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Genetic analyses reveal limited dispersal and recovery potential in the large freshwater crayfish *Euastacus armatus* from the southern Murray–Darling Basin

Nick S. Whiterod[A,E], *Sylvia Zukowski*[A], *Martin Asmus*[B], *Dean Gilligan*[C] *and Adam D. Miller*[A,D,E]

[A]Aquasave – Nature Glenelg Trust, Goolwa Beach, SA 5214, Australia.
[B]NSW Department of Primary Industries, Narrandera, NSW 2700, Australia.
[C]NSW Department of Primary Industries, Batemans Bay, NSW 2536, Australia.
[D]School of Life & Environmental Sciences, Centre for Integrative Ecology, Deakin University, Warrnambool, Vic. 3280, Australia.
[E]Corresponding authors. Email: nick.whiterod@aquasave.com.au; a.miller@deakin.edu.au

Abstract. Understanding dispersal traits and adaptive potential is critically important when assessing the vulnerability of freshwater species in highly modified ecosystems. The present study investigates the population genetic structure of the Murray crayfish *Euastacus armatus* in the southern Murray–Darling Basin. This species has suffered significant population declines in sections of the Murray River in recent years, prompting the need for information on natural recruitment processes to help guide conservation. We assessed allele frequencies from 10 polymorphic microsatellite loci across 20 sites encompassing the majority of the species' range. Low levels of gene flow were observed throughout hydrologically connected waterways, but significant spatial autocorrelation and low migration rate estimates reflect local genetic structuring and dispersal limitations, with home ranges limited to distances <50-km. Significant genetic differentiation of headwater populations upstream of barriers imposed by impoundments were also observed; however, population simulations demonstrate that these patterns likely reflect historical limitations to gene flow rather than contemporary anthropogenic impacts. Dispersal limitations, coupled with its biological traits, suggest that local populations are vulnerable to environmental disturbance with limited potential for natural recolonisation following population decline. We discuss the implications of these findings in the context of managing the recovery of the species.

Additional keywords: gene flow, genetic diversity, population fragmentation, population structure, recolonisation, spatial autocorrelation.

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Introduction

Australian aquatic fauna inhabit highly dynamic environments, with extreme hydrological conditions and climatic variability driven by sporadic rainfall, stochastic flow events, and extended drought periods (Leigh *et al.* 2015). The natural range of native freshwater species reflects historical climatic fluctuations and evidence of resistance and resilience traits associated with these highly variable environments is prevalent (Chester *et al.* 2015). However, many freshwater species have been heavily affected by anthropogenic influences such as exploitation, the introduction of invasive species and disease, and the alteration of habitat and connectivity (Dudgeon *et al.* 2006; Collen *et al.* 2014). These factors are further compounded by climate change with projections indicating reduced stream flow and increased extreme events such as droughts in the coming decades across much of the continent (Hughes 2003; Hobday and Lough 2011; Hughes 2011). It is anticipated that, although adapted to highly variable environments, some Australian freshwater species will be highly vulnerable to these rapidly changing conditions (Morrongiello *et al.* 2011; Chessman 2013; Cabrelli *et al.* 2015). The persistence of species will be largely dependent on phenotypic plasticity, genetic adaptation and the ability to disperse to more favourable habitats (Hoffmann and Sgro 2011; Beever *et al.* 2015).

The maintenance of population connectivity is critically important for the maintenance of genetic diversity and population size (Lowe and Allendorf 2010), which strongly influences population fitness (Fagan 2002; Hughes 2007), and the ability to adapt via natural selection (Hoffmann and Parsons 1997; Frankham *et al.* 2010). The fragmentation of populations acts to create small isolated populations that are prone to genetic deterioration, fitness reductions and stochastic events (Frankham *et al.* 2010; Allendorf *et al.* 2013). It is expected that ongoing fragmentation will increase the risks of local and regional

extirpation for many species, particularly those that are less vagile (Fagan 2002; Poff *et al.* 2012). Consequently, genetic tools are playing an increasing role in the conservation process. These allow for the identification of species that are likely to be vulnerable to environmental disturbance through direct estimates of population connectivity, dispersal capacity and levels of genetic diversity (Frankham *et al.* 2010; Miller *et al.* 2014; Chester *et al.* 2015; Ovenden *et al.* 2015).

Freshwater crayfish are a diverse taxonomic group, with more than 590 species currently described worldwide, and distributions across a wide range of habitats (Richman *et al.* 2015). However, at least one-third of all species are considered at risk of extinction, largely due to over-exploitation and fragmentation of habitats (Collen *et al.* 2014; Richman *et al.* 2015). Many species exhibit limited gene flow and low levels of genetic diversity (Fetzner and Crandall 2002; Dawkins *et al.* 2010; Miller *et al.* 2014), highlighting inherent dispersal limitations and susceptibility to inbreeding. These characteristics emphasise the vulnerability of freshwater crayfish to environmental disturbance (Richman *et al.* 2015). Additionally, these traits indicate that the potential for natural recovery following local population declines is likely to be limited, and assisted gene flow strategies may be needed to catalyse population recovery in some cases (Weeks *et al.* 2011; Miller *et al.* 2014).

Crayfish belonging to the genus *Euastacus* are endemic to Australia and considered the most threatened freshwater crayfish genus in the world, with ~80% of its 52 species listed under International Union for Conservation of Nature (IUCN) threat categories (Furse and Coughran 2011; Furse *et al.* 2013). The most widely distributed species in the genus, Murray crayfish *Euastacus armatus* (von Martens, 1866) has a broad historical distribution (extent of occurrence, EOO >150 000 km^2) reflecting the modern course (in place for the past 8000 years) of flowing riverine habitats across the southern Murray–Darling Basin (MDB) (Rutherfurd 1990; Morgan 1997; Furse and Coughran 2011). The species has experienced substantial and continuing declines in abundance and range due to anthropogenic impacts, such as over-exploitation, habitat degradation and disturbance, and river regulation by impoundments and weirs (Gilligan *et al.* 2007; Furse and Coughran 2011). Most recently, *E. armatus* populations in the southern MDB have suffered significant declines as a result of a severe hypoxic blackwater event, the severity of which was exacerbated by decreased flooding frequency and altered flow seasonality (Whitworth *et al.* 2012; McCarthy *et al.* 2014). The time frame of these contemporary anthropogenic impacts is short (i.e. less than century) for this late maturing (~8–9 year generation time, i.e. time to onset of sexual maturity) and long-lived (~28 years) species (Morgan 1997), and it is currently unclear how population genetic structure has been affected. Previous insights based on allozymes and mitochondrial DNA (mtDNA) suggest little genetic differentiation across sections of the species' range (Geddes *et al.* 1993; Versteegen and Lawler 1996). However, there is a need for further investigations over a broader extent of the species' range and using markers better suited for determining fine scale and contemporary patterns of genetic structure (i.e. microsatellite or single nucleotide polymorphism (SNP) markers) (Moore *et al.* 2010). In order to help guide management of *E. armatus* there is an urgent need for information on its population genetic structure to gain insights into the species' life history, and its resilience to environmental disturbance, including opportunities for natural recolonisation following major population declines.

In this study we build on findings from previous genetic research by conducting a comprehensive population genetic analysis of *E. armatus* using microsatellite markers and a spatial sampling regime covering much of the present species' range. We anticipate moderate levels of gene flow throughout connected river networks, but potentially local genetic structuring that reflects limited dispersal, small home ranges, and limited recovery potential of locally affected populations. We discuss the findings of the study in the context of the resilience of the species to environmental disturbance and guidelines to direct recovery efforts where populations have been affected. This study contributes to a growing body of literature on the genetic structure and life history traits that underpin the adaptive capacity of Australia's unique freshwater fauna, which provides a critical resource for future conservation planning.

Methods

Sample collection

Euastacus armatus individuals were sampled at 25 collection sites across 12 waterways encompassing its known present distribution as well as outlying populations to the north in the Lachlan and Macquarie catchments (Table 1; Fig. 1). Collection sites within the Edward (Deniliquin; Power Lines; Four Posts), Ovens (Wangaratta; Markwood), and upper Murray (Rocklea; Ponderosa) upper Murrumbidgee (Pine Island; Casuarina Sands) Rivers as well as Elm Grove and Wire Bridge on the Goobarragandra River were pooled for analyses (i.e. samples from 2 to 3 collection sites pooled for each waterway; five pooled sites in total) due to their close proximity and to increase sample sizes to improve statistical power rigour associated with estimates of gene flow and population structure. These site groupings were justified by Hardy–Weinberg equilibrium (HWE) estimates that indicated each pooled sample represented a randomly breeding panmictic population (Table 2). The pooled samples provided a dataset consisting of 20 site groups, hereafter 'sites'.

Euastacus armatus were targeted using a combination of trapping (hoop nets, 20 mm stretch mesh, single 0.8-m diameter steel hoop with 0.3-m drop; commercially available crab pots, 60-mm stretch mesh, 0.76-m diameter steel hoops with two eye-shaped 0.18 × 0.12-m flexible entrances), backpack electrofishing (LR-24, Smith-Root Inc., Vancouver, WA, USA) and hand netting via snorkelling (dip net, 2-mm stretch mesh, 0.10 × 0.07 m with 0.1-m drop). Genetic material was obtained from each crayfish by a small clip (~5 mm^2) of the uropod or pleopod (stored in 90% ethanol), which also acted as an identifier and precluded the possibility of sampling the same crayfish twice during sample collection on that day. All samples were collected between July 2012 and March 2015 (except for two upper Murrumbidgee sites, Pine Island and Casuarina Sands, sampled in July and August 2008). Sample sizes in some cases were constrained by low catch rates; ranging from six (Casuarina Sands) to 30 specimens (Corowa).

