



# Condition Monitoring of Threatened Fish Populations in Lake Alexandrina and Lake Albert

Report to the Murray–Darling Basin Authority and the South Australian Department for  
Environment, Water and Natural Resources

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## Summary

The objective of this condition monitoring was to assess the status of three threatened fish populations in Lake Alexandrina and Lake Albert, namely Murray hardyhead (*Craterocephalus fluviatilis*), Yarra pygmy perch (*Nannoperca obscura*) and southern pygmy perch (*N. australis*). The assessment was made partly by relating current distributions to the 2003 baseline when healthy threatened fish populations were present. The project aimed to (1) determine the Relative Abundance Index (RAI) for each threatened fish species at the icon site level, (2) determine the Young-of-the-year Index (YOYI) for each threatened fish species at the icon site level, and (3) combine the RAI and YOYI to determine the Whole of Icon Site Score (WOISS) for populations of each threatened fish species.

A new repeated sample technique was employed in 2015–16 to improve statistical robustness of the condition monitoring results, based on the outcomes of the Condition Monitoring Refinement project. Fish were sampled using seine and fyke netting on two occasions (samples A and B) at 20 sites in March 2016. Key habitat variables were also measured. A total of 16,316 fish were captured, represented by fourteen native and four alien species. Murray hardyhead was recorded in both repeated samples, with 115 fish at five sites and 40 fish at four sites in samples A and B, respectively. Southern pygmy perch was recorded in both repeated samples, with 28 fish at five sites and 12 fish at three sites in samples A and B, respectively. Murray hardyhead and southern pygmy perch were associated with non-channel and well-vegetated channel sites, respectively. Yarra pygmy perch was undetected. Alien fish dominated the catch, with high abundances of eastern gambusia (*Gambusia holbrooki*) and moderate abundances of redfin perch (*Perca fluviatilis*). Southern pygmy perch and eastern gambusia shared a preference for drainage channel habitats.

Statistical analysis of the repeated samples showed that a measure of 'true occupancy' (i.e. estimate of proportion of sites present) for Murray hardyhead was optimal with two repeated samples, and only seine netting is required. Conversely, the analysis for southern pygmy perch showed that the optimal strategy is three repeated samples with fyke netting to accurately estimate occupancy. Additional sampling effort targeting southern pygmy perch would have other benefits, including reducing variation in the YOYI and WOISS, and increasing the chance of detecting Yarra pygmy perch.

Murray hardhead and southern pygmy perch had the maximum RAI value of 1, which indicates that current populations match the baseline (i.e. the species is present in an equal proportion of sites). The index fails to indicate that, following the Millennium Drought, Murray hardyhead had not recolonised sites in Lake Albert by March 2016. Values for YOYI indicate that Murray hardyhead underwent strong recruitment over 2015–16, while recruitment in the southern pygmy perch population was limited. The WOISS  $1.00 \pm 0.42$  for Murray hardyhead meets the target (i.e. WOISS >0.5). The WOISS  $0.70 \pm 0.39$  for southern pygmy perch may be acceptable, but fails the target if the true value lies at the lower end of the tolerance. The WOISS for Yarra pygmy perch is zero, which fails the target.

## Introduction

Lake Alexandrina and Lake Albert (the 'Lower Lakes') are shallow waterbodies covering over 75,000 hectares at the terminus of the Murray–Darling Basin (MDB) (Eastburn 1990). A range of habitats fringe the Lower Lakes, including stream, river, swamp, wetland, lake and brackish water areas (estuarine conditions). The broad characteristics (e.g. salinities) of Lake Albert differ somewhat from Lake Alexandria because it has no flow through to the Coorong lagoons. The Lower Lakes harbour the most diverse fish community in the MDB, because they are inhabited by estuarine, diadromous and freshwater fishes. Also of particular interest are three threatened small-bodied (<8 cm long), short-lived species, namely Murray hardyhead (*Craterocephalus fluviatilis*), Yarra pygmy perch (*Nannoperca obscura*) and southern pygmy perch (*N. australis*) (Wedderburn and Hammer 2003; Wedderburn et al. 2012b). The status of the threatened fishes has been assessed since 2007–08 under The Living Murray (TLM) initiative of the Murray–Darling Basin Authority (MDBA).

The genetically distinct population of Yarra pygmy perch occurs nowhere else in the MDB (Hammer et al. 2010). The species was extirpated from Lake Alexandrina during the 1997–2010 drought in south-eastern Australia (Wedderburn et al. 2014; Wedderburn et al. 2012b). Yarra pygmy perch is 'Vulnerable' under the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) and 'Critically Endangered' in South Australia (Hammer et al. 2009), due to population decline and regional extinctions (Saddler et al. 2013; Wager and Jackson 1993). An attempt to reintroduce Yarra pygmy perch to the MDB in 2012–13 appears unsuccessful (Bice et al. 2014; Wedderburn 2014). A second attempt to reintroduce Yarra pygmy perch in November 2015 also had limited success, but on this occasion factors potentially associated with recovery (e.g. water levels, food availability, predation, competition) were examined through an Intervention Monitoring project (Wedderburn et al. 2016).

Southern pygmy perch is 'Endangered' in South Australia (Hammer et al. 2009). Importantly, the southern pygmy perch population in Lake Alexandrina is genetically unique from other populations in Australia (Unmack et al. 2013). The species was extirpated from the lake during the Millennium Drought (Wedderburn et al. 2014). Populations persisted in tributaries of the lake (Hammer et al. 2012; Whiterod et al. 2015), thereby providing the possibility of natural recovery. Southern pygmy perch reintroduced to the Hindmarsh Island region in 2011 and 2012 persist with limited wild recruitment (Bice et al. 2014; Wedderburn and Barnes 2014).

Murray hardyhead is endemic to the MDB, occurring in fragments upstream from Kerang in Victoria to downstream approximately 2000 km in the Lower Lakes. The small population fragments in the lower River Murray, South Australia, include isolates near Berri and Murray Bridge (Wedderburn et al. 2007). Notably, the Lower Lakes population of Murray hardyhead is genetically more diverse and distinct from populations upstream of Lock 1 in Blanchetown (Adams et al. 2011). Murray hardyhead is 'Critically Endangered' in South Australia (Hammer et al. 2009) and 'Endangered' under the EPBC Act due to severe population decline and localised extinctions during the Millennium Drought. Substantial effort was made during the drought to maintain each genetic management unit in a captive breeding program and surrogate refuge dams (Ellis et al. 2013). Likewise, a population of Murray hardyhead was conserved in a drought refuge of the lakes using environmental water allocations through the TLM initiative (Wedderburn et al. 2010; Wedderburn et al. 2013).

