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The Critical Fish Habitat Project: Reintroductions of threatened fish species in the Coorong, Lower Lakes and Murray Mouth region 2011–13

C. Bice, N. Whiterod, P. Wilson, B. Zampatti and M. Hammer

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Executive summary

Water level recession and habitat degradation (e.g. elevated salinity, loss of submerged macrophytes) in the Lower Lakes over the period 2007–2010 placed several species of threatened native fish under risk of extirpation. Thus, the South Australian Drought Action Plan (DAP) for Murray-Darling Basin (MDB) Threatened Freshwater Fish Populations was initiated with the objective of conserving threatened fish species during this period. In some cases, this necessitated removal of individuals from the wild with captive maintenance and breeding, with the objective of re-introducing fish to wild habitats upon the return of favourable conditions.

In 2010/11, broad-scale rainfall and significant inflows in the MDB resulted in increased flows to the lower River Murray, South Australia, and improved flow and habitat availability in local stream tributaries. As such, by spring 2011 water levels in Lake Alexandrina had returned to typical regulated levels (~0.75 m AHD) and aquatic habitat (i.e. submerged and emergent vegetation) was beginning to show signs of recovery. Thus, it was deemed there was the potential for reintroduction of fish captively maintained and bred since 2007.

The Critical Fish Habitat (CFH) project was developed to provide a framework to guide and undertake reintroductions of threatened fish species to the Coorong, Lower Lakes and Murray Mouth (CLLMM) region, namely Yarra pygmy perch (*Nannoperca obscura*), southern pygmy perch (*Nannoperca australis*), Murray hardyhead (*Craterocephalus fluviatilis*) and southern purple-spotted gudgeon (*Mogurnda adspersa*) with the primary objective of re-establishing self-sustaining wild populations. More specifically, the CFH project aimed to increase the likelihood for successful reintroductions by (1) identifying potential receiving sites, (2) developing and undertaking a screening process to assess the suitability of receiving sites (e.g. presence of favourable habitat, prey resources and water quality), (3) determining a method for fish release (i.e. matching the scale of captive outputs to release site number and spatial extent, transport methods, acclimatisation, hard vs. soft-release), (4) undertaking fish releases, and (5) developing and conducting a monitoring and evaluation program to assess the success of the reintroduction.

In 2011/12, approximately 10,300 fish from the four species were released at a total of nine sites across the CLLMM region. Initial post-reintroduction monitoring noted survival of low numbers of reintroduced southern pygmy perch (n = 10) and southern purple-spotted gudgeon (n = 3), with signs of wild recruitment in southern pygmy perch. Nonetheless, further reintroductions and monitoring were required in 2012/13 to work towards meeting project objectives.

In 2012/13, a total of 19 sites were deemed generally suitable for reintroductions. These sites then underwent screening to further determine their suitability, based on a series of predetermined species-

specific criteria (e.g. defined levels of habitat cover). Fish, macroinvertebrate, habitat and water quality monitoring was undertaken at these sites in spring 2012 and summer/autumn 2013, prior to reintroductions. In spring 2012, two sites were deemed suitable for reintroductions of Yarra pygmy perch, two for Murray hardyhead and one for southern purple-spotted gudgeon. No southern pygmy perch were available for reintroductions in 2012/13. In summer/autumn 2013, reintroduction involved comparatively fewer sites and numbers of fish, with one site deemed suitable for a release of Yarra pygmy perch and one site for southern purple-spotted gudgeon.

The third and fourth rounds of reintroductions under the CFH were completed in November 2012 and March 2013, with approximately 5490 individuals released across three species at four sites. Individuals were sourced from various locations including two different hatcheries (Aquasave (Todd Goodman) and the Flinders University threatened fish ARC Linkage grant genetics project) and three different surrogate dams (Crouch Dam, Munday Dam, Tupplegrove Nursery Dam). This brings the total number of fish reintroduced as part of the CFH project (2011–2013) to approximately 15,890 across 10 sites in the CLLMM region. All fish were marked with calcein prior to release to allow for differentiation of reintroduced and wild fish captured during subsequent monitoring.

Fish monitoring conducted for site assessments in spring 2012 and summer/autumn 2013 fulfilled the complementary role of post-reintroduction monitoring for previous releases. Including data from other monitoring in the region, a total of 93 threatened fish were sampled, including 69 Murray hardyhead, 11 Yarra pygmy perch, 9 southern pygmy perch and 4 southern purple-spotted gudgeon. Of the Murray hardyhead sampled at the Finniss River Junction site, the majority appear to be remnant wild fish, whilst a smaller proportion were likely recaptures of reintroduced fish. Captures of the remaining species, likely represent a mixture of recaptured reintroduced individuals and progeny of reintroduced fish. Indeed wild recruitment was observed for Murray hardyhead, Yarra pygmy perch and southern pygmy perch. Whilst positive signs of population establishment were exhibited by all species, they are present at a limited number of sites (individual sites in some cases) and in very low numbers.

The reintroduction of fish into wild habitats and restoration of viable, self-sustaining populations is a difficult task and an objective that remains aspirational in the current project. Whilst the initial results following four reintroduction rounds in 2011–2013 are encouraging, further reintroductions are likely needed to meet the primary objective of re-establishing self-sustaining wild populations. Refinements in reintroduction methodology, expansion of potential site assessment and potential site restoration/enrichment may enhance reintroduction success. Importantly, based on similar threatened fish reintroduction programs in Australia and internationally, prolonged (up to ten years) annual reintroduction efforts are most likely to result in the re-establishment of self-sustaining populations in the CLLMM region, if source fish remain available, particularly Yarra pygmy perch and Murray hardyhead.

1. Introduction

River regulation and a history of over-abstraction have greatly reduced freshwater flows throughout the Murray-Darling Basin (MDB) (Kingsford 2000). The situation was exacerbated over the period 2007–2010 when the most severe drought in recorded history was experienced in the MDB (Murphy and Timbal 2008, Potter *et al.* 2011), resulting in significantly diminished freshwater flows to the lower River Murray, South Australia. The impact was perhaps greatest in the Coorong, Lower Lakes and Murray Mouth (CLLMM) region at the terminus of the MDB, where the water level in Lake Alexandrina fell below sea level for the first time in recorded history, accompanied by significant reductions in submerged aquatic vegetation cover, disconnection of fringing vegetation habitats and elevated salinity (Kingsford *et al.* 2011). This in turn resulted in substantial declines in threatened freshwater fish species, several of which were exposed to extreme risk of local extinction (Wedderburn *et al.* 2012). Subsequently, measures were taken to prevent the extirpation of select threatened species in the CLLMM region through the South Australian Drought Action Plan (DAP) for Murray-Darling Basin Threatened Freshwater Fish Populations (Hall *et al.* 2009). In several instances this necessitated removal of individuals from the wild, captive maintenance and breeding, with the objective of re-introducing fish to wild habitats upon the return of favourable conditions (see Hammer *et al.* 2013).

Captive maintenance and breeding programs were established for at least one population of Yarra pygmy perch (*Nannoperca obscura*), southern pygmy perch (*Nannoperca australis*), Murray hardyhead (*Craterocephalus fluviatilis*) and southern purple-spotted gudgeon (*Mogurnda adspersa*) (Table 1). Captive maintenance and breeding programs utilised various ‘housing’ and rearing techniques including aquaria, pond and surrogate population (dam) establishment, and programs for different species met with varying success (Hammer 2008, Hammer *et al.* 2009a, Hammer *et al.* 2009b, Hammer *et al.* 2013). Concurrently, Flinders University, together with several other industry partners, initiated a project with the objective of enhancing the captive breeding programs by determining breeding pair choices for optimal offspring genetic fitness for each species (Carvalho *et al.* 2011, Carvalho *et al.* 2012a, Carvalho *et al.* 2012b). Captive maintenance and breeding programs involved collaboration between many different agencies including Aquasave, Native Fish Australia (SA), the Department of Environment Water and Natural Resources (DEWNR; formerly DENR), Flinders University, South Australian Research and Development Institute (SARDI), the Murray-Darling Freshwater Research Centre (Mildura), Alberton Primary School and Urrbrae Agricultural College.

Table 1. Summary of key threatened fish species in the CLLMM region and their conservation status. Conservation status is coded as Critically Endangered (CR); Endangered (E); Vulnerable (VU); Rare (R); and Protected (P) at national (*Environment Protection and Biodiversity Conservation Act 1999*), state (*Fisheries Management Act 2007*) and interim state listings (Hammer *et al.* 2009c).

Species	International (IUCN)	National (*EPBC Act*)	State Fisheries	Action Plan 09	Local significance
Yarra pygmy perch (*Nannoperca obscura*)	VU	VU	P	CR	A genetically distinct population of this species. Lake Alexandrina represents the only known MDB population.
Southern pygmy perch (*Nannoperca australis*)	-	-	P	E	SA MDB fish are genetically distinct and diverse (populations are found only in the Lower Lakes and their tributaries)
Murray hardyhead (*Craterocephalus fluviatilis*)	E	CR	P	CR	Endemic species, few populations remain (two genetically different populations in SA, one of which is present in the Lower Lakes)
Southern purple-spotted gudgeon (*Mogurnda adspersa*)	-	-	P	CR	Only known southern MDB population (present in the lower Murray region, below Lock 1, historical records in CLLMM region)

Multiple and consecutive years of below average inflows were followed in 2010/11 by broad-scale rainfall and significant inflows in the MDB. This resulted in increased flows to the lower River Murray, South Australia and improved flow and habitat availability in the CLLMM region. As such, favourable hydrology was restored to most catchments and to sites where threatened fish species were deemed at risk of extirpation between 2007 and 2010. The water level in Lake Alexandrina increased rapidly in 2010, rising above sea level and reaching typical regulated levels (~0.75 m AHD, Australian Height Datum) by September 2010 (DEWNR 2013). Increased inflows and water level restoration in Lake Alexandrina resulted in decreased salinities (DEWNR 2013) and the reconnection of formerly isolated habitats, with submerged and fringing emergent vegetation communities exhibiting signs of recovery (Gehrig *et al.* 2011). Thus, there was potential for threatened species, maintained and bred as part of the various captive breeding programs, to be reintroduced into former wild habitats.

The Critical Fish Habitat (CFH) project was developed to provide a scientifically rigorous framework to guide and undertake reintroductions of threatened fish in the CLLMM region and thus maximise the likelihood of restoring self-sustaining populations (Hammer *et al.* 2009a; Watt *et al.* 2011). This framework considered many factors including knowledge and status of threatening processes, past and current environmental conditions, and species' former range and biology, and was largely adapted from the framework of Hammer *et al.* (2009a) and a review by George *et al.* (2009). The framework aimed to enhance the likelihood of success of the current reintroductions by (1) identifying potential receiving sites, (2) developing and undertaking a screening process to assess the suitability of receiving sites, (3) determining a method for fish release (i.e. matching the scale of captive outputs to release site number and spatial extent, transport methods, acclimatisation, hard vs. soft-release), (4) undertaking fish releases, and (5) developing and conducting a monitoring and evaluation program to assess the success of the reintroductions (Hammer *et al.* 2009a, Watt *et al.* 2011).

During 2011/12, two rounds of site assessment and fish reintroduction occurred, with over 10,000 fish from the four threatened species released at nine locations around the CLLMM region (Bice *et al.* 2012). Indications of 'wild survival' were evident for several species, with recruitment observed for southern pygmy perch. Nonetheless, further reintroductions and site monitoring were required to work towards the primary objective of restoring self-sustaining wild populations. Thus, in 2012/13, site assessment and reintroduction of threatened fish to suitable habitats within the CLLMM region continued in order to meet the specific project objectives established in 2011. Specifically, the objectives for 2012/13 were to reintroduce:

- **Yarra pygmy perch** at 3 or more sites within the species' previous area of occupancy (as of 2005) in the CLLMM region. This encompasses an area from the railway bridge on the Finniss River (below Winery Road) to Wally's Wharf and Goolwa channel to the eastern side of Mundoo Island.
- **Southern pygmy perch** at 3 or more sites within the species' previous area of occupancy (as of 2005) in the CLLMM region. This encompasses an area from Pelican Lagoon in the north, near the confluence of the River Murray and Lake Alexandrina, to Hindmarsh Island in the south and Wally's Wharf on the Finniss River in the west.
- **Murray hardyhead** at 3 or more sites within the species' previous area of occupancy (as of 2005) in the CLLMM region. This encompasses Lakes Alexandrina and Albert and previous core habitat such as Hindmarsh Island, Dunns Lagoon and Waltowa.
- **Southern purple-spotted gudgeon** at 1 or more sites within the species' previous area of occupancy (as of 1960) in the CLLMM region. This encompasses the lowland braided channel and wetlands of the Finniss River from the railway bridge through to Wally's Wharf.

The present report summarises site assessments from 2012/13, as well as all reintroductions and post-reintroduction monitoring undertaken throughout the CFH project (2011–2013). As the project is due for completion in June 2013, we reflect on the success of the reintroductions, lessons learned and discuss potential recommendations for future threatened fish conservation, including reintroductions, in the CLLMM region.

2. Methods

2.1 Selection of potential reintroduction sites

A total of 21 sites across the CLLMM region were selected as potential reintroduction sites with 19 of these sites monitored in 2012/13 (Table 2; Figure 1). These sites were selected based upon knowledge of the previous presence and abundance of these species, with particular consideration of these population metrics in, or prior to, 2005 (Bice *et al.* 2012). Several sources of information are available on the pre-2005 distribution and abundance of Yarra pygmy perch, southern pygmy perch and Murray hardyhead, including Hammer *et al.* (2002), Wedderburn and Hammer (2003), Higham *et al.* (2005) and Bice and Ye (2006). Potential site selection was limited to sites where these species were previously abundant, rather than present in low numbers, to enable thorough assessment of sites with the greatest likelihood of successful reintroduction. Additionally, certain sites are notable for the former co-occurrence of these species and thus, some sites were candidates for reintroductions of multiple species (Table 2).

The selection of potential receiving sites for southern purple-spotted gudgeon followed a different approach due to the likely long-term absence (since the 1960s) of the species from the CLLMM region (Hammer *et al.* 2009a). As such, there is no contemporary data and little historical data on the distribution and abundance of this species in the CLLMM region. However, Hammer *et al.* (2009a) documented records of the species from the lower Finniss River as recently as the late 1960s, adding to earlier observations from the 1920s (Nettlebeck 1926, Rutherford 1991). Thus potential reintroduction for this species was limited to one site (Table 2).

Table 2. List of proposed receiving sites for reintroductions of southern purple-spotted gudgeon (SPSG), Yarra pygmy perch (YPP), Murray hardyhead (MHH) and southern pygmy perch (SPP, note that no SPP were available for release during 2012/13).

