Australian Government

MURRAY–DARLING BASIN AUTHORITY

Reintroduction plan for the Purple-spotted Gudgeon in the southern Murray–Darling Basin

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Cover Image: Lower Murray Southern Purple-spotted Gudgeon in captivity–adults, eggs and juveniles. Photos by Michael Hammer

Summary

The Southern Purple-spotted Gudgeon is a small, colourful freshwater fish with a distinct genetic conservation unit in the southern Murray–Darling Basin (MDB). The species underwent large declines in the region and was feared extinct, until it was recently (2002) rediscovered in the Lower Murray. The wild site has since dried (2007) owing to dramatic and rapid water level declines from the combined effects of heavy water use and prolonged drought across the MDB. Fortunately, fish were rescued from the site and these form the basis of a last resort captive breeding program. With the guidance of this document and support of stakeholders, a reintroduction program aims to re-establish the former wild site, create self-sustaining populations at other nearby sites, and develop backup refuge populations in isolated dams and wetlands. In the longer-term, sites upstream of Lock 1 would also be established to restore the former range.

The general approach for reintroduction is moving away from mass release ('hope for the best') approaches, and towards investing in fewer but 'trained' fish that have a better chance of avoiding high initial mortality and that are released as part of broader restoration programs. Accordingly, the reintroduction plan is formed by review of a range of considerations regarding: the hatchery environment and procedures; release considerations tailored to the species; aspects of sites and restoration programs that lessen ultimate threatening processes; key knowledge gaps along the way; and factors such as permit requirements and stakeholder involvement.

Recommendations for action and further investigation are made under relevant sub-headings. A specific output includes a stepwise flowchart that details likely criteria and actions between the identification of a potential release site and future establishment of a sustaining population. This is trialled to assess sites ready for immediate and future release in line with improving hatchery production and the finite life of captive broodstock.

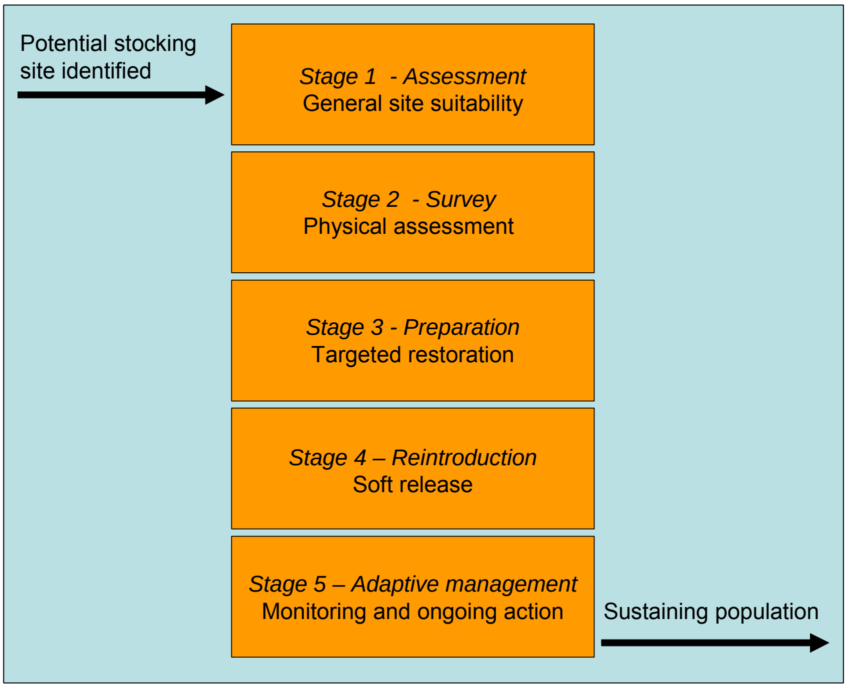


Figure 6: Summary of reintroduction process for Lower Murray Southern Purple-spotted Gudgeon

Table 1: Project stages and activities

Project component	Considerations	Criteria to move to next stage
Stage 1 - Assessment		
Survey of existing knowledge	General site suitability: • hydrology • location • habitat • water quality • fishes • stakeholders	 • has secure water supply • within natural range or is isolated • has good levels of cover • low salinity, nutrients and pollutants • no/few introduced fish known • commitment to species recovery/restoration
Stage 2 – Survey		
Specific site investigations	Physical assessment: • hydrology • location • habitat (broad) • habitat (specific) • water quality • fish survey • macroinvertebrates	 • spring inundation, stable in summer • confirm Stage 1 through ground truth • under appropriate management regime • high stable cover and aquatic plants • EC <7000; not anoxic; low P and N • no/few introduced fish found, or existing PSG • diversity and abundance of prey items

Project component	Considerations	Criteria to move to next stage
Stage 3 – Preparation		
Undertake any possible actions with appropriate approvals	Targeted restoration: • hydrology • habitat (broad) • habitat (specific) • water quality • introduced fishes • macroinvertebrates	• undertake risk assessment/permit applications • secure e-water allocation/provision • fencing/restoration of riparian zone /add physical habitat, establish aquatic plants • address point source and diffuse inputs • initiate eradication/control programs • supplement missing species/habitat diversity
Stage 4 – Reintroduction		
Reduce high initial mortality	Soft release: • permits • pre-treatment • cage • acclimatisation • release	• in place prior to release • possible reduction of fish at release site • holding cage added at selected release point/s • add fish to cage, allow adjustment period • provide exit point from cage
Stage 5 – Adaptive management		
Provide the best chance for survival	Monitoring & ongoing action: • fish research • macroinvertebrates • habitat • water quality • action	• assess survival and changes in fish community • assess temporal trends • survey physical cover and vegetation trends • ongoing testing of key parameters • use monitoring to refine management response

Contents

Abbreviations

MDB	Murray–Darling Basin
MDBA	Murray–Darling Basin Authority (formerly Commission)
SAMDBNRMB	South Australian MDB Natural Resources Management Board
DWLBC	SA Department for Water, Land and Biodiversity Conservation
NFA (SA)	Native Fish Australia (SA)
OH&S	Occupational Health and Safety
AHD	Australian Height Datum or Mean Sea Level
M. adspersa, SPSG	Southern Purple-spotted Gudgeon (*Mogurnda adspersa)*
EUS	Epizootic ulcerative syndrome

1. Introduction

The Southern Purple-spotted Gudgeon (*Mogurnda adspersa*) is a small, colourful fish that was historically widespread in two regions of the Murray–Darling Basin (MDB: Figure 1). The species remains as fragmented populations in upper tributaries of the Darling River (northern MDB) and historically occurred patchily across lowland habitats of the southern Basin (Moffat and Voller 2002; Llewellyn 2006; Lintermans 2007). The species had all but disappeared from the southern Basin by the 1970s, with only fleeting sightings at Cardross Lakes near Mildura, Victoria during the 1990s (Raadik 2001).

However, an exciting find in late 2002 documented the species from a single wetland on the River Murray between Murray Bridge and Mannum in South Australia (Hammer 2007b). Reasonably extensive searches have failed to locate any other sites in South Australia since the 1970s (Figure 2). In South Australia the species is fully protected (*Fisheries Management Act 2007*) with a conservation status of 'Endangered' (critically endangered) (Department for Environment and Heritage 2003; Hammer *et al.* 2007) and is also listed as 'Endangered' in New South Wales (*Fisheries Management (General) Regulation 2002*) and 'Threatened' (presumed extinct) in Victoria (*Flora and Fauna Guarantee Act 1988*).

Molecular genetic data confirms the new discovery as a distinctive and diverse wild population, and indeed a separate genetic conservation unit to northern Basin fish (Hammer 2008b). However, soon after rediscovery, the southern Basin population is now likely to be functionally, if not ultimately, extinct in the wild. Major water abstraction exacerbated by extended low rainfall conditions across the MDB led to significant reductions in the availability and quality of aquatic habitat along the lower River Murray and Lower Lakes. This combination of factors severely impacted native fish populations especially those which are already restricted and threatened (Hammer 2007a). In summer 2007, water levels in the single site began to drop rapidly, and by April 2007 the wetland was dry (Figure 4), with only a small amount of sub-optimal habitat in willow roots along the main channel remaining (Hammer 2007b).

With the loss from its former range, reintroduction provides the means of conserving the species across the southern Basin (e.g. Raadik *et al.* 1999). A makeshift captive population was established during the rapid wetland drying in South Australia, providing a second chance for species recovery (Hammer 2007b). Two small hatcheries with a total of some 50 wild adult fish (broodstock) have since been developed, and now have the capacity to produce fry and juveniles for release.

This plan provides a context and direction for hatchery-reared fish within species and habitat recovery. Key partners in current conservation efforts have been the Murray–Darling Basin Authority (MDBA), the SA MDB Natural Resources Management Board (SAMDBNRMB), and the SA Department for Environment and Heritage (DEH), through a 'Drought Action Plan'. It is hoped this document can guide existing efforts and attract new stakeholders and actions to progress conservation of the species and its habitat.

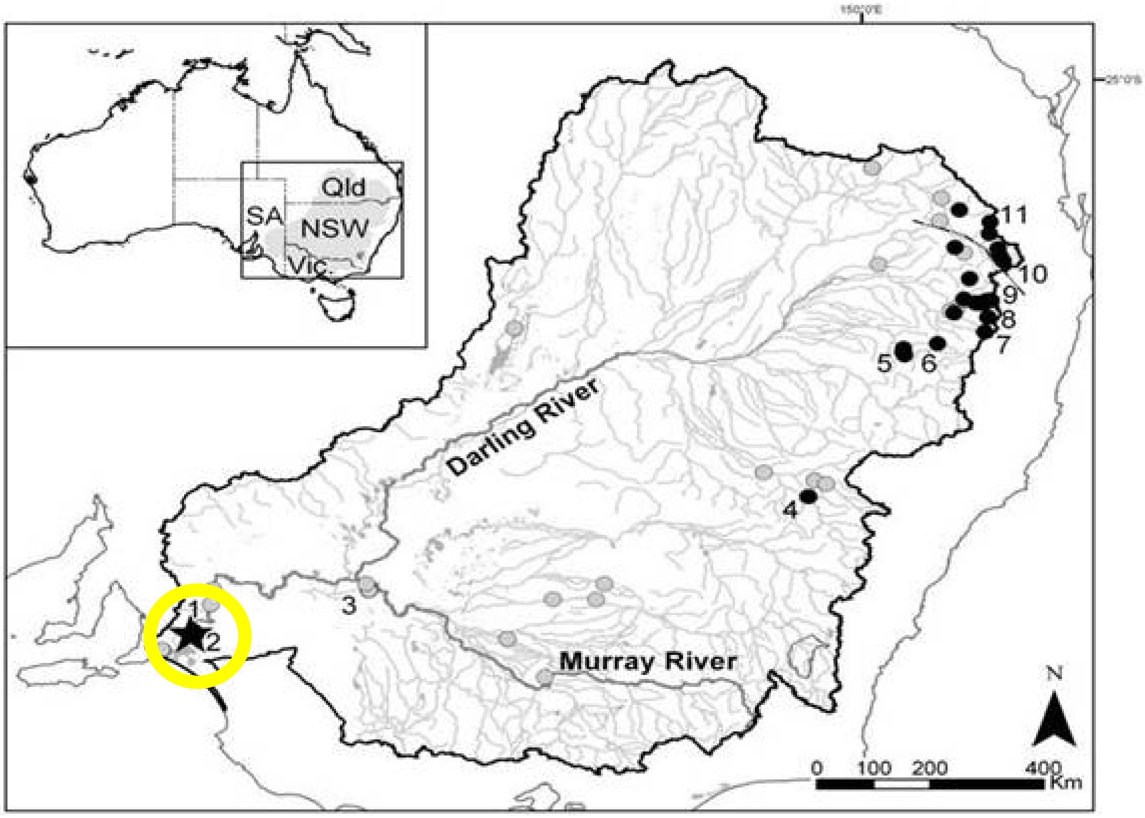


__Figure 1. Distribution records for Southern Purple-spotted Gudgeon in the Murray–Darling Basin (adapted from Lintermans 2007)__

Historic records shown with grey circles, extant populations with black circles and the recently rediscovered Lower Murray population indicated with a star. Site numbers match locations of genetic samples in Hammer (2008b).

