Conserv Genet
DOI 10.1007/s10592-016-0868-8

RESEARCH ARTICLE

Range-wide fragmentation in a threatened fish associated with post-European settlement modification in the Murray–Darling Basin, Australia

Theresa L. Cole[1,7] · Michael P. Hammer[2,3] · Peter J. Unmack[4] · Peter R. Teske[1,5] · Chris J. Brauer[1] · Mark Adams[2,6] · Luciano B. Beheregaray[1]

Received: 22 January 2016 / Accepted: 12 July 2016

Abstract Distinguishing the relative influence of historic (i.e. natural) versus anthropogenic factors in metapopulation structure is an important but often overlooked step in management programs of threatened species. Biotas in freshwater wetlands and floodplains, such as those in the Murray–Darling Basin (MDB)—one of Australia's most impacted ecosystems, are particularly susceptible to anthropogenic fragmentation. Here we present a comprehensive multilocus assessment of genetic variation in the threatened southern pygmy perch *Nannoperca australis* (578 individuals; 45 localities; microsatellite, allozyme and mitochondrial DNA datasets), an ecological specialist with low dispersal potential. We assess patterns of spatial structure and genetic diversity in populations spanning the highly fragmented MDB and test whether recent anthropogenic modification has disrupted range-wide connectivity. We detected strong and hierarchical population structure, very low genetic diversity and lack of contemporary gene flow across the MDB. In contrast, the apparent absence of pronounced or long-term phylogeographic structure suggests that observed population divergences generally do not reflect deeply historic natural fragmentation. Coalescent-based analyses supported this inference, revealing that divergence times between populations from the upper and lower MDB fall into the period of European settlement. It appears that the observed contemporary isolation of populations is partly explained by the severe modification of the MDB post-dating the onset of European settlement. Our integrated approach substantially improves the interpretation of how fragmentation impacts present-day biodiversity. It also provides novel contributions for risk-assessing management actions in the context of captive breeding and translocations of small freshwater fishes, a group of increasing global conservation concern.

Keywords Conservation genetics · Connectivity · Translocations · Ecological genetics · Climate change

Electronic supplementary material The online version of this article (doi:10.1007/s10592-016-0868-8) contains supplementary material, which is available to authorized users.

✉ Luciano B. Beheregaray
Luciano.Beheregaray@flinders.edu.au

[1] Molecular Ecology Laboratory, School of Biological Sciences, Flinders University, Bedford Park, SA 5042, Australia

[2] Evolutionary Biology Unit, South Australian Museum, North Terrace, Adelaide, SA 5000, Australia

[3] Museum and Art Gallery of the Northern Territory, PO Box 4646, Darwin, NT 0801, Australia

[4] Institute for Applied Ecology and Collaborative Research Network for Murray–Darling Basin Futures, University of Canberra, Bruce, ACT 2601, Australia

[5] Department of Zoology, University of Johannesburg, Auckland Park, Johannesburg 2006, South Africa

[6] Australian Centre for Evolutionary Biology and Biodiversity, School of Biological Sciences, University of Adelaide, Adelaide, SA 5005, Australia

[7] Department of Zoology, Otago University, PO Box 56, Dunedin, New Zealand

Introduction

Natural landscape fragmentation plays an important role in shaping metapopulation and ecosystem boundaries through its influence on many micro-evolutionary processes, including migration, genetic drift and inbreeding (Lande 1988). The dispersal of individuals between habitat patches increases the probability of species persistence by reducing

Published online: 20 July 2016

the negative demographic consequences of population fragmentation, such as genetic load, inbreeding depression and reduced genetic variation (Hanski and Gaggiotti 2004; Weeks et al. 2011). While many species are historically fragmented across landscapes, anthropogenic influences have markedly reduced population ranges and connectivity in recent decades, which in turn have negatively impacted abundance and biodiversity (Hill et al. 2006). Indeed, human-induced landscape modification and habitat loss have become a leading cause of population declines, affecting ecosystems globally (Fischer and Lindenmayer 2007).

Distinguishing the relative influence of natural versus anthropogenic factors in shaping connectivity and driving population fragmentation is an important but often overlooked step in the management of threatened species. Approaches that concomitantly infer historical and recurrent causations of population structure can improve not only the interpretation of ecological and evolutionary causes of present-day spatial differentiation (Marko and Hart 2011), but also provide novel contributions for risk assessment of conservation management actions of threatened biodiversity. The latter is particularly relevant when using translocations (i.e. the deliberate transfer of individuals and populations across the landscape) as a tool for maintaining and enhancing resilience and persistence of threatened species (Weeks et al. 2011).

Freshwater ecosystems are naturally fragmented due to geomorphological history and the hierarchical nature of riverine networks, but are also particularly vulnerable to anthropogenic landscape fragmentation and modification (Fagan 2002). Aquatic populations are often sensitive to changes in water quality and flow (James et al. 2003) and have fewer opportunities to avoid detrimental environmental conditions by seeking out other locally available micro-habitats (Hughes 2007). Recent fragmentation and poor management of freshwater ecosystems is now a major problem, not only in heavily-modified river basins, i.e., the Colorado Basin (USA; Fagan et al. 2005), Mekong Basin (Asia; Kang et al. 2009) and the Murray–Darling Basin (MDB) (Australia; Lintermans 2007), but also throughout freshwater ecosystems worldwide (Geist 2011).

The intensely regulated MDB is arguably the most important river system in Australia, contributing 50 % of the water used for agricultural irrigation and containing a range of diverse ecosystems, including 5.7 million hectares of wetlands (Leblanc et al. 2012). However, since European settlement in the region ($\sim$150 years ago), the MDB has suffered extensive urban and agricultural development (e.g. wetland reclamation), major river regulation, construction of thousands of barriers to fish passage and the introduction of exotic species (Lintermans 2007; Laurance et al. 2011). Major hydrological changes, including 18 major barriers (large weirs and dams), were initiated in the 1920s and expanded sharply in the 1950s, with irrigation and land clearing activity extending well before this time to the early-mid 1800s (Walker and Thoms 1993).The negative consequences of these alterations on its native aquatic fauna have been further exacerbated by a 13-year drought beginning in 1997 (Hammer et al. 2013).

Freshwater fishes are particularly susceptible to biodiversity loss and extinction, as they often exist naturally as small fragmented populations (Kang et al. 2009). In the MDB, over half of the native fish species are considered to be at risk of extinction, with current native fish populations estimated to represent $\sim$10 % of what existed prior to European settlement (Lintermans 2007; Leblanc et al. 2012). Until recently, management efforts aimed at restoring and protecting native fishes have mostly been directed at the few large (>20 cm length) angling species (Hammer et al. 2013). As such, many of the smaller species (<10 cm length) require urgent attention to ensure their long-term persistence in the MDB. Here we focus on one such species, the southern pygmy perch (*Nannoperca australis* Günther 1861) (Percichthyidae).

Nannoperca australis is a small bodied (<85 mm) freshwater fish distributed across southeastern Australia (Unmack et al. 2013). It inhabits environments associated with sheltered micro-habitats and aquatic macrophyte cover and is relatively short lived (3–6 years; reaches maturity within 1 year), has large demersal eggs and larva, and shows limited dispersal ability being small, localised and non-migratory (Lintermans 2007). As an ecological specialist it is an important environmental indicator and plays a role within stream and wetland ecosystems (Tonkin et al. 2008; Wedderburn et al. 2012). A recent study using allozymes, nuclear and mitochondrial (mtDNA) sequences suggested the presence of cryptic species (western and eastern taxa) (Unmack et al. 2013). It also identified two evolutionarily significant units (ESUs) within the western *N. australis,* one restricted to the inland MDB and the other occurring across coastal river basins. Historically, there is evidence *N. australis* was essentially continuously distributed within the MDB from the lower Murray in littoral, off-channel or tributary habitats along the continuous riverine corridor to the upper reaches (excluding the Darling River system), although local abundance likely varied substantially across that range (e.g. Llewellyn 1974). However populations are now only patchily distributed in the Basin (Lintermans 2007; Hammer et al. 2009). The earliest noted declines occurred in the South Australian and New South Wales sections of the MDB, where the species is considered endangered (Hammer et al. 2009), and there are efforts in these jurisdictions to actively protect the remaining populations. Notable among these efforts have been the establishment of captive breeding programs for

one near-extirpated lower Murray population and the subsequent reintroduction of fish back into the wild (Hammer et al. 2013; Attard et al. 2016).