The current monitoring program relates to the Lower Lakes, Coorong and Murray Mouth (LLCMM) Environmental Water Management Plan (MDBA 2014), and the refined LLCMM Condition Monitoring Plan (CMP) target/objective F2 to “*Ensure recruitment success of threatened small-bodied fish species in the Lower Lakes to maintain or establish self-sustaining populations*” (Maunsell 2009: currently being updated). The sampling and measures of condition for each of the threatened small-bodied fish populations varied from previous years, by using methods established through the Condition Monitoring Refinement Project (Robinson 2012; Robinson 2013; Robinson 2015; Wedderburn 2015). Two repeated samples were taken at 20 sites in March 2016 (i.e. at the end of the breeding-recruitment period) in habitats fringing Lake Alexandrina and Lake Albert. Recruitment success of the threatened fish populations in the Lower Lakes was assessed using a young-of-the-year index (YOYI). The current extent of each threatened fish population was also assessed against the 2003 baseline (Wedderburn and Hammer 2003) using a relative abundance index (RAI). The YOYI and RAI values were formulated to determine the Whole of Icon Site Score (WOISS) for each threatened fish species. Ultimately, the WOISS value determines if the EMP target has been achieved.

The objective of the condition monitoring was to assess the status of Murray hardyhead, southern pygmy perch and Yarra pygmy perch populations in the Lower Lakes by relating current population condition to the 2003 baseline populations (Wedderburn and Hammer 2003). The project aims to (1) determine the RAI for each threatened fish species at the icon site level, (2) determine the YOYI for each threatened fish species at the icon site level, and (3) combine the RAI and YOYI to determine the WOISS for each threatened fish population.

The project also monitored the distribution and abundances of other cohabiting fish species, partly to identify potential threats. For example, the presence and abundance of alien fishes are discussed, including the aggressive and prolific eastern gambusia (*Gambusia holbrooki*) and the piscivorous redbfin perch (*Perca fluviatilis*). Habitat characteristics were also measured, to identify patterns between threatened fish populations and the environmental conditions (e.g. salinity, water depth).

Data from two repeated samples were analysed, as proposed in the Condition Monitoring Refinement Project (Robinson 2015), to examine the accuracy of the findings with regards to estimating occupancy (proportion of sites occupied) of each threatened fish species. The methods are discussed in terms of how they meet the Refinement Project’s aim of developing a more accurate and statistically robust measure of condition of the threatened fish populations.

## Materials and methods

### *Fish sampling*

Twenty sites were sampled twice in March 2016 (Table 1). The second sample (sample B) at each site occurred within seven days of the first sample (sample A). Three single-leader fyke nets (5-mm half mesh) were set overnight, and placed perpendicular to the bank, or angled when in narrow channels or deep water. Grids (50-mm) at the entrances of nets excluded turtles and fish that might harm threatened fish, but are not expected to affect their ability to capture fish <250 mm long (cf. Fratto et al. 2008). Following the retrieval of the fyke nets, three seine shots were taken at each site within 10 m of the shoreline (seine net: 7 m long × 1.5 m deep; 5-mm half mesh). Fish were identified to species and counted. Total lengths (TL) of threatened fish were measured to the nearest millimetre.

Table 1. Sites that were sampled twice in March 2016 (UTM zone 54H, WGS84).

Site	Site description	Easting	Northing	Habitat type
2	Wyndgate (early reintroduction site)	309485	6066535	Drainage line
3	Hunters Creek (upstream Denver Rd)	309489	6066309	Natural channel
5	Channel off Steamer Drain	310426	6066005	Modified channel
10	Dunn Lagoon	312568	6070380	Wetland
16	Narrung (Lake Albert)	334667	6068532	Wetland
18	Goolwa Channel–Finniss River	308318	6071088	Wetland
22	Mundoo Island	311065	6064130	Drainage line
25	Dog Lake	329963	6084901	Wetland
26	Old Clayton	310519	6070104	Natural channel
30	Mundoo Island–Boundary Creek	313752	6063750	Drainage line
31	Boggy Creek	312194	6067197	Modified channel
32	Mundoo Island	312275	6064403	Drainage line
34	Shadows Lagoon	311165	6067555	Wetland
38	Black Swamp	304679	6076719	Wetland
48	Waltowa (Lake Albert)	352876	6058248	Wetland
62	Belcanoe (Lake Albert)	337274	6052900	Wetland
68	Shadows Lagoon–Hunters Creek	310784	6067009	Wetland
71	Shadows Lagoon channel (S)	311250	6067348	Artificial channel
73	Hunters Creek–Steamer Drain	310038	6066429	Natural channel
75	Currency Creek	302267	6071370	Wetland

### *Habitat measures*

Secchi depth (cm) was measured, and the following parameters were recorded using a TPS WP-81 meter:

- Salinity as Total Dissolved Solids (TDS)
- Electrical Conductivity units (EC)
- pH
- Temperature (°C)

Several other habitat variables were recorded, chosen based on their potential importance to threatened fish populations:

- Average water depth: five measures 1 m apart, beginning 1 m from the bank, or five measures equally spaced if in a narrow channel
- Bank gradient: 0–90 degrees
- Riparian vegetation: estimated percentage covering ground
- Aquatic plant cover: estimated percentage covering sediment
- Habitat type: natural channel (usually >10 m wide), modified channel (<10 m wide; includes natural drainage lines that have been excavated), lake, wetland

### *Data analyses and interpretation*

To examine the links between fish assemblages and habitat, standardised raw data in catch per unit effort (CPUE: number of fish captured/fyke net hour) were analysed by Non-metric Multi-dimensional Scaling (NMS) ordination using the Relative Sørensen distance metric in PC-ORD (ver. 6: McCune and Mefford 2011). Only fyke net data was used in the ordination, because seine net shots are inconsistent between sites.