Site #	Site name	Sub-region	Proposed species	Latitude	Longitude	Site assessment sampling		Reference
						Spring 2012	Autumn 2013	
1	Finniss above Winery Road	Lower Finniss	SPSG, YPP	35.396269 S	138.826406 E	Y	Y	(Hammer *et al.* 2009a)
2	Blue Lagoon (Pembroke)	Lower Finniss	YPP	35.429166 S	138.859059 E	Y	Y	(Wedderburn and Hammer 2003)
21	Blue Lagoon (outer site near river channel)	Lower Finniss	YPP	35.409380 S	138.839267 E	Y	Y	(Wedderburn and Hammer 2003)
3	Finniss River junction	Goolwa Channel	YPP, SPP	35.486760 S	138.893200 E	Y	Y	(Hammer 2008)
4	Hunters Creek (upstream of Denver Rd causeway)	Hindmarsh Island	YPP, SPP, MHH	35.527571 S	138.897927 E	Y	Y	(Wedderburn and Hammer 2003, Bice and Ye 2006, Bice and Ye 2007)
5	Hunters Creek (downstream of Denver Rd causeway)	Hindmarsh Island	YPP, SPP, MHH	35.527021 S	138.893191 E	Y	Y	(Wedderburn and Hammer 2003, Bice and Ye 2006, Bice and Ye 2007)
6	Eastick Creek	Hindmarsh Island	YPP, SPP, MHH	35.536366 S	138.921670 E	N	N	
7	Upper Hunters Creek (Drain behind Wyndgate)	Hindmarsh Island	YPP, SPP	35.527249 S	138.904974 E	Y	Y	(Bice and Ye 2006)
8	Natural channel connected to Hunters Creek (behind DENR-Wyndgate)	Hindmarsh Island	YPP, SPP, MHH	35.525690 S	138.898997 E	Y	Y	(Higham *et al.* 2005, Bice and Ye 2006, Bice and Ye 2007)
9	Steamer Drain	Hindmarsh Island	YPP, SPP	35.53146 S	138.90810 E	Y	Y	(Bice *et al.* 2011)
10	Holmes Creek at Eastick Creek mouth	Hindmarsh Island	YPP	35.53778 S	138.92175 E	Y	Y	(Bice and Ye 2007, Hammer 2007a, Hammer 2008)
11	Turvey’s Drain	Milang	SPP	35.39472 S	139.00804 E	Y	Y	(Bice *et al.* 2009, Hammer 2009b, Bice *et al.* 2010)
12	Currency Creek Game Reserve	Goolwa Channel	YPP	35.49335 S	138.82333 E	Y	Y	(Hammer 2008)
13	Black Swamp	Lower Finniss	YPP	35.43119 S	138.84875 E	Y	Y	(Hammer 2009b)
14	Mundoo Island Channel east*	Mundoo Island	MHH	35.54765 S	138.91821 E	Y	Y	(Wedderburn and Barnes 2009, Wedderburn and Hillyard 2010)
20	Mundoo Island Channel east 2	Mundoo Island	SPP, MHH	35.54877 S	138.92422 E	Y	Y	(Bice *et al.* 2012)
15	Mundoo Island Channel west*	Mundoo island	SPP, MHH	35.54848 S	138.91566 E	Y	Y	(Wedderburn and Barnes 2009, Wedderburn and Hillyard 2010)
16	Boundary Creek Drain*	Mundoo Island	MHH	35.55242 S	138.94520 E	N	N	(Wedderburn and Barnes 2009, Wedderburn and Hillyard 2010)
17	Boggy Creek*	Hindmarsh Island	MHH	35.52107 S	138.92888 E	Y	Y	(Wedderburn and Barnes 2009, Wedderburn and Hillyard 2010)
18	Dunn’s Lagoon*	Clayton	MHH	35.50246 S	138.93180 E	Y	Y	(Wedderburn and Hammer 2003, Bice and Ye 2007, Wedderburn and Barnes 2009, Wedderburn and Hillyard 2010)
19	Shadows Lagoon*	Hindmarsh Island	YPP	35.51738 S	138.91756 E	Y	Y	(Wedderburn and Barnes 2011)

**Denotes sites monitored by the University of Adelaide as part of the Murray-Darling Basin Authority’s ‘The Living Murray’ Program.*

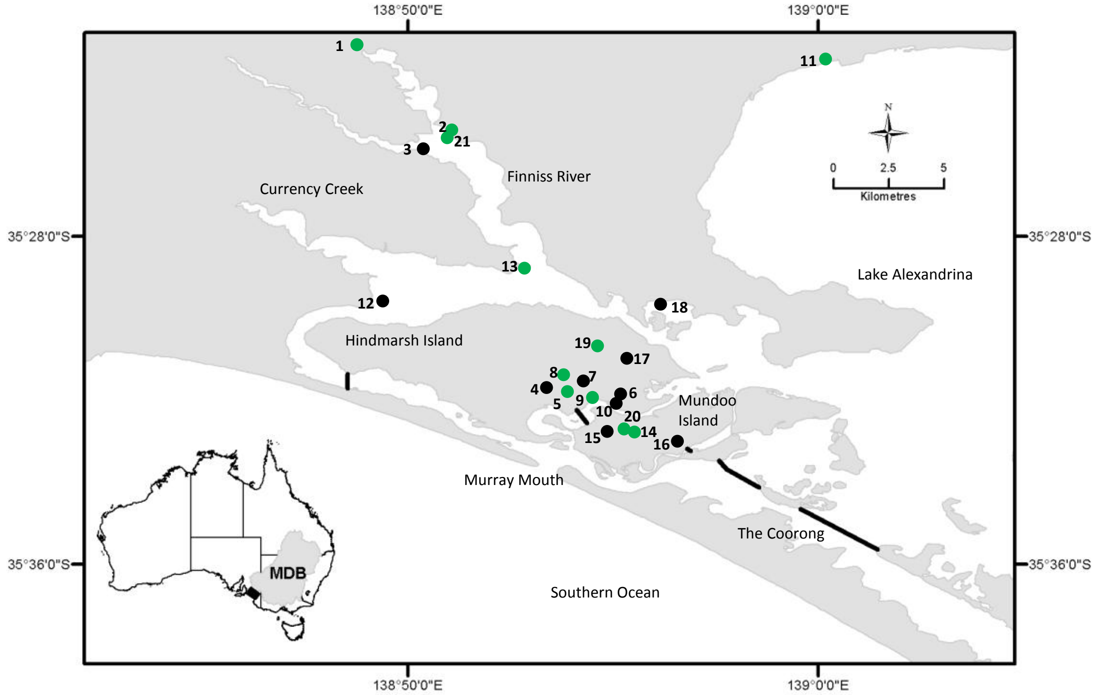

Figure 1. Summary of potential (black) and actual (green) reintroduction sites for southern purple-spotted gudgeon, Yarra pygmy perch and Murray hardyhead in the Coorong, Lower Lakes and Murray Mouth (CLLMM) region in 2012/13. Murray Barrages are indicated by bold lines. Site numbers are cross referenced in Table 2. Note: no southern pygmy perch were available for release in 2012/13.

2.2 Suitability of potential reintroduction sites

Criteria

The suitability of the potential reintroduction sites listed in Table 2 was assessed against a range of general criteria using a two-stage framework (Hammer *et al.* 2009a, Watt *et al.* 2011) (Table 3). General site suitability (stage 1) was assessed by ensuring that the key threatening process leading to the risk of extirpation was alleviated. In regards to the potential reintroduction sites across the CLLMM region and target species in the current project, the key threatening process was reduced freshwater inflows leading to significant water level recession, habitat loss (i.e. submerged vegetation) and habitat fragmentation (Kingsford *et al.* 2011, Wedderburn *et al.* 2012). Thus, water security must be favourable, particularly during seasonal dry or low-flow periods, but also over longer time scales, in order for reintroduction to occur at any given site. Other important preliminary criteria relate to the feasibility of management intervention and commitment of site landholders to species recovery and site management. If the site is deemed to be generally suitable for reintroduction, a specific site assessment was deemed necessary.

Table 3. Summary of criteria in the reintroduction framework for CLLMM region threatened species (modified from Hammer *et al.* 2009a).

Site name	Considerations	Criteria to move to next stage
General site suitability (stage 1)	Key threatening process	Key threatening process alleviated?
	Hydrology/water security	Broad-scale hydrology and water security?
	Management feasibility	Ability for individual site management intervention?
	Stakeholders	Commitment to species recovery/restoration?
Specific site assessment (stage 2)	Hydrology	Adequate water levels over the next 1-2 years?
	Fish community	Reintroduction unwarranted, if target species present? No/few introduced (namely predators) species?
	Physico-chemistry	Salinity below tolerance/within preferred range? Dissolved oxygen above tolerance? pH within suitable range?
	Habitat	Suitable composition and proportion of habitat cover for target species?
	Resources	Provision of adequate food resources for target species?

Second stage (site-specific) assessment aimed to evaluate the present suitability of sites in terms of a range of species-specific biotic and abiotic parameters. Criteria are set to ensure fish are returned to habitats that are favourable in regards to water quality, provision of resources (e.g. prey abundance) and favourable habitat (e.g. shelter and spawning habitat), whilst not placing individuals into situations of intense competition or predation; thus maximising the likelihood of success from reintroductions. For each target species, specific physico-chemical and habitat criteria, based upon published data from local sources (where possible), were evaluated to assess site suitability (Table 4). Nonetheless, expert opinion also played a significant role in the selection of reintroduction sites.

Table 4. Species-specific criteria for reintroduction of threatened fish species in the CLLMM region in 2011–2013.

Target species	Physico-chemical parameters			Aquatic habitat			Food resources	Introduced predators/competitors abundance				References
	Salinity (µScm^{-1})	Dissolved oxygen (mgL^{-1})	pH	Species composition (submerged)	Species composition (emergent)	Percentage (%) physical habitat	Presence of known prey resources (Yes or No)	Redfin perch (>120 mm)	Juvenile common carp (<100 mm)	Adult common carp (>250 mm)	Eastern gambusia	
Yarra pygmy perch	<3000	>2.0	4-10	*Myriophyllum* spp, *Ceratophyllum demersum*, *Vallisneria australis*	*Schoenoplectus validus*	>50%	Y/N	<15 per 4 nets	<30 per net	<20 caught or observed	<100 per net	(Roberts *et al.* 1995, Mittlebach and Persson 1998, Wedderburn and Hammer 2003, Bice and Ye 2006, Hammer 2007b)
Southern pygmy perch	<3000	>2.0	4-10	*Myriophyllum* spp, *Ceratophyllum demersum*, *Vallisneria australis*	*Typha* spp and overhanging and fringing grasses	>50%	Y/N	<15 per 4 nets	<30 per net	<20 caught or observed	<100 per net	(Roberts *et al.* 1995, Mittlebach and Persson 1998, Hammer 2004, Hammer 2005, McNeil and Closs 2007)
Murray hardyhead	800-25,000	>2.0	4-10	*Myriophyllum* spp, *Potamogetan pecinatus*, *Ruppia* spp., *Vallisneria australis*	*Paspalum* spp., couch, *Schoenoplectus validus*, other	>30%	Y/N	<15 per 4 nets	<30 per net	<20 caught or observed	<100 per net	(Mittlebach and Persson 1998, Wedderburn and Hammer 2003, Bice and Ye 2006, Bice and Ye 2007, Hammer and Wedderburn 2008, Wedderburn *et al.* 2008, Hammer *et al.* 2009c, Bice *et al.* 2011)
Southern purple-spotted gudgon	800-5,000	>3.0	7-10	*Myriophyllum* spp, *Ceratophyllum demersum*, *Vallisneria australis*	*Schoenoplectus validus*, *Triglochin procerum*	>30% (includes other phys habitat e.g. woody debris)	Y/N	<15 per 4 nets	<30 per net	<20 caught or observed	<100 per net	(Nettlebeck 1926, Roberts *et al.* 1995, Mittlebach and Persson 1998, Llewellyn 2006, Hammer *et al.* 2009a)

2.3 Specific site assessment

Assessment of the potential reintroduction sites against the species-specific criteria was undertaken in spring (5/11/2012 – 16/11/2012) and summer/autumn (18/02/2013 – 27/02/2013) using the following methods.

Fish monitoring

Fish assemblages were monitored at potential reintroduction sites prior to each round of releases. All sites, except Finniss River at Winery Road, were sampled with five single-winged fyke nets (four 6 m wing length, 0.6 m entry diameter and 0.003 m mesh; one 3 m wing length, 0.6 m entry diameter and 0.004 m mesh; Figure 2) set overnight. Fyke nets were set perpendicular to the bank, where possible, in habitat that was representative of the site being sampled. The Finniss River at Winery Road was sampled using a Smith-Root model LR-24 backpack electrofisher and a series of 20 box traps (0.4 m length x 0.24 m width x 0.24 m height, 0.03 m opening). This site was sampled with this technique due to its differing physical characteristics and also the potential ineffectiveness of fyke nets for sampling southern purple-spotted gudgeon in complex habitat. Several sites were monitored (using fyke nets as outlined above) by the University of Adelaide as part of the Murray-Darling Basin Authority's (MDBA) *The Living Murray Program* and data were shared between these projects (Table 2).

Figure 2. Fish sampling in the Hunters Creek (upstream of Denver Rd causeway) site in spring 2012.

All fish sampled were identified to species (Lintermans 2007) and enumerated. All threatened and non-native species (i.e. redfin perch, common carp) sampled were measured for length (mm, total length (TL) or fork length (FL) depending on tail morphology) for up to 50 individuals per species per site. Select individuals were photographed at each site as identification vouchers. Sampling was conducted under a *Section 115 permit* in accordance with the *Fisheries Management Act 2007* and PIRSA Animal Ethics Committee standards.

Macroinvertebrate monitoring

A rapid assessment of macroinvertebrate diversity and abundance accompanied fish monitoring to investigate the presence of adequate food resources for reintroduced fish. This involved the sampling of all major habitats at a site, using a 250 µm mesh dip net, for a total of 30 seconds. The contents were emptied onto a white tray, and where possible, debris and leaf litter were discarded after dislodging any attached macroinvertebrates. Macroinvertebrates were recorded to the family level using relevant identification guides (Hawking and Smith 1997, Gooderham and Tsyrlin 2002). A subjective abundance score (rare, uncommon, common, abundant, very abundant) was assigned to macroinvertebrate taxa. Macroinvertebrate sampling data were compared against known prey resources of these species (Hammer *et al.* 2009b) to subjectively determine if prey resources were adequate.

Environmental descriptors

To assess potential reintroduction sites against criteria related to habitat and water quality, the composition of physical habitat available was evaluated and water physico-chemical parameters described. Physical habitat cover was described (by visual estimation) as the proportion of aquatic habitat area (i.e. below the water surface) comprised of submerged vegetation, emergent vegetation, other physical structure (e.g. woody debris, rock) and open water. A series of random depth measures were also taken to determine mean depth at the site and a maximum depth was also determined by attempting to locate the deepest point at the site.

Various physico-chemical parameters were measured at each site. Turbidity was measured as secchi depth (m) using a secchi disk, whilst the following parameters were measured with a TPS 90-FLT water quality meter: conductivity (µS.cm^{-1}), pH, dissolved oxygen (ppm, readings at surface and at depth) and temperature (˚C).

2.4 Reintroduction methodology

Source fish hatcheries and surrogate refuges

Fish for reintroductions over 2011–2013 were sourced from a range of hatcheries and surrogate refuges formed under the DAP and related projects. Hatcheries involved the captive maintenance and/or breeding of individuals within aquaria and aquaculture tanks, whilst the surrogate refuges comprise several farm dams where founder fish were released with the objective of forming confined semi-wild self-sustaining populations (Hammer *et al*. 2009b). All hatcheries and surrogate refuges utilised as sources of fish for reintroductions under the current project, are presented in Table 5.

Table 5. Details of hatcheries and surrogate dams from which fish were sourced for reintroductions under the current project from 2011–2013.

Species	Site/stakeholder	Activity	Details
Yarra pygmy perch	Oster Dam (OD)	Surrogate refuge	A total of 70 F1 fish, sourced from Aquasave hatchery (broodstock originally from Goolwa Channel), were released in November 2008 (50 fish) and December 2008 (20 fish). Due to changing landownership attempts were made to remove all fish.
	Crouch Dam (CD)	Surrogate refuge	A total of 90 F1 fish, were released in December 2008 (20 fish: Aquasave hatchery) and April 2009 (70 fish: Cleland Wildlife Park). Broodstock fish from Goolwa Channel and Mundoo Island.
	Tupplegrove Nursery Dam (TG)	Surrogate refuge	A total of 300 F1 fish, sourced from Aquasave hatchery, were released in April 2011. Broodstock fish from Goolwa Channel, Streamer Drain and Mundoo Island.
	Flinders University (FU) hatchery	Captive maintenance and breeding	Fish bred as part of ARC Linkage project '*Restoration Genetics of Five Endangered Fish Species from the Murray-Darling Basin'.* Broodstock from Mundoo Island.
Southern pygmy perch	Flinders University (FU)	Captive maintenance and breeding	Fish bred as part of ARC Linkage project '*Restoration Genetics of Five Endangered Fish Species from the Murray-Darling Basin'.* Broodstock from Mundoo Island and Turvey Drain
	Aquasave (AQ) hatchery	Captive maintenance	A total of 30 wild fish (plus 50 F1s produced), sourced from Turvey's Drain site, were captively maintained and then incorporated into the Flinders University program or released back to the wild.
Murray hardyhead	Munday Dam (MD)	Surrogate refuge	A total of 221 fish, sourced from MDFRC (F1 and some original wild broodstock sourced from Boggy Creek and Rocky Gully), were released in May 2010 (80 fish from Boggy Creek) and May 2011 (55 juvenile and adult fish from Boggy Creek and 86 fish from Rocky Creek).
	Flinders University (FU) hatchery	Captive maintenance and breeding	Fish bred as part of ARC Linkage project '*Restoration Genetics of Five Endangered Fish Species from the Murray-Darling Basin'.* Broodstock from Boggy Creek.
Southern purple-spotted gudgeon	Aquasave (AQ) hatcheries (Adelaide and Berri)	Captive maintenance and breeding	A total of 55 fish, sourced from Jury Swamp (lower River Murray) in 2007, were captively maintained and have bred since 2008.
	Alberton Primary School (AB) hatchery	Captive maintenance and breeding	A total of 300 F1 fish, sourced from Aquasave hatcheries (broodstock source were captively maintained and released to Piawalla with some F1 fish retained and bred since 2011.
	Urrbrae Agricultural College (UC) hatchery	Captive maintenance and breeding	A total of 100 fish, sourced from Aquasave hatcheries (broodstock source were captively maintained and released to Piawalla with some F1 fish retained and bred since 2011.

