



## Regional extinction, rediscovery and rescue of a freshwater fish from a highly modified environment: The need for rapid response



Michael P. Hammer<sup>a,b,c,\*</sup>, Todd S. Goodman<sup>d</sup>, Mark Adams<sup>b,c</sup>, Leanne F. Faulks<sup>e,f</sup>, Peter J. Unmack<sup>g</sup>, Nick S. Whiterod<sup>d</sup>, Keith F. Walker<sup>c,h</sup>

<sup>a</sup> Museum & Art Gallery of the Northern Territory, GPO Box 4646 Darwin, NT 0801, Australia

<sup>b</sup> Evolutionary Biology Unit, South Australian Museum, North Terrace, Adelaide, SA 5000, Australia

<sup>c</sup> School of Biological Sciences, The University of Adelaide, Adelaide, SA 5005, Australia

<sup>d</sup> Aquasave-Nature Glenelg Trust, PO Box 2177, Mt Gambier, SA 5290, Australia

<sup>e</sup> Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia

<sup>f</sup> Molecular Ecology Laboratory, School of Biological Sciences, Flinders University, SA 5042, Australia

<sup>g</sup> Institute for Applied Ecology and Collaborative Research Network for Murray–Darling Basin Futures, University of Canberra, ACT 2601, Australia

<sup>h</sup> School of Veterinary and Life Sciences, Murdoch University, WA 6150, Australia

### ARTICLE INFO

#### Article history:

Received 24 April 2015

Received in revised form 14 August 2015

Accepted 31 August 2015

Available online xxxx

#### Keywords:

Rediscovery

Freshwater fishes

Molecular genetics

Murray–Darling Basin

Eleotridae

### ABSTRACT

There are high rates of regional and global extinctions among freshwater species and few chances for recovery. We report here on the rediscovery after 30 years of a small fish, the southern-purple spotted gudgeon (*Mogurnda adspersa*), once widespread in the southern Murray–Darling Basin of south-eastern Australia. The rediscovery was in a region, the Lower Murray, where temperate riverine and wetland habitats are modified by a broad spectrum of changes including intensive flow regulation and diversions. There was some doubt whether the rediscovered population was a true remnant or a recent introduction, particularly as there was a translocated population in a nearby artificial habitat. Fortunately, a non-government organisation acted to rescue into captivity about 50 specimens as the remaining wetland habitat dried completely, soon after rediscovery, as a consequence of a decade-long drought and water diversions. We describe the habitat and ecology of fish in the rediscovery site, and provide genetic data, both nuclear (50 allozyme loci) and mtDNA (1141 base pairs; two genes), to show that they were true remnants of the regional native population. This information allows clear planning for future recovery including reintroductions, and is a case study that provides strategies, and hope, for conservation and management concerning other modified habitats. Specifically, it highlights the need for a rapid response to conserve threatened species, the recognition of remnant natural values in altered environments, and the treatment of new finds as native until there is alternate evidence.

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### 1. Introduction

Global extinction rates are escalating in response to human impacts, and the effects are intensified in freshwater habitats (Dudgeon et al., 2006; Vörösmarty et al., 2010). As biodiversity is diminished (e.g. high extinction rates in freshwater fishes: Duncan and Lockwood, 2001), vital ecosystem processes are undermined, leading to potentially irreversible changes (Dudgeon, 2010). Occasionally, however, there may be a reprieve, and an opportunity for recovery, when species presumed extinct are rediscovered (i.e. after all reasonable searches have previously failed to locate individuals: IUCN, 2012). Most rediscoveries are reported in tropical climates (Scheffers et al., 2011), typically in remote, inaccessible or more pristine areas; on a single bush, positioned on a steep rock face, of a remote island being an extreme example (Lord

Howe Island stick-insect (*Dryococelus australis*): Priddel et al., 2003). Here we report on the rediscovery of a major regional population of a freshwater fish from a highly modified, temperate environment as an aquatic case study that epitomises issues confronted during a period of unprecedented global environmental change (Strayer and Dudgeon, 2010).

The southern purple-spotted gudgeon (Eleotridae: *Mogurnda adspersa* Castelnau, 1878) is one of many freshwater fishes to have undergone a dramatic decline in the highly-modified Murray–Darling Basin (MDB) of south-eastern Australia (Lintermans, 2007; MDBC, 2004). Until recently, it was known to occur only in a few small populations in tributaries of the Darling River in the northern MDB. The small (<150 mm Total Length) colourful species was popular as an aquarium fish, even being used as a ‘bait’ fish, and common in the southern MDB (Murray River system) until the 1970s. Subsequently, several regional surveys failed to detect the species. This is reflected in jurisdictional threatened species legislation which considers the MDB conservation

\* Corresponding author.

E-mail address: [michael.hammer@nt.gov.au](mailto:michael.hammer@nt.gov.au) (M.P. Hammer).

unit of *M. adspersa* (Adams et al., 2013; Faulks et al., 2008) as endangered (New South Wales), critically endangered (South Australia) or presumed extinct (Victoria). We report the rediscovery of southern MDB *M. adspersa* in late 2002 (Fig. 1), from a single isolated population in Jury Swamp, a small wetland alongside the River Murray between Murray Bridge and Mannum (35° 03' S, 139° 19' E), South Australia. This was 2500 km from the nearest known extant populations in the northern MDB (Fig. 2).

