and convergence was chosen for the parameter estimates.

Environmental variables. The influence of hydrology on gene flow (BAYESASS+ results) was assessed by comparison with a comprehensive summary of flow records (Kotwicki 2003). This document describes flow regime across the entire LEB. The data includes readings from hydrological gauging stations dated back to the 1940s, as well as anecdotal observations from early explorers. In addition, we used a model selection approach to identify the environmental variables influencing genetic diversity (measured as H_E) (Akaike 1973). The following variables were calculated for each riverine sampling location (not impoundments) using the stream network model developed by Stein *et al.* (2009): mean annual flow, mean summer-, autumn-, winter- and spring-time flows, coefficient of variance (CV) of mean annual flow and perenniality (proportional contribution to mean annual flow during the six driest months of the year). This model was developed using landscape and climate variables to characterize the flow regime of streams across Australia and was evaluated by comparison with data from gauging stations (Stein 2006). These flow variables were chosen to incorporate seasonal variation in flow as well as total annual flow and were thought to be most influential on the migratory and reproductive behaviour of the species. Flow values, perenniality and the CV for each sampling location were log transformed. Latitude was also included as a 'control' variable to take into consideration other unknown climatic variables that could be associated with the distribution of genetic diversity (*sensu* Banks *et al.* 2007). However, it is acknowledged that not all of the potential variables influencing genetic diversity could be included in the model selection process. For the model selection analysis, all variables were standardized to a mean of zero and standard deviation of one. The log likelihoods of the linear models of all possible combinations of the environmental variables explaining genetic diversity were estimated using the R 2.5.1 package hier part (Walsh & MacNally 2008). The best models were then selected using Akaike's Information Criterion for small sample sizes (AICc) (Akaike 1973) and the independent contribution of each variable to the model was estimated using hierarchical partitioning. Stepwise and multiple linear regressions were also conducted (Minitab 15) to attain the regression equations, adjusted R^2 and assess the level of statistical support (ANOVA) for the variables in the best models selected by AICc. In addition, we conducted a resampling-based regression analysis to estimate the effect of our environmental variables on H_E while controlling for unequal sample sizes among sites. In each of 10 000 iterations, we resampled an equal number of individuals (15) from each population, recalculated mean H_E over loci within a population and fitted a linear regression model of spring-time flow volume to the H_E data (the variable found to most influence H_E). We obtained the distribution (95% CI) of regression coefficients and P values and considered our conclusions robust if greater than 95% of P values were <0.05 and >97.5% of the coefficients were either consistently positive or consistently negative. Finally, we performed simple Mantel tests in FSTAT 2.9.3.2 (Goudet 2002) to determine the effect of riverine distance and connectivity on gene flow (estimates of migration rates from BAYESASS+). Partial mantel tests enabled the role of connectivity in shaping gene flow to be assessed while controlling for geographical distance. Riverine distances between pairs of populations were calculated using ARCMAP, and connectivity was categorized based on estimates of flooding frequency (0 = never connected, 1 = connected by annual flows).

Results

Low genetic diversity and widespread population bottlenecks

We amplified eight polymorphic loci with no consistent evidence for null alleles, large allele dropout or linkage disequilibrium. Only three of 26 populations were not in Hardy–Weinberg equilibrium using the Bonferroni procedure and nine of 26 using the B-Y procedure (Table 1). Two of these populations are stocked impoundments (Burrinjuck and Keepit) and the ongoing augmentation of these populations with hatchery reared fish of uncertain origin is a likely explanation for the observed disequilibrium. Diversity indices for each population are shown in Table 1. Overall allelic richness was relatively low (mean $A_R = 3.0$) and both allelic richness and expected heterozygosity were lower in the LEB and BULL (mean $A_R = 2.4$, $H_E = 0.38$, $P < 0.001$) than the MDB (mean $A_R = 3.5$, $H_E = 0.62$, $P < 0.001$).

Interbasin population differentiation

There was a high degree of population structure across the species range, mostly attributable to differences in variation among the basins rather than within ($F_{CT} = 0.27$ compared to $F_{SC} = 0.05$, both $P < 0.001$). Pairwise fixation indices were highest between populations

Table 1 Genetic diversity indices characterized by eight microsatellite loci for *Macquaria ambigua* populations. *n* is the mean sample size across eight loci, A_R is allelic richness standardized for sample size, H_O and H_E are observed and expected heterozygosities, HWE is the *P* value for the test of Hardy–Weinberg equilibrium with significant values after Bonferroni correction indicated in bold and after B-Y correction indicated by an asterisk