Estimation of occupancy, defined as the proportion of sites occupied by a species, was based on data from repeated samples at each site. The repeated sampling approach was necessary because species detection is generally imperfect, which can lead to the incorrect classification of occupied sites as empty. If imperfect detection is not accounted for, bias is induced in the occupancy estimator. The data were analysed using occupancy models based on zero-inflated binomial models (MacKenzie et al. 2006; Royle and Dorazio 2008). These models aim to estimate site occupancy while accounting for imperfect detection, and provide more rigorous comparisons among sites, or between sampling times at each site. The models allow the separate estimation of occupancy and detectability on a logit scale. Estimates of each parameter (and 95% confidence intervals) were then back-transformed to the probability scale.

### *Condition indicators of threatened fish populations*

Values for condition indicators of threatened fish populations were calculated based on the methods detailed in the Condition Monitoring Refinement Project (Robinson 2015; Wedderburn 2015).

#### Relative Abundance Index

The Relative Abundance Index (RAI) assesses the condition of a threatened fish population in relation to the 2003 baseline condition when healthy fish populations occurred before the impacts of the Millennium Drought (Wedderburn and Hammer 2003). The RAI uses the proportion of sites recorded in the baseline for Yarra pygmy perch (8/43 sites = 0.19), southern pygmy perch (6/43 sites = 0.14), and Murray hardyhead (10/43 sites = 0.23).

$$\text{RAI} = \text{proportion of sites recorded} / \text{proportion of sites recorded in baseline}$$

The RAI is ceiled at 1.0 to correspond with the maximum of 1.0 for the YOYI, for the purpose of aggregating the values of both indices (see below). The CMP target is RAI >0.7 for all threatened fish populations in the lakes.

#### Young-of-the-year Index

Young-of-the-year (YOY) is defined as fish in their first year of life, and total length is recorded as a surrogate measure of age: Murray hardyhead YOY <50 mm TL; southern pygmy perch YOY <40 mm TL; Yarra pygmy perch YOY <40 mm TL.

The Young-of-the-year Index (YOYI) taken in March indicates the level of successful recruitment within a threatened fish population (breeding occurs in spring for all species). The CMP target is YOYI >0.5 for Murray hardyhead (annual life cycle) and YOYI >0.3 for the pygmy perches (live for up to 4 years).

$$\text{YOYI} = \text{number of YOY} / \text{total number of fish}$$

#### Whole of Icon Site Score

A Whole of Icon Site Score (WOISS) with a tolerance (95% confidence intervals) will be determined for each threatened fish species by averaging the RAI and YOYI:

$$\text{WOISS} = (\text{RAI} + \text{YOYI}) / 2$$

The tolerance of the WOISS is calculated using the variances of the RAI and YOYI:

$$\text{WOISS Variance} = \text{VAR}(\text{RAI}) + \text{VAR}(\text{YOYI})$$

$$\text{Tolerance} = t \times \sqrt{\text{Var}(\text{WOISS})}, \text{ where } t = t_{0.05, n \text{ df}}$$

The CMP target is WOISS >0.5 for each threatened fish species.

## Results

### *Water quality*

Overall in March 2016, salinities at sites fringing Lake Albert ranged from 1.78 TDS (2730 EC) at Waltowa to 2.27 TDS (3460 EC) at Narrung (Table 2). At the same time, salinities in Lake Alexandrina varied from 0.70 TDS (1105 EC) at Dunn Lagoon to 1.95 TDS (2977 EC) at Shadows Lagoon. In sample A, the mean salinities in Lake Albert and Lake Alexandrina were 2.03 TDS (3123 EC) and 1.11 TDS (1711 EC), respectively. In sample B, the mean salinities in Lake Albert and Lake Alexandrina were 2.07 TDS (3155 EC) and 1.19 TDS (1820 EC), respectively.

Overall in March 2016, readings ranged from pH 6.40 in the channel off Steamer Drain on Hindmarsh Island to pH 8.70 at Narrung. Secchi depth varied substantially, ranging from 15 cm at Belcanoe in Lake Albert to >118 cm in Boggy Creek. Mean water temperatures were 20.1°C and 19.6°C during sample A and B, respectively.

Table 2. Water quality for samples A and B in March 2016.

Site	Salinity (TDS)		Conductivity ( $\mu$ S/cm)		pH		Secchi (cm)		Temperature (°C)	
	A	B	A	B	A	B	A	B	A	B
2	1.426	1.502	2179	2334	7.44	6.54	50	50	16.6	13.9
3	0.975	1.041	1494	1585	8.24	7.74	49	50	18.9	21.6
5	0.833	0.837	1306	1285	6.40	7.22	91	85	22.1	19.7
10	0.702	0.711	1105	1089	8.42	8.56	48	50	19.8	21.6
16	2.250	2.270	3470	3460	8.65	8.70	19	15	16.8	17.3
18	0.914	0.935	1404	1437	8.23	7.78	34	42	19.3	20.3
22	1.093	1.050	1681	1612	6.83	7.08	43	45	21.5	19.3
25	0.759	0.754	1168	1154	7.89	8.52	28	32	17.4	25.4
26	0.746	0.790	1146	1209	8.57	8.44	61	45	24.2	20.3
30	0.841	0.841	1296	1294	7.28	7.39	47	48	24.6	21.8
31	1.396	1.459	2159	2249	7.39	8.37	118	115	15.5	18.5
32	0.897	0.878	1383	1350	7.56	8.03	17	40	22.3	19.8
34	1.873	1.935	2890	2970	7.83	8.41	44	36	28.1	25.5
38	1.502	1.532	2305	2350	8.66	7.85	70	70	21.9	19.6
48	1.780	1.920	2730	2946	7.61	7.77	15	13	14.2	15.5
62	2.060	2.020	3170	3060	8.31	8.32	20	12	21.3	18.8
68	1.584	1.825	2434	2813	7.45	6.70	27	24	21.9	19.8
71	1.458	1.953	2250	2977	7.31	7.50	47	45	18	16.1
73	0.820	1.080	1249	1652	7.24	7.31	85	91	19.2	18.1
75	1.074	1.032	1640	1585	8.51	8.36	42	34	18.1	19.7

### *Physical habitat*

As reflected by the proportions of aquatic plants, most sites were well-vegetated in March 2016 (Table 3). All sites were inhabited by cumbungi (*Typha domingensis*) at the fringes, where river club rush (*Scheonoplectus validus*) often occurred. Hornwort (*Ceratophyllum demersum*) was the predominant submerged plant, and was recorded at most sites. Hornwort was especially thick in channel sites on Hindmarsh Island, where it sometimes congested most of the volume of water (e.g. sites 2 and 31). Water milfoil (*Myriophyllum* spp) was recorded at numerous sites, including all three sites in Lake Albert. Ribbon weed (*Vallisneria australis*) was common in shallow wetland sites on Hindmarsh Island, and was particularly abundant in Shadows Lagoon.

