Recent and rapid anthropogenic habitat fragmentation increases extinction risk for freshwater biodiversity

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Abstract

Anthropogenic habitat fragmentation is often implicated as driving the current global extinction crisis, particularly in freshwater ecosystems. The genetic signal of recent population isolation can however be confounded by the complex spatial arrangement of dendritic river systems. Consequently, many populations may presently be managed separately based on an incorrect assumption that they have evolved in isolation. Integrating landscape genomics data with models of connectivity that account for landscape structure, we show that the cumulative effects of multiple in-stream barriers have contributed to the recent decline of a freshwater fish from the Murray-Darling Basin, Australia. In addition, individual-based eco-evolutionary simulations further demonstrate that contemporary inferences about population isolation are consistent with the 160-year time frame since construction of in-stream barriers began in the region. Our findings suggest that the impact of very recent fragmentation may be often underestimated for freshwater biodiversity. We argue that proactive conservation measures to reconnect many riverine populations are urgently needed.

Introduction

We are now confronted by the sixth global mass extinction with the current rate of species losses far exceeding pre-anthropogenic background estimates (Barnosky *et al.* 2011). This crisis is particularly severe in freshwater ecosystems, which have shown declines of biodiversity greater than for either terrestrial or marine ecosystems (Darwall *et al.* 2018). Habitat loss and fragmentation are key factors leading to the genetic and demographic decline of populations that together threaten species persistence (Fischer & Lindenmayer 2007). Over the last century, close to one million large dams and many millions of smaller in-stream barriers have been constructed globally (Jackson *et al.* 2001; Liermann *et al.* 2012). These barriers have had devastating ecological consequences by preventing or restricting connectivity among populations, leading to higher rates of genetic drift and inbreeding. This, in turn, can lead to lower fitness due to inbreeding depression and reduced evolutionary potential due to loss of genetic diversity (Frankham 2005; Keyghobadi 2007). Additionally, small populations become more vulnerable to extirpation due to stochastic demographic events (Lande 1993) and, when this occurs on a regional scale, species extinctions are the inevitable result (Hanski 1998).

Landscape genetics provides a way to identify how human activities threaten the persistence of wild populations (Manel & Holderegger 2013). The time lag between environmental change and any detectable genetic signal resulting from this change can however make it very difficult to disentangle the effects of historical from contemporary processes (Landguth *et al.* 2010). This is particularly the case for naturally structured populations such as those found in dendritic river networks (Coleman *et al.* 2018). The progression from landscape genetics to landscape genomics has increased both the spatial and temporal resolutions at which evolutionary processes can be examined, offering a more powerful framework with which to quantify the effects of very recent disturbance on populations (Allendorf *et al.* 2010; Grummer *et al.* 2019). Previous landscape genetics studies investigating the impact of in-stream barriers have often focused on larger, migratory species or assessed only one, or a

few large barriers (Faulks *et al.* 2011; Gouskov *et al.* 2016; Torterotot *et al.* 2014). Small-bodied, but ecologically important species often receive relatively little attention from conservation managers (Olden *et al.* 2007; Saddlier *et al.* 2013) and efforts to improve fish passage and connectivity are often ineffective for these fishes (Harris *et al.* 2017). The cumulative impact of many smaller in-stream barriers (e.g. weirs, farm dams and road crossings) for small-bodied and non-migratory fishes at a basin-wide scale has been the subject of less research (Coleman *et al.* 2018; Diebel *et al.* 2015).

In this landscape genomics study, we examine the effects of recent habitat fragmentation for the southern pygmy perch (*Nannoperca australis*), a threatened small-bodied fish (<80mm) that recently experienced major demographic declines and local extinctions across the Murray-Darling Basin (MDB), Australia (Brauer *et al.* 2016; Cole *et al.* 2016; Hammer *et al.* 2013). This species is typical of many native small-bodied fishes in the region and offers a conservative model for guiding broader conservation strategies, as the impacts of fragmentation are likely to be more pronounced for larger, migratory species. The MDB has very few natural in-stream barriers, but it has been heavily modified with more than 10,000 dams, weirs, road crossings, levees and barrages constructed since the late 1850s when European settlement of this region began (Baumgartner *et al.* 2014). As such, the MDB provides a unique opportunity to examine the consequences of recent habitat fragmentation without the confounding influence of prolonged human disturbance over hundreds of years as is common to many northern hemisphere river basins (e.g. Hansen *et al.* 2014). Environmental factors, including human disturbance are known to influence genetic diversity for *N. australis* (Brauer *et al.* 2016; Cole *et al.* 2016), however little is known about the specific role that widespread habitat fragmentation has played in the species recent and rapid decline. We hypothesize that, after accounting for historical patterns of genetic structure, genetic differentiation among demes should increase with the number of in-stream barriers separating them. We also predict that populations most isolated by fragmentation would exhibit reduced effective population size (N_e) and lower levels of genetic diversity.

Additionally, we used forward genetic simulations to investigate whether high contemporary levels of genetic differentiation could have arisen in the relatively short time since the construction of in-stream barriers began in the MDB. Our results demonstrate that recent anthropogenic habitat fragmentation has contributed to the loss of genetic diversity and population isolation observed. They also suggest that proactive conservation measures to restore connectivity (e.g. environmental flows, habitat restoration) and increase evolutionary potential (e.g. genetic rescue) are urgently required for this, and potentially many other poorly dispersing aquatic species.

Methods

Sampling, ddRAD genotyping and SNP filtering

A total of 263 individuals were sampled from 25 locations, encompassing 13 catchments across the entire current MDB distribution of *N. australis* (Figure 1; Table 1). Fish were ethically euthanized using clove oil, frozen in liquid nitrogen in the field, and stored at -70°C in the Australian Biological Tissues Collection at the South Australian Museum, Adelaide.

DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit according to the manufacturers protocol. DNA integrity and purity were assessed using gel electrophoresis and a NanoDrop 1000 spectrophotometer (Thermo Scientific), respectively. Sequencing libraries were prepared in-house based on a double-digest restriction-site associated DNA (ddRAD) library protocol (Peterson *et al.* 2012). Samples were multiplexed with 48 samples per Illumina HiSeq2000 lane and sequenced as paired-end, 100-bp reads. Raw sequences were demultiplexed using the *process_radtags* module of *Stacks* v.1.04 (Catchen *et al.* 2011) before using dDocent v.1.2 (Puritz *et al.* 2014) for *de novo* reference catalogue assembly and genotyping. The data was then filtered to retain only variants present in at least 70% of individuals and in 70% of populations, retaining only one biallelic SNP per locus with a minimum minor allele frequency of 0.05.

Population structure and other demographic parameters such as effective population size should be assessed using neutral loci (Allendorf *et al.* 2010; Luikart *et al.* 2003). To define a putatively neutral dataset, F_{ST} outlier loci were detected using a Bayesian approach with BayeScan v.2.1 (Foll & Gaggiotti 2008), and the coalescent-based FDIST method (Beaumont & Nichols 1996) in Arlequin v.3.5 (Excoffier & Lischer 2010). BayeScan was run for 100,000 iterations using prior odds of 10,000. Loci different from zero with a q-value <0.1 were considered outliers. Arlequin was run specifying the hierarchical island model with 50,000 simulations of 100 demes for each of 13 populations (based on the 13 separate catchments sampled). Loci outside the neutral distribution at a false discovery rate (FDR) of 10% were considered outliers. Loci detected as outliers by either BayeScan or Arlequin were filtered. The remaining SNPs were examined for departure from expectations of Hardy-Weinberg equilibrium (HWE) using GenoDive 2.0b27 (Meirmans & Van Tienderen 2004). Finally, loci out of HWE at a FDR of 10% in more than 50% of populations were removed. Detailed information concerning library preparation and bioinformatics are described in Appendix S1.

Population structure

Pairwise F_{ST} (Weir & Cockerham 1984) was estimated among sampling sites using GenoDive (Meirmans & Van Tienderen 2004) with significance assessed using 10,000 permutations. Bayesian clustering analysis of individual genotypes was then performed using fastStructure (Raj *et al.* 2014). Ten independent runs for each value of *K* (1-25) were completed to ensure consistency and the most likely *K* was assessed by comparing the model complexity that maximised marginal likelihood across replicate runs.

Anthropogenic isolation of populations

If anthropogenic habitat fragmentation has affected population connectivity and dispersal, we should expect genetic differentiation to increase in response to the number of in-stream

barriers separating populations. To determine if local characteristics of the stream network (i.e. in-stream barriers and other local scale landscape heterogeneity) better explain population differentiation than isolation by distance (IBD), we used the StreamTree model of Kalinowski et al. (2008). Genetic distances among populations were modelled as the sum of all pairwise genetic distances that mapped to each section of the stream network. This provides a distance measure that is independent of the length of each stream section and identifies the reaches that contribute most to restricting gene flow (e.g. due to dendritic structure, in-stream barriers or other local landscape effects). Model fit was assessed by plotting the StreamTree fitted distance against observed F_{ST} and calculating the regression coefficient of determination (R^2). This model was then compared with a model of IBD calculated using multiple matrix regression with randomisation (MMRR) following the method of Wang (2013). Pairwise population distances along the river network were calculated with ArcMap v.10.2 (ESRI 2012). Model significance for the MMRR was assessed using 10,000 random permutations.

In dendritic river systems, hierarchical network structure and spatial hydroclimatic variation can also drive patterns of genetic diversity of stream-dwelling organisms (Fourcade *et al.* 2013; Hughes *et al.* 2009; Morrissey & de Kerckhove 2009; Thomaz *et al.* 2016). To evaluate the relative contributions of anthropogenic habitat fragmentation, natural stream hierarchy and environmental variation we again used MMRR. In addition to IBD, we used distance matrices calculated for the number of in-stream barriers, catchment membership, and a range of environmental variables. The number of in-stream barriers separating sites was determined using spatial data from the Murray-Darling Basin Weir Information System (Murray–Darling Basin Authority 2013). To account for the effect of dendritic stream hierarchy, a binary model matrix describing catchment membership was constructed such that pairwise comparisons of sites from within the same catchment were assigned a value of zero, and comparisons among catchments were scored as one. Finally, a subset of 40 hydroclimatic variables were obtained from the Australian hydrological geospatial fabric

(Geoscience Australia 2011; Stein *et al.* 2014). These were assigned to one of five categories describing variation in temperature, precipitation, flow regime, human disturbance and topography. Variance inflation factor (VIF) analysis was then used to exclude highly correlated variables using a VIF threshold of 10 (Dyer *et al.* 2010). The remaining variables were reduced to principal components (PCs) using the dudi.pca function in the ADE4 R package (Dray *et al.* 2016) and Euclidean distance matrices were constructed based on the PCs with eigenvalues >1 (Yeomans & Golder 1982) retained for each category. All distance matrices were z-transformed to facilitate direct comparison of partial regression coefficients (Schielzeth 2010). Each variable was initially tested in an independent univariate MMRR before significant factors were combined in a multivariate MMRR model with 10,000 random permutations used to assess significance.

