Threatened Fish Populations in the Lower Lakes of the River Murray in spring 2007 and summer 2008

Report to the South Australian Murray-Darling Basin Natural Resource Management Board

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Cover: Boundary Creek, once important habitat for threatened fish, especially Murray hardyhead (*Craterocephalus fluviatilis*; pictured).

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EXECUTIVE SUMMARY

Lakes Alexandrina and Albert (hereafter, the Lower Lakes) contain a diverse native fish community and represent one of the most significant habitat areas for threatened small-bodied fish species in South Australia. These are the nationally *vulnerable* Murray hardyhead (*Craterocephalus fluviatilis*) and Yarra pygmy perch (*Nannoperca obscura*), and southern pygmy perch (*Nannoperca australis*, considered endangered in the lower Murray-Darling Basin (MDB)). The current project aimed to provide 'condition monitoring' of threatened fish populations to assist in reaching targets specified in the Lower Lakes, Coorong and Murray Mouth Environmental Management Plan (LLCMM EMP). Specifically, the abundance, distribution and size/age structure of threatened fish species was investigated during spring 2007 and summer 2008. Seasonal change in the fish assemblage structure was also investigated along with possible relationships between fish assemblages and environmental parameters.

Prolonged drought conditions throughout the MDB have resulted in an absence of inflows and rapid recession in lake water levels since 2006, from *c.* 0.75 – *c.* -0.5 m (AHD). This has resulted in dramatic decreases in habitat availability for threatened fish species due to a total loss of favourable 'inside' habitat (off-Lake habitat) and a substantial decrease in Lake edge habitat. Secondarily, the quality of remaining habitats in the Lower Lakes has diminished substantially. Consequently, in spring 2007 and summer 2008, Murray hardyhead, Yarra pygmy perch and southern pygmy perch were collected in low to very low numbers, from restricted distributions, when compared to previous years in this region. The abundances of these species are likely to continue declining given the prevailing and long-term forecast conditions in the Lower Lakes.

Spawning and recruitment were detected for Murray hardyhead and southern pygmy perch in 2007/2008, although recruitment level was low for both species. For Murray hardyhead, likely 0+ fish represented <10% of the population compared to >50% of the population in the summers of 2006 and 2007. For southern pygmy perch 0+ individuals represented *c.* 50% of the population in summer 2008 compared to >75% in the summers of 2006 and 2007. No Yarra pygmy perch <40 mm TL were collected in spring 2007 or summer 2008 and as such, no spawning or recruitment was detected. As short-lived species, consecutive years of poor or no recruitment could lead to local extinctions of these species.

Most species increased in abundance between years. A total of 4917 (20 species) and 7607 (19 species) fish were sampled from 22 sites in spring 2007 and summer 2008 respectively. Analysis of Similarities (ANOSIM) detected a significant difference between fish assemblages sampled from sites that had water in each season (Global $R = 0.103$, $p = 0.017$). The difference between assemblages was driven by an increased abundance of small-mouthed hardyhead (*Atherinosoma microstoma*), eastern gambusia (*Gambusia holbrooki*), bony herring (*Nematalosa erebi*), lagoon goby (*Tasmanogobius lastii*), redfin perch (*Perca fluviatilis*), flat-headed gudgeon (*Philypnodon grandiceps*) and common galaxias (*Galaxias maculatus*), and a decreased abundance of Australian smelt

(*Retropinna semoni*) in summer 2008. Increased summer abundances might be attributed to crowding within remaining refuge habitats.

In spring 2007 BIOENV rank correlation analysis revealed that the fish assemblage patterns were best explained by a combination of factors including the proportion of submerged vegetation (%), conductivity (i.e. salinity) and pH ($\rho_w = 0.642$). However, in summer 2008 these environmental variables did not match as well with the recorded fish assemblage patterns ($\rho_w = 0.418$).

The abundance of Murray hardyhead, Yarra pygmy perch and southern pygmy perch in the Lower Lakes are in rapid decline. Combined with little to no recruitment and predictions of worsening habitat conditions in the Lower Lakes, these species are currently under extreme risk of local extinction. Further monitoring must be conducted to determine if there are any 'low-water' refuge sites remaining in the Lower Lakes for these species. Such sites should be conserved in-situ if possible or fish removed and conserved ex-situ where necessary.

1 INTRODUCTION

The Lower Lakes, Coorong and Murray Mouth region has been recognised as a wetland of international importance under the Ramsar Convention since 1985 and represents one of the six 'icon sites' identified across the Murray-Darling Basin (MDB) under *The Living Murray Program* (MDBC 2006). The site possesses unique ecological values that warrant its national and international status. In part, these values are a result of the small-bodied native fish community which inhabits the wetlands, fringing lake habitat and irrigation channels of the Lower Lakes (Wedderburn *et al.* 2003). The channels on the islands of the Lower Lakes and the barrage fishways maintain a link between the estuarine/marine environment of the Coorong and the freshwaters of the Lower Lakes allowing access for euryhaline fish species which coexist in this region with truly freshwater species, making the Lower Lakes region unique within the MDB (Higham *et al.* 2005).

In recent years, studies have documented the diverse and abundant fish community present in the Lower Lakes (Hammer *et al.* 2002; Wedderburn *et al.* 2003; Higham *et al.* 2005; Bice and Ye 2006) with particular attention now being afforded to rare and endangered species (Bice and Ye 2006; Bice and Ye 2007; Hammer 2007a; Wedderburn *et al.* 2007; Hammer 2008). These are the Murray hardyhead (*Craterocephalus fluviatilis*) and Yarra pygmy perch (*Nannoperca obscura*), both nationally listed as *Vulnerable* under the *Environmental Protection and Biodiversity Conservation Act 1999* (EPBC; Ebner *et al.* 2003; Hammer 2007a), and southern pygmy perch (*Nannoperca australis*; considered endangered in the Lower MDB; Lloyd and Walker 1986). All three species are 'protected' in South Australia under the Fisheries Management Act 2007.

Murray hardyhead and southern pygmy perch, once widespread in the MDB, have in recent decades suffered dramatic contractions in range (Ebner *et al.* 2003; Hammer 2005). In South Australia, known populations of Murray hardyhead exist in two salt evaporation basins near Berri (Disher Creek and Berri), in the Rocky Gully wetland near Murray Bridge (Drought Action Plan, in press) and in the Lower Lakes. All of these populations are currently under threat of extinction due to the prolonged drought conditions being experienced in the South Australian MDB, while a population in the Riverglades wetland was likely lost in 2007 when the wetland dried out. Yarra pygmy perch have a historically restricted range within the southeast of Australia and extensive habitat degradation within this limited range has led to its national classification as *vulnerable* (Allen *et al.* 2002; Hammer 2007a). Several populations exist in the southeast of South Australia, however a population at Henry's Creek has recently become extinct (Hammer 2007b), whilst the current status of other isolated populations (e.g. Mosquito Creek) is uncertain but are likely to be under threat (Michael Hammer pers. com.). The Lower Lakes population represents the only known population of this species in the MDB (Hammer 2007a) and is currently in critical decline (Bice and Ye 2007; Hammer 2008). Southern pygmy perch are present in the region as five small genetically distinct sub-populations (Hammer 2001). All of these populations are currently under threat of extinction given the prevailing drought conditions in the South Australian MDB.

Drought conditions throughout the MDB in recent years have resulted in severely diminished freshwater inflows to the Lower Lakes and a corresponding fall in Lake water levels. Lake levels in 2007/08 have fallen from *c.* 0.3 m (AHD) to *c.* -0.5 m (AHD), almost two metres below the long-term average operating level of *c.* 0.75 m (AHD; DWLBC unpublished data 2008). As Lake levels have fallen, important creek and drain habitats for threatened species have become disconnected from Lake Alexandrina and dried out (Bice and Ye 2007), whilst the area of Lake edge habitat and the quality of remaining habitat (i.e. decreased proportion of vegetation, increasing salinity) has greatly diminished. As such, the Lower Lakes populations of Murray hardyhead, southern pygmy perch and Yarra pygmy perch are under severe threat.

As one of the six icon sites identified in *The Living Murray*, ecological objectives have been established in the Lower Lakes, Coorong and Murray Mouth Environmental Management Plan (LLCMM EMP; MDBC 2006), in an attempt to maintain and enhance the ecological character of the LLCMM. To achieve these objectives, a range of targets have been developed that are specific to particular ecosystem components and processes, including *'improved spawning and recruitment success in the Lower Lakes for endangered fish species, including Murray hardyhead and Pygmy perch'* and to *'maintain and enhance habitat for native fish'*. This project involves 'condition' monitoring of fish assemblages in spring 2007 and summer 2008, and provides a baseline for future monitoring upon an 'intervention', and will assist in reaching the aforementioned targets. Specifically, the aims of this study were to:

1) Determine the distribution and relative abundance of Murray hardyhead, southern pygmy perch and Yarra pygmy perch in the Lower Lakes, and
2) Investigate seasonal variations in size/age structures of these species, and assess their spawning and recruitment success.