Water levels ranged between 21 cm in site 25 Dog Lake to 118 cm in site 38 Black Swamp in March 2016. The channel sites generally were deeper than wetland habitats, except the deeper wetland habitat in Black Swamp is a reflection the site's proximity to the channel of the Finniss River. Wetland sites in other areas of the lakes were <72 cm deep in March 2016.

Table 3. Physical habitat readings for samples A and B in March 2016.

Site	Mean depth (cm)		Aquatic plants (%)		Habitat type
	A	B	A	B	
2	45	45	90	95	Drainage line
3	38	35	87	95	Natural channel
5	73	73	90	90	Modified channel
10	38	42	17	40	Wetland
16	72	68	47	47	Wetland
18	48	40	50	50	Wetland
22	28	33	92	90	Drainage line
25	27	21	35	51	Wetland
26	37	49	60	70	Natural channel
30	76	56	52	51	Drainage line
31	104	79	97	100	Modified channel
32	90	71	25	40	Drainage line
34	40	60	60	60	Wetland
38	118	101	100	100	Wetland
48	31	31	45	45	Wetland
62	40	37	45	40	Wetland
68	43	50	49	39	Wetland
71	57	62	91	100	Artificial channel
73	73	80	100	100	Natural channel
75	38	33	55	35	Wetland

## Fish assemblages

Sampling undertaken in March 2016 recorded a total of 16,316 fish comprising of fourteen native and four alien species (Table 4). Murray hardyhead and southern pygmy perch were recorded in both repeated samples, whereas Yarra pygmy perch was undetected. Alien fish dominated the catch in March 2016 more so than any other year since 2009 (Figure 1). The high proportion of alien fish in 2016 (67% of total catch) is predominantly due to high abundances of eastern gambusia (84% of alien fish) and redfin perch (14% of alien fish). Common carp and goldfish constituted only 2% of the alien fish captured. Overall, eastern gambusia comprised 56% of the total catch of fish in March 2016. Estuarine fishes were a considerable proportion of the catch (~60%) at the end of drought in 2010, but constituted only 0.2% of the total catch in March 2016. Diadromous fishes have been a considerable component of the fish assemblage since 2013, where the proportion of common galaxias has gradually increased to 14% of the total catch in March 2016. Congolli (*Pseudaphritis urvillii*), however, was captured in lower numbers than recent years (e.g. 122 fish at 19 sites in March 2015: Appendix A), comprising only 0.5% of the total catch in March 2016.

Table 4. Number of sites recorded and total abundance of each fish species captured at 20 sites in two repeated samples in March 2016.

Common name	Scientific name	Sample A		Sample B	
		No. sites	Total abundance	No. sites	Total abundance
<b>Native fishes</b>					
Southern pygmy perch	<i>Nannoperca australis</i>	5	28	3	12
Yarra pygmy perch	<i>Nannoperca obscura</i>	0	0	0	0
Murray hardyhead	<i>Craterocephalus fluviatilis</i>	5	115	4	40
Unspecked hardyhead	<i>Craterocephalus fulvus</i>	7	105	9	157
Bony herring	<i>Nematalosa erebi</i>	12	522	11	584
Flathead gudgeon	<i>Philypnodon grandiceps</i>	15	259	15	241
Dwarf flathead gudgeon	<i>Philypnodon macrostomus</i>	6	62	9	19
Carp gudgeon	<i>Hypseleotris</i> sp.	6	39	5	16
Australian smelt	<i>Retropinna semoni</i>	10	116	8	36
Congolli	<i>Pseudaphritis urvillii</i>	7	26	9	32
Common galaxias	<i>Galaxias maculatus</i>	15	855	14	886
Smallmouth hardyhead	<i>Atherinosoma microstoma</i>	7	26	4	10
Blue-spot goby	<i>Pseudogobius olorum</i>	9	26	6	8
Tamar River goby	<i>Afurcagobius tamarensis</i>	0	0	1	1
Lagoon goby	<i>Tasmanogobius lasti</i>	0	0	1	1
<b>Alien fishes</b>					
Common carp	<i>Cyprinus carpio</i>	14	63	12	37
Goldfish	<i>Carassius auratus</i>	12	64	11	40
Redfin perch	<i>Perca fluviatilis</i>	14	422	13	557
Eastern gambusia	<i>Gambusia holbrooki</i>	20	7081	20	3830

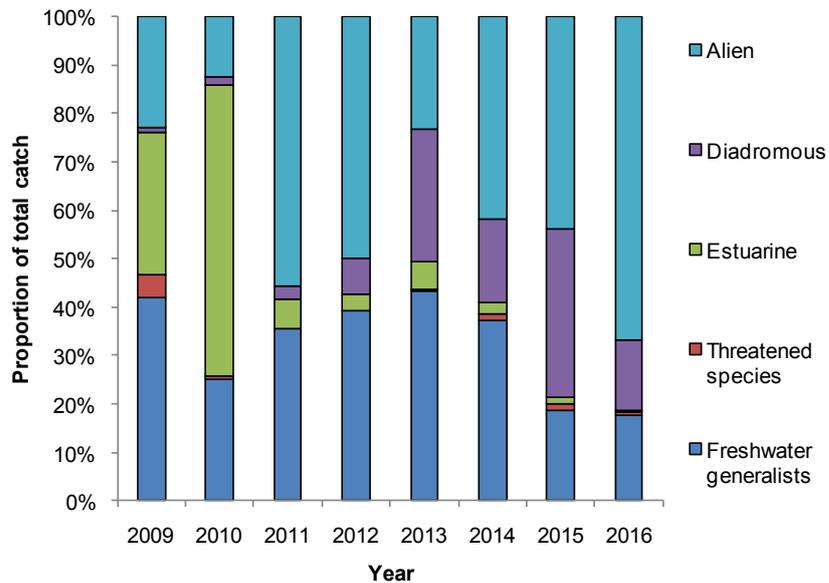


Figure 1. Proportion of major fish groups in total catches of TLM condition monitoring each March from 2009 to 2016 (data from previous condition monitoring reports; data for March 2015 was unreported but see Appendix A).