Here, we present a detailed multi-locus assessment of population structure and gene flow in the MDB ESU of *N. australis* to address two issues: one with significance to conservation management of this threatened species and the other with broader implications to freshwater biodiversity. First, we aimed to clarify spatial structure and genetic diversity in populations spanning the highly fragmented MDB through a synthesis of our primary molecular dataset (microsatellites for fine-scale contemporary population structure) with two secondary datasets (allozymes for broad-scale patterns; mtDNA for phylogeographic structure). The latter two datasets provide full integration with a previous phylogeographic study of *N. australis* across all ESUs (Unmack et al. 2013).

In addition to their practical utility in contemporary conservation biology, population genetic data can also provide insight into the timing of population fragmentation. Our secondary aim therefore was to test whether recent anthropogenic modification of the MDB has disrupted range-wide connectivity in this low-dispersal species. To do so, we combined traditional analyses of genetic variation with coalescent-based methods that provide information about historical divergence and gene flow (Marko and Hart 2011; Teske et al. 2014). These analyses were conducted to determine whether there is evidence that contemporary populations were connected in the recent past i.e. prior to the arrival of Europeans in the MDB. The null hypothesis is that isolation in this system is historic rather than recent. Historic isolation would be associated with the vast spatial scale of the MDB compared to the species' dispersal capacity and with the naturally high unpredictability of flow in Australian rivers (Puckridge et al. 1998). The latter is expected to modulate many abiotic and biotic processes, including population connectivity (Poff et al. 1997). We propose that our combined approach enables managers to risk-assess conservation actions aimed at improving persistence and resilience of fragmented populations. The latter is illustrated using results from our conservation projects that recently helped avoid regional extinctions in *N. australis* (Attard et al. 2016).

Materials and methods

Sampling

We examined 578 individuals of *N. australis*, collected between 1999 and 2011 across 45 localities. Frozen whole specimens, frozen tissue, or ethanol-preserved fin clips sampled in the field were deposited at the South Australian Museum. The region sampled encompassed the entire distribution (all known extant populations) of the MDB ESU across 13 river catchments (Fig. 1) and is herein subdivided into the Lower Murray River (LMR; Lake Alexandrina and its stream tributaries), and Upper Murray River (UMR; Murray and its tributaries including the Lachlan upstream of Swan Hill). Individuals were included in the microsatellite section only after our allozyme and mtDNA analyses confirmed their assignment to the MDB ESU of *N. australis* (sensu Unmack et al. 2013). Final sample sizes (Table 1) per molecular dataset were n = 535 for microsatellites (38 sites), n = 308 for allozymes (33 sites), and n = 161 for mtDNA (35 sites).

Allozyme analyses

Allozyme electrophoresis was undertaken as described in Unmack et al. (2013) for the same 23 variable loci screened therein. Neighbor-joining (NJ) trees or unrooted networks were used to visualize the genetic relationships among sites, also following Unmack et al. (2013).

MtDNA analyses

Sequence data for the complete cytochrome *b* gene (*cyt*b) were obtained and analysed as detailed in Unmack et al. (2013). All new sequences have been deposited in GenBank (accession numbers KX249713-KX249733). The program TCS 1.21 (Clement et al. 2000) was used to create a haplotype network based on statistical parsimony (95 % criterion) to help infer relationships among recently diverged populations.

Microsatellite analyses

Genotyping procedures

Protocols for DNA extractions and amplification of 12 microsatellite loci, developed specifically for *N. australis*, are described in Carvalho et al. (2012). Each microsatellite fragment was binned and the size scored manually in GENEMAPPER 4.0 (Applied Biosystems). Resulting profiles were checked for scoring errors caused by null alleles, stutter, and large allele dropout using MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004).

Genetic variation and bottlenecks tests

Bonferroni-adjusted statistical tests for genotypic linkage disequilibrium and for departures from Hardy–Weinberg equilibrium (HWE), expected (H_E) and observed (H_O) heterozygosities, inbreeding coefficients (F_{IS}) and measures of allelic richness (A_R) were all conducted as detailed

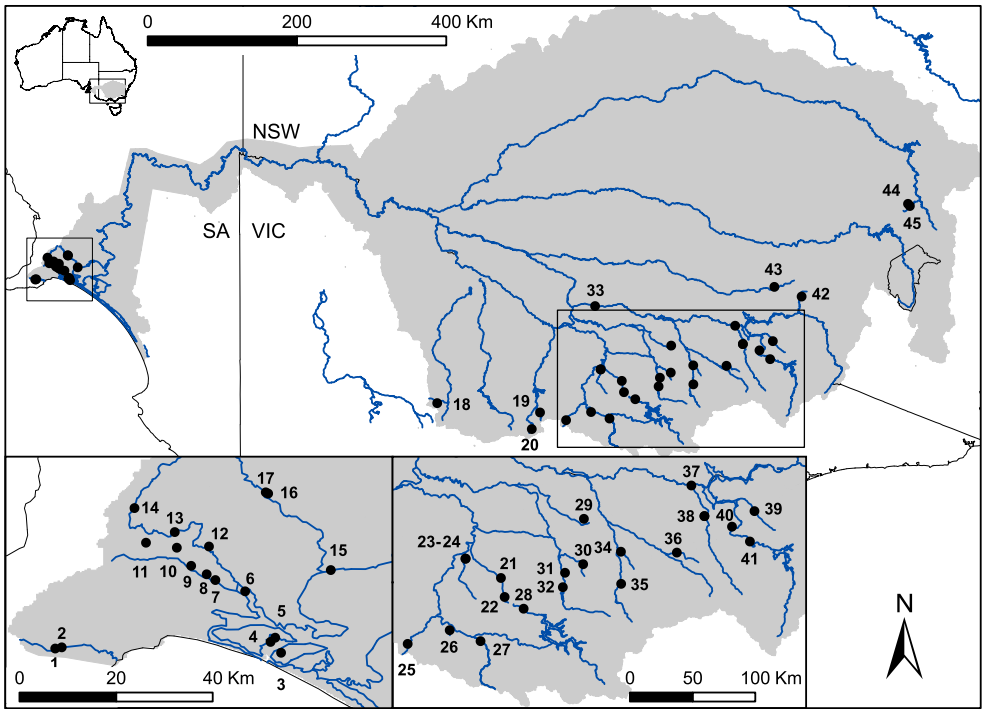

Fig. 1 Sampling sites of all *Nannoperca australis* examined. The *shaded area* identifies the known historic distribution of *N. australis* in the Murray–Darling Basin (MDB). The *insets* show details of sites sampled in the lower and upper MDB. See Table 1 for corresponding locality information

in Brauer et al. (2013). As some sites had very small sample sizes ($n = 2$), we pooled samples from sites that clustered together as indicated by the STRUCTURE analysis (below and Table 2).

We used two approaches to test for genetic signal of recent demographic reductions, or genetic bottlenecks: heterozygosity excess and the M-ratio. These tests compare observed results to theoretical expectations based on a population at equilibrium and were used to explore our data using the same parameter conditions. For the former, we used the two-phase mutation model in BOTTLENECK 1.2.02 (Cornuet and Luikart 1996). For the latter, empirical M-value and M critical were estimated in M_P_Val and used the dinucleotide microsatellite mutation rate of 5×10^{-4} as suggested by Garza and Williamson (2001). Pre-bottlenecked effective population size varied between 1000 and 10,000.

Population genetic structure and assignment tests

Global and pairwise F_{ST} and R_{ST} were calculated in ARLEQUIN, using 10,000 permutations. Choosing whether F_{ST} or R_{ST} is more appropriate is dependent on the mutation model of the microsatellite loci. We assessed the relative role of mutation or genetic drift on the inferred population structure using SPAGEDI 1.3 (Hardy and Vekemans 2002). The Bayesian clustering program STRUCTURE 2.3.4 (Pritchard et al. 2000) was used to describe and visualise population structure with default parameters and ten replicate runs with a burn-in of 10^5 and Markov Chain Monte Carlo (MCMC) of 10^6 from 38 localities. The most likely number of clusters of individuals (K) was determined following Evanno et al. (2005). Additionally, we used BAPS 5 (Corander et al. 2008) to assess population structure using default settings. BAPS also implements a Bayesian statistical model but, in contrast to STRUCTURE, provides a spatially explicit approach to identifying sub-population boundaries within the riverine dataset.

We also conducted an assignment test in GENECLASS 2.0 (Piry et al. 2004) to assess dispersal events. Here we employed the Bayesian criterion (Rannala and Mountain 1997) and a 'leave-one-out' method at the 0.05 level. Finally, ARLEQUIN was used to perform an Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992) based on the F_{ST} statistic. We examined varying hierarchical levels within the riverine network that are expected to affect on population subdivision: among and within all localities, catchments and streams (Table S1).