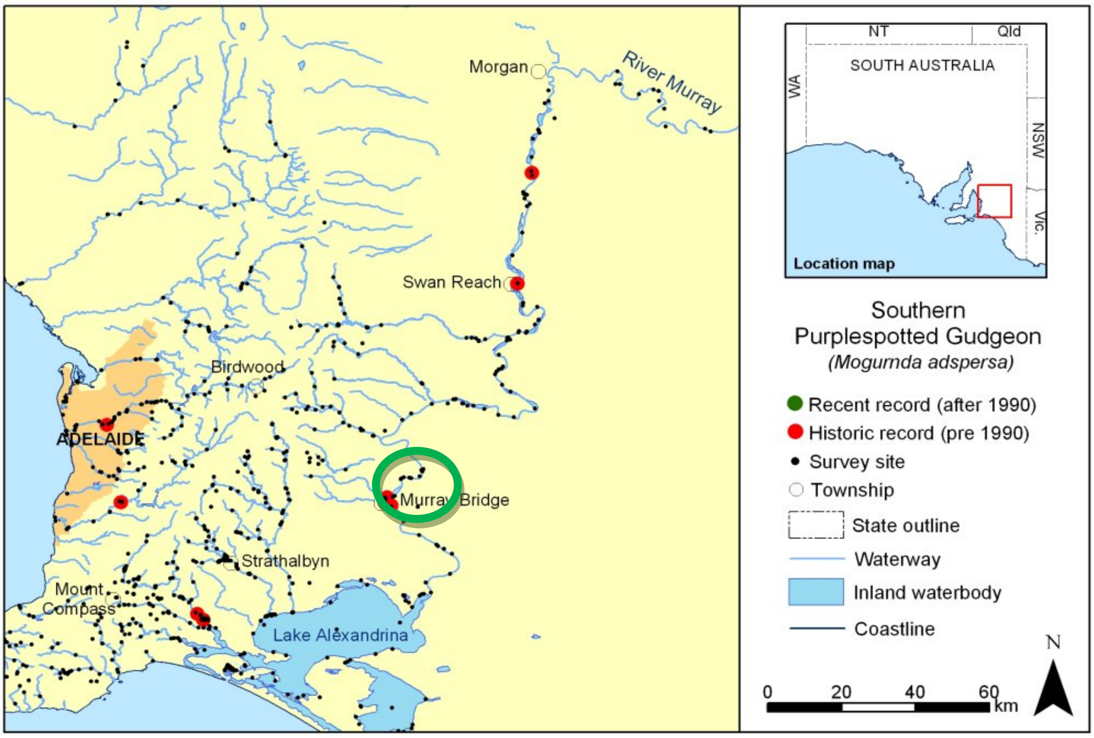


Figure 2. Distribution records for Southern Purple-spotted Gudgeon in South Australia, indicating reasonable sampling intensity and the location (exact not shown) of the remnant population (from Hammer et al. 2007)

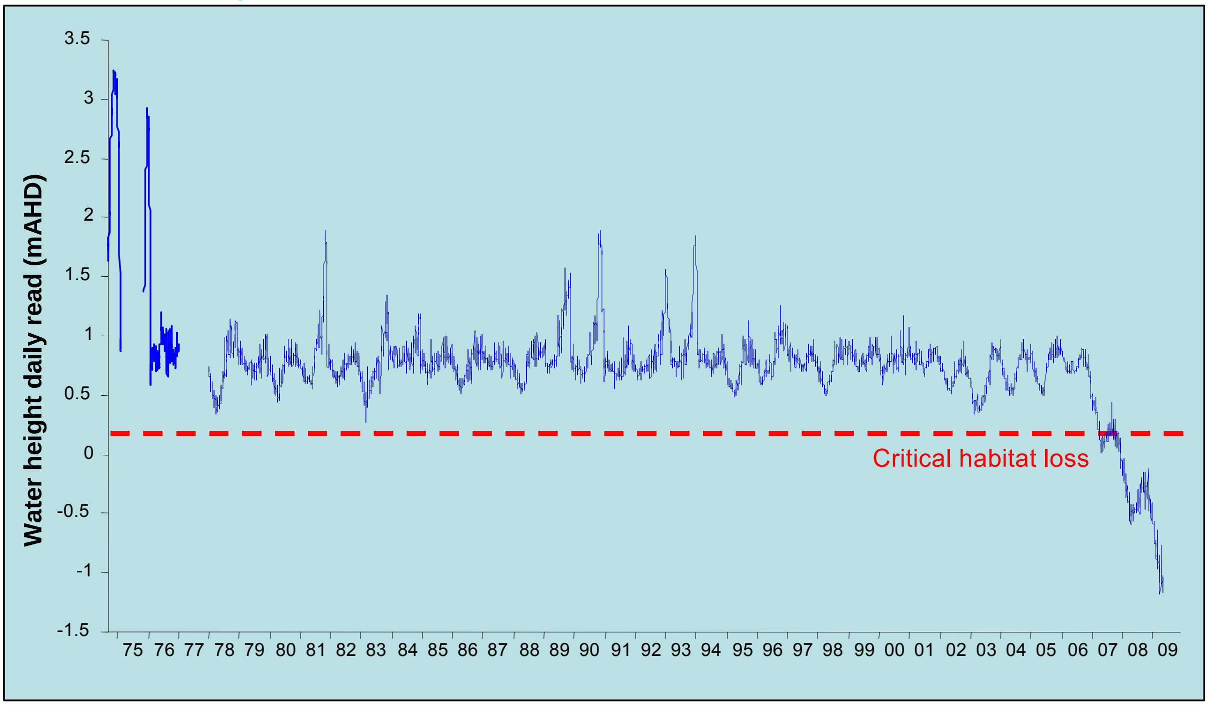


Figure 3. Indicative long-term record of water height on the Lower River Murray (metres AHD) observed at Mannum recorder A4261067 (DWLBC unpublished data)

The critically low levels after 2007 are clearly highlighted, with the level in 2009 more than one metre below average sea level. The dashed line indicates the point where the last wetlands in the region dried.

Dramatic decline of the wetland habitat supporting the only known southern Basin population of Southern Purple-spotted Gudgeon

Top: January 2007
Bottom: April 2007

2. Recovery context

2.1 Background

Captive breeding and reintroduction is a popularly quoted solution for the conservation of freshwater fishes, but there are significant risks in relying on this process alone. The notion that fish are 'saved' in captivity or can 'just be restocked if they die out' can take the onus away from protection or restoration of wild habitat and maintenance of important ecosystem and evolutionary processes (Fraser 2008). There are also high levels of risks associated with captive maintenance, such as loss of individuals or genes, and disease contamination (Philippart 1995; Frankham 2008).

Maintaining fish is time consuming, places responsibility on individuals for species survival, and requires long-term personnel and funding commitments. Ultimately, captive breeding for conservation should be a short-term (5–10 year) option, either as a last resort in face of imminent risk or applied to specific conservation (risk management) issues, such as establishing extra populations for greater species security (Margan *et al.* 1998; Trout Cod Recovery Team 2008). Captive breeding should thus fall within a broader framework of species recovery planning or ecological restoration, driven by maintaining or re-establishing sustaining populations and wild habitats (Collares-Pereira and Cowx 2004; Shute *et al.* 2005b). For example, there are numerous broader restoration actions that could match well with recovery of Southern Purple-spotted Gudgeon (e.g. Lintermans 2000; Jansen and Healey 2003; MDBC 2003; Brooks *et al.* 2004).

Useful terminology to describe different outputs of captive breeding includes:

- *Population supplementation* involves moving or releasing fish to a location where a population currently exists (e.g. to supplement genes or bolster the population number to offset threats and reach a critical mass for a sustaining population).
- *Reintroduction* involves moving or releasing fish within the natural range but where there is no extant population (e.g. re-establishing populations at historic sites).
- *Introduction*s involve moving or releasing fish outside the natural range (e.g. to farm dam refuges, but generally not into wild habitats).

Given likely extinction in the wild in the southern MDB, *M. adspersa* will need to be either reintroduced to wild sites within the former range or introduced to *ex situ* refuges. Stocked fish should ultimately be physically, behaviourally and genetically similar to those in the wild. Consequently there are particular production, ecological and behavioural considerations regarding the size, release method and numbers of fish that could be stocked, and their subsequent survival, as well as practical and legal considerations regarding implementation of reintroduction. These topics form the foundation for subsequent report sections.

2.2 Conservation objective

The working aim or recovery objective for Lower Murray Southern Purple-spotted Gudgeon should be to: **trigger immediate action for the transfer of fish from a well managed captive breeding program into less intensive, and more meaningful, wild conservation sites.** Specifically this should involve:

1. Restoration or adaptation of the last known wild site (Jury Swamp) to again be suitable for a sustaining population under different future hydrological/environmental scenarios.
2. Selection and restoration of three suitable historic sites as locations for reintroduction with ongoing stakeholder support.
3. Selection of three to five *ex situ* sites for refuge populations.

These specific reintroduction objectives fall within broader recovery planning for threatened species at the MDB level in the Native Fish Strategy (MDBC 2003), recovery objectives for the species in South Australia detailed in the draft SA Action Plan (Hammer *et al.* 2007), and planned actions of the current DEH Drought Action Plan in South Australia. Recovery plans for New South Wales and Victoria are still to be developed.

From a top-down perspective, the prime regional conservation/restoration objectives should be to improve environmental flows and management to broadly protect water levels and natural variability across release sites, which combined with habitat restoration could allow for natural recruitment and expansion towards a regionally sustaining meta-population.

2.3 Implementation of this Plan

This plan is designed to provide the necessary information on the production of fish in captivity for reintroduction, but importantly aims to detail the site-based requirements for reintroduction including targeted sites as *in situ* and *ex situ* refuges. Urgency for the uptake of recommendations comes from the potential short lifespan of the wild broodstock, the immediate availability of fish for reintroduction, and to capitalise on current investment and resources into captive breeding and other conservation planning.

There are limited specific drivers for the implementation of recovery objectives for regionally threatened fishes such as Southern Purple-spotted Gudgeon in the southern MDB (legislative focus is on nationally listed species or mitigating impacts), however specific legislation and policy aims to prevent species loss and protect biodiversity. Hence there is a target group of stakeholders that should drive the initial reintroduction process in South Australia:

- DEH: no species loss policy, threatened species conservation and Drought Action Plan
- DWLBC: implementing the River Murray Act 2003 and water licensing
- SAMDBNRMB: regional NRM Plan
- PIRSA Fisheries: protected species under the *Fisheries Management Act 2007*.

The SA DEH Drought Action Program in particular provides a strong framework for coordinating implementation, having aligned conservation goals, strong cross-agency participation, existing field monitoring, successful on-ground works program, and an *ex situ* refuge program. Nevertheless, the Southern Purple-spotted Gudgeon is a key ecological asset associated with broader preservation and restoration of biodiversity, fish communities, environmental flows and habitats, and accordingly the Reintroduction Plan could be incorporated within many projects and initiatives including:

- the Basin Plan
- the MDB Native Fish Strategy
- The Living Murray initiative
- Murray Futures
- River Murray and Eastern Mount Lofty Ranges water allocation plans
- natural resource management biodiversity and wetland programs
- proposed Wellington weir/management of reach downstream of Lock 1
- community group involvement in wetland management, monitoring and local action.

3. Hatchery production

This section is designed to consolidate and streamline hatchery operations, and thus reviews key issues for a successful captive breeding and release program.

3.1 Hatchery descriptions

Holding facilities were initially assembled *ad hoc* in response to urgent conservation measures (rescue), but more recently, two small tailored hatcheries have been developed to suit the target species and number of broodstock available. The development of twin hatcheries, run collaboratively between Aquasave and Native Fish Australia (SA) has been funded by the Murray–Darling Basin Authority, and has ongoing maintenance support through the DEH Drought Action

Plan. Another five broodstock are maintained by Native Fish Australia (SA) separately to spread risk.

All locations are temperature controlled and utilise glass aquaria for broodstock. Tanks are clearly labelled to identify individuals (permanent marker), and a log book records details of all hatchery activities. An OH&S Manual was also developed for the Aquasave/NFA(SA) hatcheries. Maintenance involves 20–30% water changes at weekly to fortnightly intervals depending on feeding regime, temperature and tank condition (e.g. more frequent during summer breeding events).

Aquasave hatchery in Adelaide

3.2 Broodstock

As of May 2009, 58 wild fish are maintained in the three locations, comprising two main hatcheries in Adelaide (n = 36 broodstock) and Berri (n = 17), and a smaller setup also in Adelaide (n = 5). Broodstock represent a range of sizes as a good demographic mix of the former wild population, with some larger fish in the program (i.e. >80 mm at time of capture) presumably already quite old (see Hammer 2007b, Appendix 1). The captive population has a matching skewed sex ratio (2:1 in favour of males), reflecting limited females in the wild at the time of rescue. As part of hatchery records maintained by Aquasave, each fish in the breeding program has an individual code which matches back to a database that records current captive location, wild collection date/location/size group, sex, and disease history (example output in Appendix 1).

Two issues relating to varying size of individuals include longevity of broodstock and pairing for breeding. General life expectancy in captivity for *Mogurnda adspersa* is 5–7 years, but has been known to extend to 10 years (MH pers. obs; D Gilligan pers. comm.). Already large (old) fish may have a short period whereby they can contribute offspring/genes. Fish with pre-existing disease or other health limitations may also make a limited contribution to offspring/genes. The second issue relates to the success of spawning and health of fish. For example, a mismatch in size in a breeding pair can create problems with aggression and result in subsequent damage or stress to one or both fish, potentially triggering the onset of disease. Furthermore, some individuals are naturally more aggressive and thus temperament can also influence possible pairings. The imbalance of males in the broodstock reduces the effective population size represented (see genetics), and further, females are most vulnerable to aggression during breeding.

Broodstock recommendations/procedures:

- review broodstock size, health condition and temperament across hatcheries to target match pairs for priority breeding
- given the scarcity of wild females, breeding pairs should be selected to minimise the potential for injury or stress (i.e. less aggressive or smaller males with larger females), and priority for general care should be on females
- make dedicated efforts to breed larger fish and those with chronic disease (see later)
- place a priority on raising small numbers of these fry (n = 20) to form replacement broodfish when required
- fry rearing should also target first generation (F1) females from older pairs to be used as additional female broodstock to improve sex bias.

3.3 Breeding protocol

As a popular aquarium fish, the breeding of *Mogurnda adspersa* including MDB populations is well established, and it is relatively straightforward to produce moderate numbers of large larvae (Blewett 1929; Llewellyn 1971; Merrick and Schmida 1984; Briggs 1998; Llewellyn 2006). Of particular relevance is a breeding program for northern Basin Southern Purple-spotted Gudgeon at the NSW Fisheries Narrandera Hatchery, where fish are produced for reintroduction. The general breeding protocol for the SA hatcheries has been developed based on the combination of literature, correspondence and visitation with the NSW program, and experience from the initial stages after rescue from the Lower Murray.

Hatchery rooms are temperature controlled, and large aquaria (200 L) house pairs for spawning. Fish can be spawned on demand during spring and summer following temperature and feeding stimuli (increases). Spawning substrate is provided in each tank (firm objects such as slate) and males and females are kept separately outside of spawning. Colour intensifies and darkens during spectacular courting displays before pairs choose a nesting site. Between 200–1,300 adhesive eggs are attached to solid surfaces which the male guards and fans until semi-pelagic larvae hatch after *c*. 10 days (Gale 1914; Blewett 1929; Llewellyn 2006). Successful spawns are allowed to hatch in the tanks and then larvae can be siphoned off after hatching for direct rearing on *Artemia* or stocked into grow-out tanks or ponds (most parents do not appear to predate heavily on fry, but fry should be siphoned off within 24 hours).