The two-dimensional ordination shows a separation of sites based on channel and non-channel habitats (Figure 2). Most of the channel sites are to the right-hand side of the plot (solid circles), and the non-channel sites (open circles) are on the left-hand side of the plot. Multi-response Permutation Procedure confirms that fish assemblages recorded in the channel and non-channel sites are significantly different ( $P < 0.001$ ). The channel sites are positively correlated with habitat complexity on Axis 1 (correlation between 'Habitat' and axis score:  $r = 0.64$ ), Secchi depth (Secchi:  $r = 0.32$ ), conductivity (EC:  $r = 0.31$ ) and water depth (Depth:  $r = 0.12$ ), while they are negatively correlated with pH ( $r = -0.40$ ). The non-channel sites show the opposite trend. The strongest correlations on Axis 2 are water depth ( $r = -0.39$ ) and conductivity ( $r = -0.17$ ), which are negatively associated with sites containing the most diverse fish assemblages including Murray hardyhead.

The relatively strong positive correlation for eastern gambusia on Axis 1 ( $r = 0.55$ ) suggests an association with channel sites of high habitat complexity, of which southern pygmy perch is also positively correlated ( $r = 0.30$ ). On Axis 1, bony herring, redfin perch and congolli are negatively correlated with channel sites and habitat complexity ( $r = -0.66$ ,  $-0.60$  and  $-0.41$ , respectively). Common galaxias, unspotted hardyhead, Murray hardyhead, western blue-spot goby and Australian smelt are negatively correlated on Axis 2 towards non-channel sites and the lowest water depth ( $r = 0.60$ ,  $0.59$ , and  $0.47$ ,  $0.35$ ,  $0.33$ , respectively).

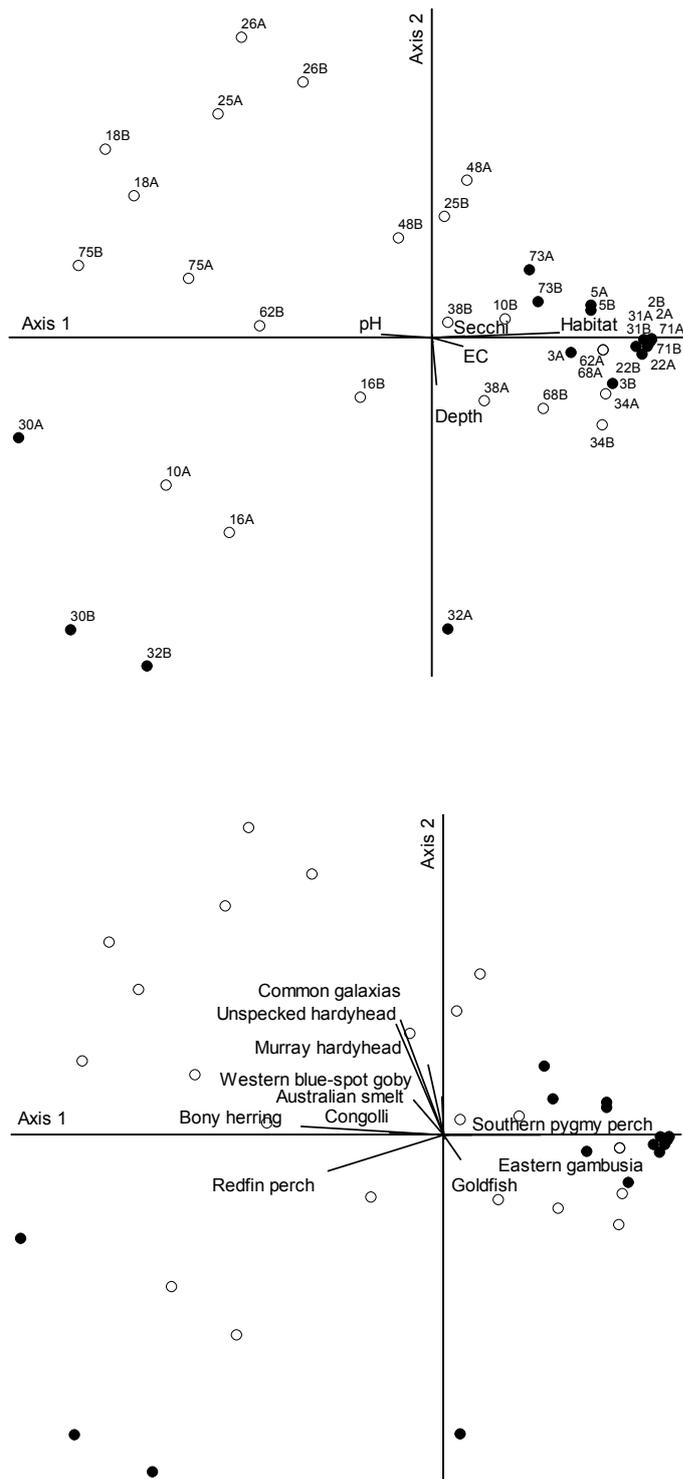


Figure 2. Two-dimensional NMS ordination (stress = 9.6%) of sites based on similarities between fish species composition and abundance. Habitat (top plot) and fish species (bottom plot) are overlaid with the vector length proportional to, and directed towards, their correlation with sites. Open dots represent non-channel sites (mostly wetlands) and solid dots represent channel sites (mostly drainage lines).

## *Condition indicators of threatened fish populations*

### Repeated sample analysis

Based on analysis of two repeated samples in the March 2016 condition monitoring, the estimate of detection for Murray hardyhead is 0.889 using a combination of seine and fyke netting (Table 5). The data verify that two replicate surveys are sufficient to minimise the variance of the estimate of occupancy for Murray hardyhead, and provide an accurate estimate of detection (Guillera-Arroita et al. 2010).