Fish maintenance

Reintroductions of threatened fish were undertaken during late spring (03/12/2012 – 05/12/2012) and early autumn (26/02/2012 – 14/03/2013) following site assessment monitoring. All fish were transported from the various hatcheries and surrogate refuges to SARDI Aquatic Sciences approximately three weeks prior to release in order to undertake calcein staining and veterinary checks. At SARDI, fish were held in a series of 5000 L (x 2) and 1000 L (x 12) aerated aquaculture pools. Temperatures were maintained between 15 and 22˚C and salinities for southern pygmy perch, Yarra pygmy perch and southern purple-spotted gudgeon maintained at approximately 1500 µS.cm^{-1}. Murray hardyhead were held at a salinity of approximately 5000 µS.cm^{-1}. Over the holding period, salinities were transitioned to the known salinity of the selected reintroduction sites.

The aquaculture pools were supplied with artificial plants to provide physical structure. Fish were fed daily, either early in the morning or late in the afternoon, on a mixed diet of live and dead foods including *Artemia*, *Daphnia*, copepods, chironomid larvae and black worms.

Soft-release enclosures

A common practise in fish reintroductions for conservation is defined as ‘soft-release’, which refers to providing fish with an acclimatisation period at the release site prior to liberation. Transportation may elevate stress and commonly results in disorientation, which may increase predation risk (Brown and Day 2002). As such allowing fish to become accustomed to the prevailing conditions and develop accompanying natural behaviours is likely to elicit a greater survival rate. Further steps in this process may include increasing the suitability of the receiving environment through local predator removal.

All fish reintroduced into the region under the current project were initially released into ‘soft-release enclosures’ (Figure 3). The enclosures were triangular in shape (~2 m x 2 m x 2 m) and clad with 6 mm stretched mesh. Prior to releases, all soft-release enclosures were sampled with a Smith-Root model LR-24 backpack electrofisher, to ensure the enclosures were predator free. Following release into the enclosures, a lid of shade cloth was fastened to the top of the enclosures to minimise predation by avian and mammalian predators. Fish were maintained in the enclosures overnight and released from the enclosures in the early morning. A period of 24 hours was chosen to allow for adequate recovery from transportation and acclimation, whilst limiting density-dependent negative impacts from holding fish for longer periods (i.e. aggression and limited dispersal) (Brown and Day 2002).

Figure 3. Soft-release enclosure at the Mundoo Channel east site, a Murray hardyhead reintroduction site.

Assessment of reintroduction success

An important component of the current project was to assess the success of reintroductions. Post-reintroduction monitoring is necessary to document (a) the presence, distribution and abundance, and (b) population demographics of reintroduced species. The ability to differentiate between wild produced (i.e. remnant wild fish or progeny of reintroduced fish) and recaptured reintroduced fish is imperative; therefore all fish reintroduced under the current project were marked with calcein prior to reintroduction.

Calcein marking

Calcein is a fluorescent chemical dye, which has been shown to be effective on a wide range of fish species and when applied through the process of osmotic induction, may produce an external and thus non-lethal detectable mark (Mohler 2003, Crook *et al.* 2009, Smith *et al.* 2010). Osmotic induction involves immersing fish in a salt bath prior to immersion in the dye solution, allowing for more efficient dye uptake (Figure 4a). The ‘calcein mark’ may then be visible upon the fish under an ultraviolet light (Figure 4b) or may be detected with the use of a fluorometer.

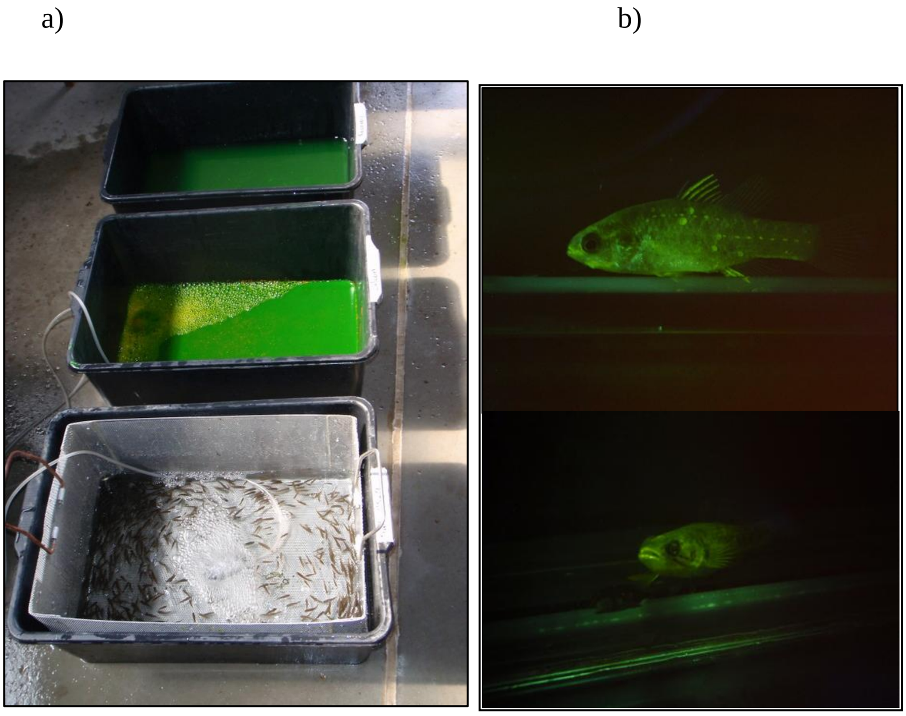

Figure 4. a) Southern pygmy perch undergoing calcein marking. Fish are in the salt bath prior to immersion in the calcein solution (next tub up) and finally bathing in a recovery tub, and b) southern pygmy perch (top) and southern purple-spotted gudgeon (bottom) as viewed under ultraviolet light 1 day post-staining.

The method of calcein marking utilised (i.e. salt bath concentrations) differed between species (Table 6). The salt concentrations used for southern purple-spotted gudgeon and Yarra pygmy perch were determined from quantitative laboratory trials (SARDI Unpublished Data; Westergaard 2013), whilst the concentration used for southern pygmy perch was based on that of the congeneric Yarra pygmy perch. The concentration used for Murray hardyhead was determined from a pilot study (~30 individuals). The calcein marking process was successful with limited mortality (associated with handling stress) and high initial calcein retention (e.g. high fluorometer readings detected for select individuals post-processing).

Table 6. Summary of calcein marking process for each target species.

Species	**Salt bath** (concentration, immersion period)	**Calcein** (concentration, immersion period)
Yarra pygmy perch	25 $g.L^{-1}$, 5 min	5 $g.L^{-1}$, 10 min
Southern pygmy perch	25 $g.L^{-1}$, 5 min	5 $g.L^{-1}$, 10 min
Murray hardyhead	50 $g.L^{-1}$, 5 min	5 $g.L^{-1}$, 10 min
Southern purple-spotted gudgeon	50 $g.L^{-1}$, 5 min	5 $g.L^{-1}$, 10 min

Post-reintroduction fish monitoring

Site assessment monitoring undertaken during the project fulfilled the complementary role of post-reintroduction monitoring (see section 2.3 for detailed methods). Data from additional monitoring undertaken in the region (e.g. University of Adelaide) is included where possible.

3. Results

3.1 Site assessment

A total of 19 sites were deemed as generally suitable for reintroductions following stage 1 assessments. The key threatening process – reduced freshwater inflows – was largely alleviated and favourable hydrology, and water security was anticipated across the CLLMM region in the foreseeable future. As such stage 2 assessments were undertaken for all sites. Tables 7–9 present the outcomes of specific site assessments in relation to the criteria established for each target species. Detailed fish, macroinvertebrate and habitat monitoring data are presented in Appendix 1 and 2. Unfortunately, no southern pygmy perch were available for releases in 2012/13 and thus all sites were monitored in regards to post-reintroduction assessment for this species. Additionally, limited resources necessitated that reintroductions in the autumn 2013 round were limited to one site each for Yarra pygmy perch and southern purple-spotted gudgeon. As such, not all assessment criteria (e.g. prey abundance) were investigated at Murray hardyhead sites.

In general, continued improvement of aquatic habitat occurred over 2012/13 following 2011/12. Favourable habitat, in the form of submerged vegetation had returned to most sites at varying levels, although recovery was limited at others (e.g. Black Swamp). Some sites, however, were increasingly dominated by either *Azolla* (i.e. Upper Hunters Creek (Drain behind Wyndgate)) or *Typha* (i.e. Mundoo Island Channel west) and management of this situation to ensure hydrological and physical connectivity, through the drain networks in particular may be required in the future.

In spring 2012, site assessment identified one site suitable for the reintroduction of southern purple-spotted gudgeon, two for Murray hardyhead and two for Yarra pygmy perch (Tables 7 – 9). In autumn 2013, one site was again deemed suitable for the reintroduction of southern purple-spotted gudgeon and one for Yarra pygmy perch (Tables 7 – 9).

Table 7. Summary of site assessments relevant to Yarra pygmy perch (YPP) and southern pygmy perch (SPP) in spring 2012 and summer/autumn 2013. Cells coloured in green indicate criteria were met, whilst red cells indicate criteria were not met.

Sites	Target species	Native species		Water quality			Food	Aquatic habitat			Introduced predators/competitors				Assessment/ comments
		Target species (Y/N)	Native spp	EC (<3,000 µS.cm^{-1})	DO (>2.0 ppm)	pH	Abundant (Y/N)	Species composition (submerged)	Species composition (emergent)	Percent (%) physical habitat	Redfin perch (>120 mm)	Juvenile common carp (<100 mm)	Adult common carp (>250 mm)	Eastern gambusia	
Spring 2012															
Blue Lagoon	YPP	N	7	1903	7.75	7.8	Y	*Myriophyllum*	*Schoenoplectus*	30	3	0	8	0	Insufficient habitat. **No release**
Blue Lagoon 2	YPP	N	10	1998	6.68	7.0	Y	*Myriophyllum*	*Schoenoplectus*	40	0	1	1	4	Insufficient habitat. **No release**
Finniss River junction	YPP	N	10	562	6.55	7.8	Y	*Myriophyllum*	*Schoenoplectus, Typha, Phragmites*	70	0	33	0	45	Recovered well (good veg, diversity of fish & prey) **MHH detected at site. No release**
Hunters Creek upstream road	YPP	N	4	783	4.46	7.7	Y	*Myriophyllum*, algae	No *Schoenoplectus* *Typha, Bolboschoenus*	40	1	6	2	1	Large adult carp observed. Site in reasonable condition **No release**
Hunters Creek downstream road	YPP	N	8	830	6.24	7.3	Y	*Myriophyllum*	No *Schoenoplectus* *Typha*	34	3	1	8	0	Vegetation proportions similar to site upstream road but littoral habitat appears favourable. **Release based on expert opinion**
Steamer Drain	YPP	Y	3	320	3.17	7.1	Y	*Myriophyllum*	*Schoenoplectus Typha*	99	2	0	0	5	YPP detected. **No further release at this time**
Natural channel north of Hunters Creek	YPP SPP	Y	4	3590	4.84	7.4	Y	*Myriophyllum, Ruppia*	No *Schoenplectus.* *Typha, Bolboschoenus, Juncus*, grasses	99	0	8	0	12	Several southern pygmy perch captured. **No further release at this time**

Table 7 continued.

Sites	Target species	Native species		Water quality			Food	Aquatic habitat			Introduced predators/competitors				Assessment
		Target species (Y/N)	Native spp	EC (<3,000 µS.cm^{-1})	DO (>2.0 ppm)	pH	Abundant (Y/N)	Species composition (submerged)	Species composition (emergent)	Percent (%) vegetated habitat	Redfin perch (>120 mm)	Juvenile common carp (<100 mm)	Adult common carp (>250 mm)	Eastern gambusia	
Spring 2012															
Holmes Creek at Eastick Creek mouth	YPP	N	7	325	9.63	8.1	Y	*Myriophyllum*	*Schoenoplectus*, *Typha*, grasses	25.1	1 (>180 juvenile)	0	3	2	No submerged vegetation. High abundance of juvenile redfin perch. **No release**
Turvey's Drain	SPP	N	4	1340	2.75	7.3	Y	*Myriophyllum*, *Ceratophyllum*	*Typha*, *phragmites*	85	0	0	0	0	Habitat favourable. **No SPP available for release**
Currency Creek Game Reserve	YPP	N	12	415	7.23	7.4	Y	*Myriophyllum*	No *Schoenoplectus*, *but Typha*, *phragmites*	70	2	0	3	8	Habitat improving and potentially favourable. **No release as another site prioritised for release**
Black Swamp	YPP	N	4	1501	7.21	7.3	N	*Myriophyllum*, *Ceratophyllum*	*Schoenoplectus*, *Typha*, *Phragmites*	30	3	0	1	0	Insufficient habitat. Little regeneration of submerged vegetation. Limited food resources. **No release**
Shadows Lagoon	YPP	Y	5	725	7.45	7.1	Y	*Vallisneria*	*Typha*, *Phragmites*	40	10 (all lengths)	50 combined		8	Vegetation slightly below threshold but appears favourable. YPP recaptured. **Further YPP release recommended**
Mundoo Island Channel west	SPP	N	1	497	1.67	8.3	Y	None	*Typha*, *Azolla*	98	3 (all sizes)	0 combined		8	Habitat favourable. **No SPP available for release**
Mundoo Island Channel east	SPP	Y	7	4780	9.36	8.19	Y	*Myriophyllum*, *Ceratophyllum*, algae	*Typha*, grasses	25	4 (all sizes)	0 combined		11	**No SPP for release**
Mundoo Island Channel east 2	SPP	Y	3	1015	6.89	9.0	Y	*Myriophyllum*, *Ceratophyllum*, algae	*Typha*, *Juncus*, grasses	95	0	0	0	4	**No SPP for release**

Table 7 continued.

Sites	Target species	Native species		Water quality			Food	Aquatic habitat			Introduced predators/competitors				Assessment
		Target species (Y/N)	Native spp	EC (<3,000 µS.cm^{-1})	DO (>2.0 ppm)	pH	Abundant (Y/N)	Species composition (submerged)	Species composition (emergent)	Percent (%) vegetated habitat	Redfin perch (>120 mm)	Juvenile common carp (<100 mm)	Adult common carp (>250 mm)	Eastern gambusia	
Autumn 2013															
Blue Lagoon	YPP	N	5	2089	7.2	8	N	*Myriophyllum*	*Schoenoplectus*	40	8 (>40 juveniles)	1	26	0	Abundant juvenile redfin and adult common carp. Inadequate food resources. **No release**
Blue Lagoon 2	YPP	N	9	2135	3.93	8.2	N	*Myriophyllum*	*Schoenoplectus*	60	3 (>40 juveniles)	0	4	9	Abundant juvenile redfin. Inadequate food resources. **No release**
Finniss River junction	YPP	N	9	674	5.6	8.3	Y	*Myriophyllum*	*Schoenoplectus* *Typha, Phragmites*	90	3	0	2	94	Was YPP release site. Site favourable but MHH were captured and other sites prioritised. **No release**
Hunters Creek upstream road	YPP SPP	N	5	761	3.46	8	Y	*Myriophyllum*	No *Schoenoplectus* *Typha, Bolboschoenus*	40	2	10 (+26 juvenile goldfish)	0	23	Limited submerged vegetation. Large adult carp observed. **No release**
Hunters Creek downstream road	YPP, SPP	N	6	779	4.2	7.3	Y	*Myriophyllum, Potamogeton crispis*	No *Schoenoplectus* *Typha*	40	0	2	1	15	Vegetation proportion similar to site u/s road but higher proportion submerged. **Release recommended**
Steamer Drain	YPP, SPP	N	3	582	5.41	7.7	N	*Myriophyllum*	*Schoenoplectus* *Typha*	80	0	3 (+15 goldfish)	0	1095	Favourable habitat, but abundant gambusia and inadequate food resources. **No release**
Natural channel north of Hunters Creek	YPP	N	2	2400	10	7.8	Y	*Myriophyllum,*	No *Schoenoplectus* *Typha, Juncus, Bolboschoenus*	98	0	0 (+3 juvenile goldfish)	0	16	Was SPP release site, with recaptures. Habitat favourable. No SPP available for release. YPP release prioritised elsewhere. **No further release at this stage**

Table 7 continued.