Basin	Sample site	*n*	A_R	H_O	H_E	HWE
FITZ	Nogoa	13	3.61	0.53	0.62	0.02
MDB	Burrinjuck	19	3.60	0.67	0.64	**High sig***
	Border Rivers	31	3.61	0.54	0.62	**High sig***
	Barwon	24	3.68	0.57	0.65	0.00*
	Castlereagh	23	3.71	0.59	0.64	0.05
	Condamine	23	3.59	0.57	0.63	0.05
	Copeton	22	3.52	0.55	0.62	0.21
	Darling	22	3.67	0.60	0.64	0.00*
	Gwydir	19	3.53	0.62	0.63	0.47
	Keepit	24	3.27	0.65	0.60	**0.00***
	Lachlan	18	3.40	0.55	0.59	0.23
	Murrumbidgee	18	3.72	0.55	0.66	0.01*
	Macquarie	15	3.28	0.54	0.57	0.17
	Murray	18	3.46	0.58	0.62	0.00*
	Namoi	22	3.58	0.59	0.62	0.23
	Paroo	23	3.40	0.62	0.62	0.27
	Windamere	24	3.50	0.63	0.62	0.05
	Wyangala	24	3.41	0.62	0.62	0.22
LEB	Barcoo	22	2.55	0.33	0.38	0.01*
	Cooper Creek	34	2.35	0.32	0.35	0.69
	Diamantina	23	2.62	0.35	0.41	0.01*
	Georgina	23	2.45	0.36	0.38	0.02
	Neales	16	2.28	0.35	0.35	0.67
	Thomson	24	2.35	0.34	0.34	0.95
	Warburton	28	2.32	0.32	0.35	0.19
BULLOO	Bulloo	20	2.74	0.54	0.52	0.82

in the LEB and all other basins: LEB vs. MDB (F_{ST} = 0.32–0.42), LEB vs. FITZ (F_{ST} = 0.34–0.40), BULL vs. LEB (F_{ST} = 0.33–0.37). Comparisons between populations found in other basins were also highly significant, but resulted in lower values of structure: FITZ vs. MDB (F_{ST} = 0.17–0.22) and BULL vs. MDB (F_{ST} = 0.12–0.22) (Table 2). Within the LEB, significant structure was also observed between the eastern (Cooper Creek, Thomson and Barcoo) and western (Diamantina, Georgina, Warburton and Neales) catchments (F_{ST} = 0.21–0.29). Populations within the MDB were much less distinct from one another (global F_{ST} = 0.02, $P < 0.001$) with the highest pairwise F_{ST} = 0.11. However, lower but significant pairwise F_{ST} values suggest some degree of population structure within the MDB, particularly in the Paroo, Keepit and Macquarie populations (Table 2). Results from the Bayesian assignment of the individuals in GENELAND were largely consistent with F_{ST} analysis, grouping populations into the four main drainage basins: FITZ, MDB, BULL and LEB (Fig. 2a). A finer scale analysis of only the LEB populations separated the eastern and western catchments (Fig. 2b). However, the GENELAND analysis within the MDB failed to resolve any intrabasin population structure.

Intrabasin dispersal

Estimates of recent gene flow indicated significant levels of dispersal among populations in the LEB, with a strong pattern of upstream dispersal within catchments and isolation among catchments (Fig. 1 and Appendix I). The proportion of migrants per generation from Cooper Ck (CO) into the Barcoo (BAR) and Thomson Rivers (THOM) was 0.28 (CI 0.21, 0.32) and 0.29 (CI 0.23, 0.33), respectively. The migration rates from Warburton (WR) into the Diamantina (DIA) and Georgina (GEO) were 0.26 (CI 0.05, 0.32) and 0.29 (CI 0.19, 0.33), respectively; given their geographical position on opposite sides of the predominantly dry Lake Eyre, there was also a surprising amount of migration from WR into Neales (NL) [0.21 (CI 0.00, 0.32)]. There was no evidence for recent gene flow between the eastern and western catchments of the LEB. Because limited preexisting population structure was found within the MDB, we were unable to resolve levels of gene flow among populations in this basin. Although these values of migration have low confidence, there was a general trend for more restricted migration upstream into impoundments than in the reciprocal direction, eg Macquarie River (MQ)−Windamere Dam (WN) = 0.002, WN−MQ = 0.023.