When the sampling methods (seine and fyke) are analysed separately, the estimate of detection of Murray hardyhead for only fyke netting appears ideal (i.e. perfect detection of 1.0), but this is misleading (i.e. sample A and B matched each time at each site). Specifically, the seine net samples revealed that Murray hardyhead was present at sites where it was not detected in fyke netting. The estimate of detection for only seine netting is acceptable with two repeated samples (Guillera-Arroita et al. 2010). Therefore, seine netting alone with two repeated samples would be sufficient if the condition monitoring program was only targeting Murray hardyhead.

Table 5. Estimates of detection and occupancy of Murray hardyhead from two repeated samples at 20 sites.

<b>Sample method</b>	<b>Detection</b>	<b>±SE</b>	<b>Occupancy</b>	<b>±SE</b>
Seine and fyke	0.889	0.110	0.253	0.098
Seine	0.750	0.171	0.267	0.106
Fyke	1.000	0.004	0.100	0.067

Based on analysis of two repeated samples in the March 2016 condition monitoring, the estimate of detecting southern pygmy perch is 0.5 (Table 6). The estimated values for detection and occupancy of the two repeated sample events at 17 sites for southern pygmy perch were used to determine the optimum number of sampling events. The data verify that three replicate surveys would minimise the variance of the estimate of occupancy and increase the detection (Guillera-Arroita et al. 2010). Ultimately, this should improve the reliability of the WOISS by reducing the tolerance (i.e. the 95% confidence intervals in which the true value of WOISS lies – see 'Condition scores' sub-section below). Importantly, there appears to be no value in seine netting to monitor southern pygmy perch.

Table 6. Estimates of detection and occupancy of southern pygmy perch from two repeated samples at 17 sites (i.e. analysis excludes the three sites in Lake Albert).

<b>Sample method</b>	<b>Detection</b>	<b>±SE</b>	<b>Occupancy</b>	<b>±SE</b>
Seine and fyke	0.500	0.217	0.400	0.179
Seine	0.026	0.046	0.973	1.470
Fyke	0.500	0.217	0.400	0.179

### Condition scores

Calculations for RAI, and therefore the WOISS, are related to the baseline condition in 2003 (Wedderburn and Hammer 2003), which was not a repeated sample strategy. Indeed, the repeated sample method has never been employed in the lakes. Therefore, the 2003 baseline did not determine an estimate of occupancy but only 'naïve occupancy' (Guillera-Arroita et al. 2010). The calculations for RAI and WOISS are unavoidably influenced by this factor to an unknown extent (Table 7). The YOYI is unaffected by this discrepancy. Nevertheless, the baseline is the best available data for scoring the current status of threatened fish populations against healthy populations that were present before the impacts of the Millennium Drought. Importantly, changes in the WOISS over future condition monitoring will provide an accurate tracking of shifts in the status of each threatened fish population. Any substantial shifts in a species' status may then be related to concurrent changes in environmental conditions at local (e.g. aquatic plant health) and broad (e.g. drought, water level management) scales.

The updated CMP target of RAI >0.7 was met for Murray hardyhead in March 2016, but with high variation ( $\pm$ SE). The YOYI value for Murray hardyhead also exceeds the minimum target of YOYI >0.7 in the updated CMP, and the low variation ( $\pm$ SE) suggests this reading is reliable. The icon site target is a WOISS >0.5 for the Murray hardyhead population. The wide tolerance suggests the true value for the WOISS for Murray hardyhead might be as low as 0.58, which still meets the updated CMP target.

The updated CMP target of RAI >0.7 was met for southern pygmy perch in March 2016. The YOYI value is relatively low for southern pygmy perch, but still exceeds the minimum target of YOYI >0.3 in the updated CMP. The high variation ( $\pm$ SE), however, suggests that the YOYI value of 0.40 is somewhat unreliable. This might also be addressed by increasing the repeated samples to three when targeting southern pygmy perch in monitoring (as described in 'Repeated sample analysis'). The icon site target is a WOISS >0.5 for the southern pygmy perch population. The wide tolerance suggests the true value for the WOISS for southern pygmy perch might be as low as 0.31, which would fail to meet the target.

The RAI and YOYI values for Yarra pygmy perch were not determined, because the species was not captured in the March 2016 condition monitoring. The icon site target is a WOISS >0.5 for the Yarra pygmy perch population. The WOISS for Yarra pygmy perch is zero, which fails to meet the target.

Table 7. Values for relative abundance index (RAI), Young-of-the-year Index (YOYI) and Whole of Icon Site Score (WOISS) for the threatened fish populations in March 2016.

Common name	RAI	$\pm$ SE	YOYI	$\pm$ SE	WOISS $\pm$ tolerance
Murray hardyhead	1.00	0.209	0.99	0.011	1.00 $\pm$ 0.42
Southern pygmy perch	1.00	0.101	0.40	0.157	0.70 $\pm$ 0.39
Yarra pygmy perch	N/A	N/A	N/A	N/A	0

## Discussion

### *Threatened fish populations*

The objective of this condition monitoring project was to determine the status of Murray hardyhead, southern pygmy perch and Yarra pygmy perch populations in the Lower Lakes by relating current population condition to the 2003 baseline (Wedderburn and Hammer 2003). Murray hardyhead and southern pygmy perch show signs of population recovery following decline during the Millennium Drought, with range expansions and recruitment over 2015–16. Yarra pygmy perch was not detected in the repeated samples in March 2016, thereby indicating that the species is again on the verge of extirpation.

The first aim of the condition monitoring project was to determine the Relative Abundance Index (RAI) for each threatened fish species at the icon site level, as a measure of their current population status. Specifically, the RAI value is the extent of a species' occurrence in 2016 as it relates to occupancy in the 2003 baseline (Wedderburn and Hammer 2003). The RAI of 1 for Murray hardyhead meets the EMP target, and suggests the species occupies the lakes at the same level as it did in 2003. A factor not considered in the RAI, however, is that Murray hardyhead has not returned to its full range, which included Lake Albert (last recorded in 2008: Wedderburn and Barnes 2009). Southern pygmy perch appears to be recovering in the south-western parts of the Lake Alexandrina (Hindmarsh Island, Mundoo Island, Black Swamp), but has yet to establish self-sustaining populations in the northern region of Lake Alexandrina. Nevertheless, the RAI of 1 for southern pygmy perch meets the target, and suggests the species now has the same population status as in 2003.