Additionally the project aimed to investigate the general fish community. Specifically to:

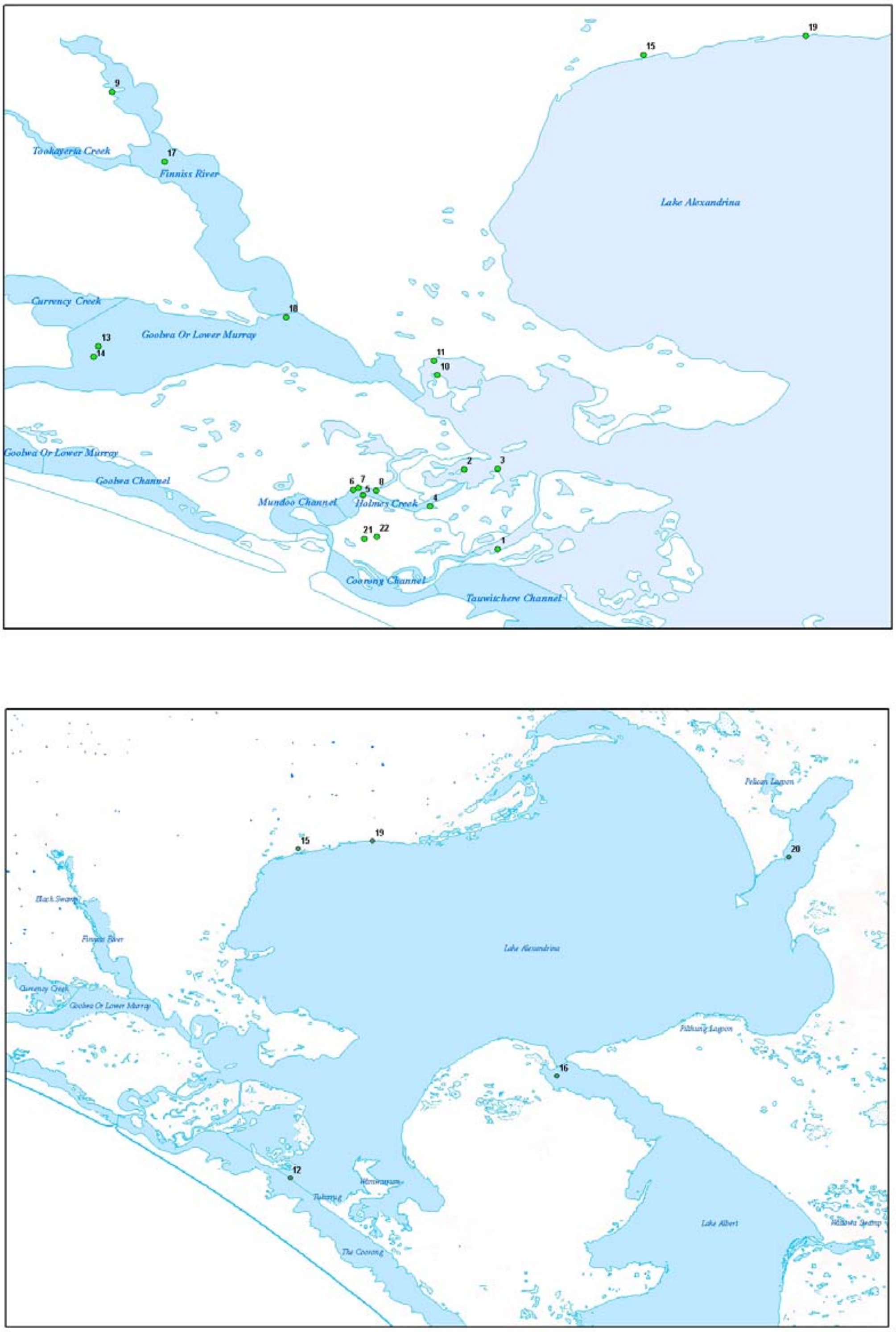
3) Investigate seasonal variation in the abundance and species composition of fish assemblages in the Lower Lakes and
4) Investigate any potential relationships between fish assemblages and environmental parameters (water quality and habitat characteristics).

2 METHODS

2.1 STUDY AREA

The Murray-Darling River system discharges into a shallow (average depth 2.9 m) terminal lake system (Lakes Alexandrina and Albert), before flowing into the Coorong and finally the Southern Ocean via the Murray Mouth. Prior to regulation and barrage construction in the 1940's, conditions within the Lower Lakes alternated between freshwater and estuarine depending upon river inflows and tidal patterns (Close 1990). The barrages, whilst disconnecting the Coorong from the Lower Lakes, created an impounded freshwater environment upstream, which led to the fixation of wetland habitats favourable for certain freshwater fish species (Wedderburn *et al.* 2003). These habitats have seemingly facilitated the persistence of many species within the region as other habitats in the Murray-Darling Basin deteriorated (Wedderburn *et al.* 2003).

Twenty two different sites across the wetlands, fringing lake habitats and irrigation channels (commonly referred to as drains) in the Lower Lakes were selected for sampling (including sites in various sub-sections of the Lakes i.e. the Finniss arm of Lake Alexandrina, Hindmarsh Island, Lake Albert, etc; Figures 1a & b; Table 1). Sites were chosen based on previous detection of Murray hardyhead, southern pygmy perch and Yarra pygmy perch and due to their perceived importance for these species within the region. As such, there was only one site in Lake Albert as these species are not widely distributed in this Lake but rather are concentrated in Lake Alexandrina. Due to diminished water levels in summer 2008, only 12 sites could be sampled in both spring and summer seasons (Table 1) and as such quantitative analysis of abundance data is limited to these sites.



(a)

(b)

Figure 1. Maps of the Lower Lakes focusing on the (a) western and (b) eastern sections of Lake Alexandrina.

Table 1. Location of sampling sites (map datum WGS84) and seasons sampled (spring 2007 and/or summer 2008). Gear types used at each site are indicated (F = fyke nets, S = seine net, B = box traps).

Site No.	Location	Latitude	Longitude	Spring gear types	Summer gear types
1	Boundary Creek	35.55214S	138.95394E	F, S, B	F, B
2	Holmes Creek (western side)	35.52702S	138.94335E	F, S, B	F, B
3	Holmes Creek (eastern side)	35.52676S	138.95387E	F, S, B	-
4	Holmes Creek, Fishtrap	35.53858S	138.93251E	F, B	F, B
5	Holmes (Southern side)	35.53506S	138.91112E	F, S, B	F, B
6	Holmes Creek (Mouth of Boggy)	35.53353S	138.90814E	F, S, B	F, B
7	Steamer Drain	35.53285S	138.90969E	F, S, B	-
8	Boggy Creek	35.53373S	138.91543E	F, B	F, B
9	Finniss River, Wallys Wharf	35.40750S	138.83153E	F, B	F, B
10	Clayton 1	35.49708S	138.93481E	F, B	-
11	Clayton 2	35.49267S	138.93364E	F, S, B	F, B
12	Tauwitcherie Barrage	35.58236S	139.00314E	F, B	-
13	Currency Creek Mouth 1	35.48791S	138.82713E	F, S, B	F, B
14	Currency Creek Mouth 2	35.49133S	138.82556E	F, S, B	-
15	Angas River	35.39588S	139.00019E	F, S, B	F, B
16	Lake Albert Entrance	35.51894S	139.18578E	F, B	-
17	Finniss River, Black Swamp	35.42959S	138.84816E	F, B	-
18	Finniss River Confluence	35.47877S	138.88672E	F, S, B	F, B
19	Bremer River	35.38972S	139.05170E	F, B	F, B
20	Pelican Lagoon	35.39007S	139.34061E	F, S, B	-
21	Hindmarsh Island Drain	35.54892S	138.91158E	-	F, B
22	Hindmarsh Island Drain 2	35.54827S	138.91556E	-	F, B

2.2 FISH SAMPLING

Fish sampling was conducted in October 2007 (spring) and February 2008 (summer) using large single-wing fyke nets (wing length 6 m, stretched mesh 8 mm, n = 3), unbaited box traps (24 x 24 x 40 cm, n = 10, wetted for 1 hour) and seine nets (7 x 2 m x 6 mm, n = 3 x 10 m hauls). Sampling across all sites took between 8 and 10 days each season.

Fyke nets were set overnight at each site on each sampling occasion. Fyke nets were set perpendicular to the bank in areas with aquatic macrophytes (where possible), as structurally complex habitats are known to provide shelter, refuge from predators (Copp 1997; Closs *et al.* 2005) and an indirect source of food to native species (Humphries 1995). Box traps were set randomly with no selection for areas with or without macrophytes. Seine net pulls were conducted at all sites where possible. This technique was not used at sites where soft sediments represented a hazard to field workers (see table 1 for gear type use at individual sites). Thus seine net data was not used for quantitative analysis of abundance data.