Male Purple-spotted Gudgeon from the Lower Murray (LM13) guarding eggs in captivity

3.4 Juvenile production

Three general scales of rearing appear useful in the captive breeding and release program.

Small scale intensive rearing: typically undertaken within the hatchery in 100 L tubs with gentle air filtration (temperature controlled). Fry are fed live foods such as *Artemia* and microworm for 1–2 months before being weaned onto aquaculture diets. This is ideal for growing batches of fry to around 20 mm before alternative arrangements need to be made. The intensive technique has so far provided fish to transfer to larger aquaria (hatcheries) or other locations such as the Adelaide Zoo, Cleland Wildlife Park (displayed as part of the Drought Action Plan) and Alberton Primary School (funded by Waterfind) for selective grow out of individuals as backup broodstock. It is resource intensive to produce large numbers of fry this way, but has advantages in being very effective so may be considered for producing fry to stock into Aquatanks.

Aquatank production: recently three 5,000 L Aquatanks have been added to the hatchery facilities with the purpose of scaling up juvenile production (currently being set up). These will be outdoor and semi-insulated with poly houses (winter) and shade cloth (summer) to provide conditions for year round growth, and will aim to rear fish either from newly hatched larvae or provide on-growing of weaned juvenile fish. These are an expansion on 200 L outdoor tubs which successfully reared smaller numbers of juveniles, and the 5,000 L tanks are very much prototypes to develop the best strategy and capacity for larger production. The medium-term vision is to develop a simple module system as a kit supplied to other volunteers and school groups who can have involvement and ownership for production and release. Key issues to be tested in the prototypes are:

- timing of introduction (larvae or juveniles) and fish density
- feed type, succession and strategy
- environmental conditions such as temperature and filtration
- mimicking wild conditions (to avoid adaptation to culture – see training later)
- production time (links to release techniques – see later).

Extensive rearing: pond rearing is undertaken at NSW Fisheries Narrandera Fisheries Centre. This involves earthen ponds (25 x 20 m, 1.5–2.0 m deep = 0.6–0.8 ML) with a good penstock/drain for easy retrieval of fish. These are filled with local river water (Murrumbidgee) two weeks prior to larval introduction. A hay bale is added to initiate the succession of zooplankton (e.g. Culver and Geddes 1993; Ingram 2009). Ponds also develop dense aquatic vegetation growth which would form the base for productivity of larger food items. Irregular small water exchanges are performed. Using this approach, 3,000–4,000 larvae stocked in spring record growth of 40–50 mm in 3 months, with good survival (~60%). A set number of larvae from each of 10–20 spawning pairs are stocked to ensure an even genetic contribution by individuals to a particular batch, with a stud book used to vary broodstock pairs. Overall, two batches of ~2,000 fish can be produced during the warmer months (Sep–Apr). Control/harvest of yabbies is recommended to reduce physical damage to fish, and bird netting is also considered essential. A downside appears to be winter stress, as allowing fish to over-winter in the ponds has a major impact (reduction) on harvest.

This example suggests that it is possible to produce fish for reintroduction at a large scale with fairly standard aquaculture techniques. There are currently no dedicated fisheries hatcheries with similar setup in close proximity to the SA hatcheries to produce fry in this way, but collaborations could be formed with NSW given their expertise. A surrogate refuge program within the Drought Action Plan may also have potential in this regard. An interesting alternative may be to develop *in situ* fry rearing wetland sites within the natural range, for example in managed wetlands or constructed ponds on Lower Murray swamps, which could act in a similar way to the Narrandera Aquaculture ponds. These would require very little water, could directly seed nearby sites, and be supported by strong community involvement.

Narrandera Fisheries Centre

Top: Aquarium room hatchery

Bottom: pond used to produce sub-adults for stocking

3.5 Disease & health issues

Because broodstock were sourced through a reactive program when wetland conditions were already sub-optimal, many fish were in poor health when collected (Hammer 2007b). Two noted parasites, the parasitic copepod *Lernaea cypracea* and a unidentified fish leech, were easily controlled in captivity by parasite medication and tank sterilisation. However, an aggressive external and internal fungus was apparent in many fish and continues to be an issue in hatchery management as some fish show chronic or occasional symptoms. Symptoms often manifest initially as small lumps under the skin or gills/stomach. These then break open to form ulcers which are susceptible to secondary fungal or bacterial attack. Definitive results are difficult given the priority on keeping broodstock alive (i.e. fish need to be sacrificed for accurate determination). However, initial fungal culture and histopathology by Vetlab and current advice from PIRSA Aquaculture Division based on provision of two infected wild fish indicates the cause to be epizootic ulcerative syndrome (EUS). EUS is a notifiable disease caused by the fungus *Aphanomyces invaderis* (actually more technically a water mould). Secondary infection is likely to include the fungus *Saprolegnia*, with quite early observations of this affecting Lower Murray fish in captivity at low temperatures (Blewett 1929).

Treatment has helped to manage but not eliminate the problem, and it is currently an extra burden on maintenance rather than a major threat to broodstock (i.e. chronic, low severity, but present). The general technique for control involves: (a) mild preventive treatment of fungal spores with disinfectant of equipment (bleach) and low doses of malachite green, (b) maintaining the general health of fish to promote their natural immune system, (c) increasing natural aspects of tanks including gravel and aquatic plants to improve point ‘b’ and allow growth of general microbial fauna to out-compete or potentially combat fungus, and (d) when lesions appear, begin treatment with malachite green, and supplement with antibiotics in severe cases – currently with Tetracycline but possibly Neomycin in the future (see Lilley and Inglis 1997). Managing the condition and stress (and hence disease) in females is critical given the small number of this sex represented in broodstock.

In general, aggression is quite high toward other individuals, and requires adequate space and separation, and careful management and observation of spawning pairs. The only other major health concern encountered was heat stress at the Berri Hatchery during a prolonged period of elevated temperatures (above 45 °C) in January 2009. The MDBA recently funded temperature control for this facility, which should eliminate future problems of this type. The jumping ability of the species has resulted in two mortalities during the two years of the program and is reported as the greatest cause of death at the Narrandera Hatchery. Management for jumping involves taping tank corners, and strict replacement of lids and minimal gap between lids at edges.

Recommendations for hatchery disease and health management:

- Seek further advice on the primary disease carried by wild Lower Murray fish, and overall review of EUS in the MBD. This will be aided by specific funding to engage expert input.
- Undertake subsequent research to assess the disease’s distribution, with specific references to areas upstream of Lock 1 through to Barmah Lakes. This research will provide feedback relating to upstream reintroductions.
- Check for transferability, presence of or susceptibility to the fungus in captive reared juveniles.
- Maintain fish health and hygiene as a preventive treatment for broodstock.
- Undertake regular hatchery site checks to ensure that jumping mortalities are minimised.

Example of a broken lesion on the dorsal surface of one of the captive broodstock

3.6 Genetics

Genetic distinctiveness and diversity are a core measure of biodiversity below the species level, and play an important part in the evolutionary history and future of species. Preservation of genetically distinct populations within species should thus be the minimum aim for natural resource management and conservation programs, and preserving within-population diversity (heterogeneity) should be a long-term goal.

The genetic distinctness of the lower Murray population follows a hierarchy of (1) belonging to a distinctive Murray–Darling Basin major lineage (Faulks *et al.* 2008), and (2) representing a distinctive major genetic population (Management Unit) that occurred at least in the Lower Murray between Murray Bridge and Cardross Lakes, possibly representing a broader single unit which occurred across the southern Basin (Hammer 2008b). Importantly, the wild Lower Murray population was classified as native, being distinct from a translocated population occurring in the nearby Army Range wetland sourced from northern Basin fish (Hammer 2008a; Hammer 2008b) (Figure 4). The within-population diversity of the Lower Murray population was gauged during rescue by testing 20 fish that died from existing disease conditions, and identified a reasonably high level of gene diversity or heterozygosity ($H_O = 0.041$).

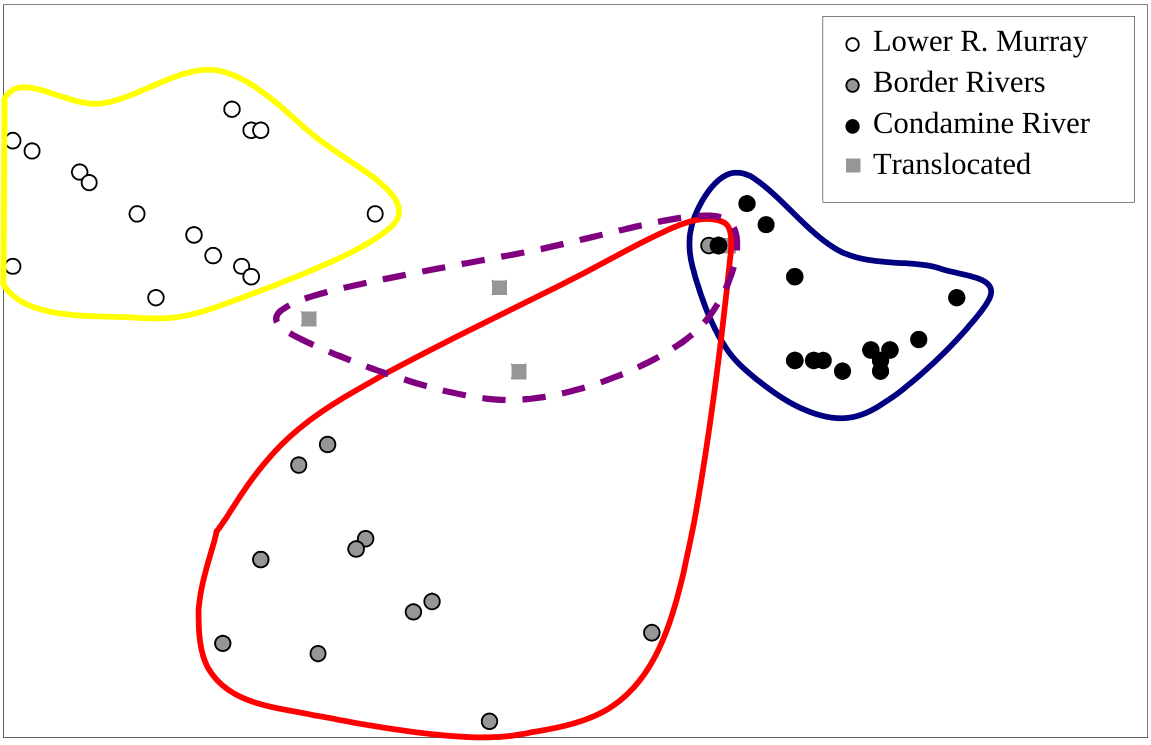


Figure 4. Visual summary of nuclear genetic information for fish from the Murray–Darling Basin

Principal Coordinates Analysis of 74 individuals, indicating the distinctiveness and diversity of the Lower Murray wild population to those in the northern Basin and the translocated population at the Murray Bridge Army Range (Hammer 2008b).

Unique forms of genes and high heterogeneity bode well for adaptation to local conditions at reintroduction sites, and imply that a standalone breeding program involving Lower Murray fish is suitable (i.e. no need for gene enhancement or mixing from northern Basin fish). Thus the primary issues for genetic management of the Lower Murray population are to maintain a representation of the wild population to protect unique genetic elements, and not to undertake mixing with northern Basin fish. In terms of maintaining within-population diversity, key considerations or risks include: the overall contribution (parentage) of older fish to the captive population – some younger broodstock could easily be juveniles of older broodstock given the small size and number of fish in the wetland where rescue occurred (Hammer 2007b); representation of fish from different parts of the wetland; the low number of females; and that not all possible male–female combinations are possible, due to differing size, temperament and potential of stress and injury to the few females held.

There are genetic techniques for the management of small numbers of breeding adults (e.g. microsatellite markers), which can identify individual relationships such as parentage and key individuals to breed from to maximise genetic diversity (e.g. Goldstein 1999). Developing markers and testing broodstock and offspring to develop a tailored breeding program thus represents an ideal research project.

Genetic management recommendations:

- Keep Lower Murray fish as a standalone breeding program.
- Preserve the genetic signatures of older fish within backup F1 juveniles that can be brought back into the breeding program.
- Fish were sourced from the north and south of the wetland: maximising the number of north–south crosses should minimise potential inbreeding.
- Use older pair crosses to bring in new F1 females to breed with smaller wild males.
- Rotate spawning partners between breeding events as best as possible.
- Allow even contribution to new populations from as many captive broodstock as possible, but with priority on older fish (e.g. NSW Fisheries collect a set number of fry from their spawning, which are mixed together and stocked into rearing ponds).
- Using allozyme electrophoresis, obtain random samples of juveniles prior to release, or from *ex situ* populations, to compare genetic diversity with the wild baseline.
- Monitor fecundity and heterozygosity every 5 years to ensure that breeders do not suffer inbreeding depression (if so, consider genetic rescue).
- Develop markers to trace the heredity of individuals to avoid sibling or parental crosses and maximise genetic diversity by pair selection (especially if heterozygosity in random samples as above is significantly lower than in the wild baseline).

4. Release considerations

Across the world, hatcheries breed, rear and release billions of fish annually, involving at least 300 freshwater species (see Brown and Day 2002). Many of these are larger recreationally valuable species such as salmonids, or in the Australian context percichthyids, but there is an increasing focus on smaller conservation listed species. One of the major problems identified with fish stocking is the often very high mortality rates observed in the initial period after release (e.g. Molony *et al.* 2004) . A comprehensive review by Brown and Day (2002) identifies key issues and approaches to improve the likelihood of successful reintroduction, and the following section draws heavily from their review and applies this to the future release program for Southern Purple-spotted Gudgeon.