Spatial analyses

We carried out spatial autocorrelation (SAC) tests in GENALEX (Peakall and Smouse 2012) to elucidate whether inferred genetic patterns are dependent on physical distances between sites. We also used Mantel tests in

Table 1 Locality, sampling date and sample size information for *Nannoperca australis*

Site #	Locality	Catchment	n	M	A	C	Lat.	Long.	Year	SAM tissue code(s)
1	Back Valley Ck #1	Inman	9	4	9	6	−35.541	138.495	2001	FISH84:ML-81+
2	Back Valley Ck #2	Inman	15	15			−35.538	138.507	2006, 2010-1	MALC:GT-82+
3	Mundoo Is., L. Alexandrina	Lower Lakes	38	38			−35.549	138.915	2006	MH-Breed1
4	Hindmarsh Is., L. Alexandrina	Lower Lakes	10	10	6	5	−35.528	138.896	2002	FISH84:ML-121+
5	Drain off Mundoo Channel	Lower Lakes	10	10	10		−35.520	138.904	2003	FISH98:LL-14+
6	Tookayerta Ck, Black Swamp#2	Lower Lakes	4	4			−35.434	138.848	2010	MALC:GT-720+
7	Tookayerta Ck, Currency Winery	Lower Lakes	18	18			−35.413	138.793	2011	MALC:GT-1198+
8	Tookayerta Ck, Black Swamp#1	Lower Lakes	11	9	11	3	−35.403	138.777	2000	FISH84:ML-16+
9	Tookayerta Ck, Deep Ck Rd	Tookayerta	20	20			−35.387	138.748	2010	MALC:GT-606+
10	Swampy Ck	Tookayerta	6	5	6	3	−35.353	138.721	2000	FISH84:ML-49+
11	Nangkita Ck	Tookayerta	21	21	9	2	−35.344	138.664	2000, 2010	FISH84:ML-54+, MALC:GT-562+
12	Finniss R., waterfalls	Finniss	23	23			−35.351	138.781	2004, 2010	MALC:GT-152+
13	Finniss R., Ashbourne	Finniss	10	10	9	5	−35.324	138.717	2000, 2011	FISH84:Fin-1+, MALC:GT-1189+
14	Meadows Ck	Finniss	20	20	10	2	−35.279	138.642	2000, 2010	FISH84:ML-60+, MALC:MAD-408+
15	Turvey's Drain, L. Alexandrina	Lower Lakes	27	27	13	6	−35.395	139.008	2000, 2008	FISH84:ML-40+, MH-Breed2
16	Angas R., Middle Ck junction	Angas	10	10			−35.252	138.891	2000, 2010	FISH84:ML-13+, MALC:GT-642+
17	Middle Ck	Angas	11	11	10	6	−35.250	138.887	2001	FISH84:ML-2+
18	Trib to Middle Ck, Warrenmang	Avoca	31	31	10	5	−37.028	143.338	1999	PU99-33
19	Jews Harp Ck, Sidonia	Campaspe	29	29	10	4	−37.139	144.578	2000	PU00-01
20	Coliban R.	Campaspe	10		10	5	−37.339	144.478	2009	PU09-135
21	Castle Ck, Telfords Bridge	Goulburn	3			3	−36.761	145.562	2003	PU-Cast1
22	Castle Ck, Killeen Hill Rd	Goulburn	3			3	−36.897	145.589	2003	PU-Cast2
23	Pranjip Ck	Goulburn	4	4	4	4	−36.623	145.309	2002	TR02-420B
24	Muddy Ck (Pranjip Ck)	Goulburn	5	5	5		−36.622	145.305	2002	TR02-410B
25	Kurkurac Ck	Goulburn	2		2	2	−37.233	144.892	2002	TR02-381B
26	Trawool Ck	Goulburn	10	10	10	5	−37.135	145.193	2002	TR02-18
27	Yea R., Yea	Goulburn	20	20	10	5	−37.213	145.414	1992	PU92-08
28	Merton Ck	Goulburn	10	10	10	5	−36.981	145.725	2009	PU09-01
29	Gnarite Ck	Broken	5		5	4	−36.336	146.157	2002	TR02-330B
30	Sam Ck	Broken	10	10	10	5	−36.661	146.152	2009	PU09-03
31	Swanpool Ck, Swanpool	Broken	14	14	10	5	−36.723	146.022	1994	PU94-43
32	Unnamed Ck, Lima South	Broken	9	9	10	5	−36.827	146.006	2009	PU09-02
33	Flat Swamp, Barmah Forest	mid Murray	5			5	−35.861	145.240	2007	PU-Barm
34	Meadow Ck, Moyhu	Ovens	20	20	10	5	−36.573	146.423	1999	PU99-79
35	King R., Cheshunt	Ovens	10	10	10	5	−36.802	146.424	2009	PU09-06
36	Happy Valley Ck	Ovens	11	10	11	5	−36.579	146.824	2009	PU09-08
37	Murray R. lagoon, Albury	Albury	21	21	10	5	−36.098	146.928	1994	PU94-47
38	Gap Ck, Kergunyah	Kiewa	20	20	20	10	−36.317	147.022	1999,2009	PU99-81, PU09-12
39	Tallangatta Ck	Mitta Mitta	10	7	10	5	−36.281	147.382	2009	PU09-17
40	Glencoe Ck	Mitta Mitta	10	10	10	5	−36.393	147.221	2009	PU09-14
41	Spring Ck	Mitta Mitta	10	10	10	5	−36.499	147.349	2009	PU09-13
42	Coppabella Ck	upper Murray	20	20	10	5	−35.746	147.729	1999	PU99-82
43	Sawyers Ck, Holbrook	Billabong	3			3	−35.630	147.400	2006	MALC:MAD-447+
44	Blakney Ck	Lachlan	8	8	8	5	−34.657	149.032	2003	FISH98:LPP-1+

Table 1 continued

Site #	Locality	Catchment	n	M	A	C	Lat.	Long.	Year	SAM tissue code(s)
45	Langs Ck	Lachlan	2	2			−34.630	149.010	2003	MALC:MAD-482+
Total			578	535	308	161				

The SAM code refers to the South Australian Museum tissue collection details. Code for sample sizes: *n* maximum available per site, *M* microsatellites, *A* allozymes, *C* cytb

Table 2 Population units (i.e. management units, MUs) identified in Murray–Darling Basin *Nannoperca australis* and summary of genetic variation based on 12 microsatellite loci

MU	n	Sites	F_{IS}	A_R	N_A	H_o	H_E
MU1	19	1 and 2	0.122	1.82	24	0.42	0.48
MU2	53	12, 13, and 14	0.296	2.11	31	0.3	0.43
MU3	21	16 and 17	0.197	1.79	24	0.34	0.42
MU4	46	9, 10, 11	0.065	3.81	56	0.52	0.55
MU5	116	3, 4, 5, 6, 7, 8, and 15	0.099	5.28	103	0.61	0.67
MU6	31	18	−0.095	1.62	24	0.51	0.47
MU7	42	23, 24 30, 31, and 32	0.232	4.02	77	0.42	0.54
MU8	40	26, 27, and 28	0.109	2.02	38	0.33	0.37
MU9	29	19	0.109	2.06	31	0.3	0.33
MU10	20	38	0.169	2.84	42	0.47	0.55
MU11	21	37	0.133	4.29	64	0.52	0.6
MU12	27	39, 40, and 41	0.128	2.33	39	0.43	0.49
MU13	40	34, 35, and 36	0.186	2.97	57	0.45	0.56
MU14	20	42	0.316	1.55	21	0.24	0.33
MU15	10	44 and 45	0.312	2	26	0.33	0.47

Table 1 sites are grouped together to form each MU in a cluster. MU is the code, n is sample size, F_{IS} is inbreeding coefficient, A_R is allelic richness, N_A is number of alleles, *Ho* and H_E are observed and expected heterozygosity, respectively

GENEPOP to assess isolation by distance (IBD) (Wright 1942). Importantly, SAC and Mantel tests might not necessarily distinguish between patterns resulting from hierarchical clustering (e.g. stream within catchments versus streams between catchments) and those resulting from IBD (Meirmans 2012). To overcome this issue, we controlled for geographic distance by doing a partial Mantel test in GENODIVE 2.0 (Meirmans and van Tienderen 2004) with standardised F_{ST} (F'_{ST}) as matrix A, clusters identified by STRUCTURE as Matrix B and riverine distance as a covariate. For all spatial analyses we estimated riverine distances in ArcMap 10 using the 9 Second DEM Derived Streams v.1.1.3 dataset. All tests were conducted over the entire study area (except for the SAC), and then separately for the LMR and the UMR.