Sites	Target species	Native species		Water quality			Food	Aquatic habitat			Introduced predators/competitors				Assessment
		Target species (Y/N)	Native spp	EC (<3,000 µS.cm^{-1})	DO (>2.0 ppm)	pH	Abundant (Y/N)	Species composition (submerged)	Species composition (emergent)	Percent (%) vegetated habitat	Redfin perch (>120 mm)	Juvenile common carp (<100 mm)	Adult common carp (>250 mm)	Eastern gambusia	
Autumn 2013															
Holmes Creek at Eastick Creek mouth	YPP	N	7	512	10.2	8.9	N	*Myriophyllum, Vallisneria*	*Schoenoplectus, Typha, Phragmites*	48	7 (+18 juvenile)	0	9 (+25, 100-250 mm)	4	Modertae abundances of redfin perch and common carp. **No release**
Turvey's Drain	SPP	N	5	789	1.46	8.6	N	*Myriophyllum, Ceratophyllum*	*Typha, phragmites*	95	1	1 (+9 goldfish)	0	317	**No SPP available for release**
Currency Creek Game Reserve	YPP	N	4	686	10.9	8.7	Y	*Myriophyllum*	*Typha, phragmites*	70	5 (+7 juveniles)	1	6 (+13 100-250 mm)	69	Favourable habitat. Other site prioritised for release **No release**
Black Swamp	YPP	N	4	1508	6.83	7.8	N	0	*Schoenoplectus, Typha, Phragmites*	40	3 (+11 juveniles)	0	0 (6 100-250 mm)	0	No submerged vegetation, poor prey resources. **No release**
Shadows Lagoon	YPP	Y	6	905	3.14	8.01	Y	*Vallisneria, Myriophyllum*	*Triglochin, Phragmites,* grasses	55	5 (all sizes)	9 combined		83	Habitat, prey and water quality favourable. YPP surviving. **Release recommended**
Mundoo Island Channel west	SPP	Y	5	753	-	7.76	-	None	*Typha, Azolla*	43	1	0 combined (+21 goldfish)		59	**No SPP available for release**
Mundoo Island Channel east	SPP	N	5	882	-	7.76	-	-	-	85	0	1 combined		144	**No SPP available for release**
Mundoo Island Channel east 2	SPP	N		1015	6.89	8.9	N	*Myriophyllum, Ceratophyllum,* algae	*Typha, Juncus,* grasses	95	0	1 (+3 goldfish)	0	953	**No SPP available for release**

Table 8. Summary of site assessments relevant to Murray hardyhead (MHH) in spring 2012. Cells coloured in green indicate criteria were met, whilst red cells indicate criteria were not met. SMHH = small-mouthed hardyhead.

Sites	Native species		Water quality			Food	Aquatic habitat			Introduced predators/competitors				Assessment
	Target species (Y/N)	Native diversity	EC (800-25000 µS.cm^{-1})	DO (>2.0 mg.L^{-1})	pH (4-10)	Abundant (Y/N)	Species composition (submerged)	Species composition (emergent)	Percent (%) vegetated habitat	Redfin (>120 mm)	Juvenile common carp (<100 mm)	Adult common carp (>250mm)	SMHH (<100 per net)	
Spring 2012														
Finniss River Junction	Y	10	562	6.55	7.8	Y	*Myriophyllum*	*Schoenoplectus, Typha, Phragmites*	70	0	33	0	0	Species present. **No release**
Boggy Creek	N	5	387	1.14	7	Y	algae	*Phragmites, Typha, Juncus, Azolla*	90	6 (all sizes)	28 combined		0	No submerged vegetation. Low salinity. **No release**
Mundoo Island Channel east	Y	7	4780	9.36	8.19	Y	*Myriophyllum, Ceratophyllum,* algae	*Typha,* grasses	25	4 (all sizes)	0 combined		10	Low levels of veg cover but favourable salinity and survival from previous release. **Release recommended**
Mundoo Island Channel west	N	1	497	1.67	8.3	Y	None	*Typha, Bolboschoenus, Azolla*	98	3 (all sizes)	0 combined		0	No submerged vegetation and low salinity. **No release**
Dunn's Lagoon	N	8	364	-	7.52	Y	-	-	76	135 (all sizes)	5 combined		0	Low salinity and high abundance of redfin. **No release**
Hunters Creek upstream road	N	4	783	4.46	7.7	Y	*Myriophyllum*	*Typha, Bolboschoenus*	40	1	6	2	0	Low salinity, abundant carp. **No release**
Hunters Creek downstream road	N	8	830	6.24	7.3	Y	*Myriophyllum*	*Typha, Bolboschoenus*	34	7	29	3	0	Adequate salinity and habitat. **Release recommended**
Natural channel north of Hunters Creek	N	2	2400	10	7.8	Y	*Myriophyllum Certaophyllum*	*Typha, Juncus, Bolboschoenus*	99	0	0 (+3 juvenile goldfish)	0	0	Adequate veg. SPP release site. Other site prioritised. **No release**

Table 8 continued

Sites	Native species		Water quality			Food	Aquatic habitat			Introduced predators/competitors				Assessment
	Target species (Y/N)	Native diversity	EC (800-25000 µS.cm^{-1})	DO (>2.0 mg.L^{-1})	pH (4-10)	Abundant (Y/N)	Species composition (submerged)	Species composition (emergent)	Percent (%) vegetated habitat	Redfin (>120 mm)	Juvenile common carp (<100 mm)	Adult common carp (>250mm)	SMHH (<100 per net)	
Autumn 2013														
Finniss River junction	Y	9	674	5.6	8.3	Y	*Myriophyllum*	*Schoenoplectus*, *Typha*, *Phragmites*	90	3	0	2	0	**No autumn releases**
Boggy Creek	N	4	606	5.45	7.8	Y	*Myriophyllum*, *Vallisneria*	*Phragmites*, *Typha*, *Ludwigia*, grasses	30	0	3 combined		0	**No autumn releases**
Mundoo Island Channel east	Y	5	882	-	7.76	-	*Myriophyllum*, *Ceratophyllum*, algae	*Typha*, grasses	85	0	1 combined		1	**No autumn releases**
Mundoo Island Channel west	N	5	753	-	7.76	-	None	*Typha*, *Azolla*	43	1	0 combined (+21 goldfish)		0	**No autumn releases**
Dunn's Lagoon	Y	11	678	-	7.19	Y	*Myriophyllum*	*Typha*, *Schoenoplectus*	68	25 (all sizes)	2 combined		35	**No autumn releases**
Hunters Creek upstream road	N	5	761	3.46	8	Y	*Myriophyllum*, *Potamogeton cripis*, algae	*Typha*, *Bolboschoenus*, grasses	40	2	10 (+26 juvenile goldfish)	0	0	**No autumn releases**
Hunters Creek downstream road	N	6	779	4.2	7.3	Y	*Myriophyllum*, *Potamogeton crispis*	*Typha*, *Bolboschoenus*, grasses	34	0	2	1	0	**No autumn releases**
Natural channel north of Hunters Creek	N	2	2400	10	7.8	Y	*Myriophyllum*,	*Typha*, *Juncus*, *Bolboschoenus*, grasses	98	0	0 (+3 juvenile goldfish)	0	0	**No autumn releases**

Table 9. Summary of site assessments relevant to southern purple-spotted gudgeon (SPSG) in spring 2012 and autumn 2013. Cells coloured in green indicate criteria were met, whilst red cells indicate criteria were not met.

Sites	Native species		Water quality			Food resources	Aquatic habitat			Introduced predators/competitors				Assessment
	Target species (Y/N)	Native diversity	EC (<3,000 µS.cm^{-1})	DO (>2.0 mg.L^{-1})	pH	Abundant (Y/N)	Species composition (submerged)	Species composition (emergent)	Percent (%) vegetated habitat	Redfin (>120 mm)	Juvenile common carp (<100 mm)	Adult common carp (>250 mm)	Eastern gambusia	
Spring 2012														
Finniss at Winery Road (Loveday)	Y	5	1896	4.2	7.7	Y	No *Myriophyllum or Vallisneria*	*Triglochin, Phragmites, Berula* and grasses	50	0	0	1	1	Generally looks good. Broad range of sub-sites, some highly vegetated. Need to consider impact of grazing. **Release recommended**
Autumn 2013														
Finniss at Winery Road (Loveday)	Y	3	2810	2.47	8.7	Y	No *Myriophyllum or Vallisneria*. *Lemna* and *Lemna*	*Triglochin, Phragmites, Typha, Berula*, grasses	70 (includes woody debris)	0	1	1	0	Generally still looks good. Recaptures indicate site is favourable. **Releases recommended**

3.2 Reintroduction summary

During 2012/13, approximately 5,490 individual fish (sourced from five different hatcheries/surrogate refuges), including ~950 Yarra pygmy perch, ~520 southern purple-spotted gudgeon and ~4,020 Murray hardyhead, were released across six sites in the CLLMM region (Figure 5 and Table 10). Due to limited resources the release in autumn 2013 was small relative to previous releases and was limited to Yarra pygmy perch and southern purple-spotted gudgeon. Fish mortality was greatest during transport of surrogate dam fish to SARDI and Aquasave-NGT (Goolwa) holding facilities. Only minor mortality (<10 fish per species) resulted from the calcein marking process. A total of ~15,840 fish from the four threatened species (~7,520 MHH at two sites, ~5,850 YPP at five sites, ~1,350 SPP at three sites and ~1,120 SPSG at one site) have now been released into the CLLMM region at 10 suitable sites across the 4 rounds of reintroductions between 2011 and 2013 (Table 10).

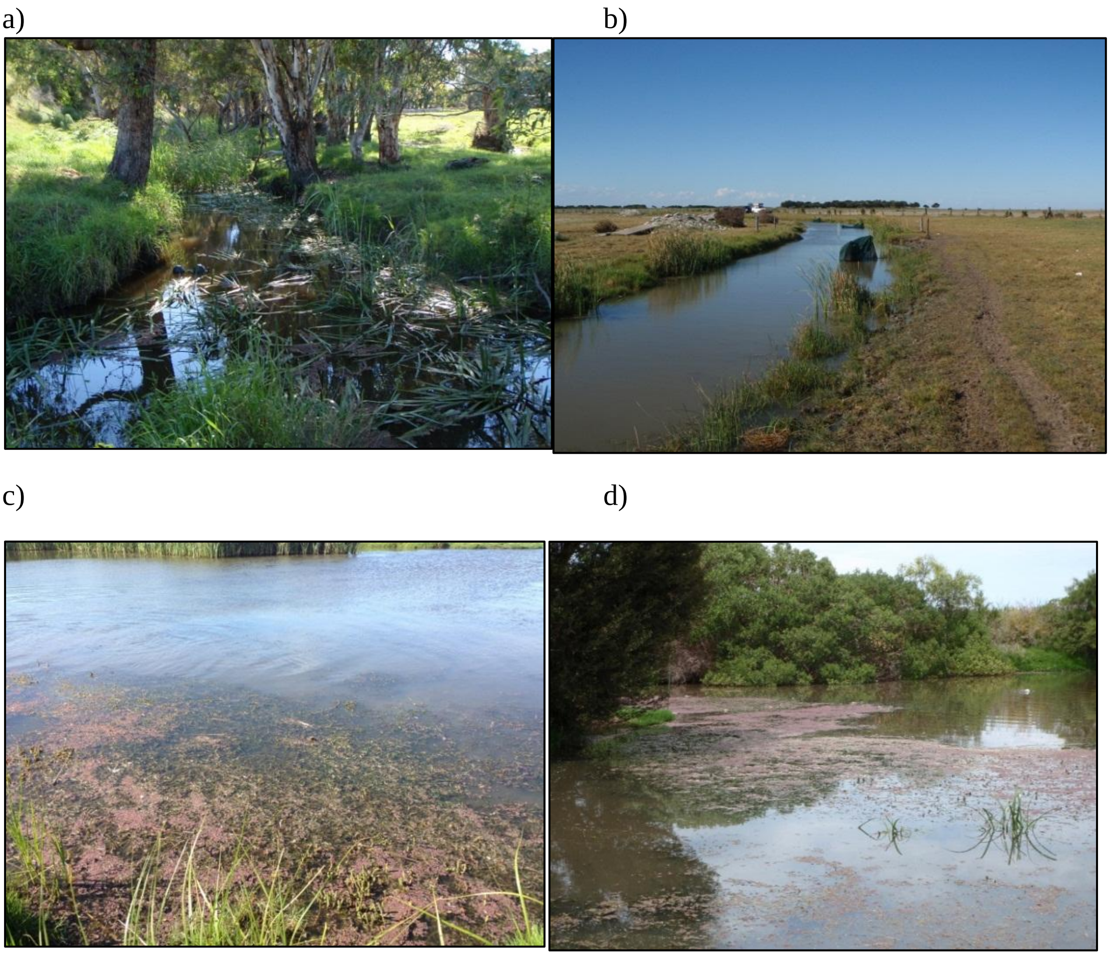

Figure 5. a) Emergent vegetation (*Triglochin procerum*) at the Finniss River southern purple-spotted gudgeon reintroduction site, b) Murray hardyhead release site at Mundoo Channel east, c) submerged aquatic vegetation (*Myriophyllum sp.*) at the Hunters Creek downstream Denver Rd site and d) Shadows Lagoon – Yarra pygmy perch reintroduction site.

Table 10. Summary of sites and numbers of Yarra pygmy perch (YPP), southern pygmy perch (SPP), southern purple-spotted gudgeon (SPSG) and Murray hardyhead (MHH) released and rationale for selection of sites for reintroductions over 2011–2013. The source of reintroduced fish is coded as either (1) surrogate dams (Crouch Dam (CD), Oster Dam (OD), Tupelo Grove Nursery (TGN) or Munday Dam (MD)), (2) Flinders University (FU, either equal contribution from broodstock (EC) or unequal contribution from broodstock (UC)), (3) the Aquasave Hatchery (AQ). See Table 5 for hatchery/surrogate dam details.

Site name	Species released	Numbers released (approx.)	Source	Justification for reintroduction
Spring 2011				
Natural channel connected to Hunters Creek	SPP	770	FU (EC)	Favourable habitat and water quality. Condition similar to pre-2007.
Turvey's Drain	SPP	300	Wild, FU (UC)	Favourable habitat and water quality. SPP remain present.
Blue Lagoon	YPP	400	CD	Favourable habitat and water quality. Diversity of favourable sites.
Finniss River junction	YPP	800	CD	Favourable habitat and water quality.
Finniss at Winery Road	SPSG	200	AQ	Broad range of sites with favourable habitat and water quality.
Autumn 2012				
Mundoo Island Channel east 2	SPP	280	FU (UC)	Favourable habitat and water quality. New site between Mundoo Channel east and Mundoo Channel west.
Streamer Drain	YPP	2200	FU (EC)	Favourable habitat and water quality. Condition similar to pre-2007.
Shadows Lagoon	YPP	1500	CD, OD, FU (UC)	Favourable habitat and water quality. Excellent broad-scale habitat. Anecdotal evidence of past presence.
Finniss at Winery Road	SPSG	400	AQ, AP	Broad range of sites with favourable habitat and water quality. Recaptures indicate site is likely favourable.
Mundoo Island Channel east	MHH	3500	MD	Favourable habitat and water quality, particularly salinity. Species abundant 2 years previous.
Spring 2012				
Mundoo Island Channel east	MHH	3500	MD	Favourable habitat and water quality, particularly salinity. Recaptures indicate site is likely favourable.
Hunters Creek downstream road	YPP	400	TGD	Site did not meet all criteria, however, both spp were formally abundant at this site.
	MHH	520	FU (EC)	
Shadows Lagoon	YPP	250	CD	Site recovered well. Recaptures indicate fish survival. Favourable vegetated habitat, high diversity of other fish & prey.
Finniss at Winery Road	SPSG	320	AQ	Broad range of sites with favourable habitat and water quality. Recaptures indicate site is likely favourable.
Autumn 2013				
Hunters Creek downstream road	YPP	300	CD	Again site did not meet all criteria, but given low number of fish for release it was decided to supplement the earlier spring release at this location.
Finniss at Winery Road	SPSG	200	AQ	Broad range of sites with favourable habitat and water quality. Recaptures indicate site is likely favourable.