4.1 Behaviour

The key behavioural aspects for the survival of stocked fish are the ‘ability to eat and avoid being eaten’. Fish are often necessarily reared on artificial diets due to the cost, limited supply and potential disease risk of wild foods, but this potentially reduces foraging efficiency (feeding) in the wild. Fortunately, captive reared Lower Murray Purple-spotted Gudgeon appear to have voracious appetites and readily consume commercially available live food when offered. Therefore in theory they could be efficiently trained to take the types of food items they are likely to encounter (dependent on location, size of stocked fish and time of year).

There are three key behaviours that are important to develop if predator-induced mortality is to be reduced: (a) avoidance strategies that reduce the probability of encountering predators (e.g. avoiding dangerous microhabitats, behaving cryptically or taking on cryptic colouration), (b) predator recognition and detection, and (c) anti-predator responses (schooling, fleeing to refuge, etc.). Each behavioural response improves with experience and hence actions to improve response should be built into the rearing process and dedicated pre-release training. Review suggests that pre-release training would not have to be extensive nor time-consuming, and may only need to be initiated shortly before release, or might be achieved more simply through acclimatisation holding pens at release sites (see below). Learning can also be increased in the presence of more knowledgeable or already trained individuals (Brown and Laland 2003).

Important behavioural observation of wild Purple-spotted Gudgeon include (a) their cryptobenthic nature in dense cover, (b) strong counter shading (dark dorsal surface) and body mottling (predator disguise) and (c) observed crepuscular activity peaks (Hammer 2007b). In contrast, captive fish are typically fearless at the surface and tame easily, can be pale, and are ready for feeding at any time in the day, thus training will be essential (cf Kelley *et al.* 2005). The placement of juveniles in dark (black) rearing containers and subsequent cryptic behaviour and darkened colouration (MH pers. obs) is a positive for prospective training. Relevant predators will vary with the size of gudgeon released, being greatest for larvae (i.e. *Hydra,* large predatory macroinvertebrates, and small fish especially *Gambusia*), moderate for 20–30 mm juveniles (e.g. micro-predatory fish, turtles, birds) and lower for 40–50 mm sub-adults (e.g. redfin and golden perch, turtles, birds).

Recommendations regarding behaviour of captive bred fish:

- Continue the practice of feeding juveniles from first feed on live food for around two months, then periodically (i.e. on *Artemia* and microworm).
- Investigate the culture of likely wild prey items to introduce for feeding at different life stages (e.g. *Daphnia*, *Partatya,* mosquito larvae).
- Identify available food resources at release sites, and capture appropriate foods as the pre-release diet (i.e. tailored feed training).
- Undertake forms of ongoing environmental enrichment that allow for both ease of culture and some behavioural response (e.g. simple structure such as rock and pipe).
- Provide a period of training in dark substrate ponds that simulate structurally complex wild habitats and expected food resources for that sized fish.
- Feed fish in the early morning and evening to simulate natural behaviour.
- Provide some form of predator or scare training to promote cryptic behaviour (e.g. scaring with nets, loud noise).
- Investigate using trained fish to increase learning (but apply caution with regard to potential aggression of more seasoned fish!).
- Experiment and assess responses adaptively to find the optimal (shortest) training time and any other methods that may be suitable for trial (e.g. conditioning with predatory fish odour).

4.2 Size, numbers and timing

As a general rule, larger fish are more likely to establish after release, and they also provide a more controlled reintroduction program (e.g. can be marked for monitoring). However, plans should be matched to individual species and receiving habitats, and there will clearly be trade-offs between the size of fish released and the time in captivity (as above). Newly hatched larvae are likely to experience much higher mortality, but attract a much lower hatchery effort. There may also be examples where available wild food resources suggest an advantage for releasing larvae and early juveniles (e.g. observed period of strong recruitment at the wild site in Spring 2005: Hammer 2007b). Most wild habitats under consideration have moderate densities of other small carnivorous fishes, where predation and competition may present a barrier to establishment from larvae. At such sites, larger fish (40–50 mm) would likely present the better reintroduction option.

The number of fish stocked is relative to post-release survival rates and so is difficult to initially define. The goal is to establish self-sustaining populations from an appropriate number of individuals representing a genetic pool of the original wild population. In the latter regard, a ‘magic number’ for survival target should be 50 fish, but in reality any breeding population established can be supplemented with ongoing release to improve diversity. A 5–10% survival rate is likely to be generous for this sort of small fish, so a starting point for release number should be 500 fish per site. This should not be tackled as a single event, as the aggressive, territorial behaviour of the target species is more suited to a multiple scatter seeding, rather than the large group introduction that may be suitable for schooling species. Persistence will likely be required, for example a program in Tennessee, America, began to document recruitment 14 years after a reintroduction program began for smaller species (Shute *et al.* 2005a, b), and locally, varying levels of long-term success have been achieved with trout cod (Trout Cod Recovery Team 2008).

The critical bottleneck observed in the pond grow-out at Narrandera, via winter mortality, suggests that cool temperatures may be a key point of mortality in the southerly distribution of the species. Releasing fish just prior to winter may not allow fish sufficient time to develop feeding behaviour, predator response or general condition to over-winter. Alternatively, spring to early summer is a time of increasing food abundance and habitat availability (e.g. growth of aquatic plants). Captive fish appear to track photo-period, regressing gonads through winter (thus providing a time to rest and improve the condition of broodstock), and then come into breeding condition with increasing ambient photoperiod. Hatchery production of larvae and young juveniles matches with spring and early summer release and on-growing of larger fish which can be over-wintered safely in captivity prior to their release at the optimal time.

4.3 Soft Release

The action of transporting fish to a site and the subsequent introduction to a new environment represents a critical point of stress and potential mortality. From experience, native fish tend to go pale and flighty during transport, which would render them vulnerable under the above discussion. The term ‘soft release’ refers to the process of allowing animals to recover from transport and to acclimatise or become accustomed to local environmental conditions. The duration of soft release has traditionally been quite short with Australian fish stocking (i.e. bags carried to site with gradual water quality adjustment prior to release). However, reintroduction theory suggests that an improved version should be considered including the provision of on-site holding pens which allow adjustment free of predators, and introduction to food resources. For aggressive species like Purple-spotted Gudgeon, holding time will be a balance between adjustment and the onset of interspecific interactions. Fish translocated from established *ex situ* refuges are likely to be better conditioned for survival in the wild and require minimal training, but the same focus on soft release should be employed.

Recommended soft release protocol:

- Use dark, aerated, and stable transport containers with hiding structure (e.g. nylon wool mops).
- Consider mild doses of anaesthetics (e.g. AQUI-S) to calm fish in transit.
- Transport outside of very cool or very warm ambient conditions.
- Develop a simple holding pen to enclose or include a section of suitable release habitat (e.g. 5 mm malleable cage wire), with a shaded roof.
- Targeted pre-surveys could remove or lessen other fishes, especially predators or *Gambusia*, within cages and the general release site to allow further time for adjustment.

- After a 3–7 day period of acclimatisation, gently lift the pen at late evening to provide small exits. Remove the cage some period later.

4.4 Post-release research

Research to address survivorship and points of mortality is critical, as it facilitates assessment of optimal release size of fish, suitable release numbers, and timing of releases to achieve the reintroduction objectives. This should be achieved through post-release research into the survivorship and biology (e.g. health, growth, habitat use) and other ecosystem components (e.g. predator gut content, vegetation health).

Tagging or radio telemetry is an ideal method for investigating many of these aspects, including monitoring movements, habitat use and investigating predator nesting sites for evidence of mortalities (e.g. Ebner and Thiem 2009). The small size of the target species (<100 mm maximum size, 20–40 mm at release) effectively excludes currently available tracking methods, but technology is improving. The small size is also preclusive of individual tags, but marking with visible implant elastomer, a type of ink visible under ultraviolet light best suited to marking batches of fish, has been developed for small bodied fish (e.g. Gallagher and Hutchison 2004). An interesting option could be documenting the patterning of purple spots on the body and fins, as this displays individual patterns that, based on observations of wild collected fish are identifiable through time for at least two years, starting as sub-adult fish 40–50 mm. At the minimum, there should be marking to distinguish between stocked and wild reared fish. There have been recent advances in chemical batch marking techniques that are simple and rapid at the hatchery rearing and release scale. Osmotic induction marking trials with alizarin red S (ARS) and Calcein have a good potential for marking and field detection (Crook *et al.* 2007).

One challenge for post-release monitoring is that the cryptic nature of Southern Purple-spotted Gudgeon makes them hard to detect (Hammer 2007b) and hence inclusion of more targeted or frequent monitoring may be required. For example, at the wild site, the species avoided fyke nets and was best captured by dip-netting or with bait traps (small entrance holes) left overnight.

Recommendations for post-release research:

- Intensive periods of observation or monitoring should follow release, including investigation of day and night behaviour in cages, gut content of predators and presence/demography, condition, and habitat use (general survivorship) of released fish.
- There is a need to review research data to assess optimum holding times in release cages, and the training and release strategy.
- Long-term monitoring should be supported at release sites, with the ultimate aim at the community and habitat level to assess improvement or decline in environmental conditions and numbers of alien species (i.e. ongoing suitability or management actions to be addressed), and any changes and potential impacts to other local species.

4.5 Management of translocations

Freshwater fishes are often highly restricted in their movement patterns owing to spatially restricted aquatic environments, specialised habitat requirements, and limited mobility of adults or juveniles especially in small native fishes (Tibbets and Dowling 1996; Hammer 2001; Knight *et al.* 2009). Isolation to particular areas can have matching genetic divergence and limits exposure to particular disease and biotic interactions such as competition and predation. Isolation within particular areas can lead to genetic divergence and limits exposure to particular disease, competition and predation. Human mediated translocations can directly interfere with or override natural processes and cause threats to local or regional populations (e.g. Arthington *et al.* 1983; Crowl *et al.* 1992; Lintermans 2004). The key issues to consider include potential for genetic, disease and biological contamination (Gillanders *et al.* 2006). The objectives of this plan are considered to minimise any impacts to wild populations through translocation of Southern Purple-spotted Gudgeon, and so align with relevant biosecurity legislation and guidelines (see below). Each state has varying requirements for reintroduction, including permits, risk assessment and protocols across multiple agencies (i.e. fisheries and relevant environment agencies). There is a *National Policy for the Translocation of Live Aquatic Organisms (1999)* and different states also have separate policies under this framework.

The Southern Purple-spotted Gudgeon is phylogenetically very distinct to other gudgeon species of the Murray–Darling Basin (Thacker and Hardman 2005), so there is limited opportunity for hybridisation and genetic contamination. There are currently five species of *Mogurnda* recognised in Australia (Allen and Jenkins 1999), and two other species do occur in more remote regions of South Australia, in the Flinders Ranges and Dalhousie Springs. The recovery planning for each occurs in distinct regions (Hammer *et al.* 2007) so the potential for hybridisation between these congeners is important but has low probability.

Genetic contamination of northern and southern Basin fish is a more pressing concern. Fortunately the genetic characterisation (Section 3.6) assists with clear boundaries for where release can occur: essentially the lower Murray main channel and directly connected wetlands and anabranches between Ovens River (NSW/Vic) and the Murray Mouth. Precautions should be taken with release into the Lachlan or Murrumbidgee systems as these have significant disconnections from the main catchment and may have been genetically distinct. Similarly, although the species occurred on the western side of the Mount Lofty Ranges (Torrens and Onkaparinga Catchments) there may have been significant isolation and related genetic divergence from the Murray, and further examination of museum material would guide the suitability of current captive stock for wild projects in this region. Other Lower Murray considerations would exclude release into the wild above natural barriers, where genetically distinct and isolated populations occur (e.g. several Eastern Mount Lofty Ranges tributaries: Hammer 2001, 2004). *Ex situ* refuges such as farm dams, artificial wetlands and ponds need careful scrutiny to ensure that overflow does not enter any of the excluded areas.

Related biosecurity issues involve the release of food prey species (e.g. glass shrimp *Paratya* and small fish) into *ex situ* locations, and should follow the precautions regarding contamination above. This is so that species do not escape into a system different to their origin and to ensure that fauna established in *ex situ* refuges in isolated areas are not mixed with the differing wild gudgeon release sites as biological contaminants (Barlow *et al.* 1987; Austin and Ryan 2002; Hughes 2003).

The disease considerations follow similar lines as described above i.e. to exclude interaction of isolated regions, but have an added complexity. The disease status of wild fish (Section 3.5) needs to be further investigated to confirm if noted infections occur more widely than the Lower Murray area (i.e. below Lock 1). Certainly the parasitic copepod *Lernaea* is carried by many alien and native fish across much of the MDB (Rowland and Ingram 1991; Harris and Gehrke 1997) and its presence is of low concern (and is unlikely to be carried by juveniles for release). Likewise the unusual fish leech is no longer prevalent in captivity. The main concern is the aggressive EUS fungus, and more detail of its identification and wider distribution needs to be confirmed before any potential reintroductions upstream of Lock 1. EUS is likely to have arrived on introduced fish, but there is little specific information on the diagnosis and occurrence of the disease elsewhere in the MDB. Harris and Gehrke (1997) indicate ulcers to be relatively common in the MDB in NSW and this may, at least in part, be attributable to EUS.

Biological contamination could include impacts within local ecosystems such as predation, competition and altered native fauna behaviour. Southern Purple-spotted Gudgeon may compete with other local gudgeons for food and space, if food resources and habitat are limited, and could have a minor predatory role on juvenile native species including hardyheads (see Section 5.2) and possibly tadpoles. Again, these would be most likely in areas outside of its natural range (so limits on release as per genetics above). Reintroduction to historic sites is unlikely to pose significant impact, but should still be considered within risk assessment before release.

Recommendations on captive fish release:

- On genetic and ecological grounds, reintroduction sites should encompass wetlands along the Murray between Barmah and the Murray Mouth. Wild sites on the SA Gulf, Eastern Mount Lofty tributaries above natural barriers, and the Lachlan/Murrumbidgee should not be stocked until further genetic information is obtained.
- Disease state may limit reintroductions above Lock 1; more detailed information from PIRSA and pathology is required.
- *Ex situ* locations need to be either within the area described above or be in isolated locations that do not flow into natural waterways.
- Appropriate permit approvals and guidelines must be followed.