All fish captured were identified, counted and length measurements were taken for Murray hardyhead (caudal fork length, FL mm), southern pygmy perch and Yarra pygmy perch (total length TL mm). Sampling was conducted under a *Section 115 permit* in accordance with the *Fisheries Act 2007* and PIRSA Animal Ethics Committee standards.

2.3 ANALYSIS OF SPAWNING AND RECRUITMENT

2.3.1 LENGTH-FREQUENCY DISTRIBUTION ANALYSIS

Seasonal variation in the size structure of Murray hardyhead, southern pygmy perch and Yarra pygmy perch populations between spring and summer was investigated through the comparison of length-frequency distributions (Kolmogorov-Smirnov goodness-of-fit-test). Length data from all sampling techniques (fyke nets, seine nets and box traps) were pooled for each species for each site on each sampling occasion to provide sufficient sample sizes to analyse length-frequency distributions. Visual analysis of length-frequency distributions may indicate the presence of 0+ cohorts (young-of-year) and hence provide a measure of spawning and recruitment.

2.4.2 OTOLITH ANALYSIS

Otolith analysis (daily growth increment counts), and a validation study of this technique to determine age for the three threatened species, was to be carried out to confirm the presence of 0+ individuals and assess recruitment success with greater confidence. However, given the small sample sizes and the current uncertain status of these species in the Lower Lakes, no fish were sacrificed for this analysis.

2.4 DATA ANALYSIS

Relative abundance data (number of fish/site/sampling occasion) for Murray hardyhead, southern pygmy perch and Yarra pygmy perch was not normally distributed between seasons. Thus, the non-parametric Kruskal-Wallace test (Kruskal and Wallace 1952) was used to compare relative abundance between seasons, because unlike parametric analysis of variance, it does not assume normally distributed populations or equal variances (Sakowicz and Zarnecki 1954). Fyke net data from 12 sites sampled each season were used for this analysis.

Differences in fish assemblages between spring and summer (seasonal variation) were examined using Bray-Curtis similarity measures (Bray and Curtis 1957), and Analysis of Similarities (ANOSIM) was used to test for significant differences (Clarke 1993). Relative abundance data was fourth-root transformed to prevent abundant species from excessively influencing the dissimilarity measure (Clarke 1993). Non-metric Multidimensional Scaling (MDS) ordination was subsequently used to visualise the temporal patterns. Species which contributed most to the differences between years were determined using SIMPER (Similarity Percentages) with a cut-off level of 60% cumulative contribution. Multivariate analyses followed the methods described by Clarke (1993) using PRIMER v.5.2 (Plymouth Routines in Multivariate Ecological Research) software package.

2.5 RELATIONSHIPS BETWEEN FISH ASSEMBLAGES AND ENVIRONMENTAL PARAMETERS

Measurement of water quality parameters was conducted at each site during the course of this study. Conductivity (micro-Siemens), pH, dissolved oxygen (ppm), water temperature (degrees Celsius; all measured with a TPS MC81 handheld multimeter) and turbidity (secchi depth) were measured at each site. Habitat variables such as, mean depth (m), wetted width (m), percentage cover of submergent and emergent vegetation and the abundance of large bodied predatory species (i.e. redfin perch, *Perca fluviatilis*) were also recorded at each site. Water quality recordings were taken prior to fish sampling at each site to reduce the influence of sediment disturbance on water quality measurements.

Relationships between fish assemblages and environmental parameters were assessed using protocols given by Clarke and Ainsworth (Clarke and Ainsworth 1993). The routine BIOENV in the software PRIMER v.5.2 was used for this correlation analysis (Clarke and Ainsworth 1993; Clarke and Warwick 2001). The rank similarity matrices (Bray-Curtis for fish assemblages and Normalised Euclidean distance for water quality and habitat parameters) were compared using the weighted Spearman rank correlation. The rank correlation coefficient (ρ_w) lies between –1 and +1, corresponding to the cases where the fish assemblage and environmental patterns are in complete opposition or complete agreement. Values around 0 correspond to the absence of any match between the two patterns (Clarke and Ainsworth 1993). Values of $\rho_w \leq 0.6$ were considered to show little correlation and were not investigated further. Symbols scaled in size to (representing the values of water quality and habitat parameters) were individually superimposed onto the two-dimensional MDS configurations of fish assemblages, to identify visual concordance when the Spearman rank correlation was considerable (Field *et al.* 1982).

3 RESULTS

3.1 LAKE LEVELS AND CONDUCTIVITY DURING SAMPLING

Lake levels throughout 2007 and 2008 have remained well below normal operating levels (c. 0.75 m; Figure 2; DWLBC unpublished data 2008). In October 2007 (spring sampling period) water levels in Lake Alexandrina, above Goolwa barrage, fluctuated between -0.1 m and 0.25 m (AHD; Figure 2). Water levels continued to fall in 2008 as a result of evaporation and diminished inflows, such that levels remained between -0.6 m and -0.23 m (AHD) during February (summer sampling period). At Lake levels below 0.35 m (AHD) significant creek and drain habitats for threatened species on Hindmarsh Island become disconnected from the Lakes and isolated from one another (Bice and Ye 2007). This critical level was reached at the end of 2006, after which the Lakes have remained below this level and continued to fall. Falling Lake levels correspond with decreases in Lake edge habitat and fragmentation of aquatic habitats.

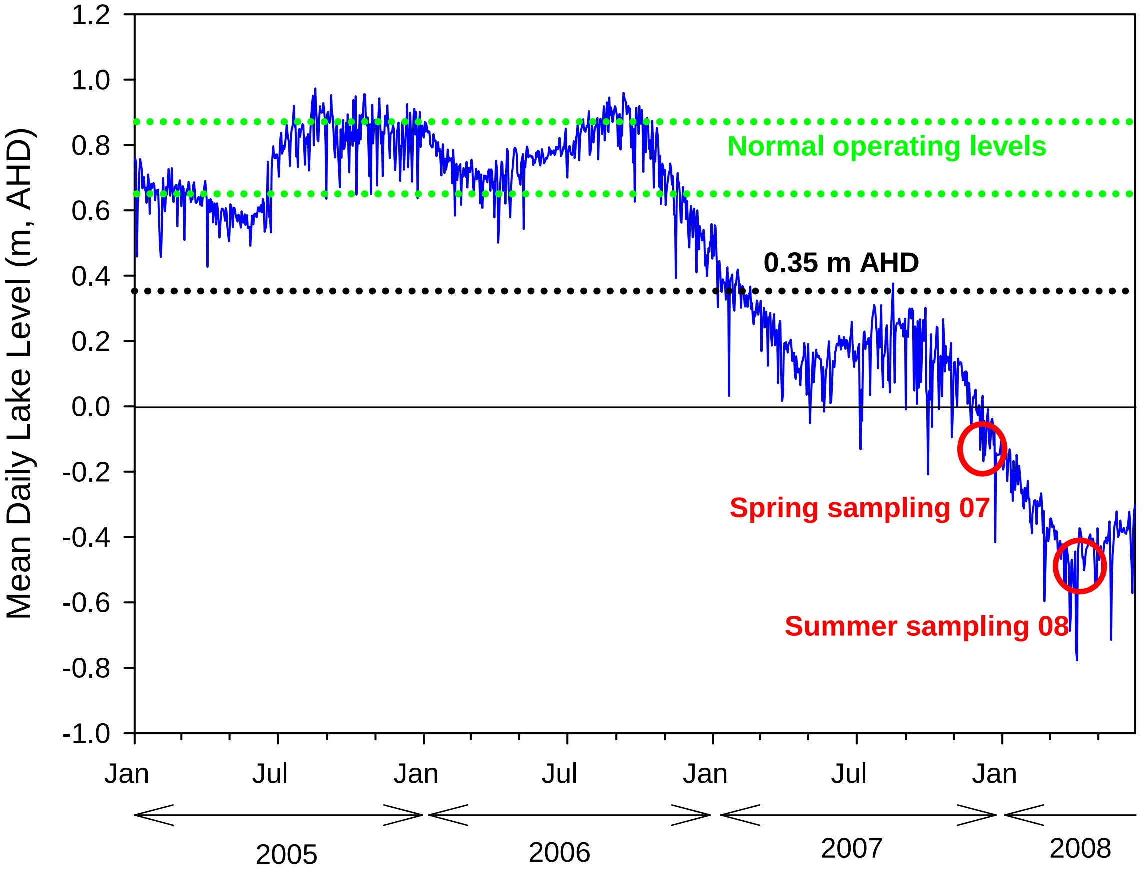


Figure 2. Water level fluctuations in Lake Alexandrina above the Goolwa barrage from January 2005-June 2008. Sampling events are indicated, as are normal operating Lake levels, the critical Lake level of 0.35 m AHD and 0 m AHD.