Habitat fragmentation, genetic diversity and population size

To test the hypothesis that the most isolated populations exhibit reduced genetic diversity we examined the relationship between population-specific F_{ST} and expected heterozygosity (H_E). Population-specific F_{ST} was estimated for each sampling site using the method of Weir and Hill (2002) and H_E was calculated using Genodive.

Effective population size was estimated using the linkage disequilibrium (LD) estimator implemented in NeEstimator 2.01 (Do *et al.* 2014). This method is based on the assumption that LD at independently segregating loci in a finite population is a function of genetic drift, and performs particularly well with a large number of loci and where population sizes are expected to be small (Waples & Do 2010). In the absence of significant F_{ST} (Table S1) Lower Murray sites MID and MUN were considered one population, and these samples were combined for the N_e estimates. NeEstimator was run assuming random mating and using a P_{crit} value of 0.075 following guidelines for small sample sizes suggested by Waples and Do (2010).

198 *Eco-evolutionary simulations*

199 Simulation studies are becoming an increasingly important part of landscape genomics as a
200 wide range of parameters can be explored for key evolutionary processes such as gene
201 flow, genetic drift, mutation and selection (Hoban *et al.* 2012). In this case, we used
202 simulations to examine whether levels of contemporary population isolation are consistent
203 with having evolved during the time since barrier construction began in the MDB. We
204 simulated three metapopulation sizes (N_e=1000, N_e=500 and N_e=100) using SLiM 3.1 (Haller
205 & Messer 2018). Each simulation was based on a 1D stepping stone population model
206 assuming equal N_e for each sub-population while maintaining a constant metapopulation
207 size to simulate a concurrent increase in the number of barriers, and reduction in habitat
208 patch size. Each simulation consisted of four 100Kb genomic elements and assumed a
209 constant mutation rate of 10^{-7} and recombination rate of 10^{-8}. Each simulation was first run
210 for a burn-in phase of 20,000 generations with a migration rate of 0.5 between adjacent sub-
211 populations to generate diversity and allow the system to reach migration–drift equilibrium
212 with F_{ST}=~0. Although this almost certainly underestimates historical population structure
213 before anthropogenic disturbance, this figure provides a conservative approach by
214 maximizing the number of generations required to evolve current levels of differentiation.
215 Following the burn-in, the construction of barriers was simulated by setting the migration rate
216 among demes to zero for 300 generations. Nine models with an increasing number of
217 demes (2-10) were simulated for each metapopulation size to examine the effect of
218 increasing levels of fragmentation (Figure S1-S3), and 100 replicate runs of each scenario
219 were completed. The --weir-fst-pop command of VCFtools (Danecek *et al.* 2011) was used
220 to calculate F_{ST} for each replicate. To estimate the time required to reach current levels of
221 observed population differentiation, assuming a generation time of one year (Humphries
222 1995), the number of generations (mean of the 100 replicates) needed to achieve F_{ST}=0.2
223 (mean contemporary F_{ST} within upper Murray catchments=0.196; Table S1) was plotted
224 against the number of fragments for each scenario for the three metapopulation models.
225 Scripts used to perform the simulations and analyses are available on Dryad: TBA.

Results

Sampling, ddRAD genotyping and SNP filtering

Following demultiplexing, 1,602,903,910 forward and reverse sequencing reads were recovered. A total of 2,589,251 variant sites were genotyped with dDocent, and after filtering 5,162 high quality SNPs were retained. We removed 873 unique F_{ST} outlier loci identified by BayeScan and Arlequin, along with a further 846 loci found to be outside HWE expectations in >50% of populations. This resulted in a final, putatively neutral dataset of 3,443 SNPs for the 263 individuals.

Population structure

High levels of population genetic structure were evident between most demes of *N. australis,* with pairwise comparisons of F_{ST} among sampling sites ranging from 0-0.79 (global F_{ST} =0.48). All pairwise F_{ST} estimates were significant (P<0.003) except between immediately adjacent lower MDB sites MID and MUN (F_{ST}= -0.002, P=0.66) (Table S1). Results from fastStructure indicated population boundaries are strongly correlated with natural riverine catchment structure, with K=13 identified as the most likely number of populations (Figure S4). This is consistent with a previous microsatellite study based on a larger sample (578 individuals; 45 localities) that inferred that, until the recent European settlement in the MDB, well-connected metapopulations of *N. australis* existed within its catchments (Cole *et al.* 2016).

Anthropogenic isolation of populations

The StreamTree model was used to identify parts of the stream network that contribute more to F_{ST} (e.g. restricted dispersal due to barriers or other local environmental conditions). Results indicated that local characteristics of the stream network better explain F_{ST} than the null hypothesis of IBD (i.e. the resistance to dispersal for any given stream section is determined by its length). Figure 1 provides a visual representation of the relationship

between StreamTree fitted distance and the density of artificial in-stream barriers, with stream sections colour coded according to F_{ST} as estimated by the model (yellow represents a modeled local F_{ST} range of 0-0.01, orange: 0.01-0.05 and red: 0.05-0.38) and the location of barriers marked with **X**. The StreamTree model was a good fit for the data and was significantly related to observed F_{ST} (R^2=0.947, β=0.986 [0.959- 1.012 95%CI], P<2X10^{-16}) (Figure 2a), whereas IBD was not significant (R^2=0.0139, β=0.108 [0.004- 0.212 95%CI], P=0.343) (Figure 2b). Although there was significant IBD within catchment groups (i.e. the first cluster in Figure 2b, R^2=0.730, β=0.0016 [0.001- 0.002 95%CI], P=6.54X10^{-8}), IBD was not significant in models across the whole basin, in contrast to models of stream hierarchy and barriers (see below). In addition, even when comparisons were limited to sites within-catchments, the number of barriers still provided a better model than IBD (R_2=0.81 vs. 0.73, respectively; Figure S5).