Environmental influences

The model that best explained the influence of environmental variables on genetic diversity included four variables: mean annual, autumn- and spring-time flows and perenniality (adj. R^2 = 0.781) (Table 3). The next two highest ranked models also accounted for a significant amount of variation in genetic diversity (adj. R^2 = 0.778 and 0.765) but were only moderately supported by ΔAICc with values of 2.610 and 2.672. These models retained the four variables of the best model, but the second also included latitude. However, spring-time flow volume was the only factor consistently significant in each model (including the full model). Hierarchical partitioning indicated that the environmental variables explaining most of the variation in genetic diversity were spring-time flow (~34%) and perenniality (~16%). Resampling analysis confirmed that our results were robust to variation in sample size, as the *P* values (both 0.002) for each regression were always <0.05 and the coefficient for spring-time flow volume was

Table 2 Pairwise F_{ST} based on eight microsatellite loci for populations of *Macquaria ambigua*. Values in bold are significant after Bonferroni correction

		FITZ	MDB																BUL	LEB						
		NOG	CN	BR	BW	PR	DR	GW	CP	NM	KP	CA	MQ	WN	LN	WY	BJ	MB	MR	BUL	BAR	CO	THO	DIA	WR	GEO	NL
FITZ	NOG	*																									
MDB	CN	**0.17**	*																								
	BR	**0.19**	0.00	*																							
	BW	**0.18**	0.00	0.00	*																						
	PR	**0.18**	**0.03**	**0.04**	**0.03**	*																					
	DR	**0.21**	0.00	0.01	0.01	**0.04**	*																				
	GW	**0.19**	0.01	0.01	0.01	**0.02**	0.00	*																			
	CP	**0.19**	0.00	0.00	0.00	**0.05**	0.00	**0.02**	*																		
	NM	**0.20**	0.00	0.00	0.01	**0.04**	0.01	0.00	0.00	*																	
	KP	**0.20**	**0.01**	**0.03**	**0.02**	**0.05**	**0.03**	**0.04**	**0.03**	**0.02**	*																
	CA	**0.18**	0.01	0.00	0.01	**0.04**	0.00	0.01	0.00	0.01	**0.01**	*															
	MQ	**0.22**	**0.03**	0.01	**0.02**	**0.11**	**0.02**	**0.05**	0.01	0.02	**0.08**	0.01	*														
	WN	**0.21**	**0.02**	0.00	0.00	**0.04**	0.00	**0.02**	**0.01**	0.00	**0.02**	0.01	**0.05**	*													
	LN	**0.22**	0.01	0.01	0.01	**0.06**	0.00	**0.03**	0.00	0.00	**0.04**	0.00	0.00	0.01	*												
	WY	**0.21**	0.01	0.01	0.00	**0.05**	0.00	**0.03**	0.00	0.00	**0.04**	0.01	**0.04**	0.00	0.00	*											
	BJ	**0.20**	0.01	0.01	**0.01**	**0.08**	0.02	**0.04**	0.01	0.01	**0.05**	0.01	**0.02**	0.02	**0.02**	**0.02**	*										
	MB	**0.20**	0.01	0.00	0.01	**0.03**	0.00	0.01	0.01	0.00	**0.04**	0.01	**0.04**	0.01	0.02	**0.02**	**0.02**	*									
	MR	**0.22**	0.01	0.00	0.01	**0.04**	0.00	**0.02**	0.01	0.00	**0.03**	0.01	**0.06**	0.00	0.01	0.00	**0.03**	0.01	*								
BUL	BUL	**0.31**	**0.15**	**0.14**	**0.13**	**0.22**	**0.13**	**0.17**	**0.13**	**0.13**	**0.16**	**0.13**	**0.12**	**0.15**	**0.12**	**0.14**	**0.13**	**0.18**	**0.16**	*							
LEB	BAR	**0.36**	**0.32**	**0.34**	**0.34**	**0.34**	**0.34**	**0.35**	**0.33**	**0.34**	**0.36**	**0.34**	**0.38**	**0.34**	**0.37**	**0.33**	**0.33**	**0.34**	**0.34**	**0.33**	*						
	CO	**0.40**	**0.35**	**0.37**	**0.38**	**0.39**	**0.38**	**0.39**	**0.37**	**0.38**	**0.39**	**0.38**	**0.42**	**0.37**	**0.41**	**0.37**	**0.37**	**0.38**	**0.38**	**0.35**	0.01	*					
	THO	**0.39**	**0.35**	**0.36**	**0.37**	**0.38**	**0.38**	**0.38**	**0.36**	**0.37**	**0.39**	**0.37**	**0.41**	**0.36**	**0.40**	**0.36**	**0.36**	**0.37**	**0.37**	**0.35**	0.01	0.00	*				
	DIA	**0.34**	**0.32**	**0.34**	**0.33**	**0.33**	**0.35**	**0.34**	**0.32**	**0.34**	**0.35**	**0.33**	**0.37**	**0.35**	**0.36**	**0.33**	**0.35**	**0.35**	**0.37**	**0.34**	**0.21**	**0.25**	**0.24**	*			
	WR	**0.38**	**0.35**	**0.37**	**0.36**	**0.37**	**0.38**	**0.37**	**0.35**	**0.37**	**0.38**	**0.36**	**0.40**	**0.38**	**0.39**	**0.36**	**0.37**	**0.38**	**0.40**	**0.37**	**0.24**	**0.27**	**0.26**	0.00	*		
	GEO	**0.35**	**0.33**	**0.35**	**0.33**	**0.34**	**0.36**	**0.35**	**0.32**	**0.34**	**0.35**	**0.34**	**0.38**	**0.35**	**0.37**	**0.33**	**0.35**	**0.36**	**0.38**	**0.35**	**0.23**	**0.27**	**0.25**	0.00	0.00	*	
	NL	**0.35**	**0.32**	**0.34**	**0.34**	**0.34**	**0.36**	**0.35**	**0.32**	**0.35**	**0.35**	**0.34**	**0.38**	**0.36**	**0.37**	**0.34**	**0.36**	**0.36**	**0.38**	**0.36**	**0.26**	**0.29**	**0.28**	0.01	0.02	0.01	*