A further consequence of falling Lake levels if rising salinity (measured as electrical conductivity, EC). Figure 3 shows the dramatic increase in salinity above the Goolwa barrage since January 2007, when Lake levels began falling rapidly (DWLBC unpublished data 2008). The conductivity levels presented are recorded at a station immediately above the Goolwa barrage and as such are highly influenced by saltwater intrusion through the barrage. Hence, conductivity levels in other areas of Lake Alexandrina are not likely to be as high, however, this data acts as a good reference for general changes within the Lake.

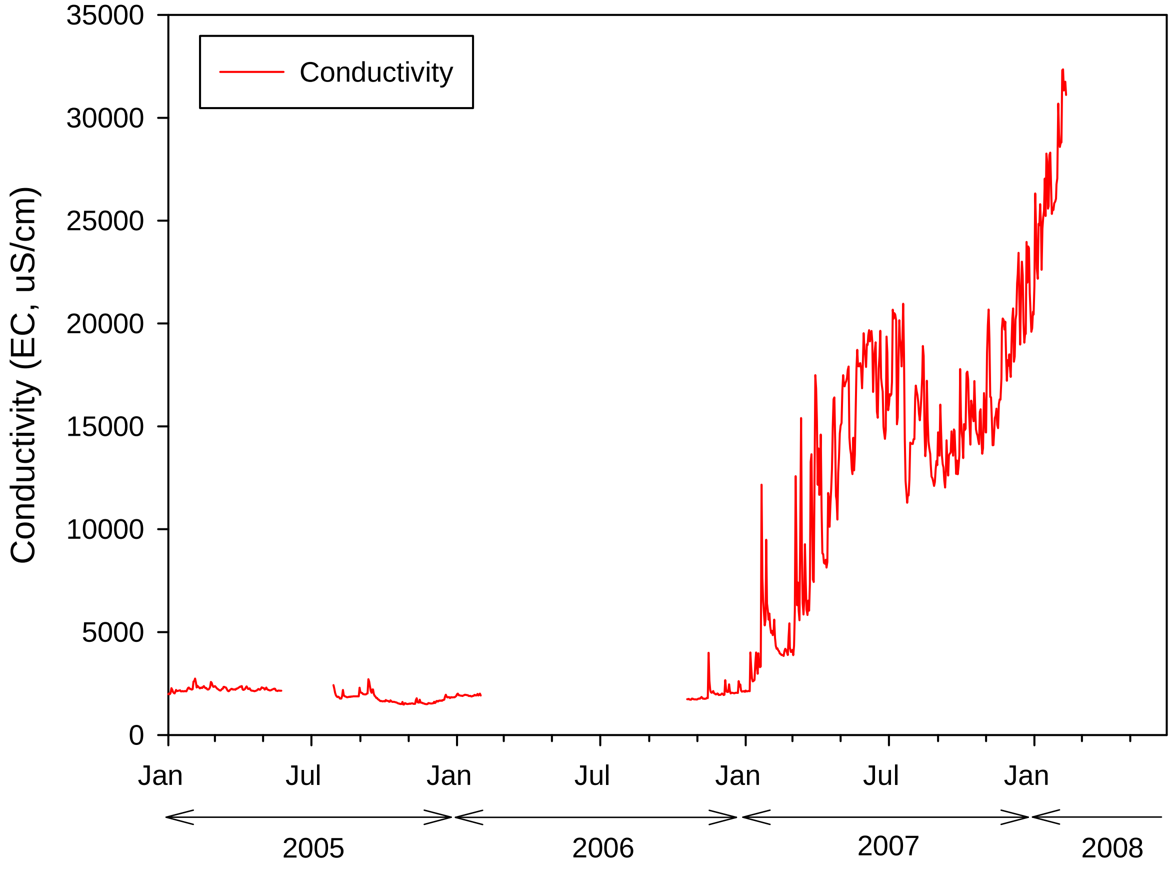


Figure 3. Conductivity (EC, uS/cm) in Lake Alexandrina above the Goolwa barrage from January 2005-June 2008.

3.2 CATCH SUMMARY

A total of 4917 (20 species) and 7607 (19 species) fish were sampled in spring and summer respectively (Table 2). Small-mouthed hardyhead, common galaxias, flat-headed gudgeon, Australian smelt and bony herring were abundant in both seasons. Bridled goby, congolli, carp, southern pygmy perch and Yarra pygmy perch were present in low numbers in both seasons. The most widespread species were common galaxias, flat-headed gudgeon, small-mouthed hardyhead, lagoon goby, Australian smelt and bony herring (a table of species presence and absence at each site sampled is given in Appendix 1).

Table 2. Total number and relative abundance of fish sampled in the Lower Lakes in spring 2007 (n=20 sites) and summer 2008 (n=14 sites). Relative abundances were calculated from fyke net data from the 12 common sites sampled in both season. Note threatened and exotic fish highlighted.

Common name	Species name	#Spring 2007	RA ± SE	#Summer 2008	RA ± SE
Murray Hardyhead@	*Craterocephalus fluviatilis*	35	0.75 ± 0.3	49	4.08 ± 2.5
Southern pygmy perch@	*Nannoperca australis*	8	0.33 ± 0.26	14	0.08 ± 0.08
Yarra pygmy perch@	*Nannoperca obscura*	2	0.08 ± 0.08	12	1 ± 1
Unspecked hardyhead@	*Craterocephalus stercusmuscarum f.*	19	0.5 ± 0.34	74	6.17 ± 4.24
Carp gudgeon@	*Hypseleotris Spp.*	6	0.33 ± 0.26	1020	3.17 ± 3.17
Flat-headed gudgeon@	*Philypnodon grandiceps*	308	6.42 ± 1.57	533	36.42 ± 12.99
Dwarf flat-headed gudgeon@	*Philypnodon macrostomus*	43	1.58 ± 0.66	5	0.17 ± 0.11
Australian smelt@	*Retropinna semoni*	2192	51.58 ± 27.92	409	34.08 ± 19.13
Bony herring@	*Nematalosa erebi*	276	14.5 ± 7.72	1083	90.25 ± 87.89
Common galaxias^	*Galaxias maculatus*	1053	29.5 ± 8.97	1261	100.58 ± 27.17
Congolli^	*Pseudaphritus urvillii*	5	0.17 ± 0.17	11	0.83 ± 0.66
Tamar river goby*	*Afurcagobius tamarensis*	52	1.58 ± 0.95	11	0.92 ± 0.43
Bridled goby*	*Arenigobius bifrenatus*	5	0.17 ± 0.11	6	0.5 ± 0.34
Western blue-spot goby*	*Pseudogobius olorum*	57	1.17 ± 0.6	63	0.75 ± 0.49
Lagoon goby*	*Tasmanogobius lasti*	194	7.83 ± 4.52	186	12.17 ± 3.69
Small-mouthed hardyhead*	*Atherinosoma microstoma*	530	16.5 ± 5.13	1840	148.67 ± 64.45
Sandy sprat*	*Hyperlophus vitattus*	3	0.08 ± 0.08	0	
Gambusia@	*Gambusia holbrooki*	32	1.17 ± 0.68	632	31.83 ± 25.22
Redfin@	*Perca fluviatilis*	86	3.92 ± 1.83	397	32.67 ± 18.61
Carp@	*Cyprinus carpio*	11	0.58 ± 0.23	1	0.08 ± 0.08
Totals		4917		7607	

@freshwater, ^diadromous, *estuarine/marine

3.3 DISTRIBUTION AND ABUNDANCE OF MURRAY HARDYHEAD, SOUTHERN PYGMY PERCH AND YARRA PYGMY PERCH

Murray hardyhead, southern pygmy perch and Yarra pygmy perch were collected in low numbers in spring and summer (Table 3). The numbers of all three species were slightly higher in summer, however, Murray hardyhead and southern pygmy perch represent a similar proportion of the total catch in both seasons (Table 3).

Table 3. Numbers of Murray hardyhead, southern pygmy perch and Yarra pygmy perch collected (all sites and sampling techniques combined) and their proportion of the Total catch, sampled from the Lower Lakes in spring 2007 and summer 2008.

	Spring 2007		**Summer 2008**	
Species	**Abundance**	**% of Total catch**	**Abundance**	**% of Total catch**
Murray hardyhead	35	0.712	49	0.644
Southern pygmy perch	8	0.163	14	0.184
Yarra pygmy perch	2	0.041	12	0.158

The relative abundance of Murray hardyhead at ‘common’ sites (*n* = 12) appears to have risen in 2008, however, statistical analysis of this data (Kruskal-Wallace rank test; Kruskal and Wallace 1952) indicated the relative abundance of Murray hardyhead did not differ significantly between seasons ($H_{1,\ 11}$= 0.026, p = 0.872; Figure 3). Large inter-site variation in abundances within each season is likely responsible for this result. A solitary Yarra pygmy perch was captured from one common site in spring and a single southern pygmy perch from one site in summer, hence statistical analysis was not performed for these species (Figure 3).