Following VIF analyses, 19 environmental variables from the five categories were retained. The first two PCs for temperature, flow and topographic variables scored eigenvalues >1 while only one component each for the precipitation and human disturbance PCAs scored an eigenvalue >1, so individual variables rather than PCs for these categories were retained. This resulted in a final list of six hydroclimatic PCs and five individual precipitation and disturbance variables (Table S2).

Assessment of the relative influence of anthropogenic habitat fragmentation, natural stream hierarchy and environmental heterogeneity indicated that population structure is driven by a combination of the effects of stream network hierarchy and the number of in-stream barriers. Univariate regressions revealed catchment membership (R^2=0.170, β=0.449 [0.336- 0.562 95%CI], P<0.0001) and the number of in-stream barriers separating sites (R^2=0.322, β=0.548 [0.458- 0.639 95%CI], P<0.0001) were both good predictors of population differentiation, while there was no evidence for isolation by environment (Table 2). Including

both significant predictors (catchment membership and number of barriers) in a multivariate model improved model fit with catchment membership, and the number of barriers each accounting for 61% and 39% of the explained variation, respectively (R^2=0.358, $\beta_{catchment}$=0.725 [0.374- 1.076 95%CI], $\beta_{barriers}$=0.462 [0.365- 0.560 95%CI], P<0.0001) (Figure 3; Table 2).

Habitat fragmentation, genetic diversity and population size

Genetic variation varied across the MDB with an average H_E of 0.161 (0.057-0.263). There was a sharp contrast between regions with average H_E of 0.253 for sites in the more connected Lower Murray wetlands, compared to 0.143 for sites in the highly fragmented upper reaches (Table 1). A strong negative relationship between population-specific F_{ST} and H_E was also evident (R^2=0.737, β=-2.05 [-2.58- -1.52 95%CI], P<1x10^{-7}) with the most isolated populations also harbouring the least genetic variation (Figure S6; Table 1). Effective population size estimates were generally low, averaging 194.75 for Lower Murray sites and 112.26 for sites in the upper reaches, with many of the latter <100 (Table 1).

Eco-evolutionary simulations

The simulations demonstrated that contemporary population differentiation among sites within catchments (mean within headwater catchments F_{ST}=0.196) could have evolved from a more connected system within the time since the construction of in-stream barriers began ~160 generations ago (Figure 4; Table S3; Appendix S3-S5). For metapopulations with an N_e of 1000, F_{ST} approached 0.2 in less than 160 generations with only three barriers fragmenting the population. Models assuming N_e=500 and N_e=100 indicated that substantially fewer generations following fragmentation were required to reach contemporary levels of F_{ST}. At N_e=500, F_{ST} =0.2 occurred after 124 generations with one barrier and after just 19 generations with nine barriers (Figure 4; Table S3). For smaller populations of

307 N_e=100 contemporary levels of differentiation evolved within 24 generations with just one
308 barrier (Figure 4; Table S3).
309
310 **Discussion**
311 Habitat fragmentation is a key process implicated in the current and unprecedented
312 worldwide loss of freshwater biodiversity (Fischer & Lindenmayer 2007). Determining the
313 contribution of recent human activities to the decline of riverine species is however
314 challenging, as the genetic signal of recent disturbance can be confounded by historical
315 patterns of dispersal shaped by hydrological network structure (Brauer *et al.* 2018; Coleman
316 *et al.* 2018; Landguth *et al.* 2010). For *N. australis*, populations most isolated by recent
317 habitat fragmentation also exhibited reduced genetic diversity and increased population
318 differentiation, and this signal remained strong after accounting for the historical effects of
319 dendritic stream hierarchy. Contemporary F_{ST} estimates were within the expected range
320 obtained by simulating the recent construction of in-stream barriers in the MDB, supporting
321 the hypothesis that anthropogenic habitat fragmentation has impacted populations since
322 European settlement of the region. Previous work based on coalescent analyses of
323 microsatellite DNA datasets has demonstrated that historical population sizes of *N. australis*
324 were much larger before European settlement (Attard *et al.* 2016), and that populations
325 across the MDB were also more connected until that time (Cole *et al.* 2016). These studies
326 support our findings and the hypothesis that the low genetic diversity, small N_e and high F_{ST}
327 observed for contemporary populations likely reflects the combined impact of both historical
328 and recent processes, rather than being due solely to natural demographic variability over
329 longer evolutionary time scales. In addition, several populations sampled for this study have
330 subsequently suffered local extirpation during prolonged drought, and the small size of most
331 remnant populations indicate they are at high risk of extinction.
332
333 Since European settlement, hydrology in the MDB has been increasingly modified due to
334 urbanisation and irrigation (Leblanc *et al.* 2012). These changes have included the