FITZ, Fitzroy basin; MDB, Murray-Darling basin; BUL, Bulloo basin; LEB, Lake Eyre basin; NOG, Nogoa; CN, Condamine; BR, Border Rivers; BW, Barwon; PR, Paroo; DR, Darling; GW, Gwydir; CP, Copeton; NM, Namoi; KP, Keepit; CA, Castlereagh; MQ, Macquarie; WN, Windamere; LN, Lachlan; WY, Wyangala; BJ, Burrinjuck; MB, Murrumbidgee; MR, Murray; BAR, Barcoo; CO, Cooper; THO, Thomson; DIA, Diamantina; WR, Warburton; GEO, Georgina; NL, Neales.

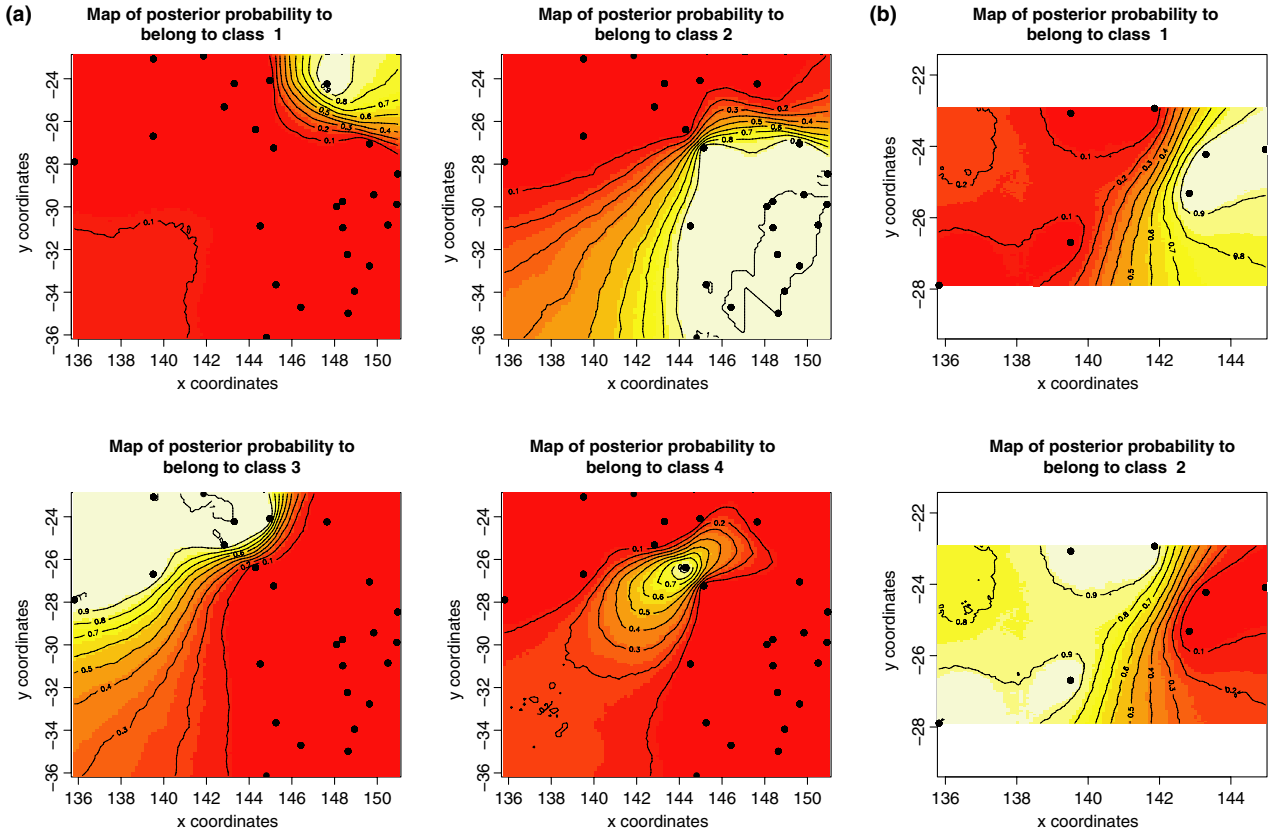

Fig. 2 Posterior probabilities for each location belonging to the groups identified in GENELAND. White indicates a high probability (>0.90). The axes indicate latitude and longitude, and the black dots correspond to our sampling locations shown in Fig 1. (a) The four groups resulting from analysis of all locations. Class 1. Fitzroy, 2. Murray-Darling Basin, 3. Lake Eyre Basin (LEB), 4. Bulloo. (b) The finer scale structure revealed within the LEB. Class 1. Eastern LEB, 2. Western LEB.

consistently positive [both 0.017 (CI 0.015, 0.020)]. A plot of the relationship between the two variables and genetic diversity (H_E) showed that genetic diversity was higher in areas with higher mean spring-time flows (Fig. 3). As the only significant gene flow detected was among populations in the LEB (see BAYESASS+ results), mantel tests were restricted to these populations. Although the combination of connectivity and riverine distance in the partial mantel test accounted for almost half the variation in gene flow (R^2 = 49.53), the correlation coefficients for these variables were not significant. However, simple mantel tests revealed that riverine distance was significantly negatively correlated with gene flow (R^2 = 41.16, b = −0.642, P = 0.001) and connectivity was significantly positively correlated with gene flow (R^2 = 39.93, b = 0.631, P = 0.0005).

Discussion

We used a landscape genetics approach to assess the interaction between ecological processes and landscape patterns in a spatially heterogeneous and temporally variable riverine network. Our investigation of a widespread Australian arid-zone freshwater fish has shown that surprisingly high levels of population connectivity occur across environments that are variable in both space and time. Identifying patterns of gene flow in *M. ambigua* has provided insight