Table 1. Site information, corresponding codes and sampling details (numbers and sex ratio) for 20 sites (including pooled collection locations) of *Euastacus armatus*

A sex ratio represented by – indicates that only females were sampled

Catchment	Waterway	Locality	Code	GPS coordinates	*n*	Sex ratio (M : F)
Murray	Murray River	Towong	TO	−36.1363, 148.002	8	–
	Murray River	Rocklea; Ponderosa	RP	−35.9838, 147.8423; −35.9988, 147.8970	18	–
	Murray River	Willow Bends	WB	−36.0596, 146.7979	29	–
	Murray River	Corowa	CO	−36.0247, 146.3788	30	–
	Murray River	Gunbower	GU	−35.9017, 144.4088	19	–
	Murray River	Pental Island	PE	−35.3840, 143.6950	13	0.4 : 1
	Edward River	Deniliquin; Power Lines; Four Posts	ED	−35.5173, 144.9616; −35.5721, 144.9943; −35.6020, 144.9932	34	–
Murrumbidgee	Murrumbidgee River	Buckingbong	BU	−34.8039, 146.6161	16	0.3 : 1
	Murrumbidgee River	Hampden Bridge	HB	−35.1056, 147.3756	28	–
	Murrumbidgee River	Alfs Bend	AB	−35.0645, 147.8355	21	–
	Murrumbidgee River	Pine Island; Casuarina Sands	PC	−35.4263, 149.0513; −35.3193, 148.9518	12	1.2 : 1
	Goobarragandra River	Elm Grove; Wire Bridge	EG	−35.4147, 148.4366; −35.3472, 148.3945	16	1 : 1
	Goodradigbee River	Sandy Flat	SF	−35.2836, 148.7384	19	0.9 : 1
	Talbingo Reservoir	Talbingo	TA	−35.6790, 148.3213	9	–
Mitta Mitta	Little Snowy Creek	Eskdale	LS	−36.4648, 147.2495	20	1 : 1
Kiewa	Kiewa River	Mongans Bridge	KI	−36.5843, 147.0929	22	0.7 :1
Ovens	Ovens River	Wangaratta; Markwood	OV	−36.2844, 146.2681; −36.4422, 146.5255	22	0.8 : 1
Goulburn	Goulburn River	Nagambie	GO	−36.7457, 145.1409	25	0.5 : 1
Lachlan	Abercrombie River	Hell Hole	LA	−34.0481, 149.5126	9	0.5 : 1
Macquarie	Cudgegong River	Gays Place	MA	−32.8529, 150.2662	20	1.2 : 1

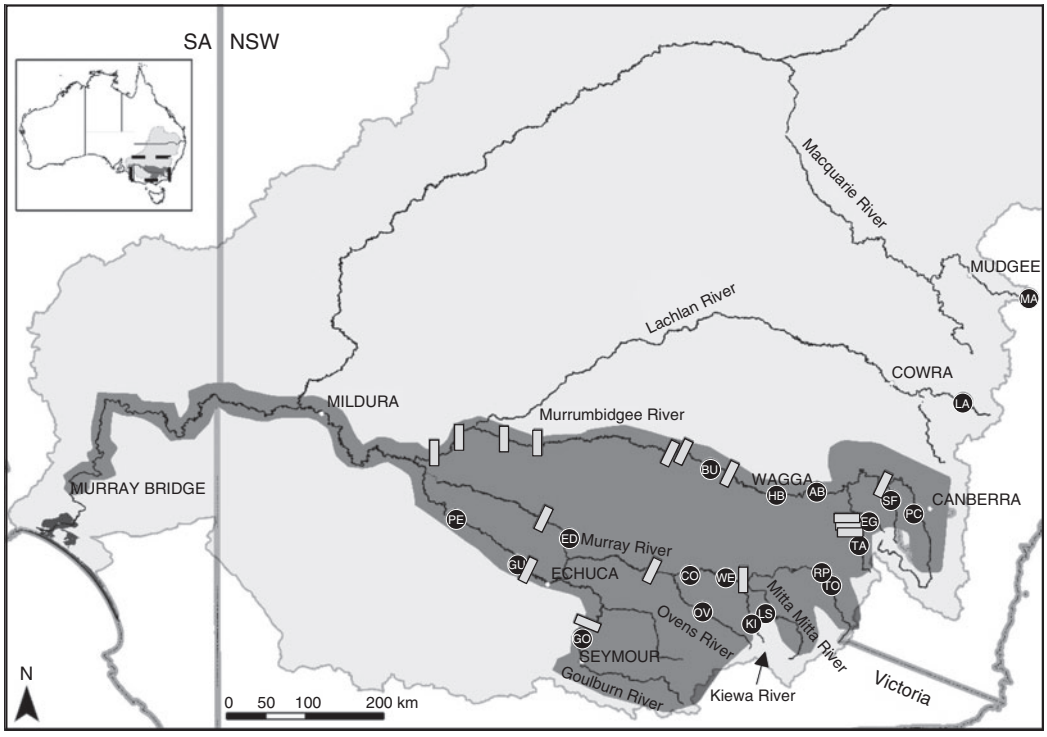

Fig. 1. Location of collection sites (black dots with site code) for *Euastacus armatus* across waterways (black lines) of the southern Murray–Darling Basin (shaded light grey). Also shown is the reported historical distribution (shaded dark grey) of the species (Gilligan *et al.* 2007) and the location of major barriers (grey bars). Pooled collection sites are denoted by grouped site code. Refer to Table 1 for details of site codes.

Table 2. Statistics for *Euastacus armatus* collection sites screened with 10 and 7 microsatellite loci respectively

Mean values over loci are presented for number of alleles (a), allelic richness (r), expected (H_E) and observed (H_O) heterozygosity, Hardy–Weinberg equilibrium P-values (HWE), and inbreeding (F_{IS}). Statistical significance ($\alpha = 0.05$) after correction for multiple comparisons is indicated by bold text. Some statistics could not be calculated at all sites because of a lack of genetic diversity across loci (denoted by NA)

Code	a	r	H_E	H_O	HWE	F_{IS}
TO	4.00, 4.71	3.64, 4.16	0.61, 0.71	0.66, 0.80	0.72, 0.82	−0.08, −0.13
RP	6.40, 7.57	4.05, 4.62	0.66, 0.73	0.56, 0.67	0.01, 0.02	0.12, 0.08
WB	6.60, 8.00	3.78, 4.46	0.61, 0.72	0.55, 0.67	0.01, 0.15	0.10, 0.07
CO	7.20, 8.29	3.97, 4.59	0.65, 0.75	0.64, 0.75	0.42, 0.18	0.00, 0.00
GU	6.11, 7.00	3.92, 4.43	0.67, 0.73	0.63, 0.72	0.37, 0.82	0.07, 0.02
PE	6.20, 7.00	4.23, 4.80	0.68, 0.75	0.63, 0.74	0.76, 0.89	0.04, 0.02
ED	7.30, 8.71	3.90, 4.54	0.64, 0.72	0.58, 0.69	0.18, 0.20	0.08, 0.04
BU	5.60, 6.71	3.66, 4.27	0.62, 0.70	0.62, 0.72	0.61, 0.42	0.00, −0.03
HB	6.20, 7.14	3.46, 3.93	0.56, 0.64	0.52, 0.61	<0.001, 0.02	0.09, 0.06
AB	4.80, 5.71	3.16, 3.60	0.49, 0.60	0.44, 0.55	0.05, 0.21	0.10, 0.07
PC	3.78, 4.43	3.19, 3.68	0.59, 0.70	0.52, 0.66	0.16, 0.94	0.14, 0.05
EG	2.89, 3.29	2.19, 2.45	0.30, 0.37	0.28, 0.34	0.59, 0.43	0.08, 0.08
SF	2.33, 2.57	2.02, 2.18	0.32, 0.36	0.21, 0.27	**<0.001**, 0.01	**0.34, 0.26**
TA	2.00, 2.14	1.79, 1.90	0.31, 0.28	0.29, 0.27	0.60, 0.93	0.12, 0.02
LS	4.89, 5.43	3.22, 3.39	0.55, 0.56	0.50, 0.55	0.04, 0.33	0.08, 0.02
KI	5.78, 6.71	3.60, 4.06	0.61, 0.68	0.62, 0.69	0.01, 0.01	−0.02, −0.02
OV	5.80, 6.86	3.89, 4.46	0.66, 0.72	0.61, 0.69	0.39, 0.28	0.06, 0.05
GO	6.90, 7.57	3.90, 4.41	0.65, 0.71	0.58, 0.69	0.06, 0.62	0.08, 0.04
LA	1.40, 1.43	1.28, 1.37	0.11, 0.11	0.12, 0.13	0.63, NA	−0.24, −0.24
MA	2.10, 2.43	1.75, 1.96	0.23, 0.31	0.21, 0.29	0.81, 0.79	0.06, 0.06

DNA extraction

Total genomic DNA was extracted using a modified Chelex extraction protocol (Walsh *et al.* 1991). Using a 0.5-mL Eppendorf tube, ~10 mg of tissue was taken from uropod clips, macerated with a scalpel, combined with 150 µL of 5% Chelex (Roche) solution and 3 µL of Proteinase K (10 mg mL^{-1}) and mixed briefly by vortex. Samples were incubated at 56°C for 2 h with periodic vortexing, followed by further digestion at 95°C for 15 min. Tissue extractions were cooled on ice for 20 min and stored at −20°C until required for analysis. Prior to PCR, Chelex extractions were homogenised by inversion and centrifuged at 13 000 rpm for 2 min (~18 900 relative centrifugal force). Supernatant was extracted for PCR from the bottom half of the supernatant, above the Chelex resin precipitate.

Microsatellite analysis

Euastacus armatus samples were genotyped at 10 microsatellite loci using procedures described in Miller *et al.* (2013). Descriptive statistics were calculated for the microsatellite data using FSTAT ver. 2.9.3 (Goudet 1995) including: (1) allelic richness per population averaged over loci, (2) Weir and Cockerham's inbreeding coefficient (F_{IS}), a global estimate of population differentiation (F_{ST}) with 95% confidence limits (Weir and Cockerham 1984), (3) population pairwise measures of F_{ST} with significance determined using permutation (10 000), and (4) tests for linkage disequilibrium between loci using a log-likelihood ratio test. Mean allelic richness and observed heterozygosity were compared among sample sites using a two-sided permutation test (10 000 permutations) also implemented in FSTAT. In order to overcome potential limitations of F_{ST} calculations using multiallelic loci (Jost 2008), additional estimates of population differentiation, global D_{est} and population pairwise measures of D_{est} (significance determined using 10 000 permutations), were generated using GenAlEx ver. 6.5 (Peakall and Smouse 2012). The software MICRO-CHECKER ver. 2.2 (Van Oosterhout *et al.* 2004) was used to assess microsatellite loci for null alleles and scoring errors using formula 1 outlined by Brookfield (1996), as evidence of null homozygotes was not apparent. The sequential Bonferroni correction (Rice 1989) was used when performing multiple simultaneous comparisons.

Estimates of observed (H_O) and expected (H_E) heterozygosity were determined using the Excel Microsatellite Toolkit (Park 2001) and deviations from HWE were determined using GENEPOP ver. 3.4 (Raymond and Rousset 1995). An analysis of molecular variation (AMOVA) was performed in GenAlEx using pairwise F_{ST} as the distance measure, with 10 000 permutations and missing data for loci set at 10%. The model for analysis partitioned variation among regions (individual waterways), among sites within regions, and within sites. Isolation by distance (IBD) analyses were performed to explore relationships between genetic differentiation and waterway distance between sites. The shortest waterway distance (in kilometres) along the river network between sites was estimated using origin–destination cost matrix analysis in ArcGIS ver. 10.3 (ESRI, Redlands, CA). Pairwise F_{ST} values were linearised (using Slatkin's linearised F_{ST} transformation ($F_{ST} \div (1 - F_{ST})$) and regressed to the natural log of waterway distance between populations (Rousset 1997) with statistical significance evaluated by regression and Mantel testing, using GenAlEx (Peakall and Smouse 2006; Peakall and Smouse 2012). Significance of Mantel tests was determined by permutation (10 000 randomisations). Spatial autocorrelation analysis was performed in

GenAlEx to assess spatial genetic structure. Distance classes for this analysis were based on 9999 permutations to test for significance. Deviation from 0 means that individuals within a given distance class are significantly more (positive values) or less (negative values) related than random.