Contemporary connectivity

BAYESASS + 3.0 (Wilson and Rannala 2003) was used to estimate contemporary gene flow across the MDB. This method uses multilocus genotypes to estimate short-term gene flow (i.e. last few generations) following a Bayesian MCMC approach. The program was run across clusters identified by STRUCTURE for 11×10^6 iterations including a burn-in of 10^6 iterations. Delta values for migration rate, allele frequencies and inbreeding coefficients were set at 0.10, 0.30 and 0.70. Estimates were considered real if they were consistent in all replicate runs.

Historical divergence

For this section we analysed the multi-locus microsatellite dataset with the coalescent-based program IMa2 (Hey 2010), which implements the isolation-with-migration model of Nielsen and Wakeley (2001). We estimated divergence time between the population from lower MDB site 9 and the population from upper MDB site 26 (Fig. 1). For comparison, we also determined the divergence time between two populations in the lower MDB (9 vs. 15). No suitable heating schemes were found for more complex

models that included >2 populations. The accuracy of demographic parameter estimations in IMa2 depends largely on the extent to which Markov chains mix, and finding a suitable heating scheme to achieve this is particularly challenging. We specified a geometric heating scheme (-function –hfg) with 150 heated chains (-hn 150), and assessed the swap rates between successive chains under 36 different combinations of heating parameters (-ha and –hb). A heating scheme with –ha 0.99 and –hb 0.8 produced the highest swap rates (between 0.76 and 0.98). We then used this heating scheme to perform four independent runs whose starting seeds differed but whose priors were otherwise identical, with results reported every hour (−l 1.0) and a burn-in prior to recording results (-b) of 100,000 steps. Upper bounds for demographic parameters were t (divergence time) = 2, θ_0 (effective population size of population 1) = 2.0, θ_1 (effective population size of population 2) = 2.0, θ_2 (population size of the ancestral population) = 60, m0 > 1 (migration into population 0) = 50 and m1 > 0 (migration into population 1) = 50. The conversion program CREATE v1.37 (Coombs et al. 2008) was used to create infiles for IMa2, and demographic estimates were converted to number of individuals (θ and m) or to time in years (t) using the program's default microsatellite mutation rate of 0.05 % per year (95 % confidence interval 0.005–0.5 % per year), a value that is very close to that reported for common carp, *Cyprinus caprio* L.; Yue et al. 2007). We assumed a generation time of one year. Coalescent genealogies generated during the four independent runs were combined in 'Load Trees' mode, and demographic parameters reported using a total of 25,694 genealogies for populations 9 versus 15, and 87 600 genealogies for populations 9 versus 26.

We recognize that the above pairwise comparisons are simplistic. However, our attempts to consider more complex histories of divergence by comparing multi-population datasets did not produce adequate results. We attempted to use the approximate Bayesian computation (ABC) approach implemented in DIYABC v2.0 (Cornuet et al. 2014) but the priors specified were not suitable to recover the observed data (results not shown). This was observed irrespectively of how broad the priors were, an issue that possibly reflects the highly structured nature of the microsatellite dataset. Nonetheless, the two population comparisons based on IMa2 are informative since they enabled us to determine whether populations in the upper and lower MDB basin diverged during the period of European settlement, or whether their genetic structure predates this period and is thus not a consequence of anthropogenic impacts on population connectivity.

Results

Allozyme analyses

The final allozyme dataset comprised 308 individuals genotyped at 23 loci (Table S2). An unrooted NJ network among all MDB sites (except site 20, which clustered closely with 'western' lineage sites; Fig. S1) revealed three broad groupings of sites, two restricted to the Goulburn catchment (site 25, not represented in the microsatellite study due to its small sample size, plus sites 26–28), and a heterogeneous third group comprising sites spread throughout the MDB and including two other Goulburn catchment sites (23 and 24; Fig. 2). Our a posteriori assessment of the extent of concordance between 15 management units (i.e. demographically distinct populations; MUs) identified by microsatellite analysis (MU1–MUl5; Table 2; details below) and the allozyme affinities of sites (Fig. 2) found good qualitative support for all MUs except for one (MU7), which appeared to be somewhat heterogeneous based on its allozyme profiles.

MtDNA analyses

The final mtDNA dataset consisted of 161 individuals sequenced for 1140 bp of the cyt*b* gene. These plus all sequences available for *N. australis* (Unmack et al. 2013) were initially analysed by generating a Maximum Likelihood tree (Fig. S1). Consistent with their allozyme profiles, the five individuals from site 20 displayed "western" clade haplotypes, whereas all other previously-uncharacterized MDB sites aligned tightly within the MDB clade. As a result, the final cyt*b* dataset for all pure MDB sites comprised 155 individuals, which together displayed 38 distinct haplotypes. Overall levels of haplotype diversity were low: 17 sites (50 %) contained a single haplotype, eight had two haplotypes, with six, two and one site with three, four and five haplotypes respectively (Fig. 3).

Overall, the TCS haplotype network (Fig. 3) points to a historically connected lineage with shallow phylogeographic structure. Most haplotypes only differ by one or two nucleotides (out of 1140 bp), except for populations from the upper Goulburn sub-basin (MU8, plus one individual from site 24), which form a distinct geographic group. Most shared haplotypes were restricted to the same sub-basin, with six haplotypes shared between sub-basins (A, B, F, J, S, X; Fig. 3). Four of the six shared haplotypes probably represent ancestrally placed haplotypes and were found in both LMR and UMR populations.

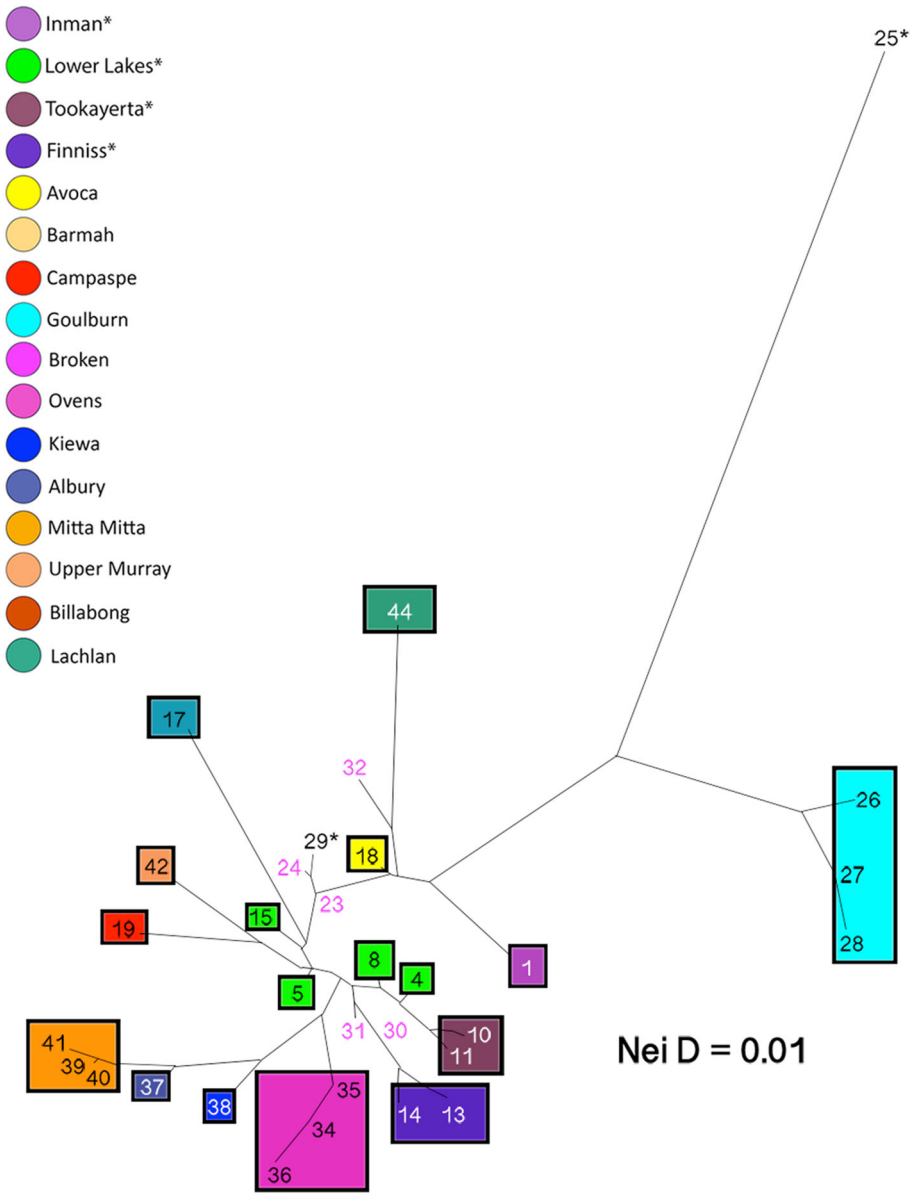

Fig. 2 Unrooted neighbor-joining network based on allozymes depicting the affinities of the 32 pure sites (as defined in Supplementary Fig. 2) surveyed for *Nannoperca australis*. Sites are enclosed in boxes which are colour-coded according to their microsatellite-assigned populations (see Fig. 5). *Asterisks* the two sites asterisked were not included in the microsatellite study due to their small sample sizes