5. Identifying potential sites

Extinction is a complex process that may be gradual or rapid due to different species traits, population characteristics, environment and threatening processes (e.g. Angermeier 1995; Lafferty *et al.* 1999; Holsinger 2000; Fagan *et al.* 2002; De La Vega-Salazar *et al.* 2003). Without addressing the causes of extinction, reintroduction programs may ultimately fail to overcome barriers to establishment or repeat natural population losses. Therefore, developing an understanding of and managing threats is perhaps the first requirement for a reintroduction program. Then by reviewing suitable environmental conditions and biological requirements, parameters can be established for selection and restoration of release sites (e.g. Raadik *et al.* 1999).

5.1 Considering underlying extinction drivers

The Southern Purple-spotted Gudgeon appeared to undergo widespread decline across the southern MDB between the 1950s and 1980s, especially in South Australia where the last verified record was in 1973 from near Blanchetown, heralding a rapid decline of a once common species. The declines were not directly observed nor investigated, and causes and related threatening processes can only be speculated on. Intensification of river regulation, felt as altered flow regimes, coincided with the noted decline in numerous native species, and in South Australia the arrival of common carp in the early 1970s may have brought new, devastating disease or contributed to declines in required environmental requirements such as submerged macrophytes (Glover 1987; Pierce 1997; Morris *et al.* 2001; Schiller and Harris 2001; Hammer *et al.* 2007).

It is now evident that a few small population isolates were preserved for a few decades longer, but eventually faced severe local events, namely extreme habitat drying, that impacted population viability (Raadik 2001; Hammer 2007b). **Reduced freshwater flow leading to rapid and catastrophic habitat loss should be considered the primary threat at any reintroduction sites (i.e. natural flows and security of environmental water).**

Other suspected threats to the species based on observations at the wild site and for extant populations elsewhere (Hoese and Larson 1980; Wager and Jackson 1993; Pierce 1997; Morris *et al.* 2001; Pusey *et al.* 2004; Hammer 2008a) include:

- loss of natural flow variability that drives the condition of habitats and recruitment
- poor water quality (e.g. low dissolved oxygen), algal blooms and increasing salinity
- factors negatively influencing aquatic macrophytes (e.g. turbidity, carp, nitrification)
- predation from introduced fishes, especially redfin (in addition to natural predators)
- aggressive interactions, competition and predation of fry by *Gambusia*
- disease.

5.1.1 Biology

There was some information gathered about the biology of Southern Purple-spotted Gudgeon from the Lower Murray wild site prior to drying (Hammer 2007b). There are also accounts of historical habitats near the wild site and the Finniss River in the late 1960s (Hammer 2004; Doyle 2005). These offer great insight into *M. adspersa* and habitats of the Lower Finniss River during the 1920s provided by a series of observations by members of the South Australian Aquarium Society (Nettlebeck 1926; Blewett 1929; Rutherford 1991). Observations from elsewhere in the MDB are important, namely a seminal study on the Murrumbidgee River (Llewellyn 2006), brief observations at Cardross Lakes (Raadik 2001), and studies of upper Darling Catchment populations (Briggs 1998; Moffat and Voller 2002; Faulks 2003).

5.1.2 Hydrology

Water regimes at MDB *M. adspersa* sites are variable, reflecting the broad spatial distribution across differing environmental regions and climates, plus the types of habitat they occur in (e.g. upland streams in the warmer northern MBD, lowland wetlands, streams and billabongs in the southern Basin). Broad (regional) scale factors that appear/appeared important to the species include spring inundation and a summer low flow period that combine to create suitable local habitat condition and heterogeneity. For example, water rises in spring inundate the edge vegetation, including grasses, allowing fish access to additional shallow dense habitat and food resources. This benefit includes the first pulse of larvae as adults come into peak spawning with the return of warmer water temperatures. River regulation and water supply for irrigation severely affect both aspects, and most recently have exacerbated low water availability to create an overriding factor of critical thresholds for habitat loss or drying (see Figure 4). In the heavily regulated Lower Murray, the impacts of locks and weirs essentially eliminate any small scale variability or seasonality. However, the section below Lock 1 and above the barrages is exposed to prevailing south-westerly or north-easterly winds that can facilitate water movement of up to 0.5 m (Figure 5 Webster *et al.* 1997). This provided local variability, and local flushing of the wild wetland is likely to have been an important factor in the persistence of *M. adspersa* since the 1970s.

The first consideration on site hydrology needs to be the security of water at a site through dry seasons (summer/autumn) or long-term low flow periods, with a water level that is sufficient to provide permanent connectivity to required dense structure (see below). A second consideration with respect to site selection and also restoration (i.e. environmental watering and water provisions), should be the timing and duration of a seasonal pulse to inundate edge habitats or provide some level of general variability.

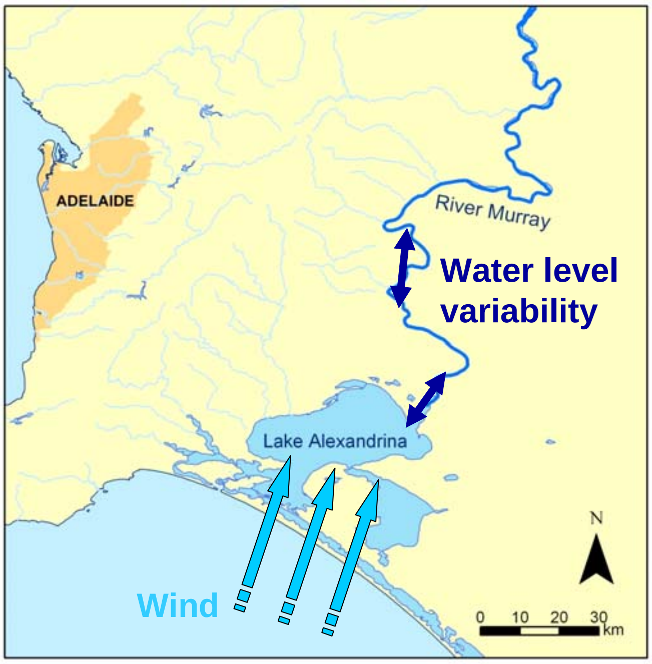


Figure 5. Schematic of the wind driven process that creates a small section of variable habitat below Lock 1 in an otherwise heavily regulated (and stagnant) Lower Murray.

Wind seiche can create natural daily changes of up to 0.5 m. Construction of a proposed weir at Wellington is likely to reduce the magnitude of this important process.

5.1.3 Habitat

The overwhelming theme of local and broader observations of habitat association of Southern Purple-spotted Gudgeon in the MDB is the presence of high levels of cover or structural integrity. The form of high cover varies, particularly between stream and wetland habitats, but primarily involves the combination of stable cover elements plus associated biological cover elements.

Stable cover elements include:

- rock such as limestone or river cobble
- woody debris (snags) and leaf litter
- tree roots
- larger solid reeds – bullrush *Typha* spp. and river club rush *Schoenoplectus validus.*

Biological cover elements include:

- submerged aquatic plants – especially ribbon weed *Vallisneria* and foxtail *Ceratophyllum*
- submerged algae and charophytes (*Chara* and *Nitella*)
- floating leaved aquatic plants – water ribbon *Triglochin*, swamp lily *Ottelia ovalifolia*
- emergent plants such as *Persicaria*
- overhanging vegetation including grasses.

Most fish from the SA wild site had a high fidelity for edge habitat, so the focus should be on a solid and complex edge habitat that interlinks with submerged cover.

Different habitat types at the wild site that should be targets for site selection and restoration:

Top: rock enforced edges with lots of crevices as habitat

Middle: dense bed of aquatic vegetation adjacent to stable cover

Bottom: inundated edge vegetation behind Schoenoplectus (bait trap entrance is just wet)

5.1.4 Food resources

Unfortunately, intensive biological study at the SA wild site was not initiated prior to habitat drying; hence an assessment of available wild prey at different times of the year is not possible. A one-off assessment at the willow habitat adjacent to the wetland in June 2009 indicated a high availability of freshwater shrimp *Paratya*, and moderate availability of other items including small fish (carp gudgeon and dwarf flathead gudgeon), *Daphnia* and *Ostracods* (Appendix 2). Casual observation identified one larger wild gudgeon to have been feeding on unspecked hardyhead, and trials on wild fish soon after capture in captivity indicated strong prey recognition for *Patatya,* small fish (especially unspecked hardyhead and sub-adult carp gudgeon), and other larger macroinvertebrates such as mayfly larvae and water boatman (Hammer 2007b). Note this mostly relates to larger adults.

Assessing remnant NSW sites on upper Darling tributaries (Faulks 2003) observed a general trend of higher macroinvertebrate diversity (family level) at sites with *M. adspersa*, with specific indicators of the presence of *M. adspersa* being higher abundances of mites, decapod crustaceans (shrimp, yabbies), snails and mussels, and damsel and dragonfly larvae. Assessment of macroinvertebrate fauna was also made within the temporary rediscovery of the species in Cardross Lakes (Raadik *et al.* 1999). Macroinvertebrate diversity and abundance was high in the general area that the few fish were recorded, being dominated by damsel flies, water boatman, backswimmers and chironomids. Summary of diet studies and observations from populations on the eastern seaboard suggests while the diet is broad and from multiple parts of the habitat (i.e. benthos, open water and surface), there is a greater reliance on aquatic insects (Pusey *et al.* 2004). Other items include terrestrial invertebrates and tadpoles.

Overall, based on available food resources and diet observations, it appears that the species has quite a generalised and opportunistic predatory feeding strategy, and as would be the case for most fishes, benefits from a greater selection (diversity) and abundance of prey items. Basic drivers for macroinvertebrate diversity and abundance include habitat diversity and type, water quality, disturbance and seasonal changes (see Ward and Stanford 1983).

Macroinvertebrate surveys to inform reintroduction assessments and monitor the availability of food for stocked fish, and also potentially as general environmental indicators for restoration, will help to identify key areas for adaptive management. Assessment of food resources at sites needs only be fairly basic to assess the general types of taxa present, with diversity within different size ranges likely important (i.e. zooplankton, aquatic insects, decapod crustaceans and small fish). A likely outcome may be the need to seed refuge sites with suitable larger prey items (especially *Paratya*).

5.1.5 Biotic interactions

In the Lower Murray, *M. adspersa* formed part of relatively diverse fish assemblages at the wild site in the early 2000s, and with stream and wetland species at the Finniss River in the 1920s (Appendix 3). They are the largest of the several gudgeon species in the region, with which they co-occurred in the same microhabitats, however the levels of competition between different species for space and food is unknown. Interestingly, there were few introduced predatory fishes in both cases (i.e. few or no redfin recorded) and this species is seen as a key threat (e.g. Hoese and Larson 1980). Both carp and *Gambusia* were present at the wild site in moderate abundance, and so some level of natural disturbance or habitat complexity allowed co-existence, particularly in regard to *Gambusia*. Natural fish predators of *M. adspersa* are likely to include predatory species such as golden perch and smaller Murray cod (*M. adspersa* was used as live bait for these species in the past), adult freshwater catfish, and congolli, although their cryptic nature and preference for shallow edges would help to avoid interactions (likewise with other aquatic and terrestrial predators).

Site selection for refuges (e.g. dams, artificial wetlands) should naturally avoid locations with introduced species or stocked larger predatory native species. When present, control of alien species should be included within site restorations, and their introduction should be avoided as high priority in areas where they do not occur.

Small fishes had abundant and sustaining populations at the wild site as an indicator of environmental health, but also as a potential key food resource for different sized M. adspersa

5.1.6 Tolerances

There is little published data on physicochemical tolerance of the species. Unpublished data from lab trials (Jackson and Pierce 1992) suggest tolerance of adults to short-term exposure to moderate salinity (~25,000 EC). However, salinities of this order are likely to have sub-lethal effects such as reduced condition, failed breeding and other indirect ecosystem impacts such as altered food resources and vegetation communities (e.g. Raadik 2001). Further, sperm and juveniles often have much lower tolerances for salinity, such that spawning and recruitment may represent more susceptible lifecycle stages. Positioning of adults in close proximity to wetland entrances in the SA wild population may suggest a preference for more oxygenated waters, or the food or habitat conditions they provide. The species handles a reasonable range of temperatures (wild site varied from 8–30 °C), but fish can become inactive and stressed by cooler temperatures (≤16 °C), and become vulnerable to disease (Blewett 1929; Merrick and Schmida 1984, pers. obs).

Captive breeding allows discovery of behavioural or life history characteristics that may constrain reproduction or recruitment of rare species (Rakes *et al.* 1999). Fish produced in captivity could allow investigation into important knowledge gaps including salinity tolerance of sperm and juveniles, spawning behaviour/success and condition of adults under different salinities, and tolerance and reaction to low oxygen levels (e.g. Whiterod and Walker 2006; McNeil and Closs 2007).

5.2 Stakeholder commitment

Stakeholder support and involvement for reintroduction will be essential, at scales from the site level to broader regional management, and across different stages of the project including:

- restoration goals and needs (e.g. Robertson *et al.* 2000)
- identification and access of potential sites
- input into assessments
- establishment of restoration initiatives and programs (and resources)
- assistance with release (permits through to activities)
- ongoing monitoring.

Raising public awareness (and hence community involvement and funding) will also be a key role for stakeholders. The Southern Purple-spotted Gudgeon is an attractive and hardy captive species, and these characteristics make it an ideal icon for the health and restoration of the River Murray and aquatic habitats in general. Indeed, several public displays have already been established, including at the new Envirodome at Adelaide Zoo and at Cleland Wildlife Park. The Alberton Primary School is involved with captive rearing and uses the species within their environmental curriculum.