Bayesian analysis of population genetic structure was also performed using the R-package software TESS ver. 2.3 (Durand *et al.* 2009). This method makes use of a geographically constrained Bayesian model that explicitly takes into account the spatial position of sampled multilocus genotypes without any prior information on the number of populations and degree of differentiation between them. K is being treated as variable enabling the determination of the modal (i.e. most likely) value. A pilot analysis was performed initially to confirm that 50 000 sweeps with a 10 000 step burn-in stabilised the likelihood. K was then determined from five independent runs where the value was allowed to vary from 1 to 11. After identifying the most likely K, 100 replicate analyses were performed using an admixture model and summarised using CLUMPP ver. 1.1.2 (Jakobsson and Rosenberg 2007).

Population simulations and tests for statistical power

Gene flow uniformity among sites within and between river systems was determined using the coalescent-based maximum likelihood method in MIGRATE ver. 3.6 (Beerli and Felsenstein 2001; Beerli 2004). Estimates of the number of migrants per generation between site pairs ($4Nm$, where N is the effective population size and m is the migration rate) were estimated using the Brownian motion model as an approximation of the stepwise mutation model. Search criteria were set at 10 short chains of 50 000 steps and three long chains of 500 000 steps with the first 10 000 iterations discarded as burn-in. Two independent runs were carried out for each comparison from different random starting seeds to check for consistency of results.

POWSIM ver. 4.0 (Ryman and Palm 2006) was used for evaluation of the α error and statistical power of the microsatellite loci for accurately detecting different levels of F_{ST}. POWSIM population simulations were also performed to determine if observed patterns of genetic structure are likely to be influenced by contemporary structures (e.g. impoundments and weirs, see Fig. 1 for locations) or historical influences. There are 16 major anthropogenic barriers within the MDB – five upland impoundments and 11 low lying weir structures – occurring between sites of the present study (Jacobs 1990). The oldest impoundment was built almost 90 years ago, equating to ~10 generations of the species. Assuming such structures provide absolute barriers to gene flow, simulations were performed under a pure drift scenario assuming zero mutation and migration between sites to determine if empirical levels of F_{ST} could be achieved within this time frame. Simulations of expected F_{ST} were performed using Ne values ranging from 1000 to 10 000, time since separation (t) ranging from 5 to 1000 generations, and allele frequencies taken from the observed data.

Results

Microsatellite analysis

A total of 391 *E. armatus* from the 20 collection sites were successfully genotyped at 10 microsatellite loci (Table 2). Marker independence was confirmed across all sample sites with linkage disequilibrium analyses indicating no significant linkage between loci. MICRO-CHECKER analyses found no evidence of scoring errors but evidence of null alleles at three loci across several sites (loci EB17, EB28, EB35). All analyses were therefore repeated excluding these potentially problematic loci but this had no significant effect on the overall patterns of genetic structure (outputs from the analyses based on seven loci are provided in the Supplementary material, Table S1).

Using the complete 10 locus dataset a total of 109 alleles were detected, with a mean of 4.91 alleles per locus over all sites. Allelic richness over all loci ranged between 1.28 and 4.23 (Table 2). Estimates for total number of alleles, allelic richness and expected heterozygosity were largely consistent across sites from the Murray, Murrumbidgee, Edward, Goulburn, Ovens Rivers and Little Snowy Creek (mean values 5.90, 3.74 and 0.62 respectively), whereas estimates from all remaining sites were notably lower (Table 2). Estimates were lowest at sites from Talbingo Dam, Lachlan, and Macquarie Rivers (number of alleles, allelic richness and expected heterozygosity; 2.00, 1.79, 0.31; 1.40, 1.28, 0.11; and 2.10, 1.75, 0.23 respectively), with permutation tests indicating these estimates are significantly lower ($P < 0.005$).

All sites were generally found to conform to HWE (Table 2), although some estimates should be interpreted with caution owing to small sample sizes. Site SF was an exception, deviating significantly from HWE and driven by a deficit of heterozygotes. HWE P-values were also marginal for several sites (RP, WB, HB, LS, KI, GO) but non-significant; however, this marginality was reduced following the exclusion of loci EB17, EB28 and EB35 ($P > 0.05$). Similarly, no F_{IS} estimates were found to be significant except for those associated with site SF (Table 2).

Global estimates of F_{ST} and D_{est} across all loci were significantly different from zero [$F_{ST} = 0.17$; 95% confidence interval (CI) = 0.12–0.23; $D_{est} = 0.29$; 95% CI = 0.17–0.40] indicating limited gene flow and genetic structuring between sampling sites. Pairwise population comparisons of F_{ST} indicate that this is driven largely by a low number of highly divergent populations (Table 3). Sites from the upper Murrumbidgee catchment (sites EG, SF, TA) were highly differentiated from all other sites, with pairwise F_{ST} estimates indicating site EG to be genetically distinct but there likely being historical gene flow between SF and TA despite geographical isolation. Pairwise F_{ST} also indicate a lack of gene flow between the Mitta Mitta (LS), Lachlan (LA) and Macquarie Rivers (MA), and all other sites included in the study. With the exception of these sites, there appears to be a general lack of genetic structure (weak and generally non-significant pairwise F_{ST} and D_{est} values) across sites from the Murray, Ovens, Edward and Goulburn Rivers. Sites from the upper Murray (TO and RP), lower Murrumbidgee (BU, HB, PC), and Kiewa Rivers (KI) appear to be weakly differentiated indicating some potential limitations to gene flow; however, estimates associated with site TO are likely to be influenced by small local sample sizes. Global F_{ST} and D_{est} across sites from the Murray (excluding site TO), Ovens, Edward, Goulburn, lower Murrumbidgee, and Kiewa Rivers indicated weak but significant genetic structuring ($F_{ST} = 0.04$; 95% CI = 0.03–0.05; $D_{est} = 0.11$; 95% CI = 0.07–0.15).

Table 3. Pairwise estimates of F_{ST} (lower diagonal) and D_{est} (upper diagonal) between *Euastacus armatus* sites
Values shown in bold are significant ($P < 0.001$) after 10 000 permutations and correction for multiple comparisons.

	TO	RP	WB	CO	GU	PE	ED	BU	HB	AB	PC	EG	SF	TA	LS	KI	OV	GO	LA	MA
TO		0.10	**0.21**	**0.19**	**0.29**	**0.25**	**0.30**	**0.30**	**0.31**	**0.33**	**0.34**	**0.62**	**0.54**	**0.59**	**0.27**	**0.22**	**0.14**	**0.34**	**0.70**	**0.71**
RP	0.05		**0.07**	**0.07**	**0.14**	**0.11**	**0.12**	**0.09**	**0.13**	**0.15**	**0.13**	**0.41**	**0.37**	**0.44**	**0.28**	**0.09**	0.05	**0.12**	**0.60**	**0.53**
WB	**0.12**	**0.05**		0.01	0.05	0.04	0.04	0.05	**0.05**	**0.04**	**0.14**	**0.36**	**0.32**	**0.38**	**0.32**	**0.08**	0.04	0.04	**0.55**	**0.48**
CO	**0.10**	**0.04**	0.00		0.05	0.04	0.05	0.06	**0.09**	**0.10**	**0.12**	**0.40**	**0.35**	**0.42**	**0.27**	**0.09**	0.03	**0.06**	**0.51**	**0.48**
GU	**0.14**	**0.07**	0.03	0.03		−0.01	0.02	0.04	**0.07**	**0.09**	**0.07**	**0.33**	**0.38**	**0.40**	**0.35**	**0.11**	0.06	0.04	**0.42**	**0.37**
PE	**0.12**	**0.06**	0.02	0.02	−0.01		0.00	0.04	0.05	0.06	0.08	**0.30**	**0.33**	**0.35**	**0.33**	**0.11**	0.02	0.03	**0.47**	**0.44**
ED	**0.15**	**0.06**	0.02	0.03	0.01	0.00		0.03	**0.05**	**0.09**	0.07	**0.36**	**0.37**	**0.40**	**0.34**	**0.07**	0.05	**0.05**	**0.48**	**0.37**
BU	**0.17**	**0.05**	0.03	0.03	0.02	0.02	0.02		0.02	0.06	0.08	**0.33**	**0.32**	**0.36**	**0.34**	**0.08**	0.07	0.02	**0.49**	**0.34**
HB	**0.19**	**0.08**	**0.04**	**0.06**	**0.04**	**0.03**	**0.04**	0.02		0.01	**0.11**	**0.26**	**0.24**	**0.27**	**0.40**	**0.10**	**0.10**	**0.06**	**0.54**	**0.33**
AB	**0.22**	**0.10**	**0.03**	**0.07**	**0.06**	**0.04**	**0.06**	0.05	0.01		**0.14**	**0.22**	**0.21**	**0.26**	**0.42**	**0.14**	**0.09**	0.05	**0.59**	**0.40**
PC	**0.18**	**0.07**	**0.09**	**0.07**	**0.04**	**0.04**	**0.04**	**0.05**	**0.08**	**0.10**		**0.32**	**0.30**	**0.36**	**0.38**	**0.15**	0.09	0.10	**0.38**	**0.32**
EG	**0.46**	**0.31**	**0.29**	**0.29**	**0.26**	**0.25**	**0.27**	**0.29**	**0.24**	**0.25**	**0.29**		**0.08**	**0.06**	**0.58**	**0.40**	**0.39**	**0.29**	**0.57**	**0.41**
SF	**0.42**	**0.29**	**0.26**	**0.26**	**0.28**	**0.27**	**0.27**	**0.28**	**0.22**	**0.23**	**0.27**	**0.15**		0.02	**0.58**	**0.38**	**0.36**	**0.29**	**0.58**	**0.47**
TA	**0.46**	**0.32**	**0.30**	**0.30**	**0.30**	**0.28**	**0.29**	**0.30**	**0.25**	**0.28**	**0.32**	**0.14**	0.05		**0.60**	**0.44**	**0.42**	**0.34**	**0.58**	**0.48**
LS	**0.17**	**0.16**	**0.20**	**0.16**	**0.19**	**0.18**	**0.19**	**0.20**	**0.25**	**0.28**	**0.22**	**0.43**	**0.43**	**0.44**		**0.26**	**0.26**	**0.40**	**0.65**	**0.57**
KI	**0.12**	**0.05**	**0.05**	**0.05**	**0.06**	**0.06**	**0.04**	**0.05**	**0.07**	**0.10**	**0.09**	**0.31**	**0.30**	**0.33**	**0.16**		**0.07**	**0.13**	**0.59**	**0.39**
OV	**0.07**	0.02	0.02	0.01	**0.03**	0.01	**0.03**	0.04	**0.07**	**0.06**	**0.05**	**0.29**	**0.27**	**0.31**	**0.15**	**0.04**		**0.09**	**0.53**	**0.52**
GO	**0.17**	**0.06**	**0.03**	**0.03**	0.02	0.02	**0.03**	0.01	**0.04**	**0.04**	**0.06**	**0.23**	**0.23**	**0.26**	**0.21**	**0.07**	**0.05**		**0.45**	**0.38**
LA	**0.58**	**0.44**	**0.43**	**0.38**	**0.35**	**0.40**	**0.37**	**0.43**	**0.45**	**0.52**	**0.39**	**0.67**	**0.65**	**0.75**	**0.52**	**0.45**	**0.41**	**0.36**		**0.41**
MA	**0.55**	**0.41**	**0.38**	**0.36**	**0.31**	**0.38**	**0.30**	**0.33**	**0.32**	**0.41**	**0.34**	**0.52**	**0.54**	**0.60**	**0.47**	**0.34**	**0.40**	**0.32**	**0.64**	