Low microsatellite variation and inbreeding but no population bottlenecks

Twelve polymorphic loci amplified successfully (Table S3), with no consistent evidence for stutter, large allele dropout, null alleles or linkage disequilibrium. All loci and sites, whether spatially or temporally separated, were in HWE after Bonferroni corrections. Allelic richness and heterozygosity were very low (mean $A_R = 2.80$, mean $H_O = 0.47$, mean $H_E = 0.53$). In the LMR, samples from MU5 had higher diversity (mean $A_R = 5.28$, $H_O = 0.61$, $H_E = 0.67$) compared to other regional localities (mean $A_R = 1.87$, $H_O = 0.37$, $H_E = 0.44$) ($P = 0.001$). Within the UMR, MU11 had the highest allelic richness ($A_R = 4.29$). We found positive and mostly significant F_{IS} values in all sites (overall mean $F_{IS} = 0.19$) suggesting that inbreeding has impacted subpopulations. Finally, our analyses revealed no consistent signal for genetic bottlenecks in *N. australis*. Only three out of fifteen populations displayed signatures consistent with bottlenecks based on the heterozygosity excess test (MU1, MU13, and MU6), whereas none of the fifteen M-ratio tests provided statistical support for population bottlenecks (Table S4).

Marked population structure

We found microsatellite population differentiation to be consistently high, based on both global F_{ST} (0.37) and R_{ST} (0.26). All subsequent analyses herein are limited to F_{ST}.

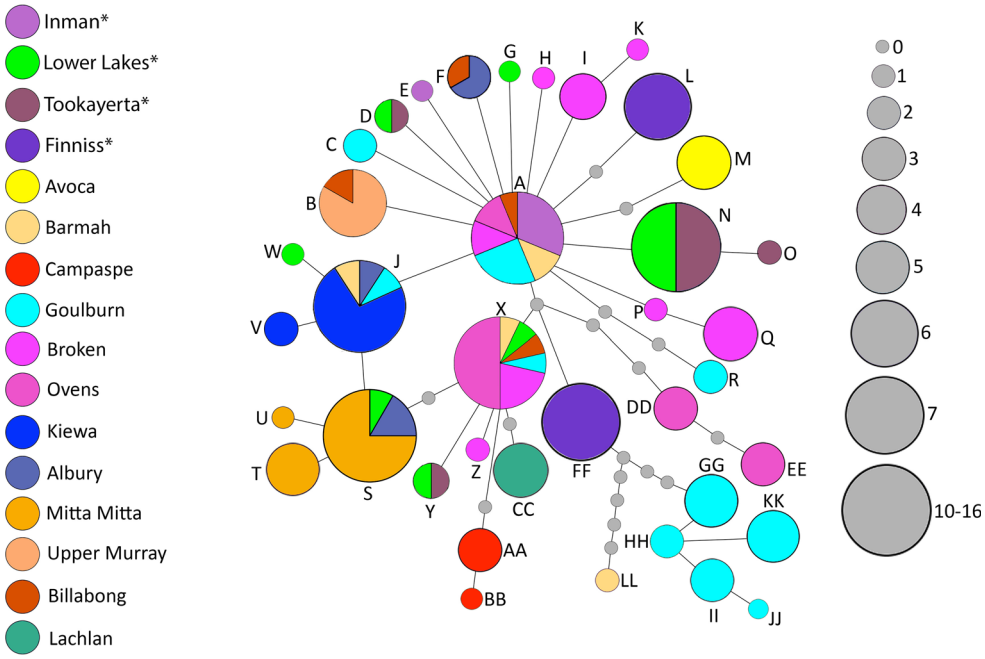

Fig. 3 Network of cytochrome *b* mtDNA sequence data for *Nannoperca australis*. Each mtDNA haplotype is colour-coded based on the catchment where it occurs. The key to river colours is presented in the *upper left corner*. *Asterisks* indicates that the river is in the Lower Murray region. Rivers are listed in their geographic order from downstream to upstream. Details for the specific geographic location for each *N. australis* haplotype are in Table 1. Circle size represents haplotype abundance; the key to circle size is in the *lower right corner*. Unsampled haplotypes are represented by *small circles*

We found >95 % of population pairwise comparisons of F_{ST} values to be statistically significant ($P < 0.05$) (Table S5). Most of these values were greater than 0.25, suggesting strong differentiation.

Bayesian clustering approaches consistently revealed marked structure between the LMR and UMR, most evident in the $K = 11$ from the spatial BAPS test (Fig. 4). Using STRUCTURE, we found 15 genetic clusters (LnP(D) = −10771.51; Fig. 5) predominantly distributed in different river catchments (Fig. 5; Table S1). These population clusters are mostly consistent with the more conservative output from BAPS and are referred hereafter as the 15 MUs identified for *N. australis* (Table 2). Interestingly, all analyses indicated admixture in MU5, comprising a group of sites within Lake Alexandrina as the lowest-elevated LMR populations before the MDB expels. Concordantly, populations from the two sites representing MU2, sampled from a tributary creek (Finniss) separated by a waterfall, appear to be genetically dissimilar.

Assignment tests corroborated the pattern of localised population structure, with most individuals (89 %) clustering according to their original collection sites. Indeed, most non-assignments were due to an individual not being assigned to any site. Results further indicate that Lake Alexandrina (a LMR site) is admixed. No LMR individuals were assigned to the UMR. Within the UMR, sites 31 and 37 appeared admixed. Finally, AMOVA provided statistically significant support for differentiation in all hierarchical groupings (Table S1; $P < 0.001$), with molecular variation explained not only by differences among catchments (25.9 %), but also among localities within the same river catchment (12.6 %). The latter is consistent with results of BAPS and STRUCTURE (Figs. 4, 5). The least amount of variation was found among localities within the same stream (3.56 %), suggesting that streams act as the smallest unit of population structuring.

Spatial differentiation

The species shows strong and significant positive spatial autocorrelation (i.e. greater-than-random genetic similarity) at lower distance classes, a pattern observed at much smaller distances in the LMR (up to 18.7 km) than in the UMR (233.9 km; Fig. S2). The IBD analysis for the entire study area approached significance ($P = 0.052$, $R^2 = 0.03$; Fig. S3) but for this particular test we used Euclidean distance since we were unable to connect the two geographical areas, nor directly connect the Lachlan, Avoca and Inman River sites to others in their regions. Testing IBD separately in the LMR and UMR using riverscape distances was considered more appropriate. For the latter, both analyses were significant ($P < 0.001$; Fig. S3b and S3c). Additionally, the partial Mantel test provided support that both hierarchical clusters and riverine distance contribute to the population structure seen in *N. australis* (Table 3); LMR (Mantel's $r = 0.499$) and in the UMR (Mantel's $r = 0.274$).

Contemporary gene flow

From a contemporary perspective, BAYESASS + 3.0 results indicate nil gene flow (migration rate $m < 0.01$) across the entire system. Critically, there was no evidence of gene flow between populations in the LMR and those in the UMR

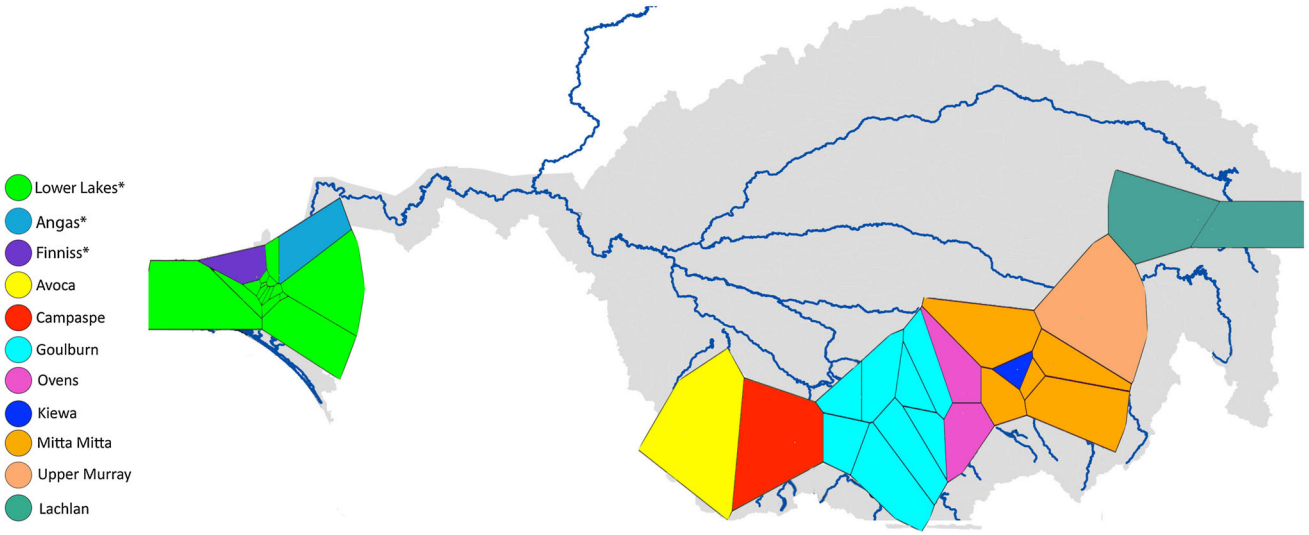

Fig. 4 Coloured Voronoi tessellation output from BAPS based on microsatellite data from 535 *Nannoperca australis* samples collected from 38 localities. The map shows the spatial scale of 11 inferred clusters in the Murray–Darling Basin. The Lower Murray River and Upper Murray River appear as clearly distinct, with no shared clusters