A fleeting sighting of southern MDB *M. adspersa* was made in 1995–1996, when a few individuals were recorded from an off-channel irrigation lake complex (Cardross Lakes near Mildura, Victoria), but subsequent intensive survey effort demonstrated that a population was not present owing to major water-level drawdown and salinization (Ellis et al., 2013; Raadik, 2001). Events such as this highlight that new finds may be short-lived, involving a few individuals in a limited area and potentially with a high risk of true extinction (Altaba, 1990; Laurance et al., 1996; Telcean et al., 2011). Information about threats and the ecology, population status and trends of rediscovered species often will be lacking, and needs to be gathered quickly to facilitate management and recovery (Ostrovsky and Popov, 2011; Wanzenböck, 2004). Indeed, only a few years after the rediscovery at Jury Swamp, the habitat dried completely, as a result of protracted drought and upstream diversions and the population was extirpated. Historic data from the southern MDB suggests the species appears to prefer slow-flowing, sheltered areas with dense aquatic vegetation (Blewett, 1929; Hammer et al., 2009).



Fig. 1. Lower Murray *Mogurnda adspersa*: inset the first fish rediscovered by T. Goodman in December 2002, top a female captured during monitoring in February 2007 and lower a captive male guarding eggs in November 2007.

A further quandary concerning rediscoveries arises in habitats modified and frequented by humans, where rediscovered species may occur through accidental or deliberate introductions (Metcalf et al., 2007) rather than having persisted despite adverse conditions. Confusion regarding origin could evoke conflicting management priorities ranging from urgent conservation action, a 'do nothing' approach, to invasive species control (Crees and Turvey, 2015). As a response to presumed regional extinction, in circa 1997, *M. adspersa* were translocated from northern MDB tributaries to the southern MDB at a small, isolated artificial wetland, the Murray Bridge 'Army Range Wetland' (Pierce, 1997) (Fig. 2). A population was established and plans were made to release some fish to the wild, but it is unknown if these were implemented (Hammer et al., 2012; Wager and Jackson, 1993). The Army Range Wetland is only 10 km from Jury Swamp, suggesting that the rediscovered fish might have been derived from the translocated population. *Mogurnda* species are also sold as aquarium fish in nearby Adelaide (population of 1.25 million people) and could have easily been transported to the Murray.

The possibility of translocation provided reason to question the origin of the rediscovered population. Furthermore the rediscovery occurred in an area of intense human activity (angling, boating, houses, dairy farms, drains, levees and introduced plants). As a consequence, government agencies were not persuaded to implement a formal conservation programme. Fortunately, during wetland drawdown some of the last remaining fish were rescued into captivity by a non-government organisation as the basis of a captive breeding programme, on the assumption they could be native to the area (Hammer et al., 2013).

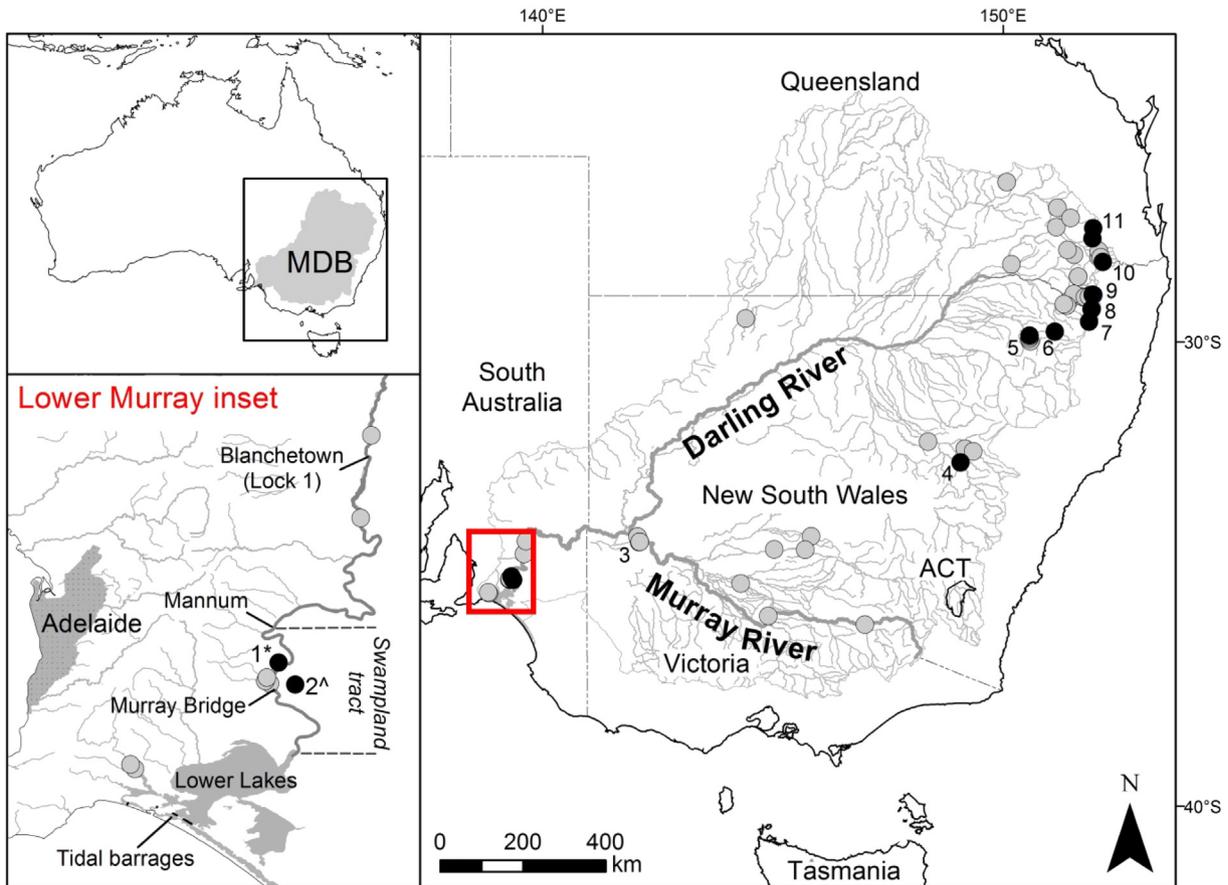
This paper documents a research programme that ran parallel to, and informed, evolving conservation measures for southern MDB *M. adspersa*. Our aims were to (1) assess the population status of *M. adspersa* in the field, and (2) investigate genetic divergence and population heterogeneity using both nuclear and mitochondrial DNA markers in order to determine population origin (Hickley et al., 2004; Miller et al., 1989; Waters et al., 2002). In retrospect, we consider how an effective precautionary management response might be developed for application to comparable situations in the future.