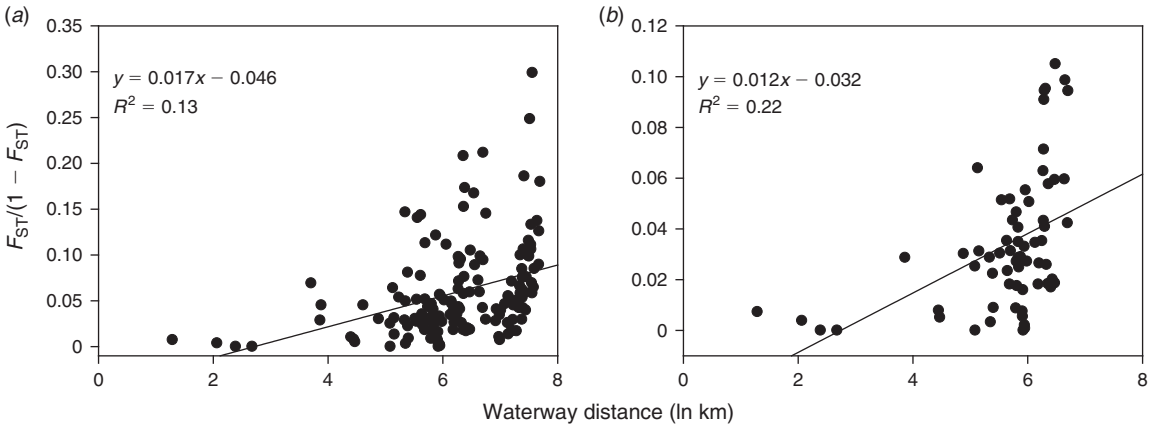

Fig. 2. Regression analysis for the *Euastacus armatus* microsatellite dataset, linearised F_{ST} against the natural log of the pairwise waterway distance (ln km). (*a*) is based on the analysis of all 25 collection sites, whereas (*b*) is based on 12 hydrologically connected sites from which gene flow is evident (based on pairwise F_{ST} estimates). These sites include those from the Murray, Goulburn, Ovens, and Kiewa Rivers. Scores for the accompanying Mantel tests were identical and $r = 0.46$ ($P < 0.001$).

AMOVA analyses indicated a high level of microsatellite variation between waterways (14%, $P < 0.01$). Within-site variation explained 82% ($P < 0.01$) of the total variation, whereas low between-site variation within waterways (4%, $P < 0.01$) indicated minimal structuring at this scale. Regression analyses and a Mantel test suggested moderate IBD. When all sites were included there was a significant association between genetic distance and waterway distance (Fig. 2*a*) with the Mantel test showing a moderate relationship between Slatkin's linearised F_{ST} and the natural log of waterway distance (Mantel $r = 0.46$, $P < 0.01$). Regression showed this relationship to be positive and linear ($R^2 < 0.13$, $P < 0.01$). A repeat analysis including only sites that are hydrologically connected, and among which gene flow was evident (sites from the Murray, Ovens, Edward, Goulburn, lower Murrumbidgee, and Kiewa Rivers; Fig. 2*b*), provided similar results (Mantel $r = 0.46$, $P < 0.01$; regression ($R^2 < 0.22$, $P < 0.01$)), suggesting moderate IBD and gene flow limitations.

A spatial autocorrelation analysis was performed using all multilocus genotypes from the Murray, Ovens, Edward, Goulburn, lower Murrumbidgee, and Kiewa Rivers (297 individuals). The relatedness coefficient (R) was calculated for all pairs of individuals, involving 26 912 pairwise comparisons across 17 distance classes, ranging from 0 to 800 km. Significant

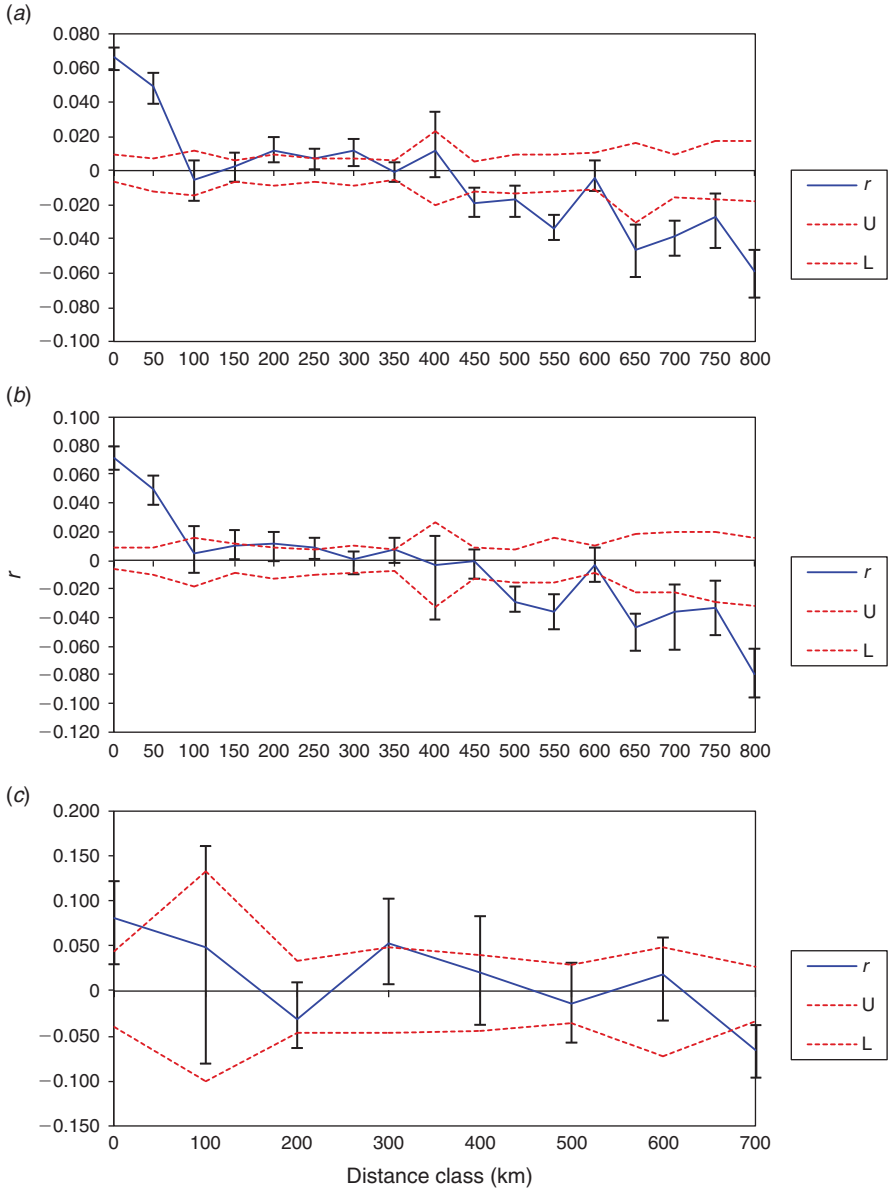

Fig. 3. Spatial autocorrelation coefficient (*r*) for microsatellite data over a range of geographic distance classes for all crayfish collected from the Murray, Goulburn, Ovens, Kiewa, and Murrumbidgee Waterways with a 95% confidence limits (U, L: upper and lower confidence limits respectively). (*a*) includes all samples from each site, whereas (*b*) and (*c*) are respectively based on analysis of female and males.

and positive spatial autocorrelation was observed up to a distance of 50 km (Fig. 3*a*), suggesting that individuals at this spatial scale are more genetically similar than would be expected at random. The autocorrelation signal becomes significantly negative at 450 km, indicating that sites separated by these distances are more unrelated than expected if random mating was occurring throughout. To determine if this pattern of spatial autocorrelation was driven by sex-biased dispersal, separate analysis of female and male only datasets were conducted. Significant spatial autocorrelation was again observed up to a distance of 50 km for the female only dataset (18 400 pairwise comparisons and 17 distance classes; Fig. 3*b*), whereas spatial autocorrelation was only observed at local scales (distance class zero) for the males (411 pairwise comparisons and 8 distance classes; Fig. 3*c*). Comparative distance classes were considerably less for males, but the male only analysis should be interpreted with some caution as the sex ratio was biased towards females for many sites (see Table 2).