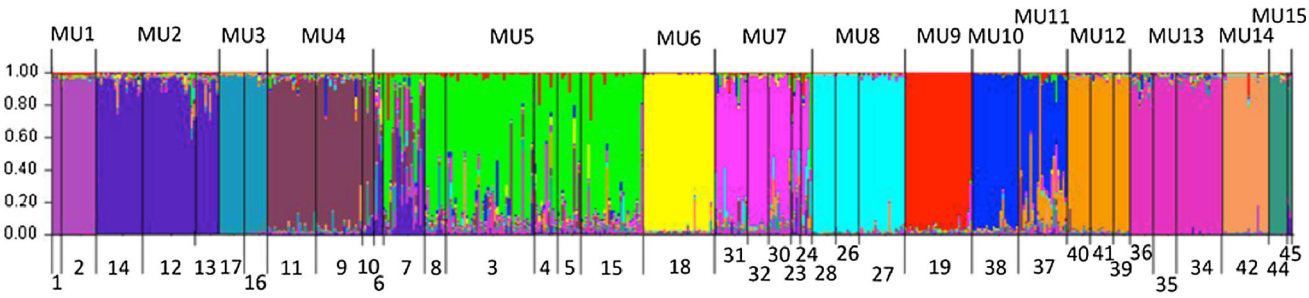

Fig. 5 STRUCTURE output based on microsatellite data showing 15 clustered populations or management units (MUs) based on 535 *Nannoperca australis* samples collected from 38 localities. Individuals are grouped by sampling location, and each individual is represented by one vertical column

(Table S6), a finding consistent with results of the various analyses of population structure. Apart from the instances of admixture within MU5, between sites 23 and 24, and among sites 30, 31 and 32, the only evidence for contemporary gene flow was between MU11 and MU10 ($m = 0.200$)—these are two UMR sites that are only ~30 km from each other.

Historic divergence between the upper and lower MDB

IMa2 estimates of divergence time between sites from the upper and lower MDB (9 vs. 26) fell into the period of European settlement (125 years), and a more recent (44 years) estimate was found for two sites in the lower reaches of the basin (sites 9 vs. 15) (Table 4). In both cases, posterior probability curves did not return to zero after reaching a peak (not shown), and higher posterior density intervals could thus not be determined (question marks in Table 4). The finding that divergence was recent nonetheless suggests that gene flow existed between populations from upper and lower reaches of the basin currently separated by a major distribution gap.

Table 3 Original and partial Mantel tests calculated using GENODIVE

	Mantel	*P* value	Partial Mantel	Significance
ALL				
Mantel r	0.115	0.052	0.364	<0.05
R^2	0.013			
LMR				
Mantel r	0.555	<0.01	0.499	<0.05
R^2	0.308			
UMR				
Mantel r	0.58	<0.01	0.274	<0.05
R^2	0.336			

Discussion

We conducted a multilocus assessment of connectivity across the distribution of the threatened freshwater fish *Nannoperca australis* in the MDB to clarify population structure and to test whether recent anthropogenic modification in the basin has disrupted population connectivity. Microsatellite DNA analyses revealed a strong and hierarchical pattern of population differentiation, low genetic diversity and nil contemporary gene flow across this highly fragmented river basin. General concordance with these findings was also provided by our allozyme dataset. Nevertheless, there was no evidence for pronounced or long-term phylogeographic structure in the mtDNA dataset. In support of this inference, coalescent-based analysis of the microsatellite dataset revealed that divergence time estimates between populations from the upper and lower MDB fall within the period of European settlement. Thus, it appears that population connectivity across the MDB probably existed until recently, and we make important recommendations in this context for conservation of the species.

Natural versus anthropogenic range-wide fragmentation

Many species are naturally fragmented across the landscape because the ecological, genetic, and evolutionary processes that affect them take place at spatial scales that are greater than the scale within which most individuals disperse (Hanski and Gaggiotti 2004). Accordingly, several examples exist of naturally fragmented freshwater populations (e.g. Huey et al. 2010). Localized population isolation potentially associated with human-induced habitat fragmentation has also been reported for freshwater organisms (Geist 2011; Dawkins et al. 2010), including MDB fishes (Faulks et al. 2010; Brauer et al. 2013). On the other hand, examples of range-wide fragmentation associated with anthropogenic influences are very rare in the literature (Hanski and Gaggiotti 2004; Pritchard et al. 2009) since they generally require evidence for rapid disruption of gene flow impacting the regional assemblage of spatially delimited local populations.

Genetic-based estimates of the timing at which populations split from one another can provide important information on whether demographic events or barriers to gene flow are attributable to natural or to anthropogenic influences (Marko and Hart 2011; Teske et al. 2014). Our genetic study strongly suggests that the observed population isolation across the range of *N. australis* in the MDB took place recently and rapidly. For instance, the age of the inferred split between two populations of the upper and lower reaches of the basin was estimated at only 125 years (Table 4). Hence, even though high levels of contemporary isolation were found among most sites (e.g. nil gene flow across the entire system; Table S6), this is probably not indicative of population divergences associated with deeply historical fragmentation across the river basin (except perhaps for a few very localized natural barriers, such as the waterfall in the Finniss River and sections of the Goulburn River). This is consistent with a recent review on the biogeography of Australian fishes, which concluded that the majority of widespread fishes in the MDB display only modest levels of phylogeographic structure across their range (Unmack et al. 2013). We argue instead that the inferred pattern can be attributed to anthropogenic modification of the MDB, which is consistent with the history of range reduction observed in *N. australis* during the last 100 years (Walker and Thoms 1993).

Nannoperca australis has recently experienced large-scale population extirpation from floodplain wetland habitat along the middle Murray River, with small population fragments now restricted primarily to tributaries of the upper Murray, and pockets of the Mount Lofty Ranges and Lake Alexandrina in the lower MDB (Cook et al. 2007; Hammer et al. 2009). Wetland habitats in the middle Murray have been dramatically altered due to significant changes in the magnitude and seasonality of flow regimes, aquatic and riparian habitat availability, water quality (increased salinity, nutrients and turbidity) and system connectivity (Lintermans 2007; Laurance et al. 2011) (Tonkin et al. 2008). Broadscale environmental changes are also likely to have affected the resilience of the MDB metapopulation, leading to a lack of replenishment to the middle Murray from upstream refuge areas.

Table 4 Divergence times, effective population sizes and migration rates estimated with IMa2 between selected sites in the Murray–Darling Basin

Population 1	Population 2	T	N0	N1	N2	M0 > M1	M1 > M0
9	26	125 (16.5–?)	96 (18–290)	164 (29–422)	1185 (0–23,745)	0.3 (0–1.0)	0.5 (0–1.6)
9	15	44 (4.5–?)	69 (14–251)	337 (71–659)	1185 (105–23,805)	0.3 (0–1.1)	1.2 (0–3.5)

Values in brackets are 95 % Highest Posterior Density Intervals. *T* divergence time between populations (in years), *N0* effective size of Population 1, *N1* effective size of Population 2, *N2* effective size of the populations' common ancestor, *M0 > M1* number of individuals received by Population 2 per generation, *M1 > M0* number of migrants received by Population 1 per generation

Conservation actions and the maintenance of evolutionary processes in a recently fragmented species

The maintenance of ecological diversity and evolutionary processes represents a challenge for conservation managers (Driscoll 1998; Moritz et al. 2002). Management strategies should capture high genetic diversity at the deme level to lower the risk of local extinction through reduced individual fitness and reduced evolutionary potential (Spielman et al. 2004). They should also consider metapopulation connectivity to allow in situ selection across landscapes (including gene flow across environmental gradients) and improve reproductive fitness of local populations due to effective selection of high fitness genotypes (Sgrò et al. 2010). In this section we use the inferred genetic architecture of *N. australis* in the MDB as a case study to inform conservation actions aimed at maintaining ecological diversity and evolutionary processes in recently fragmented freshwater fishes.