## 2. Methods

### 2.1. Study region and environmental change

The MDB is an expansive river system that covers an area of 1.06 million km<sup>2</sup> in south-eastern Australia (Fig. 2). The Murray and Darling rivers join and then flow to the Southern Ocean via the 830-km 'Lower Murray'. The Lower Murray includes four zones, namely: (1) broad floodplain tract (the 'Riverland'), (2) limestone gorge tract with a narrow floodplain, (3) swampland tract with only sparse wetland remnants in an area now reclaimed for pasture, and (4) the terminal 'Lower Lakes' region, including Lakes Alexandrina and Albert, the Murray Mouth and a coastal lagoon, the Coorong. Barrages along the seaward margins of Lake Alexandrina prevent sea water entering the Lower Lakes. The channel of the Lower Murray has a series of 10 low-level weirs, and the floodplain in some areas is protected by flood levees (Walker, 2006; Walker and Thoms, 1993).

The natural flow regime is highly variable, but has seen a dramatic alteration in streamflow volume, seasonality and flooding due to water extraction for irrigation (Leblanc et al., 2012; Walker and Thoms, 1993). Water levels in the Lower Murray below Blanchetown (Fig. 2) are influenced by wind-driven standing waves (seiches), causing changes up to ±0.3 m daily (Webster et al., 1997). These provide regular lateral river-floodplain flushing, and are unique to this river stretch, being less evident in the stable weir pools upstream (Walker, 2006). During this study (2003–2010), water in the Murray at Jury Swamp typically was turbid (Secchi transparency: 0.05–0.2 m), with relatively low salinity (electrical conductivity at 25 °C: 300–800 µS



**Fig. 2.** Distribution of *Mogurnda adspersa* in the Murray–Darling Basin (MDB), including historic (<1980) records (grey circles) and extant populations (black circles) (adapted from Lintermans, 2007). Cardross Lakes was marked as historic due to reasonable searches failing to locate individuals amid major environmental change. Site numbers correspond to Table 1. \*Location of the rediscovery site at Jury Swamp ^Known translocated population at the Army Range Wetland.

$\text{cm}^{-1}$ ), high pH and carbonate hardness, and temperatures of 10–26 °C (with values fluctuating more widely in the shallow wetland).

In 2007–2010, the combined effects of water diversions and protracted major drought ('the Millennium Drought') led to unprecedented environmental changes in the Lower Murray (Gallant and Gergis, 2011; Kingsford et al., 2011). River inflows were well below the long-term average, and there were dramatic declines in river level, to as much as 2 m below normal 'pool level' as assessed with Australian Height Datum, AHD (Fig. 3). Nearly all wetlands along the 200 km reach downstream of Blanchetown (the swampland tract and part of the gorge tract) were isolated and dried. Jury Swamp contracted over the first half of 2007, losing most of its core habitat by April, and was dry in 2008. Water levels recovered after rainfall and flooding, beginning in September 2010 (Fig. 3). Only in April 2014, some three years after the return of the Lower Murray to 'normal' pool levels did habitat begin to approach pre-drying water quality and aquatic plant density. The Jury Swamp wetland habitat would have otherwise been available continuously until the unprecedented low water levels in 2007 (Fig. 3) (Gallant and Gergis, 2011).

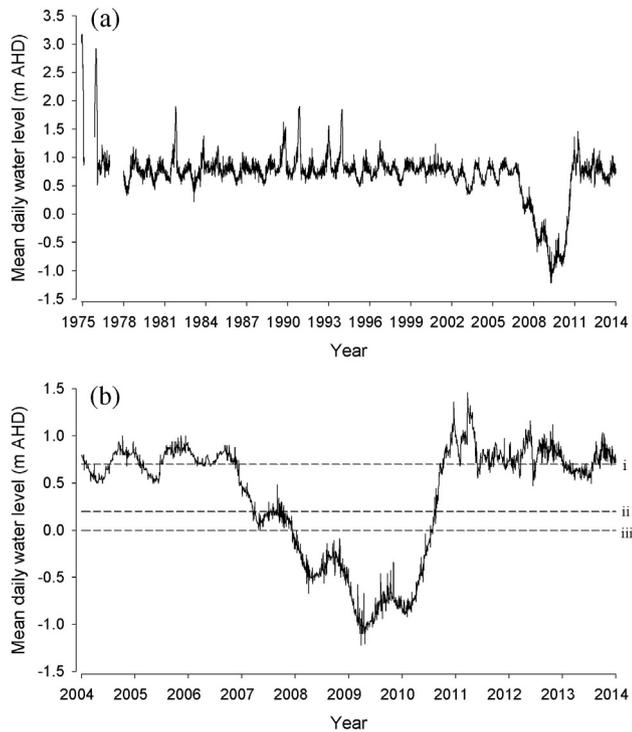
## 2.2. Field investigations

The original rediscovery prompted field observations in 2003–2006, and again more frequently in 2007–2008, as water levels fell. Sporadic visits were made after critical habitat loss during 2008–2010 (Hammer et al. 2013). Various sampling methods were trialled (i.e. fyke net, seine net and bait trap: Raadik, 2001; Smith et al., 2009), but only dip netting (0.5 m<sup>2</sup> square frame, 4 mm stretch mesh) was consistently effective in shallow areas with dense stands of aquatic plants. Bait

traps only had reasonable catches when strategically set by pushing them into dense cover in shallow water (0.3 m), and checked just after dusk and dawn. Fish were handled minimally and carefully (e.g. on wet surfaces), with total length measured and health condition assessed visually before they were returned to the point of capture, with regard for the vulnerability of both the population and habitat. Opportunistic inspections were made of 30 other potentially suitable habitats in the Lower Murray region.