TESS Bayesian clustering analysis identified five population clusters ($K = 5$). Consistent with previous analyses, crayfish from Murray, Ovens, Edward, Goulburn, lower Murrumbidgee and Kiewa sites were assigned to a single population cluster, crayfish from the upper Murrumbidgee catchment sites (EG, SF, TA) were assigned to another, whereas crayfish from the Little

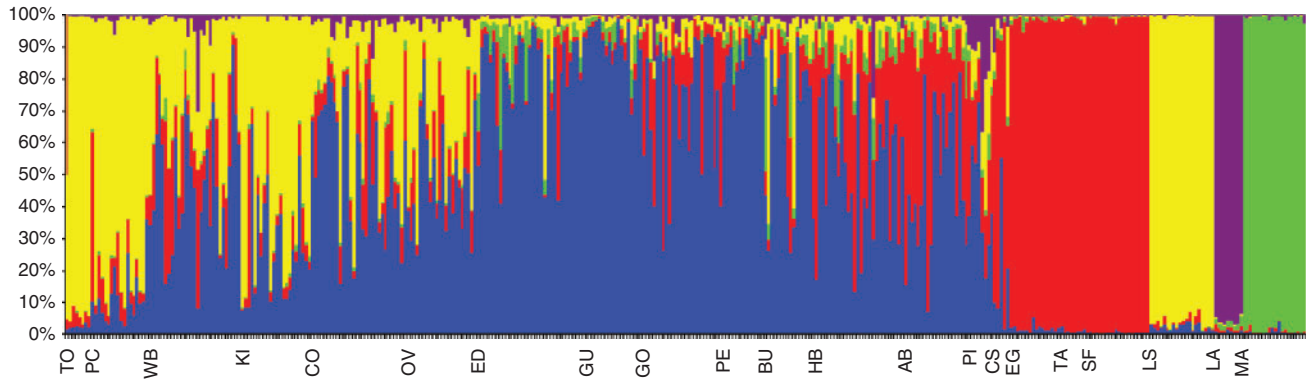

Fig. 4. TESS summary plots of the estimated membership coefficient (*y*-axis) for each individual in each of the five population clusters. Each crayfish is represented by a single vertical line broken into segments, where segments are proportional to the membership coefficient for each of the population clusters. Specimens are grouped into sites from which they were collected, and sites have been arranged in geographical order starting from the uppermost collection point on the Murray River (TO).

Table 4. Pairwise matrix of number of effective migrants per generation (4*Nm*) between populations of *Euastacus armatus* derived from MIGRATE coalescent analyses

Estimated directions of gene flow are provided, with values in the upper and lower diagonals representing sources and recipient respectively

	TO	RP	WB	CO	GU	PE	ED	BU	HB	AB	PC	KI	OV	GO
TO		1.82	0.73	1.32	0.34	0.57	1.58	0.76	0.82	1.35	0.92	0.85	1.04	1.47
RP	1.02		1.75	1.17	1.13	0.62	1.16	1.00	0.98	1.05	1.30	1.21	0.81	0.92
WB	1.54	0.75		1.06	1.40	0.53	2.00	2.27	0.93	0.52	0.58	1.45	0.48	1.11
CO	1.07	0.79	1.46		1.54	1.49	1.01	0.86	0.72	1.02	1.25	0.83	1.43	0.69
GU	0.54	0.29	0.41	1.76		1.71	1.61	1.01	0.63	1.42	0.82	0.54	1.09	0.94
PE	0.38	0.75	1.50	0.49	0.97		1.56	1.07	0.70	1.31	1.22	0.59	0.42	1.03
ED	1.06	1.05	1.08	1.17	1.63	1.29		0.87	0.92	1.16	1.20	1.44	0.76	1.11
BU	1.91	1.03	0.98	1.39	1.19	0.67	0.79		0.69	1.33	1.10	0.70	1.21	0.77
HB	1.00	0.68	0.69	1.17	0.87	0.97	1.62	1.08		0.82	1.03	0.58	0.59	0.65
AB	0.40	0.85	0.87	0.84	1.38	0.92	0.89	1.19	0.67		0.90	2.08	0.86	0.90
PC	1.17	0.88	1.76	1.04	2.01	1.19	0.64	0.57	1.06	1.00		1.60	2.40	0.87
KI	1.54	0.74	1.42	0.88	1.72	0.96	1.26	0.60	0.46	0.89	1.29		0.57	0.54
OV	1.55	0.97	0.56	1.04	0.50	1.06	0.70	2.09	1.20	0.40	1.06	1.41		0.83
GO	1.30	0.80	1.40	0.73	0.87	0.91	1.02	1.40	0.77	0.83	0.55	0.37	0.75	

Snowy Creek, Lachlan and Macquarie Rivers were assigned to their own population clusters. In Fig. 4 we provide a histogram depicting individual assignments to five population clusters. Sites are arranged in geographical order based on river distance from the uppermost Murray River catchment site (TO). Similar to the outputs from FCA and IBD analyses, this figure demonstrates a clear isolation by distance pattern with dominant ancestral genotypes (yellow and red line bar colours) at the geographic extremes of the chart, and admixed genotypes at intermediate collection sites.

Estimates of gene flow calculated with the MIGRATE software package indicated low levels of gene flow between sites. The results from each parallel run were consistent with unidirectional estimates of 4*Nm* ranging from 0.29 to 2.40 effective migrants per generation (Table 4). Overlapping 95% confidence intervals around the estimate of 4*Nm* into each population suggests gene flow directionality was likely to be symmetrical. Although estimates of 4*Nm* were low, indicating limited gene flow between sample sites, estimates were typically larger between neighbouring sites.

The statistical power of the microsatellite markers to detect various levels of true F_{ST} values between populations was tested taking into account the sample sizes, number of loci and average allele frequencies of the dataset. Results showed that the microsatellite markers will detect a true F_{ST} of 0.01 or larger with a probability of 99% or more, and an F_{ST} as low as 0.005 with 95% confidence. The α error (i.e. the probability of obtaining false significances when the true $F_{ST} = 0$) was zero. These results, coupled with the population simulations of F_{ST} (based on different combinations of *N*e and *t*), suggest that the microsatellite markers lack the power needed to detect any changes in population genetic structure due to anthropogenic barriers (i.e. impoundments and weirs). Simulations of F_{ST} assuming pure drift for 10 generations led to estimated expected F_{ST} values of 0.0054, 0.0009 and 0.0006 based on *N*e values of 1000, 5000 and 10 000 crayfish respectively. These values are

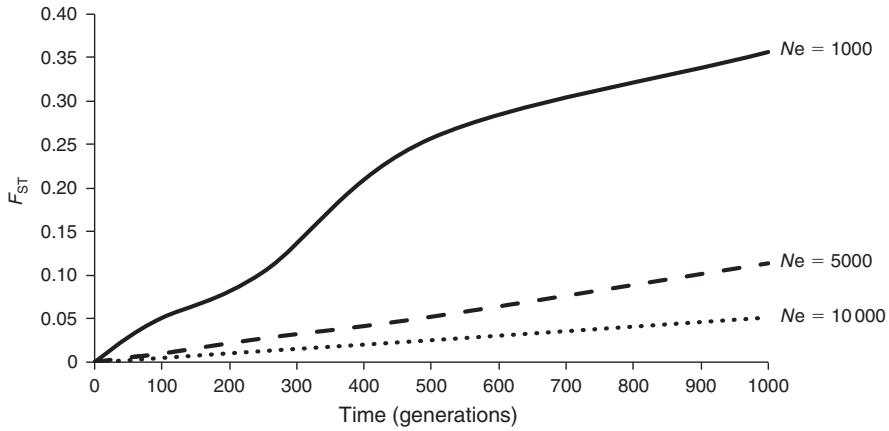

Fig. 5. Simulated F_{ST} under a pure drift scenario (assuming no mutation or migration between populations) and based on varying effective population sizes (*Ne*) and numbers of generations (*t*).

below the confident detection limits for these genetic markers. Instead, the observed levels of differentiation between sites from the Murray (excluding site TO), Ovens, Edward, Goulburn, lower Murrumbidgee, and Kiewa Rivers ($F_{ST} = 0.03$–0.10; Table 3) are likely to reflect time scales of 50–1000+ generations since the time of separation from the ancestral population (Fig. 5).

Discussion

The present study revealed a single panmictic population of *E. armatus* encompassing the majority of its present range, including the Murray, Ovens, Edward, Goulburn, lower Murrumbidgee, and Kiewa catchments (defined as the 'Murray–Murrumbidgee' grouping). Such genetic similarity – within and between catchments across river and tributaries separated by over 2000 km of waterways – is rarely observed in freshwater species, and is atypical of freshwater crayfish (Gouin *et al.* 2006; Gross *et al.* 2013) given inherent dispersal limitations, and the dendritic, and often fragmented, nature of freshwater environments. Large population sizes and sufficient gene flow among local populations has likely helped maintain high levels of genetic diversity and suppress the influence of genetic drift (Frankham *et al.* 2010; Allendorf *et al.* 2013), with the observed average allelic richness and heterozygosity ($r = 3.23$ and $H_E = 0.53$) being uncharacteristically high for freshwater crayfish (Fetzner and Crandall 2002; Gouin *et al.* 2011; Miller *et al.* 2014). In fact, populations of *E. armatus* harbour similar, or in some cases higher, levels of genetic variation compared to widespread and more vagile freshwater fish species inhabiting the MDB; such as golden perch (*Macquaria ambigua*) (Faulks *et al.* 2010), Murray cod (*Maccullochella peelii*) (Rourke *et al.* 2011) and Australian smelt (*Retropinna semoni*) (Taylor *et al.* 2010).

Despite the evidence for panmixia across much of the species' range, we suggest that the homogenisation of the gene pool is being driven by a small number of effective migrants per generation (Hedrick 2011). Significant IBD, and low migration rate estimates between sites across generations, suggest that dispersal is likely to be limited in *E. armatus*. Additionally, significant spatial autocorrelation was observed indicating that dispersal events and home ranges are likely to be restricted to distances less than 50 km. It is unlikely that these patterns are influenced by sex-biased dispersal as partitions of male and female datasets both indicated local genetic structuring, although the extent of spatial autocorrelation in males requires further investigation owing to small sample sizes.

The observed patterns are anticipated to reflect dispersal achieved through both downstream drift of very young juveniles, together with active movement by larger juveniles and adults (Robinson *et al.* 2000). Yet, an understanding of dispersal mechanisms in *E. armatus* are largely lacking. A radiotelemetry study, based solely on upland populations, demonstrated that active movements of larger juveniles and adults are restricted and home ranges small (Ryan *et al.* 2008). Lowland populations of *E. armatus* may have capacity for larger scale movements, potentially comparable or greater than those documented in the giant Tasmanian freshwater crayfish *Astacopsis gouldi* (i.e. >2 km) (Webb and Richardson 2004), which could help to explain the extent of dispersal inferred in the present study. The downstream drift of juveniles has never been quantified, but is anticipated to be critically important for survival and gene flow in the species (Gilligan *et al.* 2007; Alves *et al.* 2010). There is an obvious need to explore the dispersal of juveniles and adults to understand the recolonisation potential in the species.