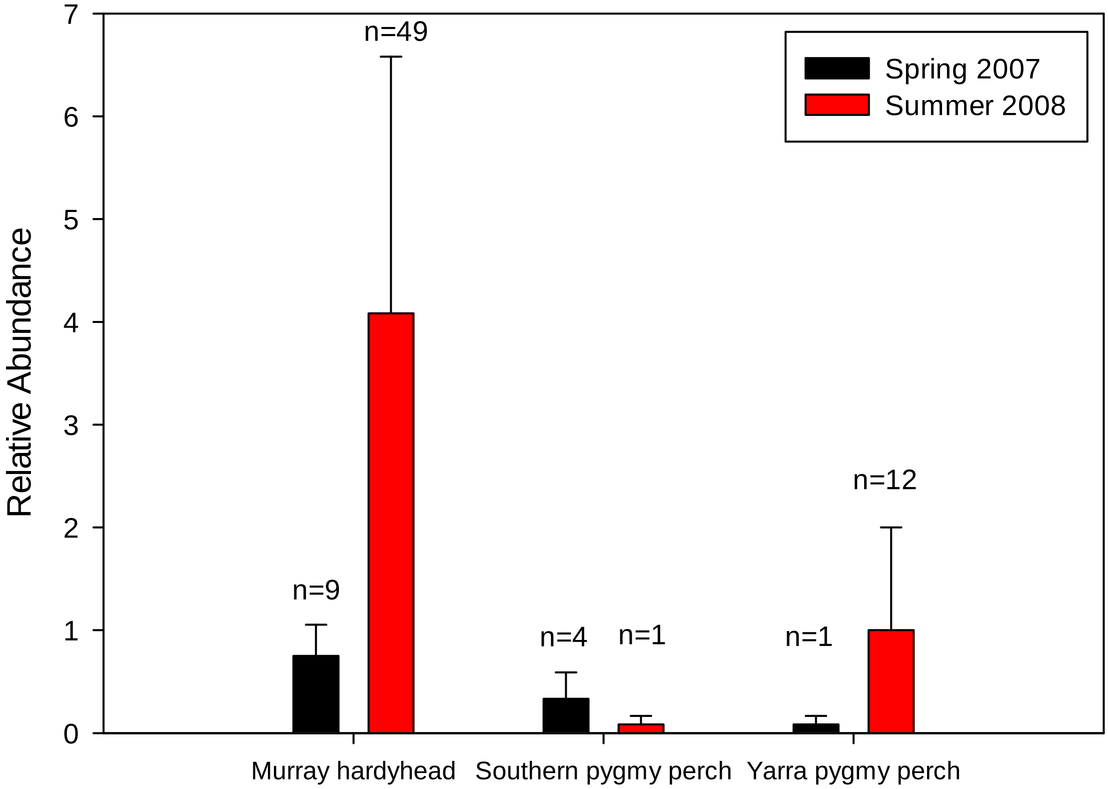


Figure 4. Relative abundance (number of fish/site/sampling occasion ± Standard Error) of Murray hardyhead, southern pygmy perch and Yarra pygmy perch in the Lower Lakes in spring 2007 and summer 2008. Note that data used was fyke net data only from 12 'common' sites sampled in both seasons.

Murray hardyhead were the most widespread of the three threatened fish species in the Lower Lakes over spring (*n* = 9 sites) and summer (*n* = 5 sites; Table 4). All three species were sampled from fewer sites in summer compared to spring, however, four sites where one or more of these species were detected in spring had completely dried out in summer and were not sampled (Table 4). Of the 20 sites initially sampled, only 12 of these were replicated in summer due to critically low water levels in the Lake. Of these sites, Clayton 2 (Dunn's Lagoon) and the mouth of the Bremer River yielded the most Murray hardyhead, whilst Boundary creek had the most Yarra pygmy perch (Table 4). In many cases, sites deemed important by Bice and Ye (2007) such as Hunters Creek and Steamer drain on Hindmarsh Island, could not be sampled in both seasons as they had completely dried (Figure 4). Hindmarsh Island drain 2, sampled in 2008 only, yielded substantial numbers of southern pygmy perch (Table 4).

Table 4. Sites and numbers of Murray hardyhead, southern pygmy perch and Yarra pygmy perch collected in the Lower Lakes region in spring 2007 and summer 2008. Sites 1, 11 and 22 (see Table 1) are highlighted based on the perceived importance of these sites for these species. ns = not sampled.

Site	Murray hardyhead Spring	 Summer	Southern pygmy perch Spring	 Summer	Yarra pygmy perch Spring	 Summer
1	**2**	**-**	**-**	**1**	**1**	**12**
2	-	-	-	-	-	-
3	-	ns	-	ns	-	ns
4	1	2	-	-	-	-
5	-	1	-	-	-	-
6	1	-	-	-	-	-
7	**13**	**ns**	**3**	**ns**	**-**	**ns**
8	1	1	3	-	-	-
9	2	-	-	-	-	-
10	11	ns	-	ns	-	ns
11	**3**	**21**	**-**	**-**	**-**	**-**
12	-	ns	1	ns	-	ns
13	-	-	-	-	-	-
14	-	ns	-	ns	-	ns
15	-	-	1	-	-	-
16	1	ns	-	ns	-	ns
17	-	ns	-	ns	1	ns
18	-	-	-	-	-	-
19	-	24	-	-	-	-
20	-	ns	-	ns	-	ns
21	ns	-	ns	-	ns	-
22	**ns**	**-**	**ns**	**13**	**ns**	**-**
Total sites	**9**	**5**	**4**	**2**	**2**	**1**

Spring 2007

Summer 2008

(a)

(b)

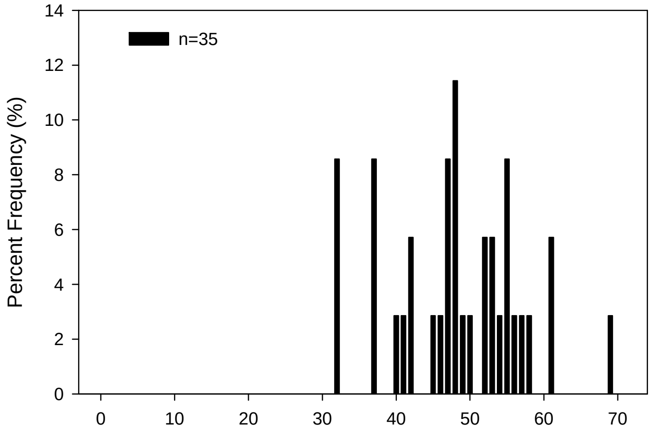
Figure 5. Steamer Drain (site 7) in (a) spring 2007 and (b) summer 2008.

3.4 SEASONAL VARIATIONS IN SIZE/AGE STRUCTURES OF MURRAY HARDYHEADS, SOUTHERN PYGMY PERCH AND YARRA PYGMY PERCH

3.4.1 Murray Hardyhead

Length-frequency distributions of Murray Hardyhead differed significantly between spring and summer (Kolmogorov-Smirnov, $D = 0.46$, $p < 0.001$; Figure 5). In spring, fish lengths ranged from 32-69 mm FL (Figure 5a) and in summer fish lengths ranged from 23-65 mm FL (Figure 5b), with mean length smaller in summer (41.1 ± 1.3 mm FL) compared to spring (48.2 ± 1.5 mm FL). Fish <30 mm FL (likely YOY fish; see Bice and Ye 2007) were not present in spring but they represented 6% of the population in 2008, indicating some level of recruitment occurred in summer 2008. Also, fish between 30-38 mm FL comprised *c.*37% of the population in summer, compared to *c.*17% in spring.