Our basin-wide study identified 15 MUs in five regional clusters, with each MU largely associated with a particular catchment. There was no evidence for contemporary connectivity between catchments, a reflection that extant populations of *N. australis* may only be connected by rare flood events. A similar result has been found in smaller-scale, catchment-wide studies of pygmy perches from southeastern Australia (Cook et al. 2007; Brauer et al. 2013). We also discovered low genetic diversity for *N. australis*, both at the deme level and across its entire range in the MDB (Tables 2, S3). The average microsatellite allelic richness ($A_R = 2.80$) was strikingly low compared to values for other freshwater fishes (average $A_R = 9.10$; DeWoody and Avise 2000). The presence of numerous positive F_{IS} values (Table 2) suggests that inbreeding has impacted many subpopulations of *N. australis*, but gives no indication of how long this impact has been operating. The phylogeographic and coalescent-based analyses suggest that *N. australis* might have persisted as a widespread metapopulation in the MDB until recently. This is consistent with the lack of signal of genetic bottlenecks (Table S4), which suggests long-term persistence of relatively small populations—a hypothesis proposed for two other threatened fishes from the MDB and coastal catchments of southeastern Australia (Faulks et al. 2011; Brauer et al. 2013). In spite of recent demographic reductions and local extinctions that took place due to habitat loss and other factors, it is conceivable that extant *N. australis* populations are naturally small and have low levels of genetic variation, likely as a result of naturally-occurring environmental fluctuations (Puckridge et al. 1998).

The emerging scenario is that the MDB lineage is composed of small and isolated demes that are presently at high extinction risk. Indeed, during sampling for this study we observed at least 10 *N. australis* sites that are now extirpated (e.g. pops 3–5, 18, 23–25, 29, 33, 34, 36, 37, 39; Table 1). We propose the following set of short, medium and long term management actions to avoid further *N. australis* extirpations in the MDB and to promote population resilience: (i) in situ habitat maintenance and restoration at the level of catchments (Table 1); (ii) ex situ genetically-informed captive breeding of selected upland and lowland MUs; and (iii) population translocations between MUs. An example of the first set of management actions has been implemented as part of an urgent plan for conservation of five threatened freshwater fishes in the lower MDB (including *N. australis*) and are detailed in Hammer et al. (2013). They include small and large scale ground works, such as habitat modification, environmental water allocation and enhancement of water quality. These actions enabled critical threshold environmental conditions to be maintained across several sites, especially core refuge habitat during drought periods. The second set of actions is particularly important when in situ species conservation is not possible and extinction is imminent (e.g. Philippart 1995). The establishment of captive populations using animals rescued from regionally distinct demes, or ecotypes, enables reservoirs of genetic diversity for short-term population augmentation, reintroduction and translocation. We have demonstrated elsewhere (Attard et al. 2016) the feasibility of a genetically-informed captive breeding program that helped to prevent regional extinction in *N. australis*. That program used 65 *N. australis* rescued from the lower MDB (MU 5 in this study) during the peak of Australia's recent decade-long 'Millennium Drought'. It produced $\sim$ 1500 F_1 offspring while minimizing their inbreeding levels and maximizing genetic diversity. Successful maintenance of genetic diversity in captivity—despite highly skewed brooder contribution to offspring—was achieved through avoiding the use of inbred brooders and carefully breeding unrelated dams and sires in 11 separate families (Attard et al. 2016). Around 1000 F_1s representing equal numbers from each family were reintroduced into restored sites in the lower MDB in 2011 and 2012 (Hammer et al. 2013). Monitoring and recapturing efforts in 2013 and 2014 demonstrated F_1 survival in the lower MDB, as well as wild spawning of captive born fish (Attard et al. 2016). This program exemplifies the concept of 'genetic capture' (sensu Weeks et al. 2011), which implements captive breeding using a relatively small number of wild caught individuals to rapidly increase effective population size and to retain >90 % of the standing genetic variation within the source population. We propose the establishment of separate, genetically-informed captive breeding programs for a minimum of one lowland and one upland MU, with additional MUs being

included in the breeding program if found to be at high risk of extinction.

Interestingly, it is the finding of recent disruption of gene flow across the basin that enabled us to better risk-assess the configuration and spatial scale of translocations for *N. australis*, which comprise our third set of management recommendations. We propose that the conservative and often limiting option of using a local source for reintroductions and augmentations (i.e. 'local is best') should not be the preferred option in recently fragmented threatened species. An emphasis on local provenance might hamper conservation efforts because (i) such populations are not expected to harbour sufficient genetic variation, (ii) it risks using inbred individuals, and (iii) it ignores the possibility that anthropogenic influence might rapidly change the local environment after population establishment (Sgrò et al. 2011), a highly feasible scenario for riverine ecosystems (Palmer et al. 2008).

Notwithstanding any ecological risks that might be identified (e.g. disease transfer), we instead propose sourcing individuals for translocations from different MUs in the MDB, targeting demes with relatively large effective sizes and, ideally connected by gene flow to other similarly large demes (see Weeks et al. 2011 for details including numerical examples). These translocations should use several individuals per generation until local effective population size is increased to a minimum threshold, such as >1000 individuals (Willi et al. 2006). This proactive strategy promotes adaptive potential for evolutionary change and the long-term persistence of translocated populations (Sgrò et al. 2011). Under this scenario, the risks of reduced fitness due to outbreeding depression are predicted to be very low because *N. australis* is represented by a single evolutionary lineage that was recently connected by gene flow across the MDB.

Our integrated approach combining population genetics and phylogeographic analyses clarifies temporal and spatial aspects of population connectivity and diversity, and therefore improves our interpretation of how natural and anthropogenic fragmentation have impacted biodiversity. It also guides the implementation of powerful management tools that target genetic issues (i.e. captive breeding, population augmentation, reintroduction and translocation) predicted to play a prominent role in conservation programs under scenarios of rapid environmental change. Conservation policies and practices rarely consider how and if wild populations are able to evolve within the new conditions faced in anthropogenically-transformed landscapes (Lankau et al. 2011). This issue is particularly critical for freshwater biotas because river systems are ranked amongst the most heavily modified ecosystems in the world (Palmer et al. 2008). Forthcoming mechanistic studies that combine information about phylogeographic divergence and adaptive genetic variation at a landscape level with experimental studies that investigate genetic signatures associated with fitness traits in small populations are expected to further improve our ability to manage adaptive resilience in highly fragmented species.

Acknowledgments We thank the many people who helped with fieldwork, and are grateful to Minami Sasaki and Jerald Johnson for laboratory assistance. Collections were obtained under permits from various state fisheries agencies and research is under Flinders University Animal Welfare Committee approval E313. Financial support was provided by the Australian Research Council (LP100200409 to LB Beheregaray, J Harris and M Adams; and FT130101068 to LB Beheregaray) and by an AJ & IM Naylon Honours scholarship (to TL Cole).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Attard C, Moller L, Sasaki M, Hammer MP, Bice C, Brauer C, Carvalho D, Harris J, Beheregaray LB (2016) A novel holistic framework for genetic-based reintroduction programs. Conserv Biol. doi:10.1111/cobi.12699

Brauer CJ, Unmack PJ, Hammer MP, Adams M, Beheregaray LB (2013) Catchment-scale conservation units identified for the threatened Yarra pygmy perch (*Nannoperca obscura*) in highly modified river systems. PLoS One 8:e82953

Carvalho DC, Hammer MP, Beheregaray LB (2012) Isolation and PCR-multiplex genotyping of 18 novel microsatellite markers for the threatened southern pygmy perch (*Nannoperca australis*). Conserv Genet Resour 4:15–17

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9:1657–1659

Cook BD, Bunn SE, Hughes JM (2007) Molecular genetic and stable isotope signatures reveal complementary patterns of population connectivity in the regionally vulnerable southern pygmy perch (*Nannoperca australis*). Biol Conserv 138:60–72

Coombs JA, Letcher BH, Nislow KH (2008) CREATE: a software to create input files from diploid genotypic data for 52 genetic software programs. Mol Ecol Resour 8:578–580

Corander J, Sirén J, Arjas E (2008) Bayesian spatial modeling of genetic population structure. Comput Stat 23:111–129

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014

Cornuet JM, Pudlo P, Veyssier J, Dehne-Garcia A et al (2014) DIYABC v2.0: a software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism, DNA se quence and microsatellite data. Bioinformatics 30:1187–1189