Three genetically divergent populations were identified from the upper sections of the Murray–Murrumbidgee grouping. The isolation of Mitta Mitta River and Goodradigbee River–Talbingo Dam populations was expected given their position above large impoundments. Impoundments, along with weirs, act to create a physical barrier as well as lentic habitats (Walker 1985) typically unsuitable for the species (Geddes *et al.* 1993; Zukowski 2012). Such features are anticipated to disrupt dispersal and genetic connectivity in *E. armatus* (cf. Alp *et al.* 2012; Baguette *et al.* 2013; Hudman and Gido 2013). Yet our population simulations indicate that the isolation of these two populations is likely to pre-date construction of the impoundments. For instance, based on the degree of differentiation between the Mitta Mitta population and the broader panmictic grouping ($F_{ST} = 0.19$), simulations indicate that under a pure

drift scenario (assuming impoundments create absolute barriers to gene flow), it would take many hundreds of generations of isolation to achieve an F_{ST} of this magnitude. These findings point to the potential limitations of microsatellite markers when assessing the influence of anthropogenic barriers in long-lived species. Additionally, it is clear that upland populations are inherently vulnerable to genetic structuring (cf. Hughes 2007; Hughes *et al.* 2009) through small population sizes and natural barriers to connectivity. The Goobarragandra River population occurs upstream of a waterfall, but it is unclear if this natural feature sufficiently disrupts dispersal and gene flow to cause the observed genetic differentiation (cf. Reis *et al.* 2015). Again, understanding of patterns of movement (including capacity for terrestrial dispersal) along with the extent of the natural fragmentation is necessary to allow further insight into the genetic status of these upland populations. High density genomic markers (SNPs) are likely to provide much greater sensitivity in resolving fine scale patterns of genetic structure and gene flow (Rašić *et al.* 2014) and should be the marker of choice for future studies.

The outlying populations in the Lachlan and Macquarie catchments were also genetically differentiated from all other populations. No private alleles were observed at these sites, and each was characterised by low levels of genetic diversity ($H_E = 0.11$ and 0.23 respectively). These patterns can arise due to historical demographic processes such as bottleneck and founder events. It is believed that these sites are outside the species' native range, and anecdotal evidence suggests that translocations of *E. armatus* into these systems occurred early in the 20th century (e.g. 100 crayfish were released to various locations in the Macquarie Catchment between 1921 and 1925: Gilligan *et al.* 2007). If these populations are a result of the translocation activities only, then founder effects would explain the low levels of genetic diversity observed. We suggest that these populations are of little conservation value in their current genetic state and management priorities should be directed towards at risk populations within the species native range. However, if the genetic condition of these populations can be improved through genetic augmentation (Weeks *et al.* 2011), these could act as security populations for the species. Broader consideration will be necessary to fully evaluate the merits and risks of this option.

Insight into resilience and recovery

Evidence of panmixia across much of the species range suggests there is sufficient gene flow to maintain population sizes and genetic diversity. However, evidence of local genetic structuring, coupled with biological traits (e.g. slow growing, late maturing), suggests that the species is vulnerable to environmental disturbance, and opportunities for large-scale dispersal and recolonisation are likely to be limited (Hughes 2007; Hughes *et al.* 2013). The natural recovery of affected populations, such as those significantly affected by recent hypoxic blackwater (McCarthy *et al.* 2014), although conceivable, is likely to be a gradual process. Although this study does not provide a reliable assessment of the impacts of anthropogenic barriers (i.e. impoundments and weirs) on contemporary gene flow, it is likely these will compromise species dispersal and the recovery of affected populations in the vicinity of these structures.

Translocations are becoming increasingly recognised as a powerful conservation tool (Weeks *et al.* 2011; Armstrong *et al.* 2015) and have long been suggested as a pragmatic solution to restore *E. armatus* populations (Geddes *et al.* 1993). In the case of *E. armatus* translocations may be necessary to combat the genetic erosion of isolated populations and to catalyse the recovery of locally affected populations. Translocations should follow the guidelines provided by Weeks *et al.* (2011) and those recently advised for the closely related Glenelg spiny freshwater crayfish (*Euastacus bispinosus*) (Miller *et al.* 2014). Outbreeding risks are likely to be minimal given the data from this and previous genetic studies, which indicate that *E. armatus* populations within the Murray–Murrumbidgee grouping are conspecific and derived from a recent common ancestor (Weeks *et al.*, 2011). However, it is recommended that the selection of translocated animals represent multiple source populations from similar habitat types to capture the greatest representation of the total gene pool and adaptive traits (Pérez-Figueroa *et al.* 2009; Coleman *et al.* 2013). Local adaptive genetic differentiation can be maintained under strong selection despite panmixia (Hess *et al.* 2013; Milano *et al.* 2014), therefore a population genomic evaluation of adaptive genetic variation within *E. armatus* could help guide management and avoid the movement of maladaptive genotypes.

Species of *Euastacus* are under threat from habitat degradation, environmental disturbance and climate change (Furse and Coughran 2011; Richman *et al.* 2015). On the basis of the present outcomes, along with those of other genetic studies focusing on *Euastacus* (Hughes 2007; Miller *et al.* 2014), it is logical to suggest that poor resilience to environmental disturbance will be a consistent trait across the genus. Multi-species genetic analyses, employing SNP loci as mentioned above, may provide more reliable insights into the effects of anthropogenic influences on population dynamics and genetic structure, and adaptive capacity in light of climate change. The availability of information for *Euastacus* species will be critical for future conservation planning, as many species occur only in restricted upland habitats (e.g. 12 species are known from only a single locality) and are of significant conservation concern (Furse and Coughran 2011).

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References

Allendorf, F. W., Luikart, G., and Aitken, S. N. (2013). 'Conservation and the Genetics of Populations.' (Wiley-Blackwell: Chichester, UK.)

Alp, M., Keller, I., Westram, A., and Robinson, C. T. (2012). How river structure and biological traits influence gene flow: a population genetic study of two stream invertebrates with differing dispersal abilities. *Freshwater Biology* **57**, 969–981. doi:10.1111/J.1365-2427.2012.02758.X

Alves, N., Merrick, J. R., Kohen, J., and Gilligan, D. (2010). Juvenile recruitment of the Murray crayfish, *Euastacus armatus* (Decapoda: Parastacidae), from south-eastern Australia. *Freshwater Crayfish* **17**, 167–175.

Armstrong, D., Hayward, M., Moro, D., and Seddon, P. (2015). 'Advances in Reintroduction Biology of Australian and New Zealand Fauna.' (CSIRO Publishing: Melbourne.)

Baguette, M., Blanchet, S., Legrand, D., Stevens, V. M., and Turlure, C. (2013). Individual dispersal, landscape connectivity and ecological networks. *Biological Reviews of the Cambridge Philosophical Society* **88**, 310–326. doi:10.1111/BRV.12000

Beerli, P. (2004). Effect of unsampled populations on the estimation of population sizes and migration rates between sampled populations. *Molecular Ecology* **13**, 827–836. doi:10.1111/J.1365-294X.2004.02101.X

Beerli, P., and Felsenstein, J. (2001). Maximum likelihood estimation of a migration matrix and effective population sizes in *n* subpopulations by using a coalescent approach. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 4563–4568. doi:10.1073/PNAS.081068098

Beever, E. A., O'Leary, J., Mengelt, C., West, J. M., Julius, S., Green, N., Magness, D., Petes, L., Stein, B., Nicotra, A. B., Hellmann, J. J., Robertson, A. L., Staudinger, M. D., Rosenberg, A. A., Babij, E., Brennan, J., Schuurman, G. W., and Hofmann, G. E. (2015). Improving conservation outcomes with a new paradigm for understanding species' fundamental and realized adaptive capacity. *Conservation Letters*. [Published online early 5 August 2015]. doi:10.1111/CONL.12190

Brookfield, J. F. Y. (1996). A simple new method for estimating null allele frequency from heterozygote deficiency. *Molecular Ecology* **5**, 453–455. doi:10.1111/J.1365-294X.1996.TB00336.X

Cabrelli, A., Beaumont, L., and Hughes, L. (2015). The impacts of climate change on Australian and New Zealand flora and fauna. In 'Austral Ark: the State of Wildlife in Australia and New Zealand'. (Eds A. Stow, N. Maclean, and G. I. Holwell.) pp. 65−82. (Cambridge University Press: Cambridge, UK.)

Chessman, B. C. (2013). Identifying species at risk from climate change: traits predict the drought vulnerability of freshwater fishes. *Biological Conservation* **160**, 40–49. doi:10.1016/J.BIOCON.2012.12.032

Chester, E. T., Miller, A. D., Valenzuela, I., Wickson, S. J., and Robson, B. J. (2015). Drought survival strategies, dispersal potential and persistence of invertebrate species in an intermittent stream landscape. *Freshwater Biology* **60**, 2066–2083. doi:10.1111/FWB.12630

Coleman, R. A., Weeks, A. R., and Hoffmann, A. A. (2013). Balancing genetic uniqueness and genetic variation in determining conservation and translocation strategies: a comprehensive case study of threatened dwarf galaxias, *Galaxiella pusilla* (Mack) (Pisces: Galaxiidae). *Molecular Ecology* **22**, 1820–1835. doi:10.1111/MEC.12227

Collen, B., Whitton, F., Dyer, E. E., Baillie, J. E., Cumberlidge, N., Darwall, W. R., Pollock, C., Richman, N. I., Soulsby, A. M., and Böhm, M. (2014). Global patterns of freshwater species diversity, threat and endemism. *Global Ecology and Biogeography* **23**, 40–51. doi:10.1111/GEB.12096

Dawkins, K. L., Furse, J. M., Wild, C. H., and Hughes, J. M. (2010). Distribution and population genetics of the threatened freshwater crayfish genus *Tenuibranchiurus* (Decapoda: Parastacidae). *Marine and Freshwater Research* **61**, 1048–1055. doi:10.1071/MF09294

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z. I., Knowler, D. J., Lévêque, C., Naiman, R. J., Prieur-Richard, A. H., Soto, D., and Stiassny, M. L. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews of the Cambridge Philosophical Society* **81**, 163–182. doi:10.1017/S1464793105006950

Durand, E., Jay, F., Gaggiotti, O. E., and François, O. (2009). Spatial inference of admixture proportions and secondary contact zones. *Molecular Biology and Evolution* **26**, 1963–1973. doi:10.1093/MOLBEV/MSP106

Fagan, W. F. (2002). Connectivity, fragmentation, and extinction risk in dendritic metapopulations. *Ecology* **83**, 3243–3249. doi:10.1890/0012-9658(2002)083[3243:CFAERI]2.0.CO;2

Faulks, L. K., Gilligan, D. M., and Beheregaray, L. B. (2010). 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*). *Molecular Ecology* **19**, 4723–4737. doi:10.1111/J.1365-294X.2010.04848.X

Fetzner, J. W. J., and Crandall, K. (2002). Genetic variation. In 'Biology of Freshwater Crayfish'. (Ed. D. M. Holdich.) pp. 291–329. (Blackwell Science: Oxford, UK.)

Frankham, R., Ballou, J. D., and Briscoe, D. A. (2010). 'Introduction to Conservation Genetics.' (Cambridge University Press: London.)