(a) Spring 2007



(b) Summer 2008

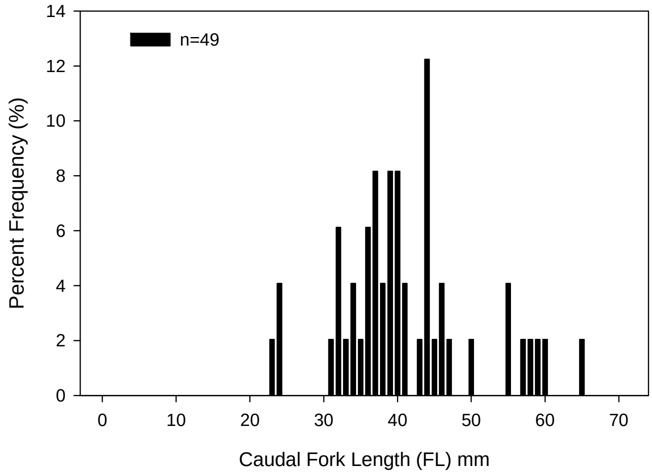
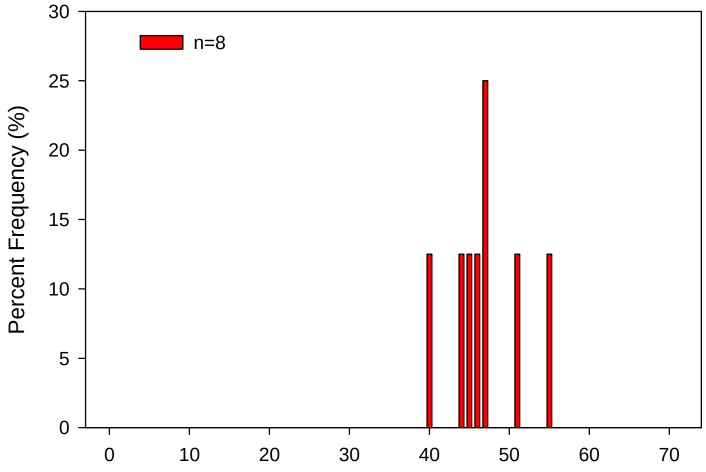


Figure 6. Length-frequency distribution of Murray Hardyhead in (a) spring 2007 and (b) summer 2008. Note that the data used for this graph were pooled for all sites and includes fyke net, box trap and seine net data. Note data are displayed as a percentage.

3.4.2 Southern Pygmy Perch

Total length (TL) of fish ranged from 40-55 mm and 30-54 mm respectively in spring (Figure 6a) and summer (Figure 6b). No statistical analysis was undertaken as the sample sizes in spring and summer were too small to allow Kolmogorov-Smirnov tests. However, no fish <40 mm TL (likely YOY fish; see Bice and Ye 2007) were present in spring but they represented *c.* 50% of the population in summer. A greater percentage of larger southern pygmy perch (>50 mm TL) were also present in summer (*c.* 43%) when compared with spring (*c.* 25%), signifying the growth of this cohort.

(a) Spring 2007



(b) Summer 2008

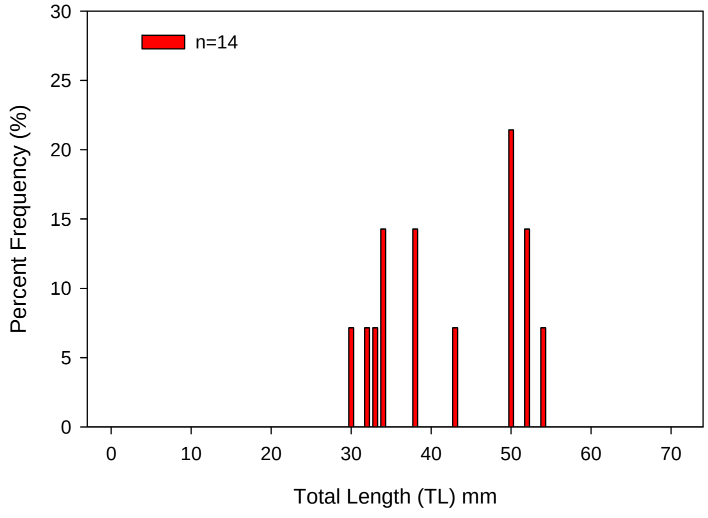


Figure 7. Length-frequency distribution of southern pygmy perch in (a) spring 2007 and (b) summer 2008. Note that the data used for this graph were pooled for all sites and includes fyke net, box trap and seine net data. Note data are displayed as a percentage.

3.4.3 Yarra Pygmy Perch

Fish ranged in length from 56-58 mm TL in spring (Figure 7a) and 47-63 mm TL in summer (Figure 7b). No statistical analysis was undertaken as the sample size in both seasons was too small. Fish <50 mm TL were absent in spring 2007 and rare in summer 2008, with no fish sampled <40 mm TL (likely YOY fish; see Bice and Ye 2007). Therefore no spawning and recruitment was detected over 2007/2008.

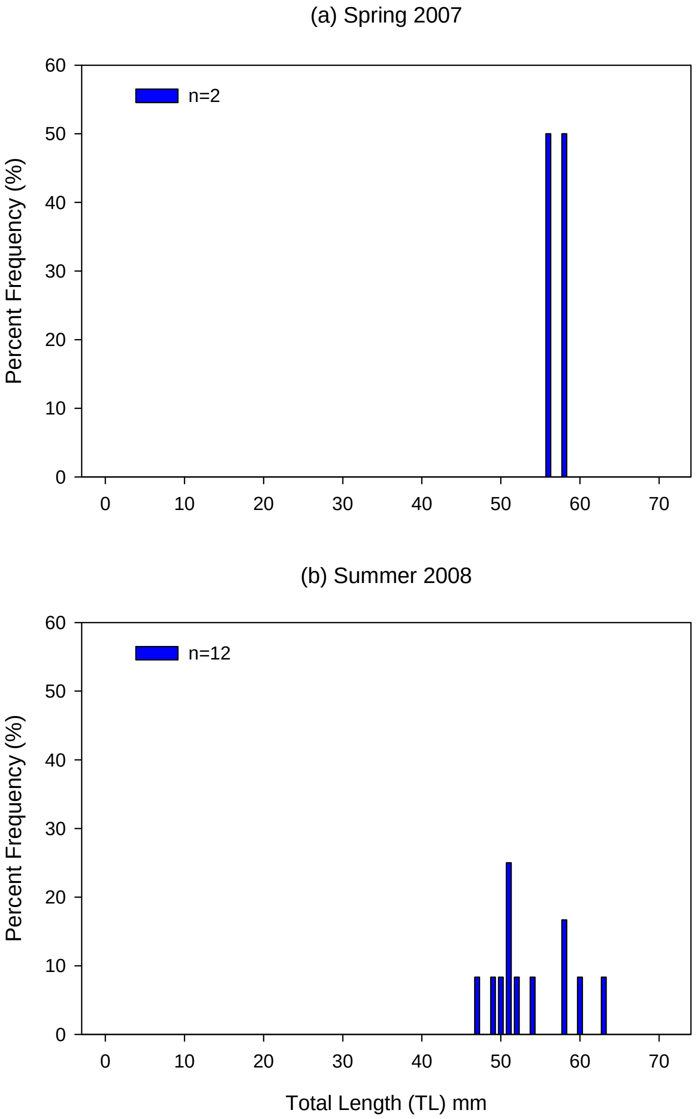


Figure 8. Length-frequency distribution of Yarra pygmy perch in (a) spring 2007 and (b) summer 2008. Note that the data used for this graph were pooled for all sites and includes fyke net, box trap and seine net data. Note data are displayed as a percentage.

3.5 SEASONAL VARIATION IN SPECIES COMPOSITION AND RELATIVE ABUNDANCE OF FISH ASSEMBLAGES

Figure 8 shows a multi-dimensional scaling (MDS) ordination of fish assemblages sampled in the Lower Lakes in spring 2007 and summer 2008. Although assemblages do not appear to form distinct groupings by season, analysis of similarities (ANOSIM) exposed a significant difference (Global $R = 0.103$, $p = 0.017$).

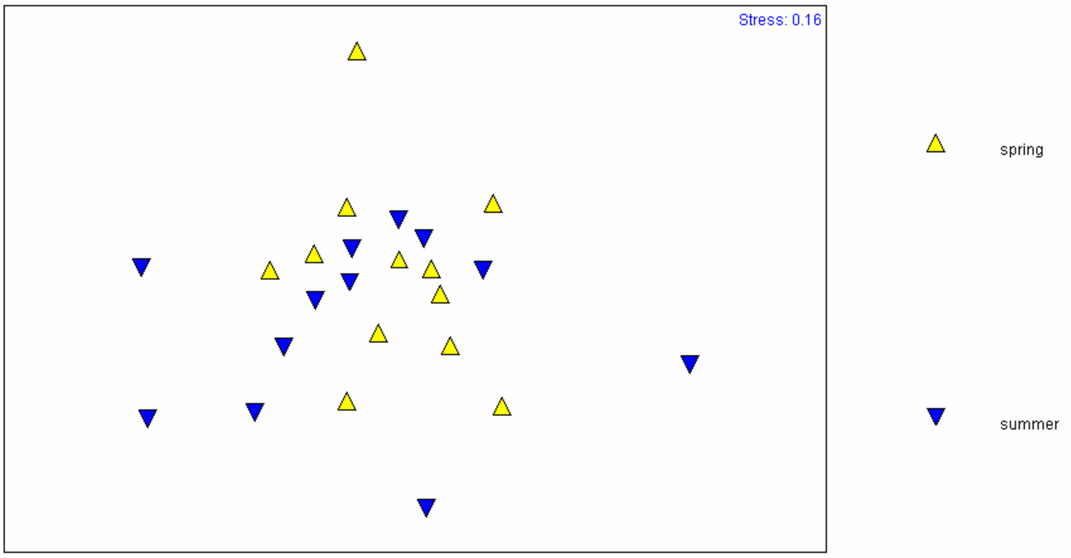


Figure 9. MDS plot (2-dimensional) for the Lower Lakes fish assemblages sampled in spring 2007 and summer 2008.