into the processes by which this freshwater fish is able to disperse across vast areas of the Australian arid zone. Despite being found in an extreme system where 'islands of water occur in a sea of dry land', *M. ambigua* takes advantage of seasonal and interannual flow events (booms) to disperse across vast areas of the Australian arid zone and to reproduce. We hypothesize that the occurrence of flow events at the start of the breeding season (spring) facilitate the connectivity of populations, increase effective population size (N_e) in spawning aggregations and increase genetic diversity. The maintenance of this natural flow regime, especially under scenarios of human-induced climate change, should be considered vital to sustaining the evolutionary potential of this and other freshwater organisms found in the Australian arid zone.

Table 3 Models of environmental variables to explain the distribution of genetic variation (H_E) at eight microsatellite loci among populations of *Macquaria ambigua*. All variables are standardized to mean of 0 and standard deviation of 1

Model rank	Annual	Summer	Autumn	Winter	Spring	CV	Perennial	Latitude	Intercept	Loglik	AICc	ΔAICc	R^2 (adj)	ANOVA	Sig variables
1	−0.078		0.044		0.110		−0.002		0.579	31.298	−47.980	0.000	0.781	$P < 0.001$	Spr and ann
2	−0.077		0.045		0.141		−0.011	0.031	0.586	32.185	−45.370	2.610	0.778	$P < 0.001$	Spr and ann
3	−0.084		0.055		0.114		−0.001		0.577	32.154	−45.307	2.672	0.765	$P < 0.001$	Spr
163 (full)	0.027	−0.073	0.017	0.012	0.143	0.009	0.005	0.095	0.593	34.184	−30.368	17.612	0.763	$P = 0.002$	Spr
214 (null)										14.573	−26.911	21.068			
HP %IC	8.668	4.332	8.627	9.414	34.497	5.824	16.418	12.220							

CV, coefficient of variance for the mean annual flow; Perennial, perenniality—proportional contribution to mean annual flow by the six driest months of the year; HP %IC, independent contribution of each environmental variable to explaining the variation in H_O; AICc, Akaike's Information Criterion adjusted for small sample sizes; R^2 (adj), R^2 adjusted for the number of variables in the multiple regression analysis; ANOVA, P value for the analysis of variation of environmental variables in the multiple regression; sig variables, variables making a significant contribution to the model according to the ANOVA.