Furse, J. M., and Coughran, J. (2011). An assessment of the distribution, biology, threatening processes and conservation status of the freshwater crayfish, genus *Euastacus* (Decapoda: Parastacidae) in continental Australia. II. Threats, conservation assessments and key findings. In 'New Frontiers in Crustacean Biology (Crustaceans Monographs 15)'. (Ed. A. Akasura.) pp. 253–263. (Brill Publishers: Leiden, Netherlands.)

Furse, J. M., Dawkins, K. L., and Coughran, J. (2013). Two new species of *Euastacus* (Decapoda: Parastacidae) from the Gondwana rainforests of central-eastern Australia. *Freshwater Crayfish* **19**, 103–112. doi:10.5869/FC.2013.V19.103

Geddes, M. C., Musgrove, R. J., and Campbell, N. J. H. (1993). The feasibility of re-establishing the River Murray crayfish, *Euastacus armatus*, in the lower River Murray. *Freshwater Crayfish* **9**, 368–379.

Gilligan, D., Rolls, R., Merrick, J., Lintermans, M., Duncan, P., and Koehn, J. (2007). Scoping the Knowledge Requirements for Murray Crayfish (*Euastacus armatus*). Fisheries Final Report Series number 89. NSW Department of Primary Industries, Sydney.

Goudet, J. (1995). FSTAT (version 1.2): a computer program to calculate F-statistics. *The Journal of Heredity* **86**, 485–486.

Gouin, N., Grandjean, F., and Souty-Grosset, C. (2006). Population genetic structure of the endangered crayfish *Austropotamobius pallipes* in France based on microsatellite variation: biogeographical inferences and conservation implications. *Freshwater Biology* **51**, 1369–1387. doi:10.1111/J.1365-2427.2006.01570.X

Gouin, N., Souty-Grosset, C., Borquez, J., Bertin, A., and Grandjean, F. (2011). Disentangling the impact of demographic factors on population differentiation of an endangered freshwater crayfish (*Austropotamobius pallipes*) using population density and microsatellite data. *Freshwater Biology* **56**, 2105–2118. doi:10.1111/J.1365-2427.2011.02629.X

Gross, R., Palm, S., Kõiv, K., Prestegaard, T., Jussila, J., Paaver, T., Geist, J., Kokko, H., Karjalainen, A., and Edsman, L. (2013). Microsatellite markers reveal clear geographic structuring among threatened noble crayfish (*Astacus astacus*) populations in Northern and Central Europe. *Conservation Genetics* **14**, 809–821. doi:10.1007/S10592-013-0476-9

Hedrick, P. (2011). 'Genetics of Populations.' (Jones & Bartlett Learning: Sudbury, MA, USA.)

Hess, J. E., Campbell, N. R., Close, D. A., Docker, M. F., and Narum, S. R. (2013). Population genomics of Pacific lamprey: adaptive variation in a highly dispersive species. *Molecular Ecology* **22**, 2898–2916. doi:10.1111/MEC.12150

Hobday, A. J., and Lough, J. M. (2011). Projected climate change in Australian marine and freshwater environments. *Marine and Freshwater Research* **62**, 1000–1014. doi:10.1071/MF10302

Hoffmann, A. A., and Parsons, P. A. (1997). 'Extreme Environmental Change and Evolution.' (Cambridge University Press: Cambridge, UK.)

Hoffmann, A. A., and Sgro, C. M. (2011). Climate change and evolutionary adaptation. *Nature* **470**, 479–485. doi:10.1038/NATURE09670

Hudman, S. P., and Gido, K. B. (2013). Multi-scale effects of impoundments on genetic structure of creek chub (*Semotilus atromaculatus*) in the Kansas River Basin. *Freshwater Biology* **58**, 441–453. doi:10.1111/FWB.12079

Hughes, L. (2003). Climate change and Australia: trends, projections and impacts. *Austral Ecology* **28**, 423–443. doi:10.1046/J.1442-9993.2003.01300.X

Hughes, J. M. (2007). Constraints on recovery: using molecular methods to study connectivity of aquatic biota in rivers and streams. *Freshwater Biology* **52**, 616–631. doi:10.1111/J.1365-2427.2006.01722.X

Hughes, L. (2011). Climate change and Australia: key vulnerable regions. *Regional Environmental Change* **11**, 189–195. doi:10.1007/S10113-010-0158-9

Hughes, J. M., Schmidt, D. J., and Finn, D. S. (2009). Genes in streams: a molecular approach to understanding movement of freshwater fauna and their riverine habitat. *Bioscience* **59**, 573–583. doi:10.1525/BIO.2009.59.7.8

Hughes, J. M., Huey, J. A., and Schmidt, D. J. (2013). Is realised connectivity among populations of aquatic fauna predictable from potential connectivity? *Freshwater Biology* **58**, 951–966. doi:10.1111/FWB.12099

Jacobs, T. (1990). River regulation. In 'The Murray'. (Eds N. Mackey and D. Eastburn.) pp. 39−60. (Murray–Darling Basin Commission: Canberra, ACT.)

Jakobsson, M., and Rosenberg, N. A. (2007). CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* **23**, 1801–1806. doi:10.1093/BIOINFORMATICS/BTM233

Jost, L. (2008). G(ST) and its relatives do not measure differentiation. *Molecular Ecology* **17**, 4015–4026. doi:10.1111/J.1365-294X.2008.03887.X

Leigh, C., Bush, A., Harrison, E. T., Ho, S. S., Luke, L., Rolls, R. J., and Ledger, M. E. (2015). Ecological effects of extreme climatic events on riverine ecosystems: insights from Australia. *Freshwater Biology* **60**, 2620–2638. doi:10.1111/FWB.12515

Lowe, W. H., and Allendorf, F. W. (2010). What can genetics tell us about population connectivity? *Molecular Ecology* **19**, 3038–3051. doi:10.1111/J.1365-294X.2010.04688.X

McCarthy, B., Zukowski, S., Whiterod, N., Vilizzi, L., Beesley, L., and King, A. (2014). Hypoxic blackwater event severely impacts Murray crayfish (*Euastacus armatus*) populations in the Murray River, Australia. *Austral Ecology* **39**, 491–500. doi:10.1111/AEC.12109

Milano, I., Babbucci, M., Cariani, A., Atanassova, M., Bekkevold, D., Carvalho, G. R., Espiñeira, M., Fiorentino, F., Garofalo, G., and Geffen, A. J. (2014). Outlier SNP markers reveal fine-scale genetic structuring across European hake populations (*Merluccius merluccius*). *Molecular Ecology* **23**, 118–135. doi:10.1111/MEC.12568

Miller, A. D., Van Rooyen, A., Sweeney, O. F., Whiterod, N. S., and Weeks, A. R. (2013). The development of 10 novel polymorphic microsatellite markers through next generation sequencing and a preliminary genetic analysis for the endangered Glenelg spiny crayfish, *Euastacus bispinosus*. *Molecular Biology Reports* **40**, 4415–4419. doi:10.1007/S11033-013-2531-5

Miller, A. D., Sweeney, O. F., Whiterod, N. S., Van Rooyen, A., Hammer, M., and Weeks, A. R. (2014). Critically low levels of genetic diversity in fragmented populations of the endangered Glenelg spiny freshwater crayfish *Euastacus bispinosus*. *Endangered Species Research* **25**, 43–55. doi:10.3354/ESR00609

Moore, A., Ingram, B., Friend, S., Hui, K. H., Robinson, N., McCormack, R., Coughran, J., and Hayes, B. (2010). 'Management of Genetic Resources for Fish and Crustaceans in the Murray–Darling Basin.' (Bureau of Rural Sciences, Australian Government, Canberra.)

Morgan, G. J. (1997). Freshwater crayfish of the genus *Euastacus* Clark (Decapoda: Parastacidae) from New South Wales, with a key to all species of the genus. *Records of the Australian Museum* **23**, 1–110. doi:10.3853/J.0812-7387.23.1997.429

Morrongiello, J. R., Beatty, S. J., Bennett, J. C., Crook, D. A., Ikedife, D. N., Kennard, M. J., Kerezsy, A., Lintermans, M., McNeil, D. G., and Pusey, B. J. (2011). Climate change and its implications for Australia's freshwater fish. *Marine and Freshwater Research* **62**, 1082–1098. doi:10.1071/MF10308

Ovenden, J. R., Berry, O., Welch, D. J., Buckworth, R. C., and Dichmont, C. M. (2015). Ocean's eleven: a critical evaluation of the role of population, evolutionary and molecular genetics in the management of wild fisheries. *Fish and Fisheries* **16**, 125–159. doi:10.1111/FAF.12052

Park, S. D. E. (2001). Trypanotolerance in West African cattle and the population genetic effects of selection. Ph.D. Thesis, University of Dublin, Dublin.

Peakall, R., and Smouse, P. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**, 288–295. doi:10.1111/J.1471-8286.2005.01155.X

Peakall, R., and Smouse, P. E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics* **28**, 2537–2539. doi:10.1093/BIOINFORMATICS/BTS460

Pérez-Figueroa, A., Saura, M., Fernandez, J., Toro, M. A., and Caballero, A. (2009). METAPOP-A software for the management and analysis of subdivided populations in conservation programs. *Conservation Genetics* **10**, 1097–1099. doi:10.1007/S10592-008-9718-7

Poff, N. L., Olden, J. D., and Strayer, D. L. (2012). Climate change and freshwater fauna extinction risk. In 'Saving A Million Species: Extinction Risk from Climate Change'. (Ed. L Hannah.) pp. 309−336. (Island Press: Washington, DC., USA)

Rašić, G., Filipovic, I., Weeks, A. R., and Hoffmann, A. A. (2014). Genome-wide SNPs lead to strong signals of geographic structure and relatedness patterns in the major arbovirus vector, *Aedes aegypti*. *BMC Genomics* **15**, 275. doi:10.1186/1471-2164-15-275

Raymond, M., and Rousset, F. (1995). An exact test for population differentiation. *Evolution* **49**, 1280–1283. doi:10.2307/2410454

Reis, K., Venere, P., Sampaio, I., Rêgo, P., Vallinoto, M., and Souza, I. (2015). Downstairs gene flow: the effects of a linear sequence of waterfalls on the only population of the endangered minnow *Astyanax xavante*. *Journal of Fish Biology* **87**, 754–762. doi:10.1111/JFB.12729

Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution* **43**, 223–225. doi:10.2307/2409177

Richman, N. I., Böhm, M., Adams, S. B., Alvarez, F., Bergey, E. A., Bunn, J. J., Burnham, Q., Cordeiro, J., Coughran, J., Crandall, K. A., Dawkins, K. L., DiStefano, R. J., Doran, N. E., Edsman, L., Eversole, A. G., Füreder, L., Furse, J. M., Gherardi, F., Hamr, P., Holdich, D. M., Horwitz, P., Johnston, K., Jones, C. M., Jones, J. P. G., Jones, R. L., Jones, T. G., Kawai, T., Lawler, S., López-Mejía, M., Miller, R. M., Pedraza-Lara, C., Reynolds, J. D., Richardson, A. M. M., Schultz, M. B., Schuster, G. A., Sibley, P. J., Souty-Grosset, C., Taylor, C. A., Thoma, R. F., Walls, J., Walsh, T. S., and Collen, B. (2015). Multiple drivers of decline in the global status of freshwater crayfish (Decapoda: Astacidea). *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **370**, 20140060. doi:10.1098/RSTB.2014.0060

Robinson, C. A., Thom, T. J., and Lucas, M. C. (2000). Ranging behaviour of a large freshwater invertebrate, the white-clawed crayfish *Austropotamobius pallipes*. *Freshwater Biology* **44**, 509–521. doi:10.1046/J.1365-2427.2000.00603.X

Rourke, M., McPartlan, H., Ingram, B., and Taylor, A. (2011). Variable stocking effect and endemic population genetic structure in Murray cod *Maccullochella peelii*. *Journal of Fish Biology* **79**, 155–177. doi:10.1111/J.1095-8649.2011.03006.X

Rousset, F. (1997). Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* **145**, 1219–1228.