Similarity of percentages analysis reveals what species are contributing to the fish assemblage differences between seasons (Table 5). The difference detected between seasons was largely driven by a decrease in abundance of Australian smelt and increases in abundances of most other species, particularly small-mouthed hardyhead, eastern gambusia, bony herring, lagoon goby, redfin perch, flat-headed gudgeon and common galaxias.

Table 5. SIMPER analysis for fish assemblages sampled from the Lower Lakes in spring 2007 and summer 2008. Results are based on fourth root transformed data. Mean abundance is number of fish per site per sampling occasion. CR (consistency ratio) indicates the consistency of differences in abundance between years, with larger values indicating greater consistency. The contribution (%) indicates the proportion of difference between years (shown by ANOSIM) attributable to individual species. A cumulative cut-off of 60% was applied. Mean dissimilarity is expressed as a percentage ranging between 0% (identical) and 100% (totally dissimilar).

Species	**Mean abundance**		**CR**	**Contribution (%)**	**Cumulative contribution (%)**
	Spring 2007	**Summer 2008**		*Mean dissimilarity = 48.80*	
Australian smelt	51.58	34.08	1.27	9.97	9.97
Small-mouthed hardyhead	16.5	149	1.34	9.78	19.76
Eastern gambusia	1.17	31.83	1.09	8.54	28.3
Bony herring	14.5	90.25	1.15	8.35	36.64
Lagoon goby	7.83	12.17	1.17	7.78	44.42
Redfin perch	3.92	32.67	1.22	7.15	51.57
Flat-headed gudgeon	6.42	36.42	1.20	6.98	58.55
Common galaxias	29.5	100.58	1.53	6.78	65.33

3.6 RELATIONSHIP BETWEEN FISH ASSEMBLAGE AND HABITAT CONDITIONS

Fish assemblages from each year were linked to multivariate environmental patterns using BIOENV rank correlation techniques in PRIMER v. 5.2 (after Clarke and Warwick 2001). Different combinations of environmental variables provide the 'best match' with assemblage patterns in different years.

In spring, a combination of the proportion of submerged vegetation (%), conductivity (i.e. salinity) and pH best matched the observed fish assemblages ($\rho_w = 0.642$). Figure 9a shows the MDS ordination of fish assemblages in spring. Site 19 (Bremer River) was separated from all other sites on the MDS ordination and exhibited a high proportion of submerged vegetation, whilst all other sites exhibited sparse submerged vegetation cover (Figure 9b). Site 19 and site 15 (Angas River) possessed the lowest conductivities (Figure 9c) and the fish assemblage at both of these sites was dominated by freshwater (e.g. flat-headed gudgeon, redfin perch) and diadromous species (i.e. common galaxias), whilst estuarine species were absent or present in very low numbers when compared to other sites. The relationship between fish assemblages and pH is unclear (Figure 9d).

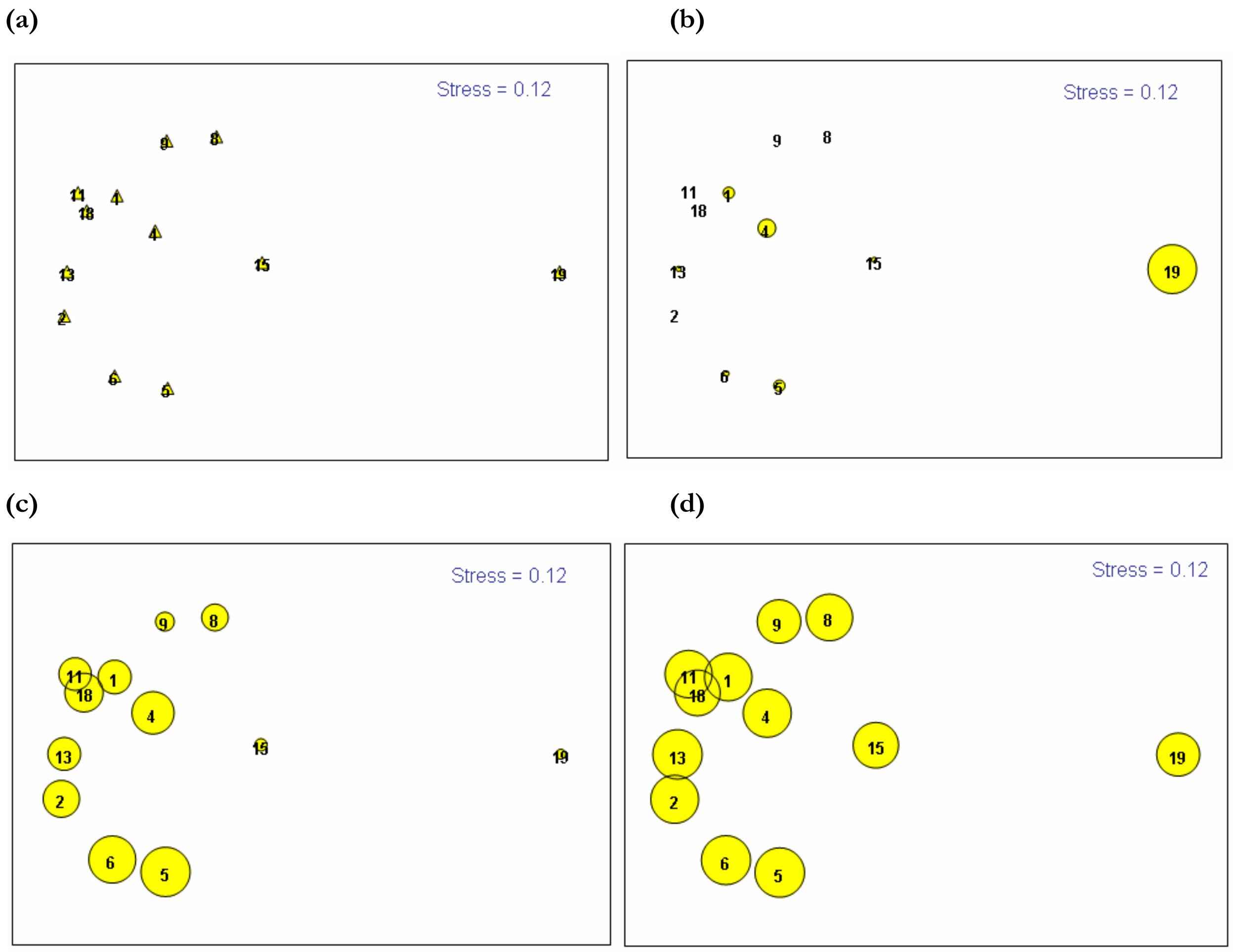


Figure 10. (a) MDS plot for fish assemblages in spring 2007. Superimposed circles represent values of (b) proportion of submerged vegetation cover (%) (c) conductivity and (d) pH at each site.

In summer environmental variables showed a very poor correlation with the observed fish assemblages (ϱ_w = 0.418). Thus, relationships were not further investigated.

4 DISCUSSION

4.1 RELATIVE ABUNDANCE AND DISTRIBUTION OF MURRAY HARDYHEAD, SOUTHERN PYGMY PERCH AND YARRA PYGMY PERCH

All three species were collected in low to very low numbers in both seasons, with Murray hardyhead the most abundant. In the spring of 2005, Murray hardyhead relative abundance, in the vicinity of Hindmarsh Island, was 35 times greater than in spring 2007 and over 150 times greater for southern pygmy perch and Yarra pygmy perch (Table 6; Bice and Ye 2006). The numbers recorded in 2005 represent some of the largest catches of these species ever recorded in the region and followed a period of medium-high Lake levels (0.8-1 m AHD). Not all sites sampled by Bice and Ye (2007) were sampled in the current study as many are now dry. However, the sheer difference in numbers does provide some insight to the current status of these species. Similarly, the abundances of Murray hardyhead, southern pygmy perch and Yarra pygmy perch in summer seasons from 2005-2008 show a marked decline for all three threatened species (Table 6; Bice and Ye 2007). The abundances of these species recorded in the Lower Lakes by Higham *et al.*(2005) in 2003 and 2004 were also far greater than those presented in the current project.

Table 6. Comparison of abundances of threatened species in spring and summer seasons of previous years. Figures for spring 2005 and summer 2005-2007 are taken from Bice and Ye (2006; 2007).