3.3 Post-reintroduction monitoring/recaptures

During spring 2012 and summer/autumn 2013, all reintroduction sites were monitored (either directly through the CFH project or Adelaide University Living Murray condition monitoring) as part of the site assessment. For completeness, the outcomes of relevant monitoring during the first year of the CFH project (previously presented in Bice *et al.* 2012) are also presented. In total, 132 individuals across the four threatened fish species were sampled from twelve sites across the region (Table 11). This monitoring is summarised for each threatened fish species below.

Table 11. Summary of the number of individual Yarra pygmy perch, southern pygmy perch, Murray hardyhead and southern purple-spotted gudgeon sampled during post-reintroduction monitoring between 2011 and 2013. *represents sites where reintroductions did not occur for that particular species.

		No. individuals sampled			
Species	**Site**	**Autumn 2012**	**Spring 2012**	**Autumn 2013**	
Yarra pygmy perch	Blue Lagoon 2	0	0	0	**12**
	Finniss River Junction	0	0	0	
	Steamer Drain	0	2	0	
	Shadows Lagoon	0	8	2	
Southern pygmy perch	Natural channel connected to hunters Creek	11	4	4	**24**
	Turvey’s Drain	1	0	0	
	Mundoo Island Channel east 2	0	1	0	
	Mundoo Island Channel east	0	2	0	
	Mundoo Island Channel west	0	0	1	
Murray hardyhead	Mundoo Island Channel east	0	4	9	**88**
	Finniss Junction*	12	7	42	
	Hunters Creek d/s road	0	0	0	
	Dunn’s Lagoon*	0	0	7	
	Old Clayton*	0	0	7	
Southern purple-spotted gudgeon	Finniss at Winery Rd	3	1	3	**7**
Total		**27**	**29**	**75**	**131**

Yarra pygmy perch

Yarra pygmy perch (~5,850) were released across five sites over the four rounds of reintroduction (Table 10) and have been detected in subsequent monitoring at two of these sites (Table 11). Initially in spring 2011, 800 and 400 individuals were released at the Finniss River Junction and Blue Lagoon sites (includes Blue Lagoon 1 and 2), but post-reintroduction monitoring has failed to detect the species at these sites. Both sites have recovered considerably since the return of favourable water levels, with the Finniss River Junction site in particular characterised by extensive beds of submerged vegetation (*Myriophyllum spp*), but the extensive nature of both sites may result in low sampling efficiency. In autumn 2012, 2,200 and 1,500 individuals were released into Streamer Drain and Shadows Lagoon, respectively. Subsequent monitoring of Streamer Drain yielded two individuals in spring 2012 – the first Yarra pygmy perch sampled in the MDB since 2007 (Figure 6) – but no fish were sampled during the summer/autumn 2013 monitoring. In spring 2012, eight Yarra pygmy perch were sampled from Shadows Lagoon and an additional 250 fish were reintroduced. In summer/autumn 2013, two fish were detected at this site. Approximately 700 individuals have also been released at Hunters Creek downstream of Denver Road, but no individuals have been detected in subsequent monitoring.

Figure 6. Yarra pygmy perch sampled from the Streamer Drain during spring 2012 – the first individual sampled from the CLLMM region (and MDB) since 2007.

In spring 2012, Yarra pygmy perch sampled from Steamer Drain and Shadows Lagoon ranged 42–55 mm TL and all but one individual exhibited fluorescence readings consistent with a calcein mark (Figure 7a and b), suggesting they were recaptures from the previous releases. The two individuals sampled from Shadows Lagoon in autumn 2013 were <40 mm TL and exhibited fluorescence readings well below that consistent of a calcein mark (Figure 7c and d). Based on length and fluorescence, these individuals are likely young-of-year (YOY) recruited in the wild following the previous spawning season. Preliminary genetic analyses of fin clip samples taken from these fish as part of the Flinders University ARC Linkage project (*Restoration Genetics of Five Endangered Fish Species from the Murray-Darling Basin*) support these results. These analyses were able to differentiate between individuals released from the Flinders University hatchery (Table 5) and those released from other hatcheries or recruited in the wild. Analyses of tissue samples from the two fish hypothesised to be YOY, confirmed they were not original fish released from Flinders University (Luciano Beheregaray, Flinders University, pers. comm.), further suggesting these individuals recruited in the 'wild'.

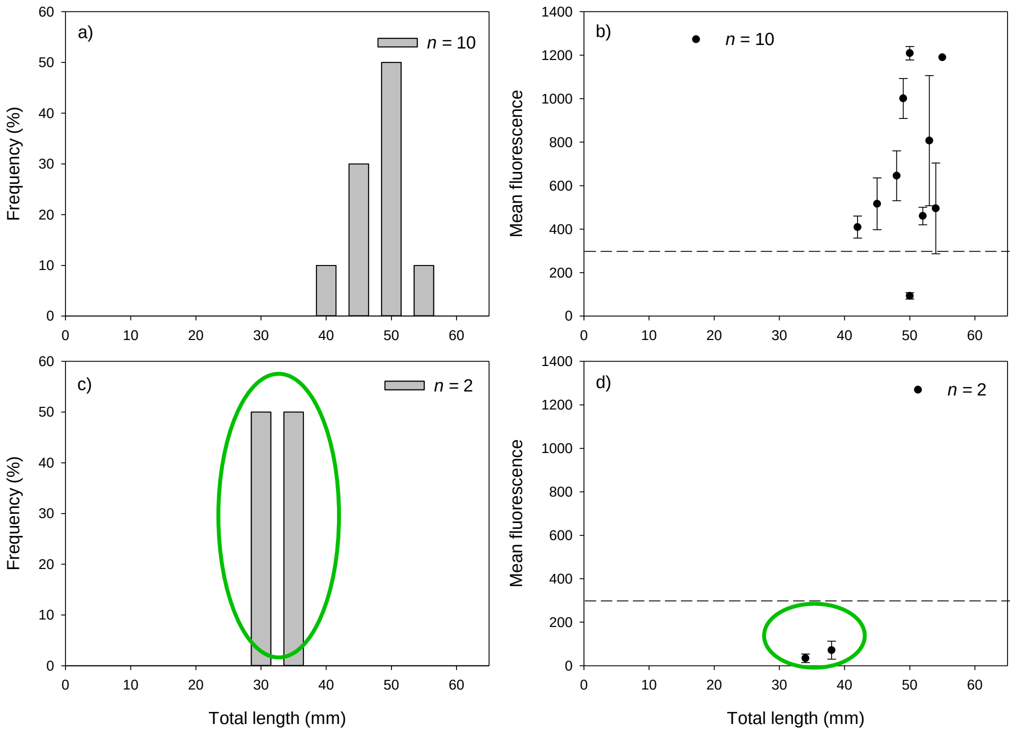

Figure 7. Length-frequency distributions and corresponding mean fluorescence (± standard error) against length plots for all Yarra pygmy perch sampled in spring 2012 (a, b) and autumn 2013 (c, d). Dashed line represents the fluorescence reading deemed to indicate a calcein mark. Green ellipses indicate likely young-of-year individuals.

Southern pygmy perch

A total of ~1,350 southern pygmy perch have been released across three sites and subsequent monitoring has detected individuals at all three sites (Table 10 and Table 11). Unfortunately, due to a lack of individuals in captivity (i.e. no surrogate refuge was established for this species) reintroductions were restricted to 2011/12. In spring 2011, ~770 and ~300 southern pygmy perch were released into the 'natural channel connected to Hunters Creek' and Turvey's Drain sites, respectively. A single southern pygmy perch was sampled at Turvey's Drain in autumn 2012, but the following two monitoring rounds failed to detect any individuals. Southern pygmy perch were detected at the 'natural channel connected to Hunters Creek' site in autumn 2012 (*n* = 11), spring 2012 (*n* = 4) and autumn 2013 (*n* = 4) (Figure 8). In autumn 2012, ~280 southern pygmy perch were released at the Mundoo Channel east 2 site, with a single fish sampled in the following two monitoring rounds in spring 2012 and autumn 2013. Individuals have also been sampled at two neighbouring hydrologically connected sites, Mundoo Channel east and Mundoo Channel west, likely suggesting small-scale dispersal from the original release site.

In autumn 2012, southern pygmy perch ranged 32–53 mm TL, with all but one fish ranging 44–53 mm TL (Figure 9a). The larger individuals all exhibited fluorescence consistent with a calcein mark, suggesting they were recaptures from the previous reintroduction. The remaining individual was 32 mm TL and exhibited low fluorescence suggesting it was a YOY recruited in the wild following the previous spawning season (2011) (Figure 9a and b). In spring 2012, a cohort of large fish was present (46–56 mm TL), with fluorescence readings similar to that indicating a calcein mark (Figure 9c and d). All of these fish were sampled from the Mundoo Island Channel group of sites and were likely recaptures from the autumn 2012 release at Mundoo Island Channel east 2. A smaller cohort of fish (18–19 mm TL; Figure 8) was sampled at the 'natural channel connected to Hunters Creek' site and exhibited very low levels of fluorescence indicating that they were recently 'wild recruited' YOY (Figure 9c and d). One individual was sampled in autumn 2013, from the Mundoo Island Channel west site, and based upon length (40 mm TL) and low fluorescence was likely a wild recruited fish from the previous spawning season (2012) (Figure 9e and f). The remaining fish (n = 4) sampled in autumn 2013, were captured from the 'natural channel connected to Hunters Creek' site during ad-hoc sampling. These fish ranged 46–52 mm TL but were not assessed for fluorescence (Figure 9e and f). As per Yarra pygmy perch, preliminary genetic analyses of tissue samples has been undertaken for some of the southern pygmy perch sampled in the last 18 months. In general, the results support the differentiation of 'recaptured reintroduced' and 'wild recruited' individuals based on length and fluorescence; all fish hypothesised as 'wild recruited' had genetics inconsistent with original release fish (Luciano Beheregaray, Flinders University, Pers. Comm.).

a) b)

Figure 8. a) Likely YOY southern pygmy perch sampled from b) the 'natural channel connected to Hunters Creek' site in spring 2012.

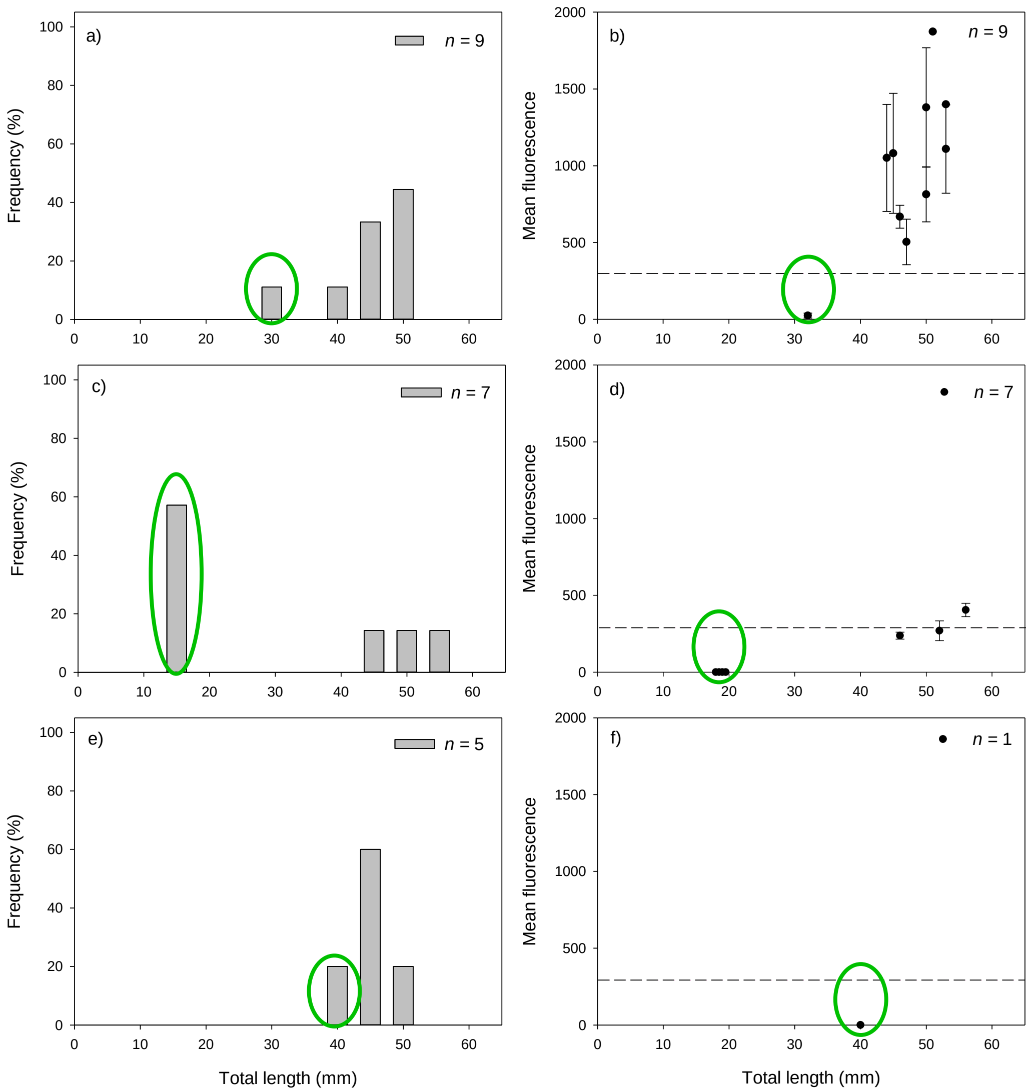

Figure 9. Length-frequency distributions and corresponding mean fluorescence (± standard error) against length plots for all southern pygmy perch sampled in autumn 2012 (a, b), spring 2012 (c, d) and autumn 2013 (e, f). Dashed line represents the fluorescence reading deemed to indicate a calcein mark. Green ellipses indicate likely young-of-year individuals.

Murray hardyhead

During autumn and spring 2012, >7,500 Murray hardyhead were released at two relatively contained sites on Hindmarsh (i.e. Hunters Creek downstream Denver Road) and Mundoo Islands (Mundoo Island Channel east) (Table 10). No individuals have subsequently been sampled at Hunters Creek downstream Denver Road, but low numbers have been sampled at Mundoo Island Channel east in both spring 2012 (*n* = 4) and autumn 2013 (*n* = 9) (Table 11). Moderate numbers of Murray hardyhead have also been sampled at two non-reintroduction sites, at the Finniss River Junction in autumn 2012 (*n* = 12; Figure 10), prior to any reintroductions under the current project, and in both spring 2012 (n = 7) and autumn 2013 (n = 42), and at Dunn's Lagoon (n = 7) and Old Clayton (n = 7) in autumn 2013 (Table 11).

In autumn 2012, Murray hardyhead ranged 22–40 mm TL (Figure 11a) and represent remnant wild fish, all being sampled from the Finniss Junction site prior to any reintroductions as part of this project. In spring 2012, Murray hardyhead sampled were larger ranging 38–58 mm TL (Figure 11a) and all fish exhibited similarly moderate fluorescence, typically just below that indicative of a calcein mark (Figure 11b). Over 70% of these fish were sampled from the Finniss River Junction. In autumn 2013, Murray hardyhead ranged 20–63 mm TL, but approximately 65% were <35 mm TL, likely representing wild recruited YOY (Figure 11c). A subsample of fish were analysed for fluorescence, with all fish displaying moderate fluorescence and many over the accepted threshold indicative of a calcein mark (Figure 11d). Due to the high variability in fluorescence readings between individuals it is difficult to determine reintroduced fish from wild fish.