5.3 Reintroduction strategy

Following the identification of a potential reintroduction site, five key stages can be recognised toward establishing a population in situ or in other refuges (Figure 6). Each is informed by specific information in the preceding sections. These are further developed Table 1 as a series of criteria and potential actions to assess or improve site suitability. Documentation of the results of assessment or actions against each criterion should be kept, and any relevant approvals and permits obtained. Ultimately, persistence at suitable sites and experimentation with release in different types of habitat will increase chances of establishing populations and provide adaptive feedback of success or failure to site selection and management.

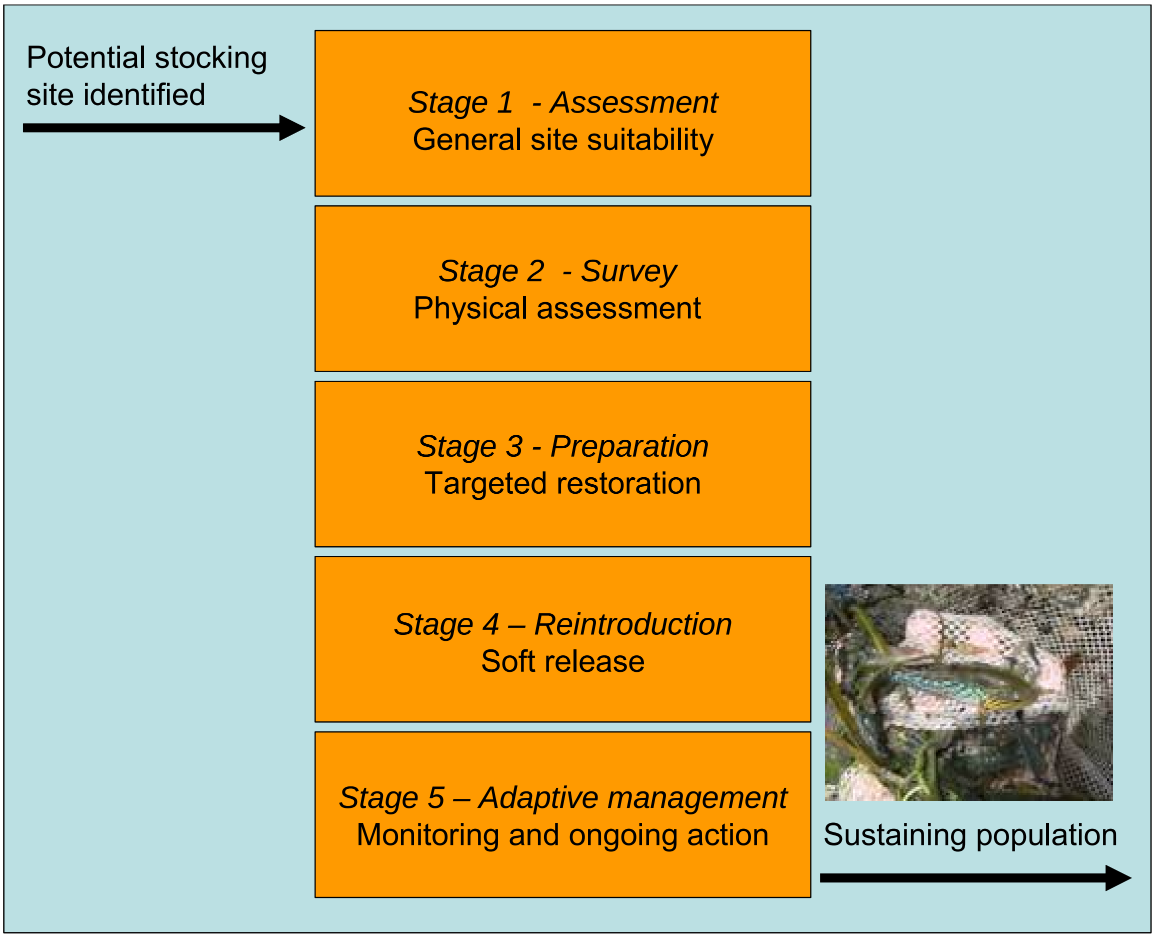


Figure 6. Breakdown of the five key stages in the process of establishing populations

Table 1. Criteria and potential actions to assess or improve site suitability as part of the reintroduction process for Southern Purple-spotted Gudgeon

Project component	Considerations	Criteria to move to next stage
Stage 1 - Assessment		
Survey of existing knowledge	General site suitability: • hydrology • location • habitat • water quality • fishes • stakeholders	 • has secure water supply • within natural range or is isolated • has good levels of cover • low salinity, nutrients and pollutants • no/few introduced fish known • commitment to species recovery/restoration

Project component	Considerations	Criteria to move to next stage
Stage 2 – Survey		
Specific site investigations	Physical assessment: • hydrology • location • habitat (broad) • habitat (specific) • water quality • fish survey • macroinvertebrates	 • spring inundation, stable in summer • confirm Stage 1 through ground truth • under appropriate management regime • high stable cover and aquatic plants • EC <7000; not anoxic; low P and N • no/few introduced fish found, or existing PSG • diversity and abundance of prey items
Stage 3 – Preparation		
Undertake any possible actions with appropriate approvals	Targeted restoration: • hydrology • habitat (broad) • habitat (specific) • water quality • introduced fishes • macroinvertebrates	 • undertake risk assessment/permit applications • secure e-water allocation/provision • fencing/restoration of riparian zone /add physical habitat, establish aquatic plants • address point source and diffuse inputs • initiate eradication/control programs • supplement missing species/habitat diversity
Stage 4 – Reintroduction		
Reduce high initial mortality	Soft release: • permits • pre-treatment • cage • acclimatisation • release	 • in place prior to release • possible reduction of fish at release site • holding cage added at selected release point/s • add fish to cage, allow adjustment period • provide exit point from cage
Stage 5 – Adaptive management		
Provide the best chance for survival	Monitoring & ongoing action: • fish research • macroinvertebrates • habitat • water quality • action	 • assess survival and changes in fish community • assess temporal trends • survey physical cover and vegetation trends • ongoing testing of key parameters • use monitoring to refine management response

6. Site assessments

Assessment of potential reintroduction sites (i.e. within the natural range) or introduction sites (i.e. refuge dams outside the natural range) followed Stages 1 and 2 of the Reintroduction Strategy and in some cases addressed Stage 3 to initiate site preparation for potential spring 2009 releases (see Section 5.4). Stage 1 involved phone or in-person interviews with land managers, and builds on and contributes to a surrogate refuge component of the DEH Drought Action Plan (some will likely be chosen as test sites within this program). Short-listed sites suitable for release were then the target of Stage 2 assessments between April and June 2009, with the best current options documented (sites are stored in a specific database: see Appendix 4). Essentially, the sites presented below represent ideal projects to be developed and taken up by groups of stakeholders.

For the presented Stage 2 assessments, site environmental measures are provided in Appendix 5. Fish survey methodology follows Hammer (2004). Macroinvertebrate surveys were a simple assessment of coarse diversity and abundance, being sampled with a standard AusRivAS 250 µm mesh dip net through the major habitats present (i.e. edge, open water, reed stems, snags), each sampled intensively for 30 seconds. Contents from the net were emptied into a white tray with most of the litter/debris discarded after shaking well to dislodge macroinvertebrates. Samples were identified and counted on site after 20–30 minutes of sorting, recording all taxa at a broad level (family generally) using a modified 'Waterwatch' catalogue (specimens were taken for later identification in some cases).

6.1 Wild site restoration

6.1.1 Jury Swamp

The obvious problem for this location as a reintroduction site is tied to the failure of water security criteria. The wetland is disconnected from the river by a water height difference of over a metre (Figure 4), with this likely to decline further this spring and beyond. The proposed Wellington Weir would stabilise the water levels to some degree (around 0.1 m AHD), but also reduce important wind seiche at the site. The basic physical cover components are still present in the wetland, but extended desiccation of macrophyte beds limits other stable cover elements, and may jeopardise the recovery of submerged aquatic vegetation. A reasonable source of small fish and macroinvertebrates is present in the edge of the main channel (Appendix 2).

A specific project should be developed to provide security of wetland habitat at the site (Hammer 2007b). This is likely to require engineering solutions such as impounding a section of the wetland then pumping into the wetland, or deepening the wetland then adding new habitat consistent to predicted water levels: the southern end is best suited to this (Figure 7). Issues include seepage through cracked clay levees, and local acid sulfate soils.

Figure 7. Arial photo of Jury Swamp, indicating the southern wetland section best suited to restoration (imagery supplied by SAMDBNRMB)

6.2 Lower Murray ready release sites

6.2.1 Lower Finniss River

The 1920s accounts of the habitat and fishes of the lower Finniss River paint the area as an aquatic paradise. Southern Purple-spotted Gudgeon were targeted from slow flowing pools with dense submerged aquatic vegetation, where they would often sun-bathe among the floating leaves of *Vallisneria* (Nettlebeck 1926; Blewett 1929; Rutherford 1991). This site is ideal for restoration for several reasons. Primarily, it has a more reliable water supply than other Lower Murray sites including summer flowing springs (i.e. Finniss River versus interstate source).

The basic habitat elements described in the 1920s also still exist – heterogeneous combination of small and large pools within red gum lined braided channels, shaded by tea tree (now *Callistemon*) and lined by reeds and rushes. The latter has been heavily degraded by stock, however there is strong landholder commitment to local restoration of one side of the stream now, and initial contact made about completing a fenced stream corridor (see green indicative line in Figure 8). Stable habitat components are still reasonable, including rocky cobble, reeds and tree roots, with small remnants of former extensive submerged aquatic plants present, including the *Vallisneria,* regionally rare *Ottelia* and *Cerataphyllum*. Perhaps the most significant change and concern is the fish community, namely the current presence of redfin, *Gambusia* and carp in different pool types

(Appendix 3). Macroinvertebrate food resources at the site were reasonably diverse and abundant (Appendix 2).

There are clearly preparation measures to be undertaken, but the assessment suggests that reintroduction to at least some of 10 release points (Figure 8) would be worth pursuing. Overall, the site presents a high prospect for a meaningful restoration program, with the added strength of providing a last refuge for biota, and future seed source, for the connected Lower Lakes.

Key restoration targets (Phase 3) include:

- Hydrology: continue prescription of Eastern Mount Lofty Ranges water resources including a focus on maintaining the duration of transition to low flows in spring and security of groundwater.
- Habitat (broad): continue fencing and general riparian restoration (may have added benefits for southern bell frog, which was also recorded here historically and may persist or could be rescued from Lake Alexandrina populations.
- Habitat (specific): increase structural complexity around key release sites with addition of local rock. Undertake restoration of submerged aquatic vegetation.
- Water quality: obtain more detailed and temporal analysis of other water quality components including heavy metals (e.g. copper).
- Introduced fishes: control programs for each species should be developed.
- Macroinvertebrates: continue to monitor changes, especially spring diversity.

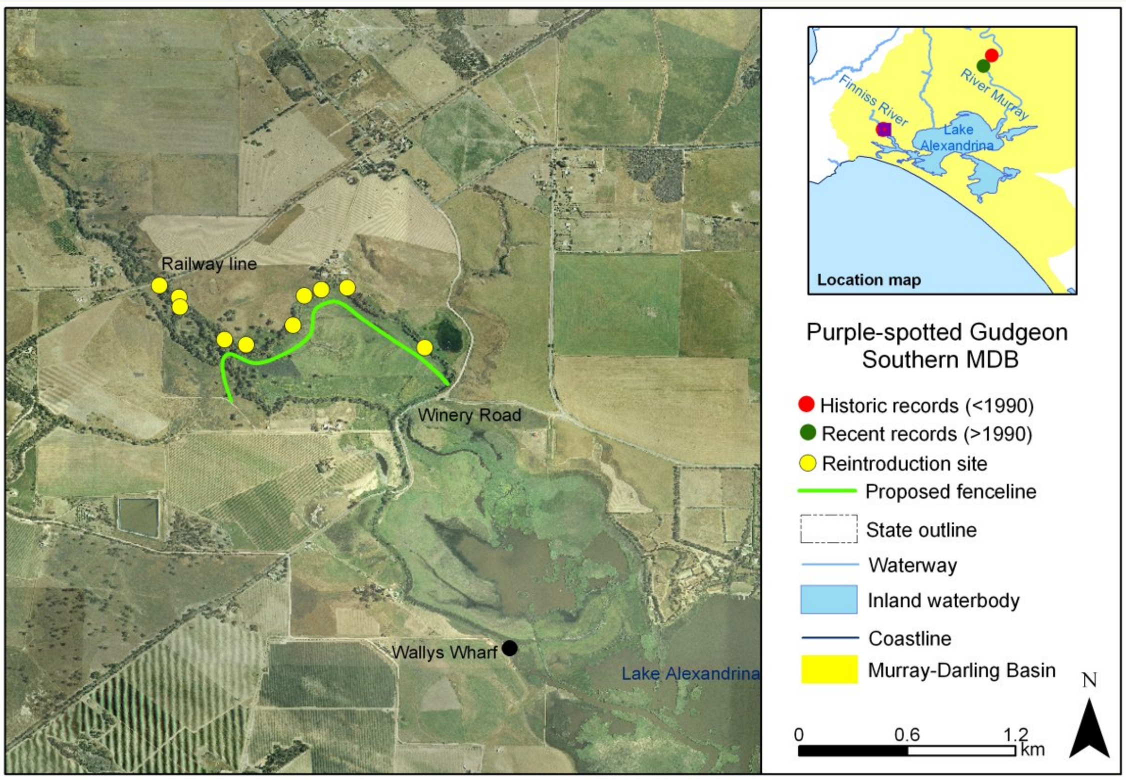


Figure 8. Location of Lower Finniss proposed reintroduction site

A representative pool within the Lower Finniss braided channel habitat

6.2.2 Piawalla Wetland

This managed wetland is effectively the only wetland habitat below Lock 1 through Wellington, receiving and holding environmental water to varying degrees since January 2007. Its importance as an *in situ* refuge for fauna and flora is very high, and securing water in the wetland through future watering is a key issue for regional conservation. Edge habitat is reasonable, with some aquatic vegetation (algae, *Myriophyllum* sp and *Crassula helmsii*) at the time of assessment. The wetland is well managed and has diverse macroinvertebrates and no introduced fish (just a few gudgeons). Piawalla is only 2 km upstream of the wild site at Jury Swamp.