The second aim of the condition monitoring project was to determine the Young-of-the-year Index (YOYI) for each threatened fish species to provide a measure of recruitment success at the icon site level. The high YOYI value of 0.99 combined with low variation in the data, and a relatively high number of Murray hardyhead captured, suggests there was strong breeding and recruitment in the population over 2015–16. Therefore, the YOYI value for Murray hardyhead meets the target. The YOYI value is relatively low for southern pygmy perch, and the high variation in the data suggests the value of 0.40 is somewhat unreliable. The high variation is likely to be partly related to the relatively low numbers of fish captured at individual sites, which could be overcome with additional sampling (see below 'Further refinement of methods'). Nevertheless, the YOYI value for southern pygmy perch meets the target.

The third aim of the condition monitoring project was to determine the Whole of Icon Site Score (WOISS) for each threatened fish species, by formulating the RAI and YOYI. As with the RAI, the WOISS for Murray hardyhead does not consider that the species has yet to recolonise Lake Albert. The WOISS of 1 (i.e. maximum score) for Murray hardyhead shows the species has recovered well in Lake Alexandrina, and meets the target for the icon site. The high WOISS for Murray hardyhead reflects its wide range and high level of recruitment in the Goolwa Channel region. The presence of Murray hardyhead in Dog Lake (northern Lake Alexandrina) also contributes to meeting the WOISS target. The WOISS of 0.7 for southern pygmy perch meets the target, but variation (tolerance) in the data makes the score somewhat unreliable. This is a reflection of the variation in the YOYI value, which might be overcome by addition sampling targeting pygmy perches (see below 'Further refinement of methods').

### *Threatened fish habitats*

There was distinct preference by Murray hardyhead for non-channel habitats with water levels in the lower range at study sites (mean depths of 21–118 cm in March 2016). Most often the preferred sites had a sandy substrate, and were open water areas with adjacent patches of water milfoil. Murray hardyhead also tended to be associated with habitats that had low numbers of alien eastern gambusia and moderate numbers of alien redfin perch. As water levels receded in summer 2015–16, during a managed drawdown of Lake Alexandrina to ~0.55 m above sea level, Murray hardyhead was still able to utilise the fringing habitats and tributaries of the Goolwa Channel. Similarly, water levels remained adequate for Murray hardyhead to persist in Dog Lake. It is undetermined, however, to what extent the water level recessions influenced recruitment of Murray hardyhead in Dog Lake, where only small numbers were captured. Conversely, relatively high numbers of Murray hardyhead were captured in some parts of the Goolwa Channel. Sites sampled in Lake Albert fit the typical habitat description for Murray hardyhead but the species was unrecorded. Notably, the species was recorded at the same sites prior to the impacts of drought.

There was distinct preference by southern pygmy perch for channel habitats with water levels of the higher range at study sites. Most often the preferred sites had a muddy, detritus-rich substrate, with abundant hornwort and cumbungi (i.e. high habitat complexity). Southern pygmy perch also tended to be associated with habitats where alien eastern gambusia was prolific, and moderate numbers of alien redfin perch were present. As water levels receded in summer 2015–16 during the managed drawdown of Lake Alexandrina, southern pygmy perch was apparently confined to the deeper areas of channel systems, such as the main channel of Hunters Creek on Hindmarsh Island. In contrast, southern pygmy perch was not captured in other parts of the connected channel system that became very shallow and thick with hornwort during the drawdown period. As with Murray hardyhead, it is undetermined to what extent the water level recessions influenced recruitment of southern pygmy perch. Further, eastern gambusia was prolific in all well-vegetated drainage channel habitats, so the alien species might also be a factor influencing the presence and recruitment of southern pygmy perch and, indeed, Yarra pygmy perch (see below 'Alien fish populations').

### *Fish assemblages*

During the last seven March monitoring events, the overall numbers of freshwater fishes peaked in 2010 and was lowest in 2012 (Wedderburn and Hillyard 2010; Wedderburn and Barnes 2012). Two ecological generalists, flathead gudgeon and bony herring, were the most numerous species in catches, excluding in March 2009. Notably, unspotted hardyhead was a substantial proportion (27%) of the native freshwater fish captured in March 2014 after being extirpated from the lakes during drought (Wedderburn and Barnes 2014). In March 2013, the threatened fishes continued to constitute a minor proportion of the fish assemblages in the Lower Lakes (Wedderburn and Barnes 2013). In March 2014, Murray hardyhead showed the first signs of population recovery, when it constituted 7% of the native freshwater fish captured (Wedderburn and Barnes 2014). In March 2016, Murray hardyhead was recorded in both repeated samples, with 115 fish at five sites and 40 fish at four sites in samples A and B, respectively. In March 2016, southern pygmy perch was also

recorded in both repeated samples, with 28 fish at five sites and 12 fish at three sites in samples A and B, respectively.

The catadromous common galaxias and congolli were the only diadromous fishes captured in TLM condition monitoring since 2008. Common galaxias remained relatively abundant from March 2008 to March 2012, but its numbers were substantially higher in March 2013 and March 2014 (Wedderburn and Barnes 2014). In March 2016, common galaxias constituted 14% of the total catch of fish, which is significant given its potential importance in the food web of the Lower Lakes (cf. Milano et al. 2013). Congolli was notably absent during the peak of drought in March 2009 and March 2010, when Lake Alexandrina was disconnected from the estuary thereby disrupting its life cycle (Zampatti et al. 2010). Relatively low to moderate numbers of congolli were captured in subsequent years, including lower numbers in the March 2016 condition monitoring.