Figure 10. Remnant 'wild' Murray hardyhead (top) with congeneric unspecked hardyhead (*Craterocephalus stercusmuscarum fulvus*; bottom) sampled from the Finniss River Junction in autumn 2012.

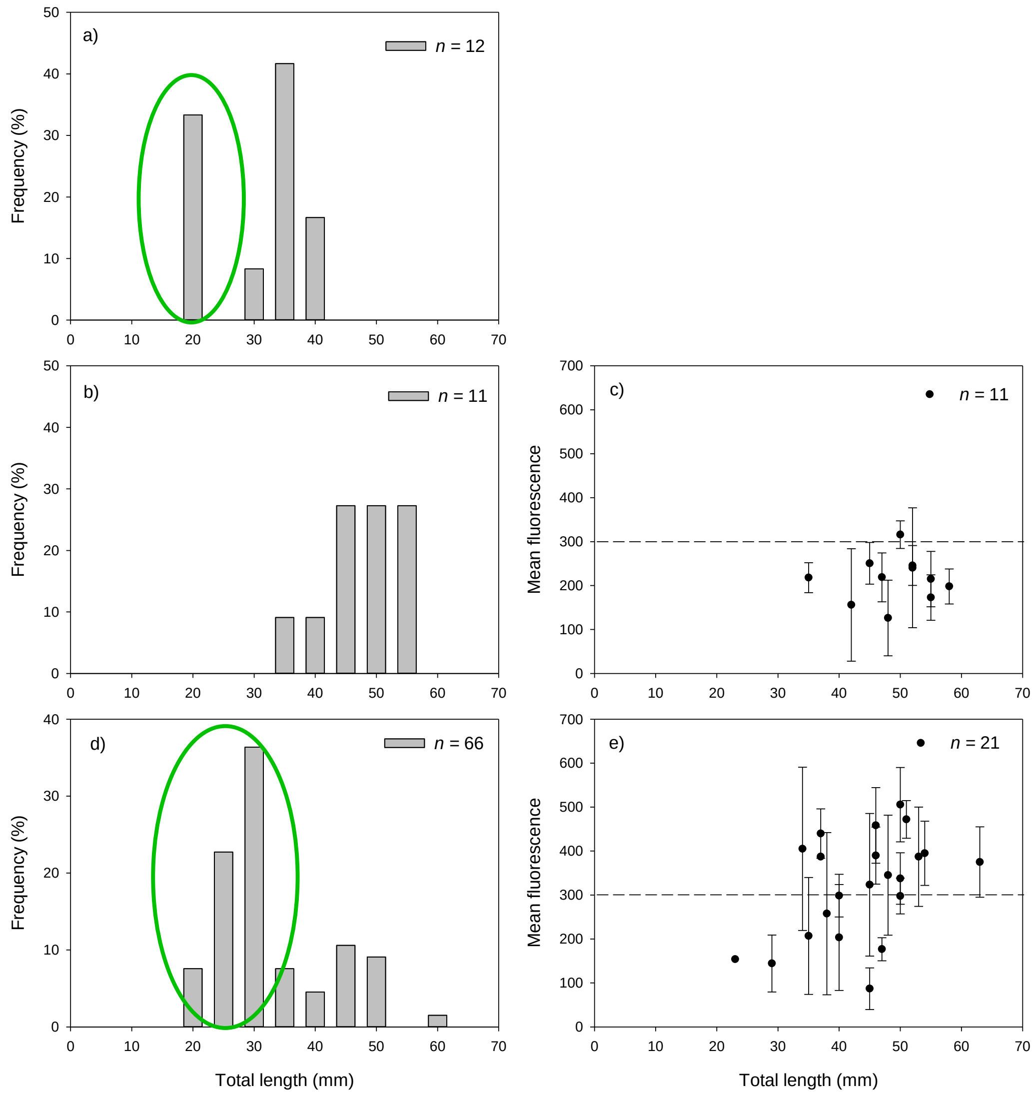

Figure 11. Length-frequency distributions and corresponding mean fluorescence (± standard error) against length plots for Murray hardyhead sampled in autumn 2012 (a), spring 2012 (b, c) and autumn 2013 (d, e). Dashed line represents the fluorescence reading deemed to indicate a calcein mark. No fluorescence readings were taken in autumn 2012. Green ellipses indicate likely young-of-year individuals.

Southern purple-spotted gudgeon

A total of 1,120 southern purple-spotted gudgeon have been reintroduced into the lower Finniss River (Winery Road) site across four events (Table 10) and low numbers have been sampled during all subsequent monitoring events (Table 11). In autumn 2012, three individuals (64 – 72 mm TL; Figure 12a) were collected, with one individual giving a reading consistent with a calcein mark, whereas the remaining two fish exhibited fluorescence inconsistent with a mark (Figure 12b). In spring 2012, one individual (70 mm; Figure 12c, 13) was sampled exhibiting no fluorescence (Figure 12d) and three individuals were sampled in autumn 2013, ranging 43–70 mm TL (Figure 12c) and again exhibited no fluorescence (Figure 12d). Two of these fish were <55 mm TL, which could represent 'wild recruited' individuals. The short-term survival of southern purple-spotted gudgeon is clearly evident at the lower Finniss site, but it is unclear whether sampled individuals represent those released during the previous (i.e. 6 months ago) or earlier (i.e. up to 18 months survival) reintroduction rounds.

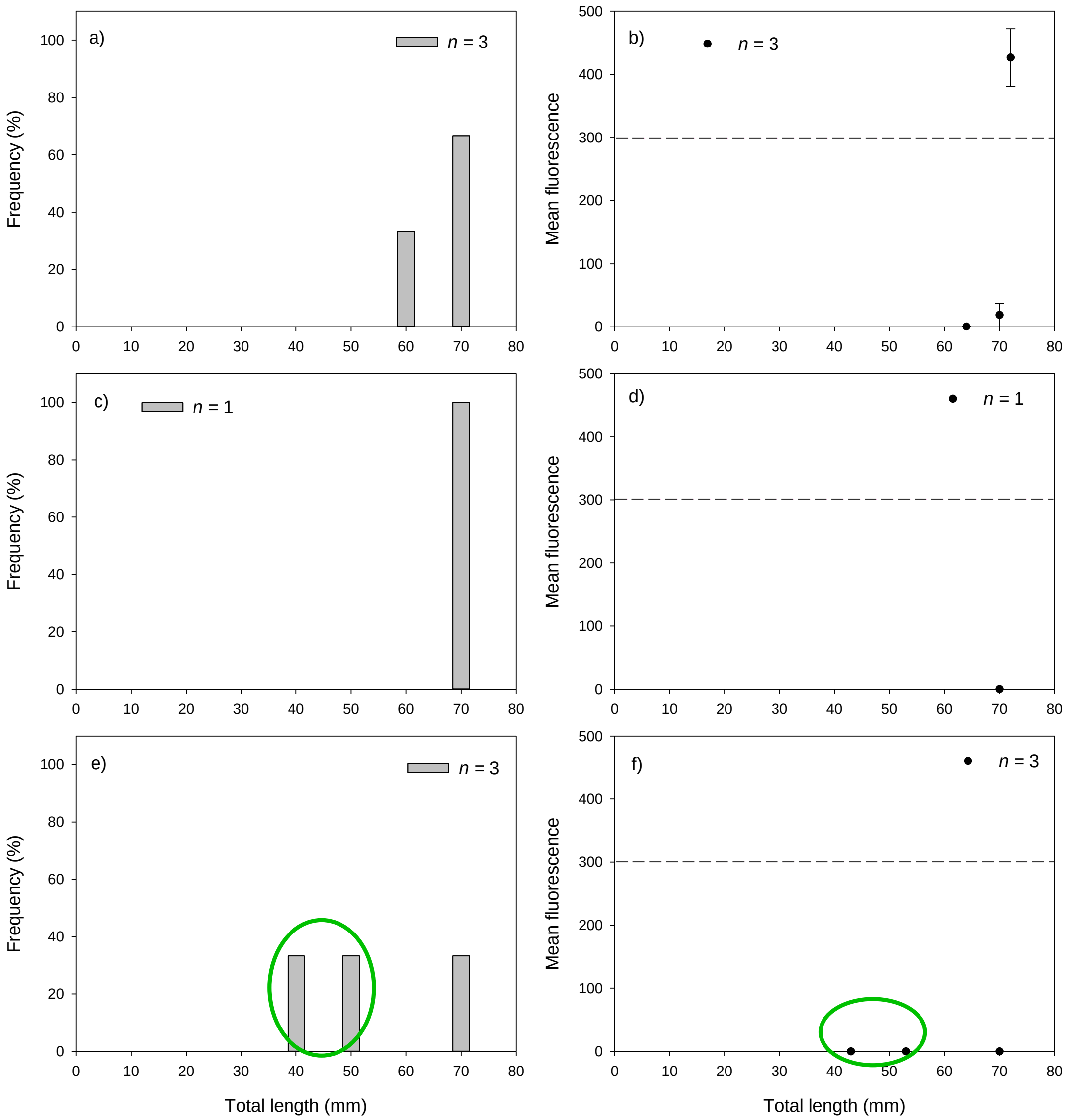

Figure 12. Length-frequency distributions and corresponding mean fluorescence (± standard error) against length plots for all southern purple-spotted gudgeon sampled in autumn 2012 (a, b), spring 2012 (c, d) and autumn 2013 (e, f). Dashed line represents the fluorescence reading deemed to indicate a calcein mark. Green ellipses indicate potential young-of-year individuals.

Figure 13. Southern purple-spotted gudgeon recaptured in spring 2012.

4. Discussion

Unprecedented drought and continued over-abstraction of water from the MDB between 2007 and 2010 placed the CLLMM region under extreme ecological pressure (Kingsford *et al.* 2011, Wedderburn *et al.* 2012). Over this period, water level recession in the Lower Lakes led to habitat fragmentation, broad-scale loss of submerged vegetation and elevated salinities, in turn resulting in significant declines in threatened fish populations (Wedderburn *et al.* 2012). Numerous urgent and ongoing conservation actions were required to avert the local extinction of Yarra pygmy perch, southern pygmy perch, Murray hardyhead and southern purple-spotted gudgeon (Hammer *et al.* 2013), and ensured a supply of individuals for reintroduction upon the return of favourable conditions.

Following broad-scale rainfall in the MDB in 2010/11 and significant inflows, the Lower Lakes returned to typical water levels (~0.75 m AHD) and there has been a gradual recovery of submerged vegetation (Gehrig *et al.* 2012), preferred habitat of the aforementioned species. Over four reintroduction events between spring 2011 and autumn 2013, ~15,840 fish, across the four species, have been released at ten suitable locations in the region. Subsequent monitoring has detected low numbers of all four species and evidence of wild recruitment in Yarra pygmy perch, southern pygmy perch and Murray hardyhead.

4.1 Status of the threatened species in the region

As of autumn 2013, following four rounds of reintroduction, Yarra pygmy perch, southern pygmy perch, Murray hardyhead and southern purple-spotted gudgeon are present within the CLLMM region. Without the conservation actions undertaken as part of the DAP, during the period of critical water shortage (see Hammer *et al.* 2013), and reintroductions as part of the current project, this outcome may not have been achieved. Whilst there have been positive indications of survival, and recruitment in some species, the aim of facilitating the establishment of self-sustaining populations is unlikely to have been met for all species at this point in time.

Reintroductions of Yarra pygmy perch have met with varying success. Following initial releases at Blue Lagoon and Finniss River Junction, no fish were subsequently detected at these sites, and recaptures occurred at Steamer Drain in the sampling round following reintroduction, but the species was not detected in autumn 2013. Yarra pygmy perch were, however, sampled at Shadows Lagoon in the last two monitoring rounds and fish sampled in autumn 2013 represent recently recruited YOY, indicating wild recruitment occurred following spawning in 2012. Whilst sampled in low numbers, these results are significant, representing the first records of Yarra pygmy perch from the Lower Lakes since February 2008 (Hammer 2008) and the first record of wild recruitment since 2007 (Bice *et al.* 2008). Despite significant sampling over the period 2008–2011 (Bice *et al.* 2009, Wedderburn and Barnes 2009, Bice *et al.* 2010, Wedderburn and Hillyard 2010, Bice *et al.* 2011, Bice and Zampatti 2011, Wedderburn and Barnes 2011, Wedderburn and Barnes 2012) no Yarra pygmy perch were detected, suggesting local extirpation (Wedderburn *et al.* 2012). Recaptures at Steamer Drain and Shadows Lagoon suggest that reintroduction success and/or efficiency of sampling to subsequently detect Yarra pygmy perch, is greatest in spatially confined sites as opposed to more 'open' sites like Blue Lagoon and Finniss River Junction. Whilst there have been initial signs of population establishment for this species (i.e. recruitment at Shadows Lagoon), further reintroductions are most likely required and should focus on spatially confined sites with favourable habitat to increase chances of success.

Southern pygmy perch underwent similar declines to Yarra pygmy perch over 2007–2011 (Bice *et al.* 2011, Wedderburn *et al.* 2012) and presence in the region has been dependent upon reintroductions under the current project. Initial reintroductions at the 'natural channel connected to Hunters Creek' site met with success, with individuals sampled and recruitment evident in all three subsequent monitoring rounds (autumn 2012, spring 2012, autumn 2013). These results are encouraging in regards to the establishment of a self-sustaining population at this site. Reintroduction at Mundoo Island Channel east 2 also met with some success, with low numbers of individuals subsequently sampled at the release site and two neighbouring, hydrologically connected sites, with recruitment evident in autumn 2013. The status of southern pygmy perch at the Turvey's Drain site remains uncertain following failure to detect any fish in the previous two monitoring rounds. Unfortunately, no surrogate refuge was established for southern pygmy perch as part of the DAP and subsequently, the limited number of individuals restricted reintroductions to 2011/12 and there is no further source of individuals for further reintroductions.

In autumn 2013, Murray hardyhead were sampled in the greatest number in the Lower Lakes since spring 2010 (Bice *et al.* 2011), with increased abundance likely a result of a combination of reintroduction success and recruitment of remnant wild fish. Whilst Murray hardyhead exhibited declines, sporadic captures of individuals were recorded between 2007 and 2010 (Bice *et al.* 2011, Wedderburn and Barnes 2011) suggesting a low number of individuals remained in wild habitats. Given the species high mobility and tolerance to elevated salinity (Wedderburn *et al.* 2008), Murray hardyhead were potentially more resilient to the prevailing conditions than both pygmy perch species. Murray hardyhead were detected at the Finniss River Junction site prior to any reintroductions (autumn 2012) and were detected at this site in the two subsequent monitoring rounds. Additionally, a low number of individuals were captured at the nearby Dunn's Lagoon and Old Clayton sites and it is hypothesised that these individuals are likely remnant wild fish. Additionally, following releases in autumn and spring 2012, individuals have been detected during both subsequent monitoring rounds at the Mundoo Island Drain east site, suggesting survival of reintroduced individuals and potentially recruitment. No individuals have yet been detected at the Hunters Creek reintroduction site. The current status of this species, however, is encouraging and with continued recovery of habitat within the Lower Lakes (Gehrig *et al.* 2012), the future sustainability of this species is promising.

Consistent sampling of low numbers of southern purple-spotted gudgeon post-reintroductions indicates continued survival of the species at the Finniss River at Winery Road site for periods of 6–18 months. Additionally, two fish sampled in autumn 2013 were <55 mm TL and may represent wild recruited individuals following the first two reintroductions. Fluorescence readings were inconsistent with a calcein mark, supporting the hypothesis that these were wild recruits, but potential issues in the reliability of detecting external marks in this species reduces the confidence in making this conclusion. Nonetheless, captures of southern purple-spotted gudgeon at this site represent the first records of the species in the CLLMM region since the 1960s (Hammer 2009a). Whilst only sampled in low numbers, habitat at the site appears favourable (i.e. abundant aquatic vegetation and woody debris) and the species stands a good chance of establishing a self-sustaining population. Nonetheless, comparatively low numbers of this species have been reintroduced and further reintroduction would likely aid population establishment.

4.2 Lessons learnt - refinement of the reintroduction framework

Reintroductions of threatened fish under the current project was guided by a scientifically rigorous framework (Watt *et al.* 2011), which was adapted from the framework of Hammer *et al.* (2009a) and a review by George *et al.* (2009). Nonetheless, in order to maximise likelihood of success, the CFH project had to be adaptive as new knowledge was gained on species ecology and the reintroduction method. Accordingly, several lessons were learnt to guide future reintroductions of these species and other reintroduction programs. These are discussed in regards to the establishment of surrogate refuges, refining fish capture and transport methods and the use of calcein as a technique for distinguishing reintroduced fish from their wild produced progeny and/or remnant wild fish.