At the site inspection in June 2009, it seemed ideal for reintroduction of *M. adspersa* larvae and adults (due in part to a high abundance of zooplankton), as well as other species such as Murray hardyhead. The key issue relates to future water security, as last year due to a lack of water supply, the wetland contracted significantly and became quite saline. Other improvements could be made including addition of edge rock sections and snags. Overall reintroduction of Southern Purple-spotted Gudgeon should be incorporated into planning, management and restoration of the wetland.

Piawalla wetland, June 2009

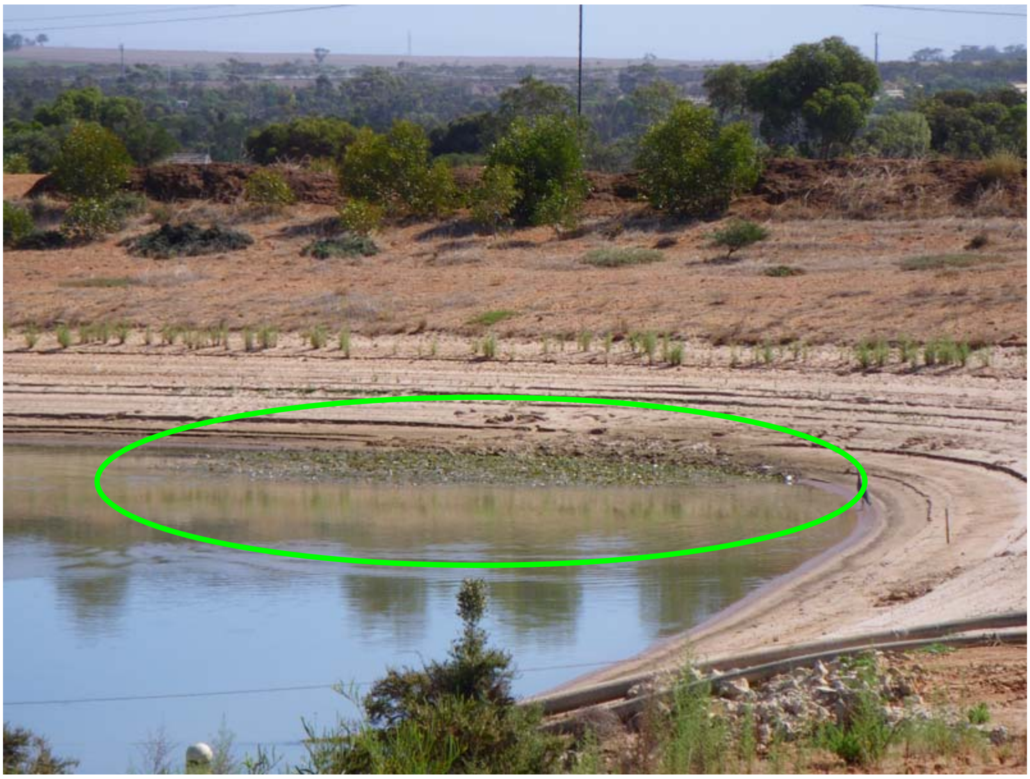
6.3 Refuge locations

No refuge is likely to be an ideal match for wild habitat, and certainly only a handful of dams met the broad criteria for serious consideration. Another wetland being developed at Warradale School has future potential. A brief description of four refuges as ready release sites is included.

6.3.1 Murray Bridge stormwater wetland

This is the closest refuge found to the wild site and has several positive aspects. Firstly, it is under supportive and proactive management by the Murray Bridge Council and is in a secure (fenced) site. Water permanency appears secure even through the recent dry period and is fresh (300–500 µS), but quite turbid after rain (overall similar to the wild habitat). The site has only recently been established, however *Vallisneria* and *Potamogeton ochreatus* is already becoming established to supplement edge planting and rock habitat installed by the Council. Successful establishment of stocked flathead gudgeon and carp gudgeon suggest the general suitability of the site for fish (no exotic fish are present), and a base food resource for larger *M. adpsersa*. The site could be enhanced by:

- addition of simple rock piles extending as longitudinal transects, to provide cover at varying water heights (some variability has been noted)
- further establishment of submerged aquatic vegetation
- addition of shrimp from the Murray (food resource).



Murray Bridge Stormwater Wetland (Vallis circled)

6.3.2 Leigh Dam

A small, spring fed and heavily vegetated dam near Dawesley, unlikely to overflow into natural waterways due to the Brukunga Mine arrangements. The dam is fenced and managed for conservation. It has abundant carp gudgeon (stocked) and some shrimp, with a reasonable abundance of macroinvertebrates (Appendix 2). There are a few larger catfish, which the owner has offered to remove to aid establishment of stocked fish, and areas of rock could be added. The dam was moderately saline in autumn (EC 4.72 mS), but overall release should be trialled with minimal restoration works required. Stocking larger *M. adspersa* is recommended.

The small spring fed dam, near Dawsley, owned by Chris Leigh

6.3.3 Munday Dam

This site is in upper Reedy Creek, and the dam and indeed the sub-catchment have no fishes present. This dam is spring fed and has thick aquatic plants (including *Vallisneria* and a filamentous algae) and abundant food resources. The site was noteworthy in June 2009 for its extremely high *Ostracod* density as well as the presence of *Daphnia* and some larger prey items. Again, this water was slightly saline (EC 4.44 mS in June) and the site could be improved by the addition of sections of rock extending from reed edge cover. However, it is basically ready for stocking any sized *M. adspersa*.

The Munday Dam as viewed from the small jetty. Note the presence of submerged aquatic plants that would provide habitat for small fish and macroinvertebrates.

6.3.4 Beyond Today residential development wetland

This site is a recently developed but established stormwater wetland in an isolated sub-catchment (housing development) just outside the MDB near Victor Harbour. It has all the basic habitat elements, and vegetation is continually improving. It was slightly saline in autumn, but this may improve as the local disturbance settles. This wetland site could be improved with addition of local *Paratya* to increase the weighting of larger prey items and perhaps addition of pipes, snags and other habitat elements to increase habitat heterogeneity.

Further assessment in June 2009 indicated ECs ranging from 2.2–5.64 mS across the three pools sampled. This wetland supported a moderately diverse macroinvertebrate assemblage, with very high densities of *Ostracods* and *Daphnia* in all three pools as well as a reasonable abundance of larger prey items such as damsel fly larvae (particularly in the top pool). The Beyond Development Wetland would appear to be ready for stocking with juvenile or larger fish with the proviso of the improvements recommended above.

The top pool of the Beyond Today residential development wetland

6.4 Lower Murray restoration sites

Small projects to develop sites with secure water using existing infrastructure or simple new structures may provide ideal reintroduction sites. The aim with this type of site is to link into existing projects and restorations, and perhaps provide a renewed focus for other planned initiatives. There are likely to be numerous locations that could be assessed and developed by stakeholders using the simple steps and criteria of Figure 6. Breakdown of the five key stages in the process of establishing populations

Table 1.

As an example, a small channel running adjacent to that at Rocky Gully wetland (on the eastern side of the main levee) is a known historic site from the 1970s (Doyle 2005). In 2007, the site still had very similar habitat conditions including dense submerged vegetation and reeds, but has since dried. There is currently a bank on the Rocky Gully outlet drain to improve water security, with environmental water pumped from the Murray over the levee. Given the very close proximity of the two drains, the small dry site could easily become a small restoration site by adding a bank at the river end and filling using existing watering infrastructure (very little water required).

Small channel running alongside Rocky Gully Wetland outlet channel – the green circle indicates the temporary levee and pumping infrastructure in very close proximity

6.5 Release sites upstream of Lock 1

The longer-term aim of species recovery should be to restore the natural range by extending reintroductions above Lock 1. This will have the key advantage of protecting the species from critical conditions being experienced in the lower river reach, but will presumably require sites that retain some level of variability (can be tested by trial and error). As highlighted in Section 4.5, further research into the distribution of the EUS carried by broodstock from the wild is required prior to any reintroductions above Lock 1.

There is unlimited potential, with some ideas for further development including:

- The renowned Banrock Station has the ability to manage water inputs and levels, and initial discussion suggests a smaller pool near the inlet could be developed to provide refuge during dry phases, which might then reseed the larger wetland or exit channel. An effective carp management program and water regimes promoting dense aquatic vegetation growth should be implemented.
- Other managed wetlands either as sustaining habitat or *in situ* aquaculture habitats (e.g. Acoona Station, Little Duck Lagoon and Brenda Park).
- Berri Marina is an easily accessible site with reasonable edge structure (more could be added), extensive aquatic plant growth, and diverse and rich food resources. This wetland is not managed.
- The Chowilla Icon site could provide opportunities for broader restoration works to suit the species, including controlled wetlands that may act as aquaculture rearing ponds.

- Kings Billabong, Victoria, was assessed as a reintroduction site in 1999 and shown to have a good potential based on secure water regime, abundant edge and submerged macrophyte cover, and diverse and abundant food resources (Raadik *et al.* 1999). Redfin were present. A more recent assessment is in preparation (Murray–Darling Freshwater Research Centre, Mildura) and opportunistic visits in 2008 (MH) suggest it to still be an ideal candidate site.
- A smaller site (Sandilong Creek) in the Riverside Golf Course at Mildura also shows high potential (Raadik *et al.* 1999).
- Potential reintroduction sites in NSW include Washpen Creek (a refuge currently maintained for freshwater catfish) and Thegoa Lagoon (D. Gilligan pers. Comm.).

Boardwalk at Banrock Station wetland

Kings Billabong, Victoria

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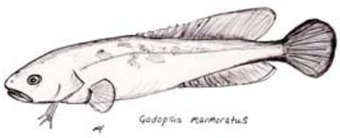

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9. Appendices

Appendix 1. Details of captive wild broodstock

Hatchery	Fish_#	Sex	Locn	Wild in	07 age
SARDI	LM01	Female	South	26/02/2005	4+
SARDI	LM02	Female	South	07/11/04	3+
SARDI	LM04	Male	South	07/11/04	3+
SARDI	LM05	Male	South	07/11/04	3+
SARDI	LM06	Male	South	07/11/04	3+
Aquasave	LM07	Male	South	Jan-07	4+
Aquasave	LM08	Female	South	Jan-07	4+
Aquasave	LM09	Male	South	Apr-07	4+
Aquasave	LM10	Female	South	Apr-07	4+
Aquasave	LM11	Male	South	Apr-07	1+
Aquasave	LM12	Female	South	09/04/07	3or4+
Aquasave	LM13	Male	South	Jan-07	2+
Aquasave	LM14	Female	South	Jan-07	2+
Aquasave	LM15	Male	North	09/04/07	2+
Aquasave	LM16	Female	South	Jan-07	3+
Aquasave	LM17	Male	South	Jan-07	2+
Aquasave	LM18	Female	South	Jan-07	2+
Aquasave	LM19	Female	South	Jan-07	2+
Aquasave	LM20	Male	South	Apr-07	0+ or 1+
Aquasave	LM21	Female	North	09/04/07	1+
Aquasave	LM22	Male	South	Apr-07	0+ or 1+
Aquasave	LM23	Female	South	09/04/07	4+
Aquasave	LM24	Male	South	Apr-07	0+ or 1+
Aquasave	LM25	Female	South	Apr-07	0+ or 1+
Aquasave	LM26	Male	North	09/04/07	0+
Aquasave	LM27	Female	North	20/01/08	0+
Aquasave	LM28	Male	South	Apr-07	0+ or 1+
Aquasave	LM29	Female	South	Apr-07	0+ or 1+
Aquasave	LM30	Male	North	Apr-07	0+
Aquasave	LM31	Female	North	Apr-07	0+
Aquasave	LM32	Female	South	Apr-07	0+
Aquasave	LM33	Male	Levee	Apr-07	0+
Aquasave	LM34	Male	North	09/04/07	1+
Aquasave	LM35	Male	North	09/04/08	1+
Aquasave	LM36	Male	South	Apr-07	0+ or 1+
Aquasave	LM37	Male	South	Apr-07	0+ or 1+
Aquasave	LM38	Male	South	Apr-07	0+ or 1+
Aquasave	LM39	Male	South	Apr-07	0+ or 1+
Aquasave	LM40	Male	South	Apr-07	0+ or 1+
Aquasave	LM41	Male	South	Apr-07	0+ or 1+
Aquasave	LM62	Male	South	26/03/09	0+
Berri	LM45	Female	Levee	Apr-07	2+
Berri	LM46	Male	South	Apr-07	4+
Berri	LM47	Female	South	Apr-07	4+
Berri	LM48	Male	South	Apr-07	4+
Berri	LM49	Female	South	Apr-07	3+
Berri	LM50	Male	South	Apr-07	4+
Berri	LM51	Female	South	Apr-07	4+
Berri	LM52	Male	South	Apr-07	3+
Berri	LM53	Male	South	Apr-07	3+
Berri	LM54	Female	South	Apr-07	3+
Berri	LM55	Male	South	Apr-07	3+
Berri	LM56	Female	South	Apr-07	3+
Berri	LM57	Male	South	Apr-07	0+
Berri	LM58	Male	South	Apr-07	0+
Berri	LM59	Male	Levee	12/02/09	0+
Berri	LM60	Male	Levee	12/02/09	0+
Berri	LM61	Male	Levee	12/02/09	0+