	2005	**2006**	**2007**	**2008**
Species		**Spring season**		
Murray hardyhead	35.45 ± 33.5	Not sampled	0.75 ± 0.3	Not sampled
Southern pygmy perch	113.8 ± 86.3	Not sampled	0.33 ± 0.26	Not sampled
Yarra pygmy perch	41.2 ± 33.7	Not sampled	0.08 ± 0.08	Not sampled
		Summer season		
Murray hardyhead	16.1 ± 5.2	18.6 ± 21.1	7.4 ± 4.5	4.08 ± 2.5
Southern pygmy perch	10.6 ± 6.5	6.5 ± 7.2	3.8 ± 6.8	0.08 ± 0.08
Yarra pygmy perch	2.8 ± 1.6	6.7 ± 12.2	1.8 ± 3.2	1 ± 1

Declines in the abundance of Murray hardyhead, southern pygmy perch and Yarra pygmy perch are due to the extremely low water levels in the Lower Lakes throughout 2007 and 2008. Water levels below 0.35 m (AHD) are considered to be unfavourable for these species, resulting in habitat fragmentation and isolation of important habitats on Hindmarsh Island from Lake Alexandrina (Higham *et al.* 2005; Bice and Ye 2007). Lake levels began falling below 0.35 m (AHD) in January 2007 and have continued to fall due to prolonged drought conditions and minimal or no inflows. During summer 2008, Lake levels consistently fell below -0.5 m (AHD) resulting in a dramatic decrease in Lake volume/area, near total loss of off-channel habitats, decrease in lake edge habitat (access to emergent vegetation) and general decrease in aquatic habitat quality (e.g. desiccation of submerged vegetation) including increased salinity. This dramatic constriction of habitat area has likely forced Murray hardyhead, southern pygmy perch and Yarra pygmy perch into sub-optimal habitats (i.e. open water) where they may encounter increased competition for diminishing resources with other small-bodied species (native and exotic) and potentially increased predation pressure from piscivorous fishes (i.e. redfin perch, etc.).

In summer 2008, Murray hardyhead were collected in their greatest numbers from Dunn's Lagoon at Clayton (*n* = 21) and the mouth of the Bremer River (*n* = 24). Murray hardyhead have been consistently recorded from Dunn's Lagoon in recent years (Wedderburn *et al.* 2003; Higham *et al.* 2005; Bice and Ye 2007; Wedderburn *et al.* 2007) and it is likely this area represents an important refuge for this species particularly during the current low water levels. Bice and Ye (2007) reported an increase in the abundance of Murray hardyhead at 'outside' or lake habitat sites in 2007 (e.g. Dunn's Lagoon) as opposed to 2006 and 2005, as water levels began to fall and off-channel habitats dried up. This may explain the persistence of the species at Dunn's Lagoon and its appearance at the mouth of the Bremer River, a locality where this species has not previously been recorded.

Southern pygmy perch were found in the greatest numbers (*n* = 13) in a drain on private property on Hindmarsh Island, whilst Yarra pygmy perch were found in greatest numbers (*n* = 12) in Boundary Creek. Boundary Creek has subsequently dried up and the status of the Hindmarsh Island drain is unknown.

4.2 SPAWNING AND RECRUITMENT OF MURRAY HARDYHEAD, SOUTHERN PYGMY PERCH AND YARRA PYGMY PERCH: LENGTH-FREQUENCY DISTRIBUTION ANALYSIS

In summer 2006 and 2007, Murray hardyhead 17-31 mm FL represented the 0+ cohort and constituted *c.* 80% and 50% of the population, with fish 33-40 mm FL representing an older cohort (Bice and Ye 2007). In summer 2008, fish <31 mm FL represented just 6% of the population. Fish of this size were not present in the population in spring 2007 indicating that they are newly recruited to the population; nevertheless, the level of recruitment is very small. Some individuals between 31-38 mm FL may represent 0+ fish, however, this is unlikely given the length distributions for 2007 presented in Bice and Ye (2007), but was not confirmed with otolith analysis.

Murray hardyhead exhibit a protracted spawning season from September through till January in Lakes Cardross and Hawthorn in Victoria (Ellis 2005) and this pattern appeared similar in the Lower Lakes in 2005 and 2006 (Bice and Ye 2007) when length distributions showed no distinct divisions between cohorts. In summer 2007, there was a distinct division between cohorts which may have been a result of a restricted spawning season in 2006/07 (Bice and Ye 2007). This pattern was repeated in summer 2008 although the proportion of the population representing new recruits was very small (<10%).

Previous work in the Lower Lakes has suggested that southern pygmy perch <40 mm TL in summer represent new recruits (Bice and Ye 2006; Bice and Ye 2007), with a division between cohorts typically occurring around this length. In spring 2007 no fish <40 mm TL were recorded but constituted *c.* 50% of the population in summer 2008, signifying that some level of recruitment had occurred. In the summers of 2007, 2006 and 2005 this cohort represented >75% of the population. Thus the recruitment witnessed in 2008 was well below that of previous years.

Similar to southern pygmy perch, Yarra pygmy perch <40 mm TL in summer are considered to be 0+ individuals (Bice and Ye 2006; Bice and Ye 2007). This cohort typically represents >80% of the population during summer (Bice and Ye 2007) but was absent in both spring 2007 and summer 2008. Thus, no spawning and recruitment was detected, although this species was caught in very low numbers.

Fish lengths from all sites and sampling gear types were pooled for length frequency distribution analyses, to provide maximum sample sizes due to the low numbers of threatened species collected in spring 2007 and summer 2008. As such, inconsistent fishing effort between seasons (gear types used) may affect length-frequency distributions. Nevertheless, given the extremely low numbers of fish collected, this analysis is necessary to provide any indication of population size structure.

The continued rapid recession of water levels in the Lower Lakes has most likely affected the spawning and recruitment success of these species. This has resulted in a loss of optimal off/channel and 'drain' habitat and a dramatic decrease of lake edge habitat, and consequently limited access to emergent vegetation. Both pygmy perch species typically spawn in spring (Humphries 1995; Kuiter *et al.* 1996; Bice and Ye 2006) which normally coincides with times of high Lake levels (Lake levels are typically surcharged to compensate for summer evaporative losses), allowing access to newly inundated spawning and nursery habitat. Juvenile Murray hardyhead have also been recorded colonising newly inundated habitats on Hindmarsh Island (Wedderburn *et al.* 2003). These highly vegetated, structurally complex habitats would provide shelter, refuge from predators (Copp 1997; Closs *et al.* 2005) and provide an indirect source of food for larval and juvenile fish (Humphries 1995). The loss of such habitats has likely resulted in the decreased spawning and recruitment success of these species. As short-lived species successive years of poor recruitment represents an extreme threat to these populations.

4.3 SEASONAL VARIATION IN SPECIES COMPOSITION AND RELATIVE ABUNDANCE OF FISH ASSEMBLAGES

The fish assemblages recorded in spring and summer differed significantly. This difference was driven largely by a decrease in abundance of Australian smelt and increases in abundance for most other species, particularly, small-mouthed hardyhead, eastern gambusia, bony herring, lagoon goby, redfin perch, flat-headed gudgeon and common galaxias. Such increases in abundance are likely due to the prevailing low-water level conditions in the Lower Lakes. As Lake levels have receded, off-channel habitats have been lost and the available lake/open water habitat area has decreased significantly, potentially forcing fish to live at greater densities. Most of these species are considered to be habitat generalists (e.g. small-mouthed hardyhead, bony herring and flat-headed gudgeon; Mallen-Cooper 2001), have previously been recorded in high abundances in lake or open water habitats (e.g. common galaxias and redfin perch; Bice and Ye 2006; Bice and Ye 2007) or are considered highly tolerant of adverse environmental conditions (e.g. eastern gambusia; McKinsey and Chapman 1998; Arthington and Marshall 1999; McNeil and Closs 2007) and consequently may possess competitive advantages to other species (e.g. pygmy perch *Spp.*) in these remaining habitats.

4.4 RELATIONSHIPS BETWEEN FISH ASSEMBLAGES AND HABITAT CONDITIONS

Relationships between fish assemblages and environmental parameters in spring and summer were inconsistent. In spring 2007, a combination of the proportion of submerged vegetation cover (%), conductivity (salinity) and pH exhibited relationships with fish assemblages ($\rho_w = 0.642$). Site 19 (mouth of the Bremer River) was separated from all other sites in the MDS ordination and possessed the greatest proportion of submerged vegetation. Along with site 15 (the mouth of the Angas River) this site exhibited the lowest conductivity. The fish assemblages at these two sites were dominated by freshwater species (i.e. flat-headed gudgeon, redfin perch) while estuarine species were rare or absent. The relationship between fish assemblages and pH was unclear.

Fish assemblages from summer exhibited a poor correlation with