## 2.3. Fish rescue

As it became apparent that water levels in Jury Swamp were becoming critical to population survival, a last-resort effort was made to rescue a representative of remaining fish into captivity (February–April 2007). Makeshift facilities comprising glass aquaria were assembled, with all new or sterilised equipment used. Fish were sampled with dip net and seine net and carefully transferred to aerated 20 L buckets for transport. Badly diseased fish (see section 3.1) that were not deemed recoverable were euthanized, and others treated immediately with a combination of proactive and reactive commercial medications. *M. adspersa* is well suited to captive husbandry due to its small size, broad diet, and ease of spawning/rearing; eggs are deposited on solid surfaces and guarded by the male, the larvae are briefly semi-pelagic and accept large live foods such as brine shrimp (*Artemia*) nauplii at first feed (Leggett and Merrick, 1987; Llewellyn, 2006). The initial intention was for rescued fish to be held in captive maintenance and returned to the wild when water returned. However, the length of the critical water shortage (3 years) and the subsequent protracted recovery time from the impacts of desiccation (e.g. loss of aquatic plants) meant that a long-



**Fig. 3.** Mean daily water level (metres above Australian Height Datum, AHD) for the Lower Murray at Mannum (station A4261067), indicating (a) long-term variability and (b) water-level decline (Department of Environment, Water and Natural Resources, unpublished) linked to field observations: (i) inundation of emergent vegetation, (ii) loss of core *Mogurnda adspersa* habitat and (iii) total loss of wetland habitat.

term captive breeding programme was required. Two dedicated temperature controlled hatcheries were developed and linkages made with schools to develop additional support hatcheries (see Hammer et al., 2012).

#### 2.4. Genetic analyses

A previous molecular assessment of geographically-dispersed, remnant populations in northern Darling tributaries revealed strong sub-structuring (Faulks et al., 2008), providing a testable hypothesis that distinct gene pools should exist in the southern and northern MDB. Thus, genetic comparisons with the Jury Swamp population could clarify their origin, either via translocation of northern MDB fish

or conservation units/congeners from north-eastern Australia (Adams et al., 2013; Hughes et al., 2012).

The locations and sample sizes of populations for genetic analyses are shown in Fig. 2 and Table 1. We obtained material from the Jury Swamp population as fin clips from the field (sample size ( $n$ ) = 2, date of capture 2003) and as snap-frozen muscle ( $n$  = 19, 2007) from rescued fish that were terminally affected by disease. A further frozen sample was taken from translocated fish from the Army Range Wetland ( $n$  = 10, 2003). All fish retained were euthanized using 0.1% clove oil in water and stored at  $-70$  °C in the Australian Biological Tissues Collection (ABTC) at the South Australian Museum, Adelaide. Additional samples from the northern MDB were available as frozen material in the ABTC (six sites:  $n$  = 1–31, 1995–1997) and fin clips (four sites:  $n$  = 7–17, 2000–2003) integrated from Faulks et al. (2008). Finally, a small tissue sample was obtained from a single fish from Cardross Lakes, an irrigation area fed from the Murray, Victoria (1995), preserved in alcohol and held at the Museum Victoria, Melbourne. The method of preservation dictated the numbers of fish available for allozyme analysis.

Muscle homogenates were subjected to allozyme electrophoresis on cellulose acetate gels (CelloGel®), following the principles and procedures of Richardson et al. (1986). Thirty-four enzymes or non-enzymatic proteins displayed allozymically-interpretable banding patterns after histochemical staining: ACON, ACP, ADA, ADH, AK, ALD, CA, CK, ENOL, FDP, FUM, GAPD, GLO, GOT, GP, GPI, GSR, IDH, LDH, MDH, ME, MPI, NDPK, PEPA, PEPB, PEPD, PGAM, 6PGD, PGK, PGM, PK, SORDH, TPI and UGPP. Details of enzyme and locus abbreviations, enzyme commission numbers, electrophoretic conditions, stain recipes and allozyme nomenclature are shown in Hammer et al. (2007).

For the allozyme data, we used Principal Co-Ordinates analysis (PCO) to assess the genetic affinities of individual fish independently of their geographic origin or mtDNA profiles. We also examined the genotypic data statistically for evidence of deviations from Hardy-Weinberg expectations or linkage disequilibrium within populations, and heterogeneity of allele frequencies between populations, using pairwise comparisons (corrected for multiple tests) plus a global test across all polymorphic loci based on Fisher's method (Fisher, 1948).  $F$ -statistics were used also as overall measures of within-site variability and between-population divergence, and observed heterozygosity levels ( $H_o$ , direct count method) were calculated as a measure of within-population diversity. These methods are described more fully by Hammer et al. (2007).