High dispersal of a freshwater fish across the arid zone

Spatial heterogeneity is predicted to influence the ability of organisms to move across the landscape, therefore affecting population genetic structure. However, the degree of this effect may be determined by other factors such as a species dispersal ability or reproductive biology. For strong dispersers like *M. ambigua*, variation in the landscape may only be effective at large spatial scales. The spatial genetic structure observed surprisingly implies that dispersal occurs quite readily in *M. ambigua*, even among locations that are often isolated by hundreds of kilometres of dry riverbed. Although the GENELAND output displays population groups in areas outside of the riverine network, the delineation of basin boundaries is clear; in fact, during flooding events, fish may be able to migrate across these normally dry areas. It appears that only at larger catchment and basin scales, gene flow becomes restricted in this species. Within the LEB, just two major catchment divisions were distinguished: the eastern group (Cooper Creek, Barcoo and Thomson) and the western group (Warburton, Diamantina, Georgina and Neales) (Fig. 2). An examination of the drainage network (Fig. 1) and the hydrological characteristics of the LEB provide insight into the processes behind these patterns.

Lake Eyre lies ~15 m below sea level and is encircled by catchments creating a large endoreic basin (Kotwicki 2003). The Cooper Creek catchment (eastern group) drains into Lake Eyre on the central eastern side, whereas Warburton Creek drains into the northern part of the lake and the Neales into the north western. Given that Lake Eyre is predominantly dry, the eastern and the western groups identified only have the potential to be connected during large flood events when the lake is inundated to a depth sufficient enough for *M. ambigua* to be able to swim across it. Events of this magnitude occur at a frequency of about once every 25 years and prior to the floods of early 2009, last occurred in 1974 when the lake was at its highest level since European settlement (Kotwicki 2003). Therefore, connectivity across the lake is relatively rare, especially for a fish like *M. ambigua* that has a life expectancy of 25–30 years (Mallen-Cooper & Stuart 2003). This means that, at best, fish are only likely to experience one opportunity during their lifetime to migrate across the lake. Additionally, migration across Lake Eyre during sporadic filling events may be further compromised for fishes because of the generally high levels of salinity (Ruello 1976; Kotwicki 2003). In contrast, connectivity via flooding events within the two groups occurs more frequently. Annual seasonal flows can connect waterholes within a reach and major flooding events within the catchments

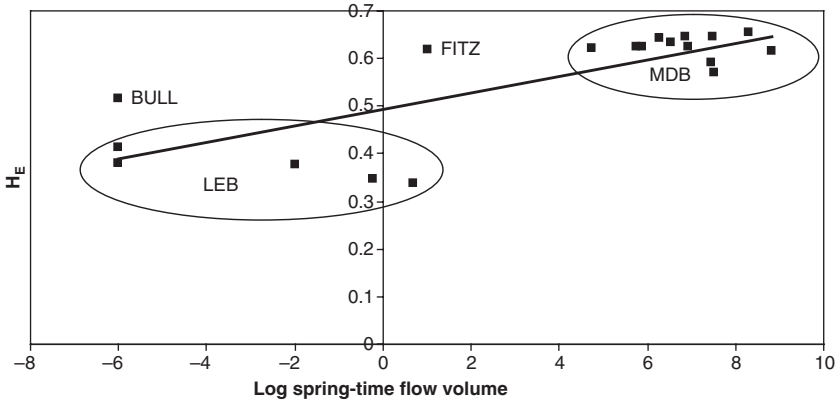

Fig. 3 Relationship between genetic diversity (H_E) and the log of mean spring-time flow volume.

generally occur once a decade (Kotwicki 2003), facilitating the upstream migration of fish into isolated waterholes in the upper reaches of catchments as indicated in Fig. 1.

Unexpectedly, high levels of dispersal across fragmented landscapes have been observed in other aquatic taxa. For example, salamanders and caddisfly larvae utilize 'stepping stone' habitats such as riparian corridors and networks of geographically proximate ponds to enhance dispersal ability (Wilcock *et al.* 2007; Purrenhage *et al.* 2009; Wang *et al.* 2009). In contrast, creek chub use intermittent waterways to overcome in-stream barriers (Boizard *et al.* 2009), exemplifying the role of temporal environmental variation in population connectivity. This temporal variation can be likened to spatial variation, for example flow events on the rivers in the Australian arid zone can act as 'stepping stones' of dispersal in time.

In contrast to the LEB, there was an overall higher gene flow and a lack of population genetic structure within the MDB. We infer that this is because of the lack of any large isolating feature such as Lake Eyre in the LEB, as well as a less variable hydrological regime. The MDB has a higher annual rainfall and is less seasonal than the LEB (Stein *et al.* 2009), and this more consistent hydrological connectivity is then expected to facilitate dispersal and prevent population differentiation within the MDB. The lack of substantial population structure within the MDB (F_{ST} = 0.02, P < 0.001) precluded estimates of recent gene flow in the basin. The only indication of genetic structure in the MDB was the significant pairwise fixation indices for the Paroo, Macquarie and Keepit populations (Table 2). The Paroo is the most north western catchment of the basin and experiences a variable hydrological regime similar to the LEB. This catchment is only connected to the remainder of the basin through a series of terminal wetlands during large flood events and genetic differentiation of populations from this catchment have been observed in previous studies of *M. ambigua* (Musyl & Keenan 1992) and other freshwater taxa (Hughes & Hillyer 2003; Haynes *et al.* 2009). The Macquarie also has a series of often dry terminal wetlands that may reduce migration to and from the catchment (Kingsford & Auld 2005). The third distinct population unit, Lake Keepit, is a man made impoundment that has been stocked with *M. ambigua* for recreational fishing purposes. Our estimates of gene flow did suggest that the dam wall acts as an effective barrier for upstream dispersal (migration rate CI = 0.0018–0.0073), and downstream dispersal is limited to times of dam overflow (migration rate CI = 0.0018–0.051).