Rutherfurd, I. (1990). Ancient river young nation. In 'The Murray'. (Eds N. Mackay and D. Eastburn.) pp. 17–36. (Murray–Darling Basin Commission: Canberra, ACT.)

Ryan, K. A., Ebner, B. C., and Norris, R. H. (2008). Radio-tracking interval effects on the accuracy of diel scale crayfish movement variables. *Freshwater Crayfish* **16**, 87–92.

Ryman, N., and Palm, S. (2006). POWSIM: a computer program for assessing statistical power when testing for genetic differentiation. *Molecular Ecology Notes* **6**, 600–602. doi:10.1111/J.1471-8286.2006.01378.X

Taylor, E., Woods, R. J., Macdonald, J. I., Crook, D. A., Schmidt, D. J., and Hughes, J. M. (2010). Contemporary and historical patterns of connectivity among populations of an inland river fish species inferred from genetics and otolith chemistry. *Canadian Journal of Fisheries and Aquatic Sciences* **67**, 1098–1115. doi:10.1139/F10-043

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M., and Shipley, P. (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**, 535–538. doi:10.1111/J.1471-8286.2004.00684.X

Versteegen, M., and Lawler, S. (1996). Population genetics of the Murray River crayfish *Euastacus armatus*. *Freshwater Crayfish* **11**, 146–157.

Walker, K. F. (1985). A review of the ecological effects of river regulation in Australia. *Hydrobiologia* **125**, 111–129. doi:10.1007/BF00045929

Walsh, P. S., Metzger, D. A., and Higuchi, R. (1991). Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques* **10**, 506–513.

Webb, M., and Richardson, A. (2004). A radio telemetry study of movement in the giant Tasmanian freshwater crayfish, *Astacopsis gouldi*. *Freshwater Crayfish* **14**, 197–204.

Weeks, A. R., Sgro, C. M., Young, A. G., Frankham, R., Mitchell, N. J., Miller, K. A., Byrne, M., Coates, D. J., Eldridge, M. D. B., Sunnucks, P., Breed, M. F., James, E. A., and Hoffmann, A. A. (2011). Assessing the benefits and risks of translocations in changing environments: a genetic perspective. *Evolutionary Applications* **4**, 709–725. doi:10.1111/J.1752-4571.2011.00192.X

Weir, B., and Cockerham, C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370. doi:10.2307/2408641

Whitworth, K. L., Baldwin, D. S., and Kerr, J. L. (2012). Drought, floods and water quality: drivers of a severe hypoxic blackwater event in a major river system (the southern Murray–Darling Basin, Australia). *Journal of Hydrology* **450-451**, 190–198. doi:10.1016/J.JHYDROL.2012.04.057

Zukowski, S. (2012). Impacts of fishing regulations on the sustainability of Murray crayfish (*Euastacus armatus*), Australia: social and biological perspectives. Ph.D. Thesis, Charles Sturt University, Albury, NSW.

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Supplementary material

Genetic analyses reveal limited dispersal and recovery potential in the large freshwater crayfish *Euastacus armatus* from the southern Murray–Darling Basin

Nick S. Whiterod[A], *Sylvia Zukowski*[A], *Martin Asmus*[B], *Dean Gilligan*[C] *and Adam D. Miller*[A,D,E]

[A]Aquasave – Nature Glenelg Trust, Goolwa Beach, SA 5214, Australia.

[B]NSW Department of Primary Industries, Narrandera, NSW 2700, Australia.

[C]NSW Department of Primary Industries, Batemans Bay, NSW 2536, Australia.

[D]School of Life & Environmental Sciences, Centre for Integrative Ecology, Deakin University, Warrnambool, Vic. 3280, Australia.

[E]Corresponding author. Email: a.miller@deakin.adu.au

Table S1. Pairwise estimates of F_{ST} (lower diagonal) and D_{est} (upper diagonal) between *Euastacus armatus* collection sites based on seven microsatellite loci

Values shown in bold are significant ($P < 0.001$) after 10 000 permutations and correction for multiple comparisons

	TO	RP	WB	CO	GU	PE	ED	BU	HB	AB	PC	EG	SF	TA	LS	KI	OV	GO	LA	MA
TO		**0.16**	**0.38**	**0.32**	**0.45**	**0.40**	**0.45**	**0.46**	**0.51**	**0.54**	**0.42**	**0.69**	**0.63**	**0.65**	**0.29**	**0.33**	**0.21**	**0.53**	**0.78**	**0.82**
RP	0.05		**0.12**	**0.12**	**0.24**	**0.21**	**0.20**	**0.15**	**0.22**	**0.23**	**0.18**	**0.43**	**0.43**	**0.48**	**0.36**	**0.16**	0.08	**0.21**	**0.73**	**0.65**
WB	**0.13**	**0.04**		0.01	0.05	0.05	0.03	0.04	**0.09**	**0.08**	**0.12**	**0.32**	**0.33**	**0.38**	**0.38**	**0.11**	0.05	0.04	**0.62**	**0.52**
CO	**0.10**	**0.04**	0.00		0.08	0.08	0.08	0.08	**0.15**	**0.16**	**0.13**	**0.40**	**0.40**	**0.44**	**0.33**	**0.14**	0.05	**0.10**	**0.59**	**0.55**
GU	**0.14**	**0.08**	0.02	0.03		–0.01	0.03	0.07	0.10	**0.11**	0.09	**0.29**	**0.41**	**0.42**	**0.45**	**0.18**	**0.10**	0.07	**0.51**	**0.44**
PE	**0.13**	**0.07**	0.02	0.03	0.00		0.00	0.08	0.09	0.07	0.09	**0.28**	**0.37**	**0.37**	**0.43**	**0.18**	0.04	0.07	**0.55**	**0.53**
ED	**0.15**	**0.07**	0.01	0.03	0.01	0.00		0.05	**0.07**	**0.09**	**0.11**	**0.34**	**0.41**	**0.42**	**0.44**	**0.11**	**0.08**	**0.08**	**0.61**	**0.47**
BU	**0.16**	**0.05**	0.02	0.03	0.03	0.03	0.02		0.03	0.05	**0.13**	**0.31**	**0.35**	**0.37**	**0.43**	**0.13**	0.11	0.04	**0.62**	**0.42**
HB	**0.20**	**0.09**	**0.04**	**0.06**	**0.04**	**0.04**	**0.03**	0.01		0.02	0.12	**0.20**	**0.23**	**0.24**	**0.51**	**0.15**	**0.17**	**0.09**	**0.63**	**0.33**
AB	**0.23**	**0.10**	**0.04**	**0.07**	**0.05**	**0.04**	**0.04**	0.03	0.01		0.10	**0.14**	**0.19**	**0.23**	**0.52**	**0.17**	**0.13**	0.06	**0.64**	**0.38**
PC	**0.15**	**0.06**	**0.05**	**0.05**	**0.03**	**0.03**	**0.04**	**0.05**	**0.05**	0.05		**0.28**	**0.29**	**0.36**	**0.50**	**0.21**	0.10	**0.15**	**0.51**	**0.46**
EG	**0.40**	**0.25**	**0.19**	**0.22**	**0.19**	**0.18**	**0.20**	**0.21**	**0.15**	**0.13**	**0.21**		0.11	0.09	**0.76**	**0.39**	**0.37**	**0.25**	**0.60**	**0.37**
SF	**0.39**	**0.26**	**0.21**	**0.22**	**0.26**	**0.24**	**0.24**	**0.24**	**0.18**	**0.17**	**0.22**	**0.16**		0.03	**0.75**	**0.42**	**0.38**	**0.30**	**0.63**	**0.48**
TA	**0.41**	**0.28**	**0.23**	**0.24**	**0.26**	**0.24**	**0.24**	**0.25**	**0.18**	**0.20**	**0.26**	**0.15**	0.05		**0.77**	**0.46**	**0.43**	**0.35**	**0.62**	**0.48**
LS	**0.15**	**0.17**	**0.17**	**0.14**	**0.20**	**0.19**	**0.19**	**0.20**	**0.25**	**0.27**	**0.23**	**0.46**	**0.47**	**0.49**		**0.31**	**0.31**	**0.52**	**0.77**	**0.68**
KI	**0.12**	**0.06**	**0.04**	**0.05**	**0.07**	**0.07**	**0.04**	**0.06**	**0.07**	**0.09**	**0.09**	**0.25**	**0.27**	**0.30**	**0.16**		**0.11**	**0.21**	**0.73**	**0.47**
OV	**0.07**	0.03	0.02	0.02	**0.04**	0.01	0.03	0.04	**0.07**	**0.06**	**0.04**	**0.22**	**0.24**	**0.26**	**0.15**	**0.04**		**0.15**	**0.63**	**0.63**
GO	**0.17**	**0.07**	0.02	**0.03**	0.03	0.03	**0.03**	0.02	**0.04**	0.03	**0.06**	**0.16**	**0.20**	**0.22**	**0.23**	**0.08**	**0.05**		**0.55**	**0.45**
LA	**0.54**	**0.43**	**0.38**	**0.35**	**0.35**	**0.39**	**0.36**	**0.43**	**0.42**	**0.47**	**0.40**	**0.62**	**0.62**	**0.73**	**0.54**	**0.46**	**0.39**	**0.36**		**0.56**
MA	**0.49**	**0.38**	**0.31**	**0.31**	**0.29**	**0.34**	**0.28**	**0.30**	**0.26**	**0.32**	**0.34**	**0.42**	**0.49**	**0.53**	**0.47**	**0.32**	**0.37**	**0.29**	**0.64**	