Our mtDNA sequences (~850 bp *ATP6/ATP8* plus ~400 bp *CR*) for southern MBD samples (GenBank Accession numbers: DQ219325–26) were generated following Faulks et al. (2008), and thereafter used to extend the existing mtDNA dataset for MDB *M. adspersa* (GenBank

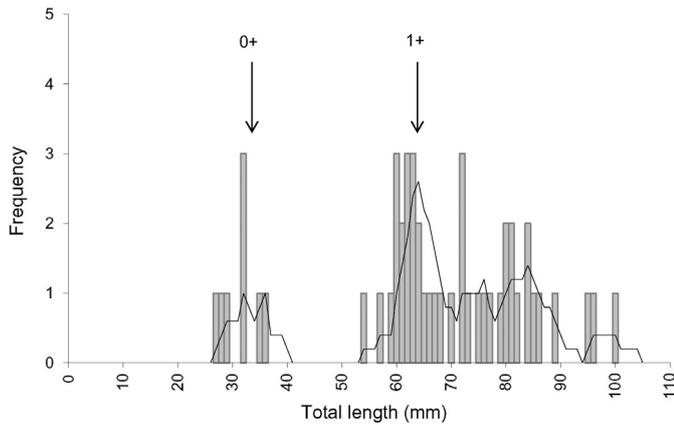
**Table 1**  
*Mogurnda adspersa* localities and sample sizes for genetic analyses. Site numbers match Fig. 2.

Site	Field code	Locality	Drainage	State	Latitude (S)	Longitude (E)	n Allozymes	n mtDNA
1	FISHY4	Jury Swamp <sup>a</sup>	Murray R.	SA	35°03'	139°19'	19	16
2	FISH98	Army Range Wetland <sup>b</sup>	Murray R.	SA	35°08'	139°21'	10	9
3	MVA22791	Cardross Lakes	Murray R.	Vic.	34°18'	142°07'	–	1
4	LF1	Wuluuman Ck	Macquarie R.	NSW	32°36'	149°04'	–	17 <sup>c</sup>
5	PU97-38	Halls Ck, Bingara	Border rivers	NSW	29°52'	150°35'	1	14 <sup>c</sup>
6	FISH19	Inverell	Border rivers	NSW	29°47'	151°07'	4	4 <sup>c</sup>
7	PU5	Deepwater R.	Border rivers	NSW	29°18'	151°55'	5	3 <sup>c</sup>
8	PU4	Severn R.	Border rivers	NSW	29°34'	151°52'	3	2 <sup>c</sup>
9	PU3	Tenterfield Ck	Border rivers	NSW	28°59'	151°57'	1	17 <sup>c</sup>
10	PU97-41	Farm Ck	Condamine R.	Qld	28°17'	152°10'	31	2 <sup>c</sup>
11	LF2	Toowoomba	Condamine R.	Qld	27°33'	151°57'	–	7 <sup>c</sup>

<sup>a</sup> Site of rediscovery.

<sup>b</sup> Known translocated population.

<sup>c</sup> Samples previously included in Faulks et al. (2008); this study extends the mtDNA analysis and adds nuclear data.



**Fig. 4.** Demographic data (with 5-point moving average) for Lower Murray *Mogurnda adspersa* in January/February 2007 ( $n = 50$ ). Two peaks in length frequency are arrowed: juveniles spawned in spring 2006 (0+) and a strong cohort presumably from the previous spring (1+).

Accession numbers: CR6, DQ219317–18, DQ219324, DQ21927–31, EF548159). Genealogical relationships within MDB *M. adspersa* were investigated by constructing a statistical parsimony haplotype network (see Faulks et al., 2008).

**3. Results**

**3.1. Ecological observations**

Contemporary records of *M. adspersa* in the southern MDB, following rediscovery in 2002, are restricted to Jury Swamp in the swampland tract of the Lower Murray. Their local distribution was limited to two inlets to the wetland and a nearby drain entrance, all connected to the main channel (river length 600 m; estimated area of occupancy <0.05 km<sup>2</sup>). Searches at other locations (including known historic sites) in the swampland and gorge tracts were not successful, although critical water shortage had eliminated most apparently-suitable habitat by summer 2007–2008. Over a five-year period, 160 specimens of *M. adspersa* were captured from Jury Swamp, although these were likely to include

repeat captures. In January 2007, when the wetland habitat started to dry, sampling became easier and in the order of 100–200 fish were present. By April 2008, no water remained in any previously-occupied wetland areas, and subsequent monitoring events along the adjoining river edge sporadically revealed individual specimens only, with all records ceasing after spring 2009.

The species evidently requires dense cover in the form of physical elements and aquatic vegetation, in off-channel habitats. Wild fish were detected in a variety of microhabitats. Core microhabitat prior to 2007 was along shoreline constructed rock walls among overhanging grasses and emergent vegetation (*Triglochin*), among dense stands of emergent and submerged macrophytes (e.g. *Ceratophyllum*, *Schoenoplectus*, *Vallisneria*). A secondary channel microhabitat under a stand of willows (*Salix* spp.) had abundant submerged woody debris and tree roots. As water levels fell during 2007, rocky edges and emergent vegetation were isolated (critical level 0.2 m AHD: Fig. 3), with submerged plants and rocks used increasingly as cover. By late May 2007 ( $\leq 0.1$  m AHD), aquatic vegetation had been virtually eliminated. Willow-lined river-edge microhabitats were unoccupied until late autumn 2007, when two individuals were recorded (the main river channel is typically wide, >3 m deep and steep sided). Most adults and juveniles were captured in shallow water (0.1–0.5 m), among dense plant cover in areas with slow-flow (0.0–2.0 m s<sup>-1</sup>) where water exchanged between the main river and the wetland, driven by water-level fluctuations in the swampland tract of the Lower Murray (see section 2.1).