Appendix 2a. Macroinvertebrate & water quality data – Murray and refuges

Site	Wild site	Piawalla	Leigh Dam	Munday Dam	Beyond Dam
Date	4/06/2009	4/06/2009	23/05/2009	22/06/2009	17/06/2009
Zooplankton					
Seed shrimp (ostracod)	18	300		4400	5000
Copepod	5		300	200	200
Waterflea (*Daphnia*)	8	1000		170	6000
Macroinvertebrates					
Freshwater limpet				5	1
Pouch snail		15			19
Little basket shell					
Non-biting midge larvae	3			6	
Segmented worm					
Biting midge larvae		1			4
Leech					
Roundworm					
Flatworm					
Cranefly larvae					
Mosquito larvae					
Mosquito pupae					
March fly larvae					
Black fly larvae					
Hydra					
Soldier fly larvae					
Scud (amphipod)			10	70	300
Isopod					
Water mite		5	15		13
Fishing spider					
Water boatman		2		4	24
Back swimmer		1			10
Water strider					
Small water strider					
Water measurer					
Needle bug					
Water scorpion					
Water scavenger beetle			1		2
Predacious diving beetle	1	20	28	65	97
Whirligig beetle					
Crawling water beetle					
Stonefly nymph					
Damselfly nymph	1	3		7	207
Mayfly nymphs			1	5	
Riffle beetle larvae					
Marah beetle larvae					
Water scavenger beetle larvae					
Whirligig beetle larvae					
Dragonfly nymph		3			1
Predacious diving btl larvae					
Springtail					
Caddisfly larvae					20
Decapod crustaceans & fish					
Glass shrimp (*Paratya*)	17		1	39	
Freshwater prawn	2				
Yabby	2				
Freshwater crab					
Small fish	9		2		
Gambusia					
Water Quality					
pH	8.0	8.8	7.4	8.5	6.9
EC (mS)	0.73	2.50	4.72	4.44	5.64
Transparency (m)	0.3	0.5	0.8	0.7	0.6
DO (surface)	8.6	6.9	6.1	13.2	8.1
Temperature (oC)	15.9	14.1	12.8	10	4.48
Nitrate ppm	0	0	0	0	0
Phosphate ppm	0	0	0	<0.25	<0.25
Time	1040	1218	1430	1400	1200

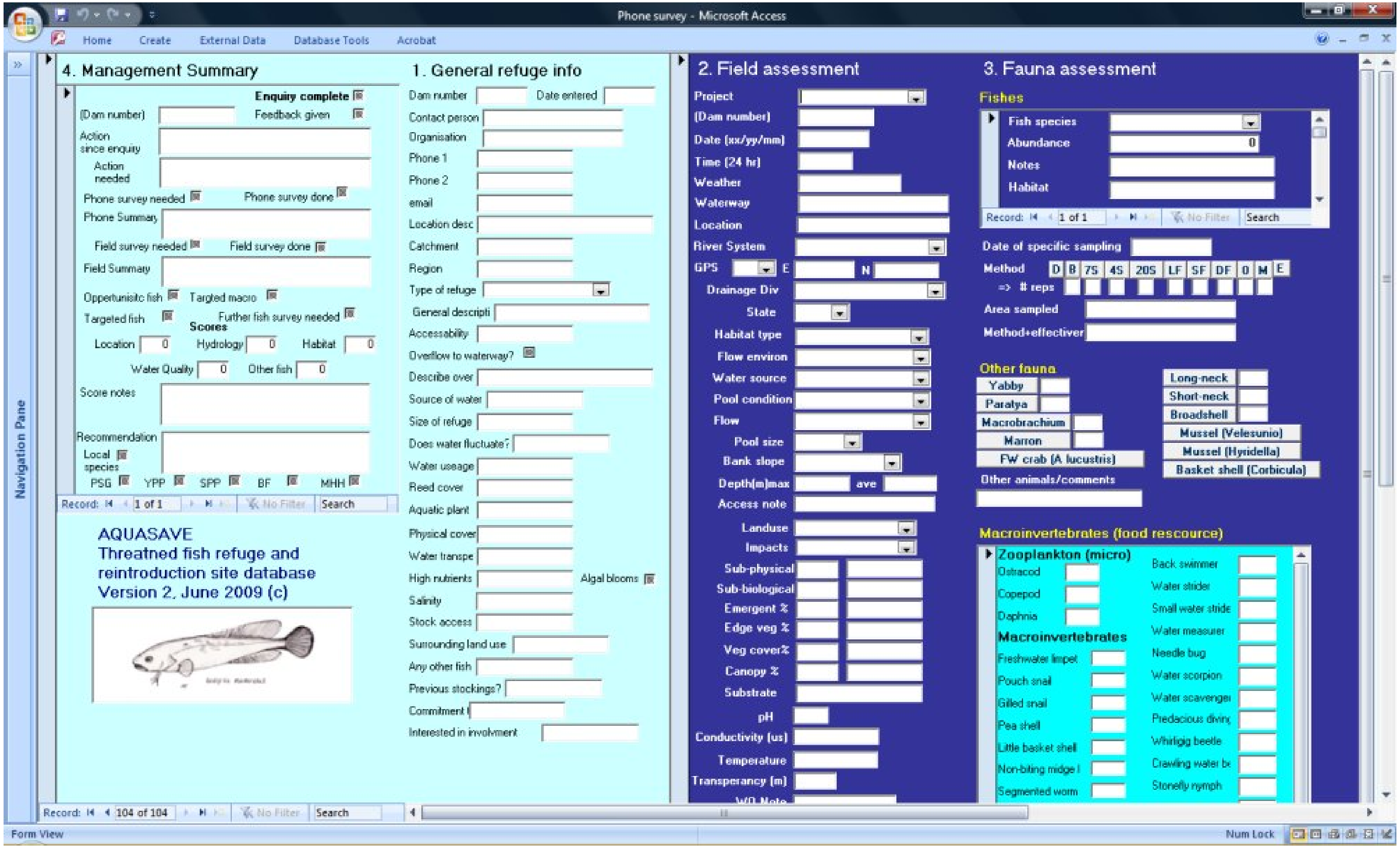
Appendix 2b. Macroinvertebrate & water quality data – Finniss River

Site	Finniss 1	Finniss 2	Finniss 3	Finniss 4	Finniss 5	Finniss 6	Finniss 7	Finniss 8	Finniss 9	Finniss 10
Date	26/05/09	26/05/09	26/05/09	26/05/09	26/05/09	26/05/09	26/05/09	26/05/09	26/05/09	26/05/09
Zooplankton										
Seed shrimp (ostracod)	50	400	20	300	300	20	10		20	20
Copepod	2		3			1			1	
Waterflea (*Daphnia*)	2					1				
Macroinvertebrates										
Freshwater limpet										
Pouch snail	1	2			2	1	4		3	3
Little basket shell										
Non-biting midge larvae	3	2		12	5	3	1	2	1	3
Segmented worm										
Biting midge larvae		2								
Leech							1			1
Roundworm										
Flatworm										
Cranefly larvae										
Mosquito larvae	1	1			1					
Mosquito pupae										2
March fly larvae										
Black fly larvae										
Hydra										
Soldier fly larvae										
Scud (amphipod)	5		10	100	5	10	50	60	20	80
Isopod										
Water mite	50	2			4	350				
Fishing spider										
Water boatman				3						11
Back swimmer		2					3		4	11
Water strider										
Small water strider		1			1	6		1	3	
Water measurer										
Needle bug										
Water scorpion										
Water scavenger beetle										
Predacious diving beetle	250	100	60	20	10	50	40	10	20	70
Whirligig beetle										
Crawling water beetle										
Stonefly nymph				10		1				
Damselfly nymph				10	7	3				30
Mayfly nymphs		400	40		6	1	5		20	
Riffle beetle larvae										
Marah beetle larvae										
Water scavenger beetle larvae										
Whirligig beetle larvae										
Dragonfly nymph			1	3						1
Predacious diving btl larvae										
Springtail										
Caddisfly larvae	4		3			12		5	1	
Decapod crustaceans & fish										
Glass shrimp (*Paratya*)	5		1							
Freshwater prawn										
Yabby				1						1
Freshwater crab	1				1					1
Small fish								1		
Gambusia		1					4	1	5	
Water Quality										
pH	7.8	7.6	7.6	7.3	7.3	7.2	7.2	7.4	7.2	7.0
EC (mS)	2.82	2.90	2.83	2.89	2.83	2.84	2.85	2.83	2.85	2.74
Transparency (cm)	50	60	60	40	60	60	80	60	25	70
DO (surface)	7.5	4.1	6.7	0.2	3.5	3.5	6.3	5.0	5.3	6.0
Temperature (oC)	13.6	13.5	13.4	13.1	13.7	13.2	14.2	13.9	13.7	13.2
Nitrate ppm	0	0	0	0	0	0	0	0	0	0
Phosphate ppm	0	0	0	0	0	0	0	0	<0.5	0
Time	1005	1100	1300	1400	1410	1415	1500	1613	1630	1700

Appendix 3. Summary of fish records at Lower Murray Southern Purple-spotted Gudgeon sites, past and present (Nettlebeck 1926; Hammer 2004, 2007b)

Common name	Scientific name	Jury swamp 2000s	Finniss 1920s	Finniss 2000s
Native species				
Freshwater catfish	*Tandanus tandanus*	Rare, juveniles in wetland	Common	Absent
Bony herring	*Nematalosa erebi*	Common wetland entrance	Not mentioned	Absent
Smelt	*Retropinna semoni*	Common	Present	Rare
Common galaxias	*Galaxias maculatus*	Rare, mainly willows	Common	Becoming rare
Mountain galaxias	*Galaxias olidus*	-	Common	Rare
Murray rainbowfish	*Melanotaenia fluviatilis*	Common	Common	Absent
Murray hardyhead	*Craterocephalus fluviatilis*	Rare, shallow habitats	Likely present	Rare, downstream
Unspecked hardyhead	*Craterocephalus stercusmuscarum fulvus*	Common wetland	Likely present	Rare, downstream
Smallmouthed hardyhead	*Atherinosoma microstoma*	-	Likely present	Rare, downstream
Agassiz's glassfish	*Ambassis agassizii*	-	Rare, wetland downstream	Absent
River blackfish	*Gadopsis marmoratus*	-	Common at times	Absent
Murray cod	*Maccullochella peelii peelii*	-	Present - larger pools	Absent
Murray-Darling golden perch	*Macquaria ambigua ambigua*	Rare, channels	Present - larger pools	Possibly present
Southern pygmy perch	*Nannoperca australis*	-	Common	Absent
Yarra pygmy perch	*Nannoperca obscura*	-	Common	Absent
Silver perch	*Bidyanus bidyanus*	Rare, main channel	Present periodically	Absent
Congolli	*Pseudaphritis urvillii*	-	Common	Rare, declining
Western carp gudgeon	*Hypseleotris klunzingeri*	Rare wetland and channel	?	Absent
Midgley's carp gudgeon	*Hypseleotris* sp. 1	Common	?	Absent
Murray Darling carp gudgeon	*Hypseleotris* sp. 3	Common	Common	Rare, downstream
Hybrid forms (e.g. Lake’s carp gudgeon)	*Hypseleotris* spp.	Common	?	Absent
Southern purple-spotted gudgeon	*Mogurnda adspersa*	Rare, mainly wetland	Common, variable w. season	Absent
Flathead gudgeon	*Philypnodon grandiceps*	Common	Not mentioned	Common
Dwarf flathead gudgeon	*Philypnodon* sp.	Moderately common	?	Common
Western bluespot goby	*Pseudogobius olorum*	-	Present	Rare, downstream
Alien species				
Goldfish	*Carassius auratus*	Rare	Not mentioned	Present
Common carp	*Cyprinus carpio*	Common incluidng lg adults	Not present	Present
Brown trout	*Salmo trutta*	-	In deeper pools	Present larger pools
Eastern gambusia	*Gambusia holbrooki*	Common	Not mentioned	Modertae in shallows
Redfin perch	*Perca fluviatilis*	-	Not mentioned	Common larger pools

Appendix 4. Database for managing data generated from different stages of reintroduction site selection and assessment



Appendix 5. Environmental measures taken at potential reintroduction site assessments

Location (description and GPS-WGS 84 datum, zone 54H): waterway, weather, land use, potential impacts and environmental characteristics were recorded for each sampling site to assist with the interpretation of results and future replication. Digital photos were taken of all sites. Environmental characteristics included details of aquatic and interlinked riparian condition under the following categories:

- General descriptors: habitat type (i.e. stream, wetland, instream dam).
  - pool size as an estimation of surface area
  - bank slope (e.g. steep = 45°, vertical = 90°)
  - depth (maximum and average)
  - substrate type (e.g. sand, gravel, mud).
- Flow environment:
  - a temporal measure of connectivity based on seasonal conditions and local landholder input (e.g. ephemeral, six months flow connection, or permanently connected), plus comments such as whether the area is spring fed
  - specific note of potential overflow was made for artificial refuges.
- Pool condition and flow:
  - a measure of water level in comparison to the normal bank level of a pool (e.g. concentrated, bank level, in flood) and recording of flow at the time of sampling, ranked relative to magnitude: low = <10 L/sec; medium = 10–100 L/sec; high = 100–200 L/sec; very high = >200 L/sec.
- Contributions to cover (% of volume occupied and type):
  - submerged physical (e.g. snags, leaf litter, rock)
  - submerged biological (e.g. aquatic plants, *Chara*, other algae)
  - emergent (e.g. reeds, rushes and sedges, tea tree)
  - fringing vegetation within two metres of the water's edge (particular note of small amphibious species on the bank such as *Crassula*, *Centella*, *Ranunculus*)
  - canopy – measure of overhanging vegetation (shade)
  - general surrounding terrestrial vegetation cover.
- Water quality:
  - TPS meters taken at 0.3 m depth recording (a) temperature, (b) conductivity (k = 10 probe, range 200–200,000 μS = μScm^{-1}), (c) pH, and (d) dissolved oxygen
  - test kits were used to assess the levels of phosphate, nitrate and ammonia
  - water transparency measured *in situ* against a white object, with comments on contributions to low values such as natural tannin, colloids or algae.