335 construction of thousands of barriers to fish passage across the basin (Baumgartner *et al.*
336 2014) and it is now considered one of Australia's most fragmented and degraded
337 ecosystems (Davies *et al.* 2010; Kingsford 2000). The focus of most barrier mitigation
338 actions in the MDB to date has been on restoring passage across larger dams along the
339 main river channel (Barrett & Mallen-Cooper 2006). Although some fishways have been
340 designed to facilitate movement of smaller fish, they have predominantly targeted large-
341 bodied, highly mobile species (Baumgartner *et al.* 2014). Furthermore, the spatial scale of
342 dispersal for many small-bodied MDB fishes often restricts their movements to headwater
343 streams and wetlands away from the main channel (Harris *et al.* 2017). Habitat loss and
344 fragmentation associated with the thousands of smaller barriers in headwater streams have
345 therefore likely contributed to the widespread decline of many smaller and more sedentary
346 MDB fishes, including *N. australis* (Brauer *et al.* 2018; Cole *et al.* 2016; Hammer *et al.* 2013;
347 Huey *et al.* 2017). It is perhaps surprising then, that there have been relatively few studies
348 explicitly testing the genetic effects of anthropogenic fragmentation on small-bodied fishes in
349 the MDB. One recent example in the neighbouring Yarra River catchment however
350 combined a large empirical dataset with spatially explicit simulations to examine the role of
351 artificial barriers in driving local-scale patterns of genetic variation for river blackfish
352 (*Gadopsis marmoratus*), a small and sedentary species also found in the MDB (Coleman *et*
353 *al.* 2018). Based on eight microsatellite loci, genetic diversity was found to be lower for
354 populations above barriers in small streams, with several isolated populations also exhibiting
355 signs of inbreeding. In addition, their simulations demonstrated that power to detect recent
356 impacts of barriers could be improved by increasing the number of loci used, highlighting the
357 benefit of modern genomic data for conservation genetics.
358
359 An unprecedented severe and prolonged drought between 1997 and 2010 caused
360 catastrophic loss of habitat and local extirpation for some *N. australis* populations,
361 particularly in the lower Murray (Hammer *et al.* 2013; Wedderburn *et al.* 2012). In response
362 an emergency conservation-breeding and restoration program was implemented in the lower

363 MDB (Attard *et al.* 2016; Hammer *et al.* 2013), and additional breeding and translocations
364 among several headwater populations have been initiated (P. Rose, *personal*
365 *communication*). As the impacts of climate change intensify, proactive conservation
366 management interventions such as those already underway for *N. australis*, will be
367 increasingly considered for other species inhabiting the MDB and fragmented freshwater
368 ecosystems elsewhere in the world. Indeed, a recent study incorporating physiological and
369 functional traits with species distribution models for 23 fish species predicted severe
370 declines in taxonomic and functional diversity of MDB fish communities in the coming
371 decades due to climate change (de Oliveira *et al.* 2019). Managing regulated river systems
372 to provide environmental flows, habitat restoration and other measures to re-establish
373 connectivity among habitat patches (e.g. installation of fishways) have the potential to
374 address some impacts and should continue to be priorities for conservation and water
375 management. Nonetheless, these long-term, landscape-scale measures are often
376 constrained by competing interests related to political and socio-economic issues (Davis *et*
377 *al.* 2015).
378
379 Additionally, many species may be already depleted to the point where improved
380 environmental conditions alone will not be sufficient to facilitate recovery. In this case,
381 genetic rescue offers a potential solution for a broad range of threatened taxa (Ralls *et al.*
382 2018; Whiteley *et al.* 2015). Despite strong evidence supporting the benefits of genetic
383 rescue for fragmented populations however, conservation managers are often reluctant to
384 adopt these measures (Frankham 2015). We suggest that the impacts of recent habitat
385 fragmentation may have been underappreciated for many species, and that estimates of
386 population structure solely attributed to historical evolutionary processes have potentially led
387 to management frameworks that actually reinforce fragmentation and isolation at the
388 expense of species-level genetic variation (sensu Coleman *et al.* 2013). There is also
389 increasing evidence that natural selection can influence the evolutionary trajectory of small
390 and fragmented populations (Brauer *et al.* 2017; Fraser 2017; Wood *et al.* 2016). Critically

391 for conservation, this indicates that adaptive divergence of small populations can occur
392 quickly following fragmentation (Brauer *et al.* 2017), and that even very recently isolated
393 populations may harbor novel adaptive diversity. It is therefore important to build
394 evolutionary resilience by facilitating genetic exchange among isolated populations to
395 restore natural evolutionary processes and maintain species-level genetic variation,
396 potentially valuable under a range of future selection regimes (Webster *et al.* 2017; Weeks
397 *et al.* 2016).
398
399 There is a global biodiversity crisis unfolding in freshwater ecosystems with aquatic
400 vertebrate populations declining by 80% over the last 50 years (Darwall *et al.* 2018).
401 Restoring functional connectivity for whole aquatic communities across entire river basins
402 via traditional mitigation approaches is simply not feasible within the time frame required to
403 enable many currently threatened species to persist. There is also now strong empirical
404 evidence that several long-established beliefs central to prevailing conservation practices
405 are overly cautious, and that the current local-is-best approach increases the prospect of
406 managing species to extinction (Frankham *et al.* 2017; Pavlova *et al.* 2017; Weeks *et al.*
407 2016). Given widespread fragmentation, habitat loss, and the ongoing global decline of
408 freshwater biodiversity, a rapid paradigm shift is needed to empower conservation
409 practitioners to take action before a threatened species demographic situation becomes
410 critical. There are risks associated with any proactive management intervention such as
411 translocation or genetic rescue. These risks however need to be weighed against the ever-
412 increasing risk of doing nothing.
413

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422

423 **Data Archiving Statement**

424 Reference sequences, SNP genotypes, sample coordinates and environmental data used in
425 analyses are available on Dryad: TBA

426

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627

628 Table 1. Sample size (N), expected heterozygosity (H_E), population-specific F_{ST} (Weir & Hill
629 2002) and effective population size estimates (N_e). Lowland wetland sites referred to as
630 Lower Murray in the text are indicated in bold. *MID and MUN samples combined for N_e
631 estimation.