The establishment of surrogate refuges for Yarra pygmy perch, Murray hardyhead and southern purple-spotted gudgeon under the DAP (Hammer *et al.* 2009b) was not a novel approach, but has been utilised in several instances in the United States for small-bodied threatened species such as pupfishes (*Cyprinodon* spp.) (Winemiller and Anderson 1997, Karam *et al.* 2012, Schaeffer *et al.* 2012). In comparison to the hatchery rearing of fish, such an approach allows fish to be reared in a semi-natural setting with exposure to some level of predation pressure, live foods and climatic variation, potentially producing individuals better suited to reintroduction and wild survival. Additionally, hatcheries are often expensive to operate and are limited by space and resources, which in turn may limit fish production. The use of surrogate refuges, however, may alleviate these issues. In the current project >65% of all reintroduced individuals have been sourced from surrogate refuges and the populations of Yarra pygmy perch at Crouch Dam and Tupplegrove Nursery, and Murray hardyhead at Munday Dam, remain abundant and exhibit regular recruitment (Hammer *et al.* 2013). For southern purple-spotted gudgeon, short-term survival has been observed in a recently established surrogate refuge (a further surrogate refuge will be established in the coming months) indicating a potentially viable reintroduction source for this species (Kate Mason, pers. comm.). Thus, the use of surrogate refuges (i.e. establishment of self-sustaining ex-situ populations) has been invaluable in the current project, representing a low cost medium-long term strategy in threatened fish conservation, with these refuges providing an ongoing source of large numbers of individuals for reintroduction. Nonetheless, issues of diminished genetic diversity and genetic drift need to be taken into account due to low numbers of original founder fish and increasing temporal isolation from wild populations.

Stress associated with transport (e.g. during stocking programs) has been shown to impact the condition and subsequent survival of a range of fish species (Portz *et al.* 2006). Indeed, during the present reintroductions, the capture and transport of fish from surrogate refuges was at times problematic. Yarra pygmy perch and Murray hardyhead are both highly susceptible to handling stress and the movement of individuals from nets to buckets and aerated holding tubs before transportation back to holding facilities (~1.5 hr journey) often resulted in moderate levels of mortality (up to ~25%) in the subsequent 2–3 days. Transportation of these species from hatcheries and other holding facilities, however, resulted in very low transport-related mortality rates, likely as a result of comparatively lesser physical stress. Thus, any future capture of individuals from surrogate refuges should be undertaken in a manner that minimises handling, injury and stress.

Calcein and other chemical dyes, have been used for some time in the United States (Mohler 1997), and more recently in Australia (Crook *et al.* 2009), for non-lethally differentiating stocked and wild individuals of recreationally important freshwater fish species and is now becoming increasingly common, for the same purpose, in ‘conservation stocking’ (i.e. reintroduction) programs. Several factors influence the usefulness of this technique including fish growth and degradation of mark intensity by sunlight (Honeyfield *et al.* 2008, Westergaard 2013). In the current project, using calcein marking to non-lethally differentiate between recaptured reintroduced individuals and wild spawned fish was successful for both Yarra pygmy perch and southern pygmy perch, but potentially unsuccessful for southern purple-spotted gudgeon and Murray hardyhead. Large individuals (>40 mm TL) of both pygmy perches sampled at reintroduction sites exhibited fluorescence consistent with a calcein mark, indicating these individuals were recaptures from previous reintroductions. Several individuals were also sampled for both species, which based on length-frequency data, were hypothesised to be YOY and low fluorescence readings confirmed this hypothesis. Alternatively, all southern-purple-spotted gudgeon sampled throughout the project, with one exception, exhibited fluorescence levels inconsistent with a calcein mark. Whilst it is possible some fish sampled were wild recruited fish, captures in autumn 2012 (and most likely spring 2012) were undoubtedly recaptures of reintroduced fish based upon their size. Variable calcein mark detection between species was likely the result of behavioural differences between species and environmental conditions at reintroduction sites. Sites where pygmy perch were sampled were generally more turbid and heavily vegetated, and both species possess a high affinity for vegetated habitat, rarely venturing into open habitats. Conversely, the southern purple-spotted gudgeon are known to bask in direct sunlight and the site on the Finniss River has a high transparency, potentially resulting in relatively greater exposure to UV light and subsequent mark deterioration. Westergaard (2013) found similar degradation of mark intensity in Yarra pygmy perch when exposed to high levels of sunlight. Interestingly, Westergaard (2013) also found that maintaining Yarra pygmy perch in a low-light environment for two weeks post-staining decreased the degradation of calcein marks upon subsequent exposure to sunlight. This approach may be adopted for southern purple-spotted gudgeon for future reintroductions and may enhance mark retention. Despite the observed external mark degradation in southern purple-spotted gudgeon, we suggest continued use of this technique as calcein also marks internal bony structures (e.g. otoliths). Given the potential life-span of the species (potentially >5years), longer-term assessment of reintroduction success could be made in the future should the population reach a size adequate to allow the sacrifice of low numbers of individuals for otolith microstructure analysis.

The use of calcein marking for differentiating reintroduced and wild recruited Murray hardyhead was ineffective due to potential natural fluorescence in this species. Individuals sampled from the Finniss River Junction, Dunn's Lagoon and Old Clayton sites, which based upon location and length, were unlikely to be reintroduced individuals, exhibited fluorescence similar to that indicative of a calcein mark. Ad hoc measurements taken simultaneously from the congeneric unspecked hardyhead (*Craterocephalus stercusmuscarum fulvus*) also presented fluorescence levels consistent with a mark, suggesting natural fluorescence may be common to the genus. Given the inability to differentiate the source of fish based upon fluorescence and the short life span of the species (1–2 years), we suggest future reintroductions may forego calcein marking. Nonetheless, other techniques, such as otolith microchemistry, may be effective in differentiating reintroduced and 'wild recruited' individuals, but would require sacrificing individuals.

4.3 The way forward - future conservation of threatened fish species

Whilst considerable time and effort have been directed towards the reintroduction of threatened species under the current project and positive signs of survival, and in some cases recruitment, have been exhibited by all species, meeting the objective of self-sustaining wild populations that require minimal management attention, remains aspirational. All species are present at a limited number of sites (in some cases individual sites), and with the exception of Murray hardyhead, in low numbers, meaning they are highly vulnerable to stochastic events. When viewed in light of other such reintroduction programs for threatened fishes both in Australia (Lyon *et al.* 2012) and internationally (Shute *et al.* 2005, Rakes and Shute 2006, Bezold 2007, George *et al.* 2009), effort expended in the current project has been comparatively minimal. Reintroductions of European sturgeon (*Acipenser sturio*) and lake sturgeon (*Acipeser fulvescens*) in Germany and the United States, respectively, have involved the reintroduction of tens of thousands of individuals over multiple years (Bezold 2007). Reintroductions of small-bodied threatened species, such as darters (Percidae) and madtoms (Ictaluridae), in the south-eastern United States have released similar numbers of fish to the current project, but reintroduction programs have occurred over periods of up to 20 years (George *et al.* 2009). Re-establishment of yellowfin madtom (*Noturus flavipinnis*), smokey madtom (*Noturus baileyi*) and Citico darter (*Etheostoma sitikuense*) into Abrams Creek, Tennessee (Shute *et al.* 2005) is often viewed as a success story for threatened fish reintroductions; nevertheless, it took five years before there were any recaptures of reintroduced fish and ten years before wild recruitment was observed (George *et al.* 2009). Lyon *et al.* (2012) suggest the long-term (10 years) stocking program for trout cod (*Maccullochella macquariensis*) in the Ovens River, in the MDB, provided the opportunity for some cohorts to encounter favourable conditions for survival, whilst fish stocked in other years did not survive and contribute to future populations. Such projects suggest further reintroductions of the target species in the current project are likely required to meet the objective of re-establishing self-sustaining wild populations. Indeed, common to all the aforementioned programs is the annual release of small-medium number of individuals over multiple years, which may have resulted in the success of these programs. Sources of Yarra pygmy perch and Murray hardyhead remain abundant in the surrogate refuges and present an opportunity to continue reintroductions on an annual basis.

Potential future site assessments and reintroductions should expand their scope from the current list of selected historic long-term monitoring sites. Several of the current sites were sampled as part of general fish assemblage monitoring projects or represented marginal habitat that fish were forced into during drought. Future assessments could be expanded to further sites that pass stage 1 assessment and appear to be highly suitable for reintroduction, based on expert opinion (e.g. Finniss River immediately upstream Wally's Wharf). The potential success of future reintroductions could also be further enhanced through habitat restoration and enrichment. Several drain habitats on Hindmarsh and Mundoo Islands have become 'overgrown' with emergent vegetation (particularly *Typha domingensis*), restricting water flow and potentially fish movement through the drain networks.

Works to partially clear some of this vegetation may increase habitat suitability and aid dispersal to neighbouring sites.

Ongoing monitoring of the threatened fish populations (both reintroduced and remnant populations) throughout the CLLMM region, is integral, firstly to provide specific assessment of the success of fish reintroductions to-date and secondly to evaluate temporal changes in condition across the CLLMM region. Targeted post-reintroduction monitoring is required to determine the survival of reintroduced fish and their distribution (i.e. measures of population extent and dispersal) and provide information on population demographics (i.e. age structure and recruitment), as well as monitoring threats (e.g. changes in habitat water levels, introduced species) at the reintroduction sites and more broadly. Importantly, any future monitoring and management should incorporate knowledge gathered and lessons learned during the drought and associated DAP project, and subsequently throughout the period of reintroductions under the current project, on efforts required to conserve threatened fish species.

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6. Appendices

Appendix 1. Species and abundance of fish sampled from all sites in spring 2012

	Murray hardyhead	Southern pygmy perch	Yarra pygmy perch	Southern purple-spotted gudgeon	Unspecked hardyhead	Bony herring	Golden perch	Australian smelt	Flat-headed gudgeon	Dwarf flat-headed gudgeon	Carp gudgeon spp.	Common galaxias	Congolli	Lagoon goby	Tamar River goby	Bluespot goby	Small-mouthed hardyhead	Sandy sprat	Redfin perch	Eastern gambusia	Common carp	Godfish	Native species richness	Non-native species richness
Finniss above Winery Road	0	0	0	1	0	0	0	0	1	1	0	1	70	0	0	0	0	0	0	1	1	0	**5**	**2**
Blue Lagoon	0	0	0	0	0	0	0	4	67	5	21	0	27	8	0	0	0	13	21	0	10	0	**7**	**2**
Blue Lagoon 2	0	0	0	0	1	0	3	5	91	11	43	28	16	2	0	4	0	0	23	4	10	0	**10**	**3**
Finniss River junction	7	0	0	0	15	0	3	2	51	12	20	114	21	9	0	0	0	0	9	45	25	8	**10**	**4**
Hunters Creek (us Denver Rd causeway)	0	0	0	0	0	0	0	0	26	0	3	1053	10	0	0	0	0	0	3	1	9	4	**4**	**4**
Hunters Creek (ds Denver Rd causeway)	0	0	0	0	0	0	0	2	31	9	3	650	18	6	0	0	0	1	6	0	31	1	**8**	**3**
Natural channel connected to Hunters Creek (behind DENR-Wyndgate)	0	4	0	0	0	0	0	0	3	0	0	48	0	0	0	0	0	0	0	12	8	8	**3**	**3**
Steamer drain	0	0	2	0	0	0	0	0	1	0	1	32	3	0	0	0	0	0	6	5	3	0	**5**	**3**
Holmes Creek at Eastick Creek mouth	0	0	0	0	1	0	3	11	27	0	0	73	5	8	0	0	0	0	183	2	10	1	**7**	**4**

Appendix 1 continued.

	Murray hardyhead	Southern pygmy perch	Yarra pygmy perch	Southern purple-spotted gudgeon	Unspecked hardyhead	Bony herring	Golden perch	Australian smelt	Flat-headed gudgeon	Dwarf flat-headed gudgeon	Carp gudgeon spp.	Common galaxias	Congolli	Lagoon goby	Tamar River goby	Bluespot goby	Small-mouthed hardyhead	Sandy sprat	Redfin perch	Eastern gambusia	Common carp	Godfish	Native species richness	Non-native species richness
Turvey's drain	0	0	0	0	0	0	0	0	0	2	9	0	0	0	0	0	0	0	1	0	4	0	**2**	**2**
Currency Creek Game Reserve	0	0	0	0	0	0	5	0	12	1	0	109	8	2	0	0	0	0	3	8	12	2	**6**	**4**
Black Swamp	0	0	0	0	0	0	2	0	30	2	10	0	3	0	0	0	0	0	5	0	5	0	**5**	**2**
Mundoo channel east 2	0	1	0	0	0	0	0	0	0	0	5	1	0	0	0	0	0	0	0	4	0	1	**3**	**2**
Mundoo channel east	4	2	0	0	0	0	0	1	41	0	129	0	1	0	0	0	0	0	4	11	0	1	**6**	**3**
Mundoo channel west*	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3	0	8	1	**1**	**3**
Dunn's Lagoon*	0	0	0	0	1	3	0	4	2	0	3	51	9	6	0	0	0	0	135	4	5	0	**8**	**3**
Boggy Creek*	0	0	0	0	0	0	0	0	31	1	15	4	3	0	0	0	0	0	6	0	28	0	**5**	**2**
Shadows Lagoon*	0	0	8	0	0	0	0	0	132	0	60	19	1	0	0	0	0	0	10	8	50	9	**4**	**4**

Appendix 2. Species and abundance of fish sampled from all sites in autumn 2013

	Murray hardyhead	Southern pygmy perch	Yarra pygmy perch	Southern purple-spotted gudgeon	Unspecked hardyhead	Bony herring	Golden perch	Australian smelt	Flat-headed gudgeon	Dwarf flat-headed gudgeon	Carp gudgeon spp.	Common galaxias	Congolli	Lagoon goby	Tamar River goby	Small-mouthed hardyhead	Sandy sprat	Redfin perch	Eastern gambusia	Common carp	Godfish	Native species richness	Non-native species richness
Finniss Winery Road	0	0	0	3	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	5	0	**2**	**1**
Blue Lagoon	0	0	0	0	0	16	1	0	7	3	0	0	9	0	0	0	0	64	0	38	2	**5**	**3**
Blue Lagoon 2	0	0	0	0	30	193	4	1	272	6	5	17	16	0	0	0	0	63	9	9	8	**9**	**4**
Finniss River junction	43	0	0	0	0	0	0	0	97	6	7	21	12	0	0	0	0	5	94	7	6	**6**	**4**
Hunters Creek (us Denver Rd causeway)	0	0	0	0	0	0	0	0	6	5	2	100	33	0	0	0	0	2	23	14	26	**5**	**4**
Hunters Creek (ds Denver Rd causeway)	0	0	0	0	0	0	0	0	381	4	8	42	16 8	0	0	0	0	1	15	3	0	**5**	**4**
Drain behind Wyndgate	0	0	0	0	0		0	0	3	0	1	5	7	0	0	0	0	0	0	5	3		
Natural channel connected to Hunters Creek (behind DENR-Wyndgate)	0	0	0	0	0	0	0	0	0	0	0	13	0	0	0	0	0	0	16	1	3	**1**	**3**
Steamer drain	0	0	0	0	0	0	0	0	39	1	1	53	2	0	0	0	0	1	1095	3	16	**5**	**4**
Holmes Creek at Eastick Creek mouth	0	0	0	0	82	1	4	5	313	0	1	47	8	0	0	1	0	25	4	34	2	**9**	**4**
Turvey's drain	0	0	0	0	0	0	0	0	4	3	5	0	0	0	0	0	0	1	317	2	9	**3**	**4**
Currency Creek Game Reserve	0	0	0	0	47	5	5	1	27	2	1	19	16	0	0	0	0	12	69	20	2	**9**	**4**
Black Swamp	0	0	0	0	0	4	1	0	139	1	1	0	1	0	0	0	0	14	0	6	0	**6**	**2**

Appendix 2 continued.