Dawkins KL, Furse JM, Wild CH, Hughes JM (2010) Distribution and population genetics of the threatened freshwater crayfish genus *Tenuibranchiurus* (Decapoda: Parastacidae). Mar Freshw Res 61:1048–1055

DeWoody JA, Avise JC (2000) Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. J Fish Biol 56:461–473

Driscoll DA (1998) Genetic structure, metapopulation processes and evolution influence the conservation strategies for two endangered frog species. Biol Conserv 83:43–54

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491

Fagan WF (2002) Connectivity, fragmentation, and extinction risk in dendritic metapopulations. Ecology 83:3243–3249

Fagan WF, Aumann C, Kennedy CM, Unmack PJ (2005) Rarity, fragmentation, and the scale dependence of extinction risk in desert fishes. Ecology 86:34–41

Faulks LK, Gilligan DM, Beheregaray LB (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*). Mol Ecol 19:4723–4737

Faulks LK, Gilligan DM, Beheregaray LB (2011) The role of anthropogenic vs. natural in-stream structures in determining connectivity and genetic diversity in an endangered freshwater fish, Macquarie perch (Macquaria australasica). Evol Appl 4:589–601

Fischer J, Lindenmayer DB (2007) Landscape modification and habitat fragmentation: a synthesis. Glob Ecol Biogeogr 16:265–280

Garza JC, Williamson EG (2001) Detection of reduction in population size using data from microsatellite loci. Mol Ecol 10:305–318

Geist J (2011) Integrative freshwater ecology and biodiversity conservation. Ecol Ind 11:1507–1516

Hammer MP, Wedderburn S, Van Weenen J (2009) Action Plan for South Australian Freshwater Fishes. Department for Environment and Heritage, Government of South Australia. Native Fish Australia (SA) Inc., Australia

Hammer MP, Bice CM, Hall A, Frears A, Watt A, Whiterod NS, Beheregaray LB, Harris JO, Zampatti BP (2013) Freshwater fish conservation in the face of critical water shortages in the southern Murray–Darling Basin, Australia. Mar Freshw Res 64:807–821

Hanski I, Gaggiotti O (2004) Ecology, genetics, and evolution of metapopulations. In: Hanski I, Gaggiotti O (eds) Elsevier. Academic Press, Amsterdam, p 696

Hardy OJ, Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol Ecol Notes 2:618–620

Hey J (2010) Isolation with migration models for more than two populations. Mol Biol Evol 27:905–920

Hill JK, Hughes CL, Dytham C, Searle JB (2006) Genetic diversity in butterflies: interactive effects of habitat fragmentation and climate-driven range expansion. Biol Lett 2:152–154

Huey JA, Baker AM, Hughes JM (2010) High levels of genetic structure in the Australian freshwater fish, *Ambassis macleayi*. J N Am Benthol Soc 29:1148–1160

Hughes JM (2007) Constraints on recovery: using molecular methods to study connectivity of aquatic biota in rivers and streams. Freshw Biol 52:616–631

James KR, Cant B, Ryan T (2003) Responses of freshwater biota to rising salinity levels and implications for saline water management: a review. Aust J Bot 51:703–713

Kang B, He D, Perrett L, Wang H, Hu W, Deng W, Wu Y (2009) Fish and fisheries in the Upper Mekong: current assessment of the fish community, threats and conservation. Rev Fish Biol Fish 19:465–480

Lande R (1988) Genetics and demography in biological conservation. Science 241:1455–1460

Lankau R, Jørgensen PS, Harris DJ, Sih A (2011) Incorporating evolutionary principles into environmental management and policy. Evol Appl 4:315–325

Laurance WF, Dell B, Turton SM, Lawes MJ, Hutley LB, McCallum H, Dale P, Bird M, Hardy G, Prideaux G, Gawne B, McMahon CR, Yu R, Hero J, Schwarzkopf L, Krockenberger A, Douglas M, Silvester E, Mahony M, Vella K, Saikia U, Wahren C, Xu Z, Smith B, Cocklin C (2011) The 10 Australian ecosystems most vulnerable to tipping points. Biol Conserv 144:1472–1480

Leblanc M, Tweed S, Van Dijk A, Timbal B (2012) A review of historic and future hydrological changes in the Murray–Darling Basin. Global Planet Chang 80:226–246

Lintermans M (2007) Fishes of the Murray–Darling Basin: An introductory guide. Murray–Darling Basin Authority, Canberra

Llewellyn LC (1974) Spawning, development and distribution of the southern pigmy perch *Nannoperca australis australis* Gunther from inland waters in eastern Australia. Mar Freshw Res 25:121–149

Marko PB, Hart MW (2011) The complex analytical landscape of gene flow inference. Trends Ecol Evol 26:448–456

Meirmans PG (2012) The trouble with isolation by distance. Mol Ecol 21:2839–2846

Meirmans PG, Van Tienderen PH (2004) GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. Mol Ecol Notes 4:792–794

Moritz C, McGuigan K, Bernatchez L (2002) Conservation of freshwater fishes: integrating evolution and genetics with ecology. In: Collares-Pereira MJ, Coelho M (eds) Freshwater fish conservation. Options for the future. Fishing News Books, Blackwell Science, Oxford, pp 293–310

Nielsen R, Wakeley J (2001) Distinguishing migration from isolation: a Markov chain Monte Carlo approach. Genetics 158:885–896

Palmer MA, Reidy Liermann CA, Nilsson C, Flörke M, Alcamo J, Lake PS, Bond N (2008) Climate change and the world's river basins: anticipating management options. Front Ecol Environ 6:81–89

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28:2537–2539

Philippart JC (1995) Is captive breeding an effective solution for the preservation of endemic species? Biol Conserv 72:281–295

Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L, Estoup A (2004) GENECLASS2: a software for genetic assignment and first-generation migrant detection. J Hered 95:536–539

Poff NL, Allan JD, Bain MB, Karr JR, Prestegaard KL, Richter BD, Sparks RE, Stromberg JC (1997) The natural flow regime. Bioscience 47:769–784

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Pritchard VL, Metcalf JL, Jones K, Martin AP, Cowley DE (2009) Population structure and genetic management of Rio Grande cutthroat trout (*Oncorhynchus clarkii virginalis*). Conserv Genet 10:1209–1221

Puckridge JT, Sheldon F, Walker KF, Boulton AJ (1998) Flow variability and the ecology of large rivers. Mar Freshw Res 49:55–72

Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. Proc Natl Acad Sci 94:9197–9201

Sgrò CM, Lowe AJ, Hoffmann AA (2011) Building evolutionary resilience for conserving biodiversity under climate change. Evol Appl 4:326–337

Spielman D, Brook BW, Frankham R (2004) Most species are not driven to extinction before genetic factors impact them. Proc Natl Acad Sci 101:15261–15264

Teske PR, Sandoval-Castillo J, Waters JM, Beheregaray LB (2014) Can novel genetic analyses help to identify low-dispersal marine invasive species? Ecol Evol 4:2848–2866

Tonkin Z, King AJ, Mahoney J (2008) Effects of flooding on recruitment and dispersal of the Southern Pygmy Perch (*Nannoperca australis*) at a Murray River floodplain wetland. Ecol Manag Restor 9:196–201

Unmack PJ, Hammer MP, Adams M, Johnson JB, Dowling TE (2013) The role of continental shelf width in determining freshwater phylogeographic patterns in south-eastern Australian pygmy perches (Teleostei: Percichthyidae). Mol Ecol 22:1683–1699

Van Oosterhout C, Hutchinson WF, Wills DP, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Walker KF, Thoms MC (1993) Environmental effects of flow regulation on the River Murray, South Australia. Regul Rivers 8:103–119

Wedderburn SD, Hammer MP, Bice CM (2012) Shifts in small-bodied fish assemblages resulting from drought-induced water level recession in terminating lakes of the Murray–Darling Basin, Australia. Hydrobiologia 691:35–46

Weeks AR, Sgró CM, Young AG, Frankham R, Mitchell NJ, Miller KA, Byrne M, Coates DJ, Eldridge MDB, Sunnucks P, Breed MF, James EA, Hoffmann AA (2011) Assessing the benefits and risks of translocations in changing environments: a genetic perspective. Evol Appl 4:709–725

Willi Y, van Buskirk J, Hoffmann AA (2006) Limits to the adaptive potential of small populations. Annu Rev Ecol Evol Syst 37:433–478

Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163:1177–1191

Wright S (1942) Isolation by Distance. Genetics 28:114–138

Yue GH, David L, Orban L (2007) Mutation rate and pattern of microsatellites in common carp (*Cyprinus carpio L.*). Genetica 129:329–331