Catchment	Site	N	H_E	F_{ST}	N_e (95% CI)
Tookayerta (TOO)	**TBA**	**7**	**0.227**	**0.059**	**∞**
Lower Lakes (LMR)	**ALE**	**10**	**0.263**	**0.066**	**198.6 (158.6–264.9)**
	MID	**7**	**0.262**	**0.092**	**190.9 (163.3–229.4)***
	MUN	**6**	**0.260**	**0.034**	
Angas (ANG)	MCM	9	0.097	0.555	76.3 (61.0–101.3)
Avoca (AVO)	MIC	11	0.114	0.409	13.7 (13.2–14.4)
Campaspe (CAM)	JHA	12	0.091	0.364	393.8 (184.0–∞)
Upper Goulburn (UGO)	MER	17	0.075	0.467	70.4 (61.4–82.2)
	TRA	10	0.075	0.433	50.7 (41.2–65.3)
	YEA	8	0.087	0.364	260.4 (111.1–∞)
Lower Goulburn (LGO)	PRA	9	0.243	0.179	114.9 (98.4–137.9)
	SEV	11	0.218	0.119	54.8 (50.8–59.4)
Broken (BRO)	BEN	10	0.236	0.159	117.2 (101.7–138.2)
	SAM	10	0.234	0.188	124.7 (108.0–147.2)
	LIM	18	0.118	0.337	99.1 (88.5–112.5)
Ovens (OVE)	KIN	16	0.104	0.297	69.9 (62.1–79.8)
	HAP	9	0.114	0.369	∞
	MEA	8	0.158	0.245	53.4 (45.7–64)
Kiewa (KIE)	GAP	12	0.168	0.305	122.5 (105.3–146.2)
Albury (ALB)	ALB	12	0.226	0.299	305.4 (241.8–413.4)
Mitta Mitta (MIT)	SPR	10	0.152	0.262	98.1 (80.5–125)
	GLE	10	0.143	0.408	51.1 (46.1–57.2)
	TAL	7	0.164	0.479	31.9 (29.1–35.2)
Upper Murray (COP)	COP	16	0.133	0.297	118.7 (102.2–141.1)
Lachlan (LAC)	LRT	8	0.057	0.672	18.1 (15.3–21.8)

632

633 Table 2. Results of multiple matrix regression with randomisation (MMRR) tests for the
634 relationship between pairwise genetic distance (F_{ST}) and geographic distance, catchment
635 membership, number of in-stream barriers and environmental distances. Pairwise
636 environmental distances between each site were calculated as Euclidean distance for each
637 environmental variable and principal component (PC) described in Brauer *et al*. (2016). *P*-
638 values <0.0001 are indicated in bold.

Model	Variable	Coefficient	95%CI	*P*-value	R^2	Model *P*-value
	Distance	0.108	0.004- 0.213	0.3340	0.014	
	Catchment	0.449	0.336- 0.562	**0.0001**	0.170	
	Barriers	0.548	0.458- 0.639	**0.0001**	0.322	
	TempPC1	-0.130	-0.233- -0.028	0.2465	0.021	
	TempPC2	0.180	0.077- 0.282	0.1443	0.039	
	CATCOLDQRAIN	0.098	-0.007- 0.202	0.3813	0.011	
	CATDRYQRAIN	-0.061	-0.170- 0.043	0.5515	0.004	
	STRWETQRAIN	-0.058	-0.162- 0.046	0.5496	0.004	
	FlowPC1	-0.053	-0.158- 0.051	0.6698	0.003	
	FlowPC2	-0.125	-0.227- -0.023	0.3520	0.019	
	CDI	0.037	-0.068- 0.142	0.6571	0.002	
	FRDI	-0.087	-0.190- 0.015	0.4603	0.009	
	TopoPC1	-0.121	-0.225- -0.017	0.2368	0.017	
	TopoPC2	0.021	-0.083- 0.125	0.8644	0.001	
Catchment+Barriers					0.358	**0.0001**
	Catchment	0.725	0.374- 1.076	**0.0045**		
	Barriers	0.462	0.365- 0.560	**0.0001**		