	Murray hardyhead	Southern pygmy perch	Yarra pygmy perch	Southern purple-spotted gudgeon	Unspecked hardyhead	Bony herring	Golden perch	Australian smelt	Flat-headed gudgeon	Dwarf flat-headed gudgeon	Carp gudgeon spp.	Common galaxias	Congolli	Lagoon goby	Tamar River goby	Small-mouthed hardyhead	Sandy sprat	Redfin perch	Eastern gambusia	Common carp	Godfish	Native species richness	Non-native species richness
Mundoo Island Channel east*	9	0	0	0	0	0	0	0	32	0	7	0	1	0	0	1	0	0	144	1	0	**5**	**2**
Mundoo Island Channel east 2	0	0	0	0	0	0	0	0	0	0	29	0	0	0	0	0	0	0	953	1	3	**1**	**3**
Mundoo Island Channel west*	0	0	0	1	0	1	0	0	1	0	5	29	0	0	0	0	0	1	59	0	21	**5**	**3**
Boggy Creek*	0	0	0	0	0	0	0	0	14	0	21	3	1	0	0	0	0	0	197	3	1	**4**	**3**
Dunn's Lagoon*	7	0	0	0	38	154	1	2	24	5	14	16	8	0	0	1	0	25	35	2	0	**11**	**3**
Shadows Lagoon*	0	0	2	0	0	0	0	0	84	1	1	2	6	0	0	0	0	5	83	9	2	**6**	**4**

*denotes sites sampled by Wedderburn and Barnes (2012).

Appendix 3. Macroinvertebrate families and subjective abundance scores (R – rare, C – common, A – abundant, V – very abundant) from sampling conducted during fish monitoring in spring 2012.

	Damselfly nymph	Dragonfly nymph	Mayfly nymph	Caddisfly nymph	Paratya	Macrobrachium	Yabby	Snail (*Physa*)	Snail (gilled)	Water strider	Predacious diving beetle	Water scavenger beetle	Whirligig bettle	Crawling water beetle	Beetle larvae	Corixids (boatmen)	Notonectid (backswimmers)	Amphipod	Non-biting midge larvae	Biting midge larvae	Mosquito larvae	Copopoda	Cladocera	Ostracoda	Fishing spider	Freshwater limpet	Freshwater crab	Roundworm
Finniss Winery Road			R		A		C	R		R						R		C	R	C	R	C	C	R			A	
Blue Lagoon					V	V	C		R									A		C		V						
Blue Lagoon 2	C		R	R	V	V		R								R			R	C	R	A						
Finniss River junction	C				V	V	A		R							R	C	A	R	C	R	C	C	C				
Hunters Creek (us Denver Rd causeway)	C				R			R								V	V	A	C			A	C			R		
Hunters Creek (ds Denver Rd causeway)	C				R					R						C	C	A	C	C		C	C			R		
Drain behind Wyndgate							V	R		R						R		A		R		A						R
Natural channel connected to Hunters Creek (behind DENR-Wyndgate)	C										C			C		A	A	V		A	A	C	C	C				
Steamer drain	A	C				A	C					R				C		C	R	C			C	C				R
Holmes Creek at Eastick Creek mouth	R							R			R					C	C	A		A		R	R	R				
Turvey's drain	A	A					V				V						A	C	A			C						
Currency Creek Game Reserve	A	R			A		R	R			R						C	A		A		C	C	C				
Black Swamp	C	C	R	R	C													C	C	C		C		C		R		

	Damselfly nymph	Dragonfly nymph	Mayfly nymph	Caddisfly nymph	Paratya	Macrobrachium	Yabby	Snail (*Physa*)	Snail (gilled)	Water strider	Predacious diving beetle	Water scavenger beetle	Whirligig bettle	Crawling water beetle	Beetle larvae	Corixids (boatmen)	Notonectid (backswimmers)	Amphipod	Non-biting midge larvae	Biting midge larvae	Mosquito larvae	Copopoda	Cladocera	Ostracoda	Fishing spider	Freshwater limpet	Freshwater crab	Roundworm	Lepidopteran larvae
Mundoo Island Channel east*	A				C												A	A		R		C	R	C		C			
Mundoo Island Channel east 2	V	C						R		R			R					V	A		C	C		A					
Mundoo Island Channel west*		R						R			R					R	C	V		C		C		A	R				
Boggy Creek*	C							C		C	C					C		A	A	C		C	R	C				R	
Dunn's Lagoon*	R									A	R					C			V	A			C	V				R	
Shadows*	A				A											A	C	V	C	A		V	C			C			C

Appendix 4. Macroinvertebrate families and subjective abundance scores (R – rare, C – common, A – abundant, V – very abundant) from sampling conducted during fish monitoring in autumn 2013.

	Damselfly nymph	**Dragonfly nymph**	**Mayfly nymph**	**Caddisfly nymph**	**Paratya**	**Macrobrachium**	**Yabby**	**Snail (*Physa*)**	**Snail (gilled)**	**Water strider**	**Predacious diving beetle**	**Water scavenger beetle**	**Whirligig bettle**	**Crawling water beetle**	**Beetle larvae**	**Corixids (boatmen)**	**Notonectid (backswimmers)**	**Amphipod**	**Non-biting midge larvae**	**Biting midge larvae**	**Mosquito larvae**	**Copopoda**	**Cladocera**	**Ostracoda**	**Leech**	**Fishing spider**	**Freshwater limpet**	**Roundworm**	**Water measurer**
Finniss Winery Road	A		R	R			C			V								A		A			C						
Blue Lagoon	C		C	C	V	V										C		C	C										
Finniss River junction		R	A		A	A	C										A			A		C	C						
Hunters Creek (us Denver Rd causeway)	V	R	R		A		A			C	C					A		R	R			A	C		R			R	R
Hunters Creek (ds Denver Rd causeway)	C		A		V	C	C				C					C	V	A	A										
Drain behind Wyndgate			R				V			C	A					A	A	R		C		C							
Natural channel connected to Hunters Creek (behind DENR-Wyndgate)		C					V			C	V			A				A	C		C		C	A		A			
Steamer drain	C						V	C			R	R		C					C					R					
Holmes Creek at Eastick Creek mouth	R				A	V	R									R	A	C		A									
Turvey's drain	R				R		V				A			C		A							R						
Currency Creek Game Reserve			R	R	V	V	C										C	C		C		C	C						
Black Swamp	C				V	V												R		R		R					R		

Appendix 4 continued.

	Damselfly nymph	Dragonfly nymph	Mayfly nymph	Caddisfly nymph	Paratya	Macrobrachium	Yabby	Snail (*Physa*)	Snail (gilled)	Water strider	Predacious diving beetle	Water scavenger beetle	Whirligig betle	Crawling water beetle	Beetle larvae	Corixids (boatmen)	Notonectid (backswimmers)	Amphipod	Non-biting midge larvae	Biting midge larvae	Mosquito larvae	Copopoda	Cladocera	Ostracoda	Leech	Fishing spider	Freshwater limpet	Roundworm	Water measurer
Mundoo Island Channel east 2	A						V	C			A						R	R						A					
Mundoo Island Channel west*																													
Boggy Creek*	A		C	C	C									C			A	A	R	C				A					
Dunn's Lagoon*					C												C	A	C	C	C			C				C	
Shadows Lagoon*	A				V									C		C	C	A				C	C						

Appendix 5. Habitat cover and physico-chemical parameters measured at all sites during sampling in spring 2012.

	Habitat				Physico-chemical parameters							
	Submerged (%)	**Emergent (%)**	**Physical (%)**	**Open water (%)**	**DO surface (ppm)**	**DO depth (ppm)**	**pH**	**Temp (˚C)**	**Conductivity (µS.cm^{-1})**	**Secchi depth (m)**	**Mean depth**	**Max depth**
Finniss Winery Road	0	45 (*Typha, Phragmites, Triglochin, Azolla, Berula,* grasses)	5 (snag, woody debris)	50	4.2	3.31	7.66	21.3	1896	0.35	0.46	0.65
Blue Lagoon	1 (*Myriophyllum, Potamogetan crispus*)	29 (*Typha, Schoenoplectus,*)	0	70	7.75	6.73	7.82	20.6	1903	0.22	0.52	0.6
Blue Lagoon 2	1 (*Myriophyllum, Potamogetan crispus*)	39 (*Schoenoplectus, Typha, Phragmites*)	0	60	6.68	0.81	7.03	22.2	1998	0.1	0.56	0.75
Finniss River junction	45 (*Myriophyllum*)	25 (*Typha, Phragmites, Schoenoplectus*)	0	30	6.55	4.32	7.77	22.1	562	0.22	0.56	0.65
Hunters Creek (us Denver Rd causeway)	15 (algae, *Myriophyllum, Potamogetan crispus*)	25 (*Typha, Bolboschoenus,* grasses)	0	60	4.46	3.89	7.71	20.9	783	0.22	0.9	1.2
Hunters Creek (ds Denver Rd causeway)	15 (*Myriophyllum, Potamogetan crispus*)	19 (*Typha, Bolboschoenus,* grasses)	1 (snag, rock)	65	6.24	2.17	7.26	20	830	0.35	0.68	0.75
Natural channel connected to Hunters Creek (behind DENR-Wyndgate)	70 (*Myriophyllum, Ceratophyllum,* algae)	29 (*Typha, Juncus, Bolboschoenus,* grasses)	0	1	4.84	-	7.42	21.3	3590	>depth	0.58	0.75
Steamer drain	85 (*Myriophyllum,* algae)	14 (*Typha, Bolboschoenus, Juncus, Azolla,* grass)	0	1	3.17	1.36	7.1	19.8	320	0.15	0.84	1.0
Holmes Creek at Eastick Creek mouth	0.1 (*Myriophyllum*)	25 (*Typha, Schoenoplectus,* grass)	0	74.5	9.63	9.37	8.13	22	325	0.25	0.65	0.9
Turvey's drain	65 (*Myriophyllum, Ceratophyllum*)	20 (*Typha, Phragmites,* grasses)	0	15	2.75	0.8	7.32	24.1	1340	0.7	0.92	1.5
Currency Creek Game Reserve	25 (*Myriophyllum*)	45 (*Typha, Phragmites*)	0	30	7.23	4.63	7.44	17.5	415	0.25	0.57	0.76
Black Swamp	1 (*Myriophyllum, Ceratophyllum*)	29 (*Typha, Phragmites, Schoenoplectus*)	0	70	7.21	0.87	7.29	20.8	1501	0.3	1.08	1.4

Appendix 5 continued

	Habitat				Physico-chemical parameters							
	Submerged (%)	**Emergent (%)**	**Physical (%)**	**Open water (%)**	**DO surface (ppm)**	**DO depth (ppm)**	**pH**	**Temp (˚C)**	**Conductivity ($\mu S.cm^{-1}$)**	**Secchi depth (m)**	**Mean depth**	**Max depth**
Mundoo Island Channel east*	10 (*Myriophyllum, Ceratophyllum*, algae)	13 (*Typha*, grasses)	2 (rock)	75	9.36	9.42	8.19	24.2	4780	0.2	0.91	1.3
Mundoo Island Channel east 2	55 (*Myriophyllum, Ceratophyllum*, algae)	40 (*Typha, Juncus*, grasses)	0	5	6.89	2.65	8.97	25	1015	>depth	0.64	0.9
Mundoo Island Channel west*	0	98 (*Typha, Bolboschoenus, Azolla*, algae)	0	2	1.67	0.59	8.3	19.9	497	0.25	0.93	1.4
Boggy Creek*	5 (algae)	90 (*Azolla, Typha, Phragmites, Juncus*, grasses)	0	5	1.14	0.8	6.99	18.6	387	0.3	0.68	1.2
Dunn's Lagoon*					6.91	4.76	7.09	23.7	725	0.25	0.62	0.75
Shadows*	35 (*Vallisneria*)	5 (*Typha, Azolla, Phragmites, Boobealla, Meulaleuca*, grasses)	0	60	7.45	4.67	7.09	23.7	725	0.25		

Appendix 6. Habitat cover and physico-chemical parameters measured at all sites during sampling in autumn 2013.

	Habitat				Physico-chemical parameters							
	Submerged (%)	**Emergent (%)**	**Physical (%)**	**Open water (%)**	**DO surface (ppm)**	**DO depth (ppm)**	**pH**	**Temp (˚C)**	**Conductivity (μS.cm^{-1})**	**Secchi depth (m)**	**Mean depth**	**Max depth**
Finniss Winery Road	0	60 (*Typha, Phragmites, Triglochin, Lemna, Berula*, grasses)	10 (snag, woody debris)	30	2.47	-	8.71	21	2810	>depth	0.5	0.7
Blue Lagoon	5 (*Myriophyllum, Potamogetan crispus*)	35 (*Typha, Schoenoplectus*, grasses)	0	60	7.2	-	8	30.4	2089	0.35	0.67	0.85
Blue Lagoon 2	5 (*Myriophyllum, Potamogetan crispus*)	55 (*Schoenoplectus, Typha, Phragmites*)	0	40	3.93	-	8.19	30.1	2135	0.35	0.56	0.6
Finniss River junction	60 (*Myriophyllum*)	30 (*Typha, Phragmites, Schoenoplectus*)	0	10	5.6	0.3	8.3	20.1	674	0.43	0.57	0.7
Hunters Creek (us Denver Rd causeway)	10 (*Myriophyllum*,)	30 (*Typha, Azolla, Bolboschoenus*, grasses)	0	60	3.46	3.25	7.98	21.1	761	>depth	0.53	0.8
Hunters Creek (ds Denver Rd causeway)	20 (*Myriophyllum, Potamogetan crispus*)	10 (*Typha, Bolboschoenus*, grasses)	0	70	4.2	4.22	7.28	25.7	779	0.35	0.62	0.7
Natural channel connected to Hunters Creek (behind DENR-Wyndgate)	70 (*Myriophyllum*, algae)	28 (*Typha, Bolboschoenus*, grasses)	0	2	9.96	1.56	7.84	25.2	2400	>depth	0.41	0.7
Steamer drain	55 (*Myriophyllum*, algae)	25 (*Typha, Bolboschoenus*, grass)	0	20	5.41	2.74	7.65	23.1	582	0.25	0.6	0.9
Holmes Creek at Eastick Creek mouth	2 (*Myriophyllum, Vallisneria*)	44 (*Typha, Phragmites, Schoenoplectus, Bolboschoenus*)	2 (rock)	52	10.19	-	8.89	22.6	512	0.35	0.67	1.2
Turvey's drain	55 (*Myriophyllum, Ceratophyllum*)	40 (*Typha, Phragmites, Azolla* grasses)	0	5	1.46	0.74	8.58	20.3	789	>depth	0.96	1.5
Currency Creek Game Reserve	30 (*Myriophyllum*)	40 (*Typha, Phragmites*)	0	30	10.88	10.48	8.74	22.1	686	0.35	0.64	1.2
Black Swamp	0	40 (*Typha, Phragmites, Schoenoplectus*)	0	60	6.83	6.49	7.83	24.2	1508	0.25	1.17	1.5

Appendix 6 continued.

	Habitat				Physico-chemical parameters							
	Submerged (%)	**Emergent (%)**	**Physical (%)**	**Open water (%)**	**DO surface (ppm)**	**DO depth (ppm)**	**pH**	**Temp (˚C)**	**Conductivity (µS.cm^{-1})**	**Secchi depth (m)**	**Mean depth**	**Max depth**
Mundoo Island Channel east*												
Mundoo Island Channel east 2	50 (*Myriophyllum*)	45 (*Typha*, *Bolboschoenus*, grasses)	0	5	0.82	0.33	8.87	19.7	669	>depth	0.66	0.8
Mundoo Island Channel west*	0	98 (*Typha*, *Bolboschoenus*, *Azolla*, algae)	0									
Boggy Creek*	5 (*Vallisneria*, *Myriophyllum*)	25 (*Typha*, *Phragmites*, *Ludwigia*, grasses)	0	70	5.45	4.28	7.8	22	606	0.47	0.81	1.5
Dunn's Lagoon*					7.75	5.43	8.74	22.5		0.35	0.56	
Shadows*	45 (*Vallisneria*, *Myriophyllum*)	10 (*Phragmites*, *Triglochin*, *Boobealla*, *Meulaleuca*, grasses)	0	45	3.14	2.6	8.01	21.6	905	0.25	0.54	1

*denotes sites sampled by Wedderburn and Barnes (2012).