Survival at the southerly latitude of Jury Swamp may be promoted by the thermal buffer afforded by the Murray as a large riverine habitat. Feeding and reproduction in highly turbid water are also unique to the Lower Murray population, and may represent a local adaptation. Fish appeared to position themselves in shallow areas, especially at night, to prey upon larger macroinvertebrates and small fish. An 80-mm male was captured with an atherinid (*Craterocephalus fulvus*) in its mouth, and in captivity rescued fish fed readily on smaller fish, atyid shrimp (*Paratya australiensis*), juvenile parastacid crayfish (*Cherax destructor*), small palaemonid prawns (*Macrobrachium australiense*), odonate larvae and dytiscid beetles. Other species recorded in high abundance alongside *M. adspersa* included eleotrids (*Philypnodon* spp., *Hypseleotris* spp.), *C. fulvus* and an alien poeciliid (*Gambusia holbrooki*), with some 20 species recorded as part of the local wetland fish community. Fine-meshed fyke nets set near known edge-habitat (day and

**Table 2**

Allele frequencies at variable allozyme loci for all Murray–Darling Basin *Mogurnda adspersa* populations sampled. Also shown are the observed heterozygosity values ( $H_o$ ; direct count method, across all 50 loci) for each site (S.E. = standard error). Site codes match those used in Table 1 and Fig. 2. Sample sizes are shown in parenthesis. The following 35 loci were invariant: *Acp1*, *Acp2*, *Adh1*, *Adh2*, *Ak*, *Ald1*, *Ald2*, *Ca2*, *Ck*, *Enol*, *Fdp*, *Fum*, *Gapd*, *Got1*, *Gp*, *Gpi1*, *Gpi2*, *Ldh*, *Mdh1*, *Mdh2*, *Me1*, *Mpi*, *Ndpk1*, *PepA1*, *PepA2*, *PepB*, *PepD1*, *Pgam*, *6Pgd*, *Pgk*, *Pgm1*, *Pgm2*, *Pk1*, *Pk2*, and *Ugpp*.

Region	Jury Swamp	Border rivers					Condamine River	Army Range translocated
	1 (19)	5 (1)	6 (4)	7 (5)	8 (3)	9 (1)	10 (31)	2 (10)
<i>Acon1</i>	b	b	b	b	b	b	b <sup>96</sup> ,a	b
<i>Acon2</i>	d <sup>94</sup> ,b <sup>3</sup> ,a	d	d	d	d	d	d	d
<i>Ada</i>	b <sup>91</sup> ,c	b	b	b	b	b	b	b
<i>Ca1</i>	b	b	b <sup>87</sup> ,a	b	b	b	b	b
<i>Glo</i>	c	c	c <sup>75</sup> ,a	c	c	c	c	c
<i>Got2</i>	b	b	b	b	b	b	b <sup>84</sup> ,a	b
<i>Gsr</i>	b	b	b	b	b	b	b <sup>97</sup> ,c	b
<i>Idh1</i>	b	a <sup>50</sup> ,b	a	a <sup>67</sup> ,b	–	b	b	b <sup>95</sup> ,a
<i>Idh2</i>	b <sup>97</sup> ,a	a <sup>50</sup> ,b	b	a <sup>90</sup> ,b	a <sup>83</sup> ,b	b	b	b
<i>Me2</i>	d <sup>50</sup> ,b <sup>31</sup> ,a	b	b	b	b	b	b	b
<i>Ndpk2</i>	b <sup>90</sup> ,a	b	b	b	b	b	b	b
<i>PepD2</i>	c <sup>75</sup> ,d	c	c	c	c	c	c	c
<i>Sordh</i>	a <sup>87</sup> ,b	a	a	a <sup>50</sup> ,b	a	b	b	b <sup>70</sup> ,a
<i>Tpi1</i>	c	c	c	c <sup>90</sup> ,d	c	c	c	c
<i>Tpi2</i>	c <sup>97</sup> ,d	d	c <sup>50</sup> ,d	d <sup>83</sup> ,c	–	c	c	c
$H_o$	0.041	0.040	0.035	0.040	0.009	0.000	0.010	0.010
S.E.	0.018	0.028	0.023	0.019	0.009	0.000	0.010	0.008

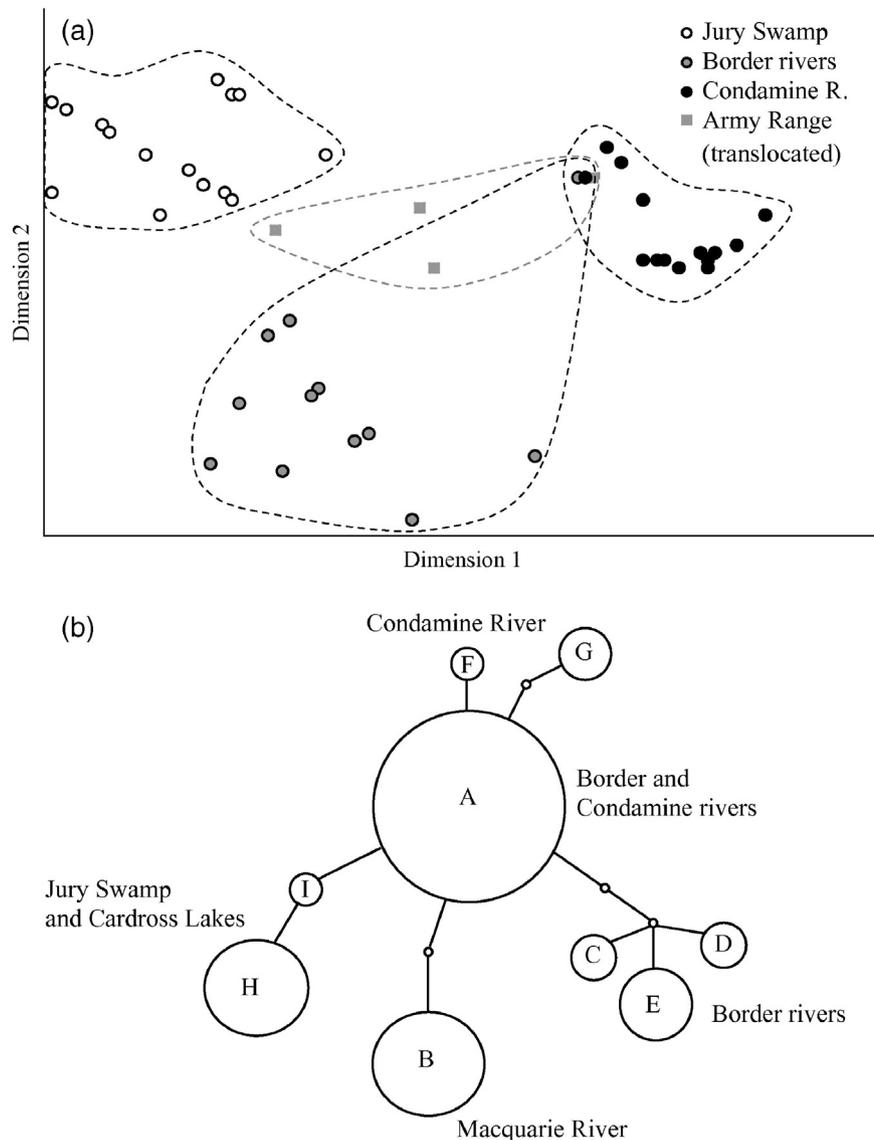
night) seldom captured fish, suggesting that there was minimal movement during stable conditions. As water levels receded, however, the species made forced local excursions away from cover (tens of metres), although they retained strong fidelity for cover when the water returned temporarily during daily fluctuations.