639

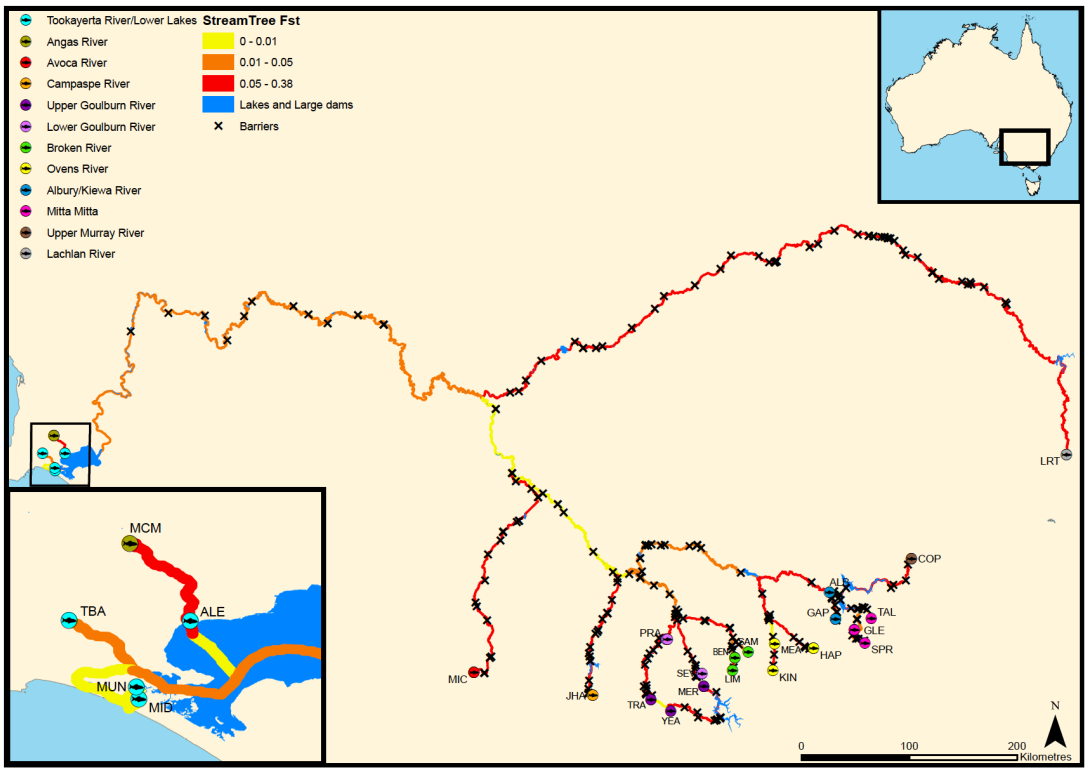

Figure 1. *Nannoperca australis* sampling locations in the Murray-Darling Basin (MDB). Stream sections are colour coded according to F_{ST} estimated using the *StreamTree* model (Kalinowski *et al.* 2008). Cross markers represent the location of artificial in-stream barriers.

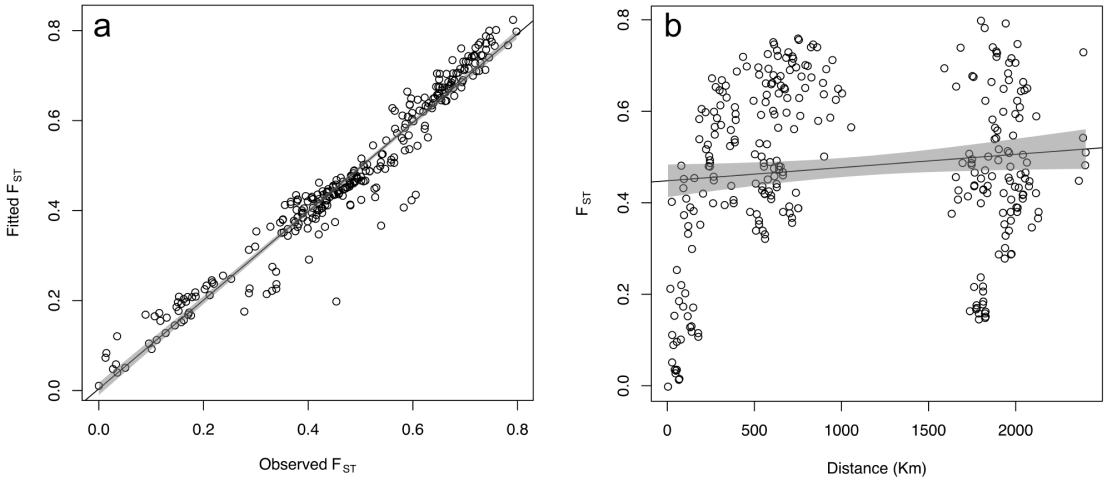

Figure 2. Plots of a) *StreamTree* analyses and b) isolation by distance (IBD) for *Nannoperca australis* in the MDB. The *StreamTree* plot compares fitted F_{ST} based on the *StreamTree* model with observed pairwise F_{ST} values (R^2=0.947, β=0.986 [0.959- 1.012 95%CI], $P<2X10^{-16}$). The IBD plot depicts the relationship between pairwise F_{ST} and riverine distance between sampling sites (R^2=0.0139, β=0.108 [0.004- 0.212 95%CI], P=0.343). Shaded area represents the 95% confidence interval.