Spring-time flow as a driver of genetic diversity

It is well recognized that spatially fragmented populations are expected to contain lower levels of genetic diversity (e.g. Bergl *et al.* 2008; Reid *et al.* 2008), with one recent example suggesting that even taxa with good dispersal abilities can still be at risk of genetic deterioration (Dixo *et al.* 2009). In this study, we have demonstrated that populations can also experience temporal fragmentation, causing similar consequences for genetic diversity as spatial fragmentation. This process could have implications for the conservation of genetic diversity and evolutionary potential in a suite of taxa where population connectivity is variable over time, particularly in freshwater environments. Temporally variable hydrological regimes in freshwater systems can result in cycles of population booms and busts that can produce population bottlenecks, reduce N_e and consequently lower genetic diversity (Huey *et al.* 2008). An investigation of the effect of historical processes on genetic diversity across the species range found that the phylogeographical history of the MDB and LEB was similar, with a series of population expansions and connectivity facilitated by moister

Pleistocene climate conditions (Faulks *et al.* 2010). Therefore, we conclude that the differences in microsatellite genetic diversity between these two basins are a consequence of contemporary rather than historical processes. In addition, as the phylogeographical patterns of the MDB (stocked) and LEB (unstocked) are similar, we conclude that fish stocking has had minimal effect on the genetic architecture of the MDB. Here, we considered the effect of eight factors related to hydrological variability in determining microsatellite genetic diversity. These factors were chosen to test the effect of hydrological variability on the genetic diversity of *M. ambigua* populations at various temporal scales: annual (annual flow, CV and perenniality) and season specific (summer, autumn, winter, spring). From these, hierarchical partitioning and model selection indicated that the most influential factor was mean spring-time flow volume (Table 3). Therefore, we hypothesize that variation in the flow regime at the start of the species' reproductive season, spring, influences genetic diversity. Variation in this variable across the distributional range of *M. ambigua* is caused by the influence of different climate systems. In the northern part of the species range (LEB, BULL and FITZ), monsoonal climate patterns with high summer rainfall dominate, while further south (MDB) the monsoon influence dissipates and Mediterranean climate patterns produce higher winter rainfall (Linacre & Hobbs 1977). This produces a cline of increasing spring-time flow volume from north to south, a pattern well reflected in our observations of genetic diversity across the species range (Fig. 3). In particular, this pattern is driven by strong differences between the LEB and MDB. Within basin trends are less obvious, possibly because there is a lack of power to detect a correlation between differences in H_E and spring-time flow volume at this scale. A greater flow volume during spring may increase the connectivity of populations, perhaps promoting the migration of more fish and/or fish from a larger area of the drainage basin. Survival and dispersal of larval/juvenile fish may also be enhanced as inundated floodplains provide additional food and shelter and flow carries pelagic eggs downstream (Lake 1967; Arumugam & Geddes 1987). This mixing of potentially large numbers of fish from a variety of regularly isolated populations during spawning aggregations may lead to a greater N_e and increased genetic diversity.

Management

Previous studies have suggested the need for a taxonomic revision of *M. ambigua*, as populations from each of the four drainage basins are evolutionary distinct (Musyl & Keenan 1992; Faulks *et al.* 2010). Our microsatellite data support these findings with no evidence for contemporary gene flow among the four drainage basins of the species natural range. In addition, we observed two differentiated groups within the LEB, suggesting that separate management units could be designated in this basin. All drainages in the western group (Warburton, Diamantina, Georgina and Neales) should be maintained as an independent group from the rivers to the east (Cooper Creek, Barcoo and Thomson). In contrast, the lack of structure observed in the MDB suggests the designation of management units within this basin is not necessary. Clarification of the taxonomy and designation of evolutionary significant units (ESUs) (Moritz 1994) will help prioritize management resources to maximize the evolutionary potential of the species. Swamping wild populations with large numbers of hatchery reared fish produced from a few matings has been shown to reduce genetic diversity (Reisenbichler & Phelps 1989; Nicod *et al.* 2004; Ward 2006). Although stocking activities within the MDB have been common, there is little evidence of their impact on the natural levels of genetic diversity, as indicated by both the mtDNA (Faulks *et al.* 2010) and microsatellite data. The demand for stocking activities in the MDB will continue to be high and maintaining natural levels of genetic diversity is of importance. This can be achieved by using adequate numbers of broodfish (NSW Fisheries 2003) from a range of locations across the basin.