Demographic data were gathered to examine life history, track population trends and identify flow-ecology relationships, from late summer/early autumn catches ( $n = 93$ ) including a more complete assessment as part of rescue operations in summer 2007. Fig. 4 shows young-of-year fish (0+) clustered at 20–40 mm, and subsequent peaks show a strong group between 50 and 70 mm (1+; out-rearing in ponds of captive-spawned larvae matched this group) and a group of older fish to a maximum size of 100 mm. Recruitment occurred at Jury Swamp in all years studied prior to wetland drying (2003–2007), with a particularly strong cohort in 2007 following spawning in spring 2005 (Fig. 4). The hydrograph (Fig. 3) showed higher minimum annual water levels in spring through autumn 2005–2006 compared to preceding years (0.7 m AHD), and this may have promoted strong recruitment, indicating a dynamic population response under favourable aspects of

the flow regime. The sex ratio of the population was skewed 2:1 toward males.

Fish health was examined visually. Initially, all were healthy, with low incidence of external parasites (i.e. individuals with the parasitic copepod *Lernaea* and small, unidentified leeches). As stressors relating to low water level developed in 2007 (e.g. temperature extremes, lack of habitat and concentration of inter- and intra-specific competitors), the incidence of disease increased. In January 2007, one of 33 fish captured (a 61-mm male) was diseased, with a large ulceration on its flank. A month later, as water levels in most of the wetland fell (0.2 m AHD) and summer advanced, the number of diseased fish increased to 38% of sampled individuals (i.e. 16 of 42). Diseases included severe fungal infections (probably *Saprolegnia*) and fin rot and/or lesions diagnosed as Epizootic Ulcerative Syndrome (EUS), a disease caused by *Aphanomyces invadans* (Vetlab Adelaide, pers. Comm.).

Some 55 wild adult fish were rescued into captivity prior to the wetland drying. Ongoing disease treatment was required for some of these fish along with appropriate hatchery design and management protocols (Hammer et al., 2012).



**Fig. 5.** Genetic analyses for *Mogurnda adspersa* in the Murray–Darling Basin, showing (a) Principal Coordinates Analysis of allozyme data; relative scores are plotted for the first and second dimensions, explaining 38% and 23% of the total variance, respectively, and (b) haplotype network based on mtDNA control region and ATPase 6/8; letters A–I correspond to unique haplotypes (Table 5), the sizes of circles reflect the overall frequency of each haplotype, each discrete line indicates a single mutational difference, and small circles along these lines represent missing haplotypes (not sampled or extinct).



and ongoing survey effort, using a diversity of approaches, is potentially required to rediscover presumed extinct aquatic taxa (Miller et al., 1989). For example Marsh et al. (2003) suggested that the rediscovery of loach minnow (*Tiaroga cobitis*) after some 25 years of relatively intensive sampling must have resulted from dispersal from an adjacent unsampled area; Hödl and Eder (1996) employed a different approach on survey methods and timing to rediscover several species of clam shrimp (Branchiopoda); and surveys in new areas outside the known or expected range have also been successful (e.g. golden topminnow (*Fundulus chrysotus*): Wills et al., 1998). In the current example, dip netting proved the most effective sampling method for *M. adspersa*. However, this is seldom used in surveys as it is difficult to standardise effort, thus prompting a re-think of future survey design. Freshwater habitats are well suited to environmental DNA approaches (Thomsen et al., 2012), and this promises to be an additional search tool for presumed extinct taxa.

Rediscovery has also occurred under a somewhat unique set of circumstances for several aquatic species, being located in refuges within artificial environments. Brook silverside (*Labidesthes sicculus*) were rediscovered in a Texas reservoir, occurring only in particular sheltered areas that included marinas, which were thought to provide physical habitat and feeding opportunities (light-attracted insects) (Pratt et al., 2002); the giant European freshwater pearl mussel (*Margaritifera auricularia*) was rediscovered in constructed Spanish canals running parallel to the main river (see Araujo and Ramos, 2000); and a freshwater snail (*Notopala hanleyi*) considered extirpated in the southern MDB following changes in foodweb dynamics, was rediscovered in irrigation pipelines that provided suitable microbial films (Sheldon and Walker, 1993). All show parallels with *M. adspersa* found refuging in the highly modified local and regional habitat at Jury Swamp. With 95% of swamp-land tract wetlands lost, the rediscovery site combines rare remnant habitat with suitable cover, albeit the base element (rock walls) being artificial.