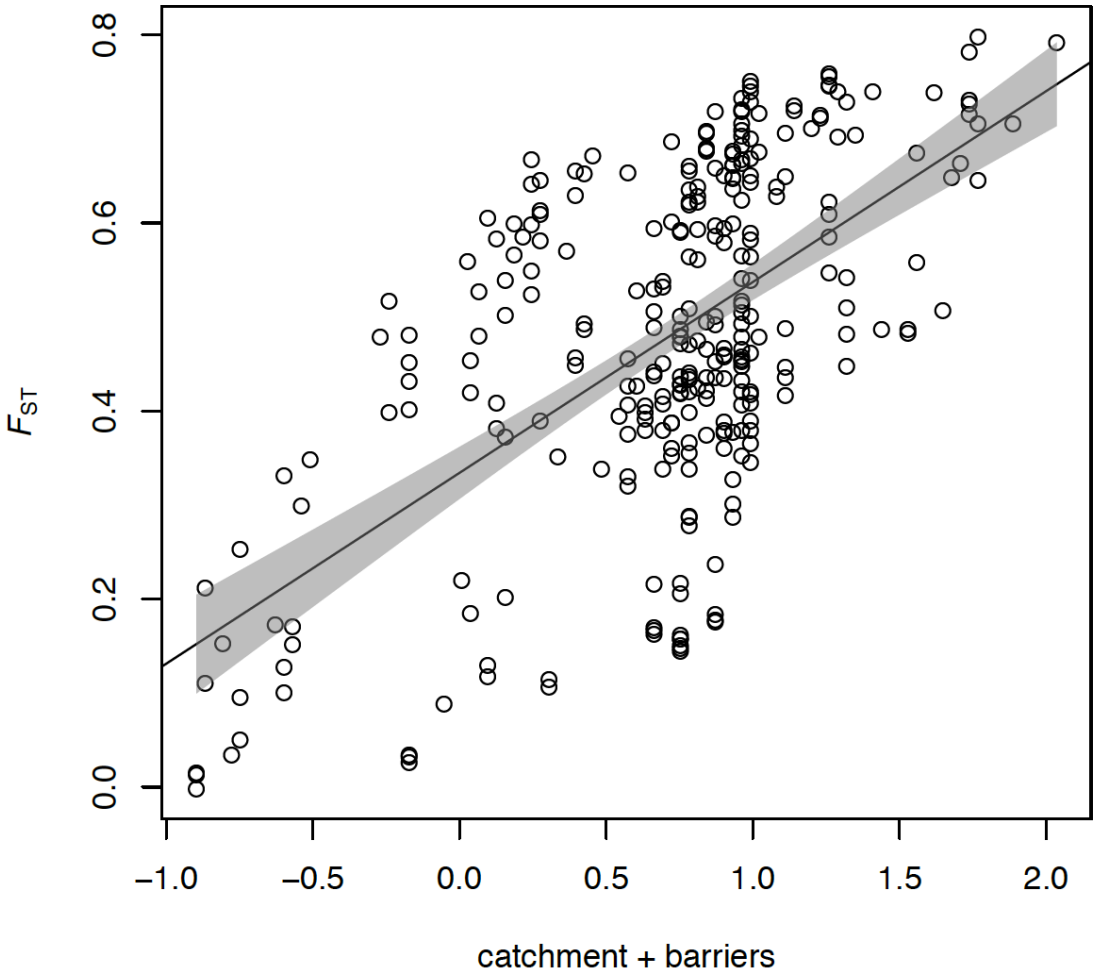

Figure 3. Multiple matrix regression with randomisation (MMRR) plot for the combined effects of natural stream hierarchy (model matrix of catchment membership) and number of barriers on F_{ST} (R^2=0.358, $\beta_{catchment}$=0.725 [0.374- 1.076 95%CI], $\beta_{barriers}$=0.462 [0.365- 0.560 95%CI], P<0.0001). Shaded area represents the 95% confidence interval.

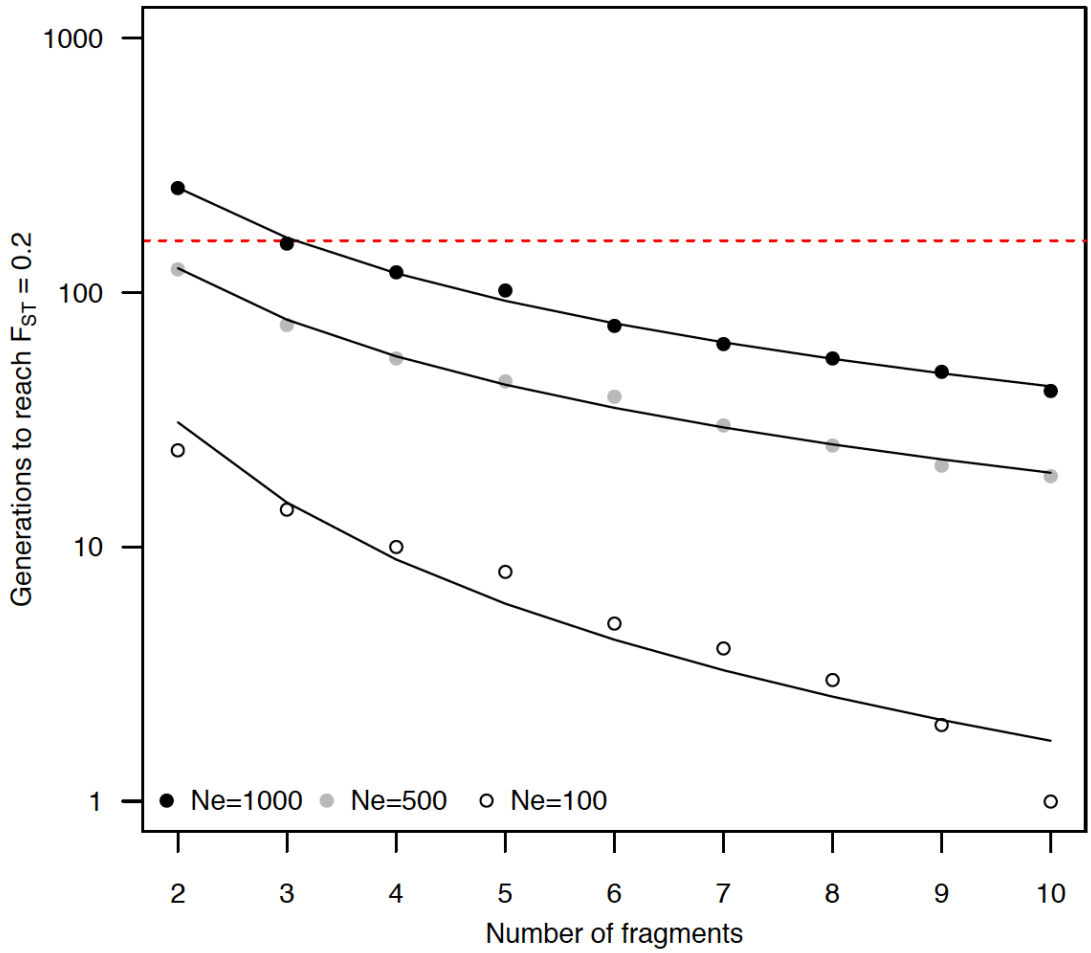

Figure 4. Number of generations (log scale) for global F_{ST} to reach 0.2 with increasing levels of habitat fragmentation for simulated *N. australis* metapopulations of N_e=1000, N_e =500 and N_e =100. Simulations were based on a stepping stone model assuming equal N_e for each sub-population and were allowed to run for 20,000 generations with a migration rate of 0.5 between adjacent demes before 300 generations with no migration. Red dashed line indicates the approximate number of generations since construction of in-stream barriers began in the MDB (160 generations).