Our inferences suggest that population genetic structure and diversity in *M. ambigua* is significantly influenced by the hydrological regime of freshwater environments across its distribution. Therefore, it is pertinent to consider how these conditions may alter under future scenarios of climate change. It is anticipated that inland Australia will experience increases in temperature and evaporation rates and reduced rainfall, resulting in a spread in the area and intensity of aridification (Meehl *et al.* 2007). However, changes in the frequency and magnitude of annual flow regimes and extreme flooding events are difficult to predict. In particular, the changes to ENSO, which governs the interannual hydrological regimes of inland Australia, are unknown (Meehl *et al.* 2007). Increased aridification will lead to further isolation of populations; if flooding events connecting these populations are reduced, then genetic drift could lead to decreased genetic diversity. Under this scenario, it will become increasingly important to facilitate the migration of fish during flow events. Barriers to dispersal such as weirs and impoundments are known to restrict the movement of freshwater fish (Gehrke *et al.* 1995). However, construction of fishways can help mitigate this problem and restore connectivity

among populations (Mallen-Cooper *et al.* 1995; Stuart *et al.* 2005). Providing freshwater fish with an environment in which natural levels of gene flow can be maintained is essential for the conservation of natural population processes. The landscape genetics approach used here can provide a holistic view of the processes that are vital to the management of Australia's unique freshwater environments. In addition, our study contributes to our understanding of the influence of spatial and temporal heterogeneity on population and landscape processes, one of the research priorities in landscape ecology.

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This study forms part of Leanne Faulks' PhD research, supervised by Luciano Beheregaray and Dean Gilligan. Leanne Faulks is interested in evolutionary biology and its application to the conservation of freshwater fauna in Australia. Dean Gilligan has broad research interests in freshwater biodiversity and management. Luciano Beheregaray works on conservation genetics, phylogeography and speciation of aquatic animals, especially fishes.

Appendix I

Estimates of proportion of migrants per generation in Lake Eyre basin populations of *Macquaria ambigua* as calculated in BAYESASS+. Values are from the location in each row to the location in each column and 95% confidence intervals are shown in brackets. Significant amounts of migration are highlighted. The proportion of 10 separate runs returning the displayed estimates is shown in the last row.

From/To	BAR	CO	DIA	GEO	NL	THO	WR
BAR	0.687 (0.667, 0.745)	0.002 (0.000, 0.016)	0.007 (0.000, 0.035)	0.006 (0.000, 0.035)	0.009 (0.000, 0.048)	0.006 (0.000, 0.034)	0.002 (0.000, 0.016)
CO	0.281 (0.205, 0.324)	0.990 (0.960, 1.000)	0.007 (0.000, 0.032)	0.006 (0.000, 0.033)	0.008 (0.000, 0.042)	0.288 (0.229, 0.325)	0.002 (0.000, 0.015)
DIA	0.006 (0.000, 0.034)	0.002 (0.000, 0.012)	0.679 (0.667, 0.713)	0.006 (0.000, 0.032)	0.008 (0.000, 0.042)	0.006 (0.000, 0.032)	0.002 (0.000, 0.016)
GEO	0.006 (0.000, 0.032)	0.002 (0.000, 0.013)	0.006 (0.000, 0.031)	0.680 (0.667, 0.714)	0.007 (0.000, 0.043)	0.005 (0.000, 0.031)	0.002 (0.000, 0.015)
NL	0.006 (0.000, 0.033)	0.002 (0.000, 0.011)	0.036 (0.000, 0.239)	0.011 (0.000, 0.075)	0.748 (0.667, 0.995)	0.006 (0.000, 0.030)	0.011 (0.000, 0.176)
THO	0.007 (0.000, 0.039)	0.002 (0.000, 0.013)	0.007 (0.000, 0.033)	0.006 (0.000, 0.035)	0.008 (0.000, 0.045)	0.680 (0.667, 0.713)	0.002 (0.000, 0.013)
WR	0.007 (0.000, 0.037)	0.002 (0.000, 0.013)	0.258 (0.054, 0.324)	0.285 (0.190, 0.326)	0.213 (0.000, 0.319)	0.008 (0.000, 0.039)	0.979 (0.802, 1.000)
Consistency	0.80	0.70	0.70	0.80	0.70	0.80	0.70