#### 4.2. Fish rescue

Population rescue was considered a last-resort conservation option for southern MDB *M. adspersa* when all efforts to preserve the population in situ failed (Hammer et al., 2013). The sheer magnitude of environmental change rapidly eliminated all suitable habitat and led to extinction in the wild of the regional population. The recovery of another species from the southern MDB, the Yarra pygmy perch (*Nannoperca obscura*), under similar circumstances during the critical water shortage, was also solely dependent on outcomes relating to rescued fish. The chance for their rescue was taken just in time before regional extirpation (Hammer et al., 2013). Changes in population status at regional or species level can happen rapidly, with little opportunity, or the prior perceived need/resources, for more proactive captive breeding approaches. Globally, 69 animals and plants are currently assessed as extinct in the wild (i.e. only known in captivity or naturalized outside its past range: IUCN, 2015), and some form of conservation intervention has ultimately provided future recovery options for these species. The Lake Pedder galaxias (*Galaxias pedderensis*) is a prime example of successful recovery following rescue. It initially boomed, then declined rapidly, following the flooding of its only habitat in Tasmania, Australia for hydroelectric power generation. Timely and critical decisions before extinction lead to the rescue a handful of fish, which were translocated to two other areas where populations are now established (Chilcott et al., 2013).

#### 4.3. Population origin

Ecological and genetic data were gathered at a critical time, before the rediscovered Jury Swamp population was extirpated (Ostrovsky and Popov, 2011; Wanzenböck, 2004). The ecological data indicated habitat preferences and cryptic behaviour that conceivably could have

enabled the population to persist undetected prior to rediscovery. The nuclear and matrilineal genetic data showed that the rediscovered population belonged to a MDB conservation unit, but was not derived from fish translocated from the northern MDB, including via the Army Range Wetland. Instead they were distinctive, with a high frequency of a mtDNA haplotype found only in one other extirpated southern MDB population (Cardross Lakes, Victoria). While heterozygosity levels were relatively low, they were similar or higher than in other wild MDB populations, implying that the Jury Swamp population was unlikely to have come from an inbred aquarium strain. Distinctive Lower Murray populations exist for at least five other fish species (see Adams et al., 2011; Hammer et al., 2014), providing indirect support for the conclusion that Jury Swamp represented a remnant of a former southern MDB population (Husemann et al., 2012). Other potential sub-division in the southern MDB could not be assessed herein due to a lack of contemporary samples. More specialised techniques using primers designed for short but informative mtDNA portions may help in future to reconstruct the historic phylogeny (e.g. based on museum specimens: Metcalf et al., 2012).

#### 4.4. Conclusion

It is an interesting reality that at least some threatened species can persist at altered sites, amid peri-urban development and under intensive recreational and agricultural activity. In this example, the modifications include rock walls that offset losses of natural habitat and barrages that buffer water diversions. Thus investigating the presence of ecological assets and recognising their conservation needs in altered environments, and not just aesthetically pleasing or 'pristine' areas, can be an important part of natural resource management (Dudgeon and Smith, 2006; Hammer et al., 2013). Moreover, species in closer proximity to human populations can act as icons for community engagement and potential ecosystem recovery (Dudgeon et al., 2006), as was the case here for *M. adspersa* which eventually garnered widespread support from management agencies, researchers, schools and non-government organisations.

Being localised and exposed to critical threats, rediscovered populations typically require immediate intervention to ensure their long-term survival (Scheffers et al., 2011). The rediscovered population of *M. adspersa* had such elements, including skewed sex ratios, habitat loss, alien species and disease, as well unprecedented environmental changes (Soulé, 1987). These coincident pathways reinforce the need to act rapidly, as the window of opportunity for recovery and conservation may be brief (Laurance et al., 1996). The capacity within agencies to undertake such actions can be hindered by bureaucratic process and time lag, and hence rapid response strategies that include diverse stakeholders and contingency funds can build flexibility and improve chances for success (Lintermans et al., 2014).

The imperative for action, following rediscovery, may be undermined by doubts over the origin of the population. This study suggests that rediscoveries should be treated as 'native' until clarification is possible, no matter how implausible that hypothesis initially seems. We were able to acquire genetic and ecological data within two years of the first signs that the critical habitat was regressing, prompting a collaborative conservation programme which has included captive breeding, establishment of surrogate locations, habitat restoration and planned reintroductions (Hammer et al., 2012; Hammer et al., 2013). Given quick diagnosis and triage, and with ongoing commitment to the species, we trust that in this region the immediate future for Lower Murray *M. adspersa* has been secured.

#### Acknowledgements

Numerous people provided field assistance or other support. Special thanks to S. Angley, L. Beheregaray, C. Bice, D. Bray, G. Doyle, S. Doyle, R. Foster, A. Frears, D. Gilligan, A. Goodman, D. Goodman, A. Hall, M.F.

Hammer, J., Higham, A., Kessel, K., Mason, L., Piller, T., Raadik, D., Short, D., Sortino, M., Treanor, M., Tucker, Q., Ye, S., Wedderburn, S., Westergaard and S. Wright. Permits for field research were obtained from PIRSA Fisheries with approval of the Animal Ethics Committee at The University of Adelaide. Three anonymous reviewers helped to greatly improve a draft version of the manuscript.

Role of the funding source.

Financial support was provided to MPH through an Australian Post-Graduate Award (# 1063724) and a supplementary scholarship from the CRC for Freshwater Ecology (E.822). Funding to support parts of the field study was provided by the South Australian River Murray Catchment Water Management Board (now Natural Resources, SA Murray–Darling Basin) (project 181C). Funding for conservation actions are covered elsewhere (Hammer et al., 2013). Study design, analysis and reporting, and the decision to submit this article for publication, were undertaken by the authors independently of the funding bodies.

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