### *Alien fish populations*

Eastern gambusia was the most numerous alien fish species in March 2013 and March 2014 when river flows had stabilised following drought (Wedderburn and Barnes 2014). By March 2016, eastern gambusia was prolific in well vegetated drainage channel sites, which were also occupied by much lower numbers of southern pygmy perch. In the MDB, eastern gambusia can shape wetland fish assemblages by influencing the physical condition of native fish, which might be significant for ecological specialists in their obligate habitat (Macdonald et al. 2012). In this regard, southern pygmy perch and Yarra pygmy perch are ecological specialists requiring habitats that are occupied by large numbers of eastern gambusia by mid-summer. A previous intervention monitoring project found that Murray hardyhead can cohabit in drought refugia with eastern gambusia if food resources are abundant (Wedderburn et al. 2010). Intervention monitoring that examined ecological issues related to Yarra pygmy perch reintroductions in late 2015 found that eastern gambusia consumed some of the same prey items as the threatened species (Wedderburn et al. 2016), suggesting competition for food might be a factor impacting on pygmy perch populations. The aggressive nature of eastern gambusia might also be a factor, whereby the alien species excludes threatened fishes from preferred habitats (Alcaraz et al. 2008). The impacts of eastern gambusia on the presence and recruitment of small-bodied threatened fishes in the lakes have yet to be fully examined.

Redfin perch is introduced to the Southern Hemisphere where it has been implicated in the extirpation of small-bodied native fishes, mostly through predation (e.g. western pygmy perch *Nannoperca vittata* in WA: Hutchison 1991). A previous intervention monitoring project by Wedderburn et al. (2012a) found that redfin perch >9 cm long were preying on small-bodied native fishes in Lake Alexandrina. The prey was mostly gobies and flathead gudgeon in autumn 2011. During this time, the threatened fishes were essentially absent so were not expected in the diet of redfin perch. Nevertheless, subsequent analysis found that the redfin perch had switched to piscivory at <6 months of age (Wedderburn and Barnes 2016). This is significant, given that juvenile redfin perch occur in the obligate habitat of the threatened small-bodied fishes. Adding to concern is that redfin perch was the fourth most abundant fish species captured in March 2016. Therefore, it is possible that redfin perch is an impediment to the population recovery of the threatened fish populations. Notably,

however, intervention monitoring in late 2015 found no evidence that juvenile redbfin perch had preyed on reintroduced Yarra pygmy perch (Wedderburn et al. 2016).

The cyprinids, common carp and goldfish, were in relatively low abundances in March 2014, where they made up a small proportion of the alien fish numbers. Adult common carp were frequently observed at many sites, but they were too large to be captured in the gridded fyke nets. The cyprinids are likely to be the alien species of least concern with regards to their direct impacts on the three threatened small-bodied fishes. Their indirect impacts (e.g. influence on habitat condition), however, might be significant.

### *Further refinement of methods*

The first use of the refined condition monitoring method demonstrates that a repeated sample strategy provides an estimate for occupancy that is more accurate than naïve occupancy obtained in single sample monitoring. The repeated sample method also increases detection of rare fish species. The sampling design used in March 2016 was optimal for Murray hardyhead with three seine shots at 20 sites in two repeated samples. Fyke netting was unnecessary for monitoring Murray hardyhead. Conversely, the design was lacking somewhat for monitoring southern pygmy perch, which is a reflection of its ecological differences from Murray hardyhead (see Figure 2). An optimal sampling design for southern pygmy perch is three repeated samples from at least 17 sites (exclude Lake Albert) using fyke nets. Seine netting was unnecessary for monitoring southern pygmy perch. The absence of data for Yarra pygmy perch in March 2016 means no calculations can be performed for the species.

The reliance on an earlier single-sample survey to calculate RAI detracts somewhat from the accuracy of the RAI and, therefore, the WOISS. This might be a factor causing some of the variation (tolerance), but it is unavoidable. Shifts in the status of each threatened fish population can be accurately tracked in future condition monitoring. Any substantial shifts in a species' status can then be related to concurrent changes in environmental conditions at local and broad scales. The high variation in the YOYI for southern pygmy perch also adds to the variation in its WOISS, and is probably a reflection of the low numbers recorded. This point further highlights the benefits of a third repeated sample for southern pygmy perch, because more fish would be included in the YOYI calculation.

The following summarises the broad fish sampling methods required for future condition monitoring of threatened small-bodied fishes in the Lower Lakes:

- (1) Separate sampling gears target ecologically different Murray hardyhead (seine only) and pygmy perches (fyke only).
- (2) Statistical analysis showed that two repeated samples with seine netting are required to optimise the estimate of detection and occupancy for Murray hardyhead.
- (3) Statistical analysis showed that three repeated samples are required to optimise the estimate of detection and occupancy for southern pygmy perch.
- (4) By increasing effort for southern pygmy perch to three repeated samples, the variation around the YOYI value will be reduced, thereby reducing the tolerance of the WOISS.
- (5) By increasing effort to three repeated samples for southern pygmy perch, there is a greater chance of detecting Yarra pygmy perch.

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## Appendix A. TLM condition monitoring fish catch summary for March 2015.

Common name	Scientific name	24 sites sampled	
		No. sites captured	Abundance
<b>Native fishes</b>			
Southern pygmy perch	<i>Nannoperca australis</i>	2	37
Yarra pygmy perch	<i>Nannoperca obscura</i>	0	0
Murray hardyhead	<i>Craterocephalus fluviatilis</i>	7	56
Unspecked hardyhead	<i>Craterocephalus fulvus</i>	10	101
Bony herring	<i>Nematalosa erebi</i>	17	664
Flathead gudgeon	<i>Philypnodon grandiceps</i>	21	605
Dwarf flathead gudgeon	<i>Philypnodon macrostomus</i>	13	33
Carp gudgeon	<i>Hypseleotris</i> sp.	9	155
Australian smelt	<i>Retropinna semoni</i>	10	35
River Murray rainbowfish	<i>Melanotaenia fluviatilis</i>	2	15
Golden perch	<i>Macquaria ambigua ambigua</i>	2	3
Congolli	<i>Pseudaphritis urvillii</i>	19	122
Common galaxias	<i>Galaxias maculatus</i>	23	2861
Smallmouth hardyhead	<i>Atherinosoma microstoma</i>	5	11
Blue-spot goby	<i>Pseudogobius olorum</i>	8	67
Tamar River goby	<i>Afurcagobius tamarensis</i>	2	6
Lagoon goby	<i>Tasmanogobius lasti</i>	5	42
<b>Alien fishes</b>			
Common carp	<i>Cyprinus carpio</i>	11	60
Goldfish	<i>Carassius auratus</i>	13	33
Redfin perch	<i>Perca fluviatilis</i>	15	169
Eastern gambusia	<i>Gambusia holbrooki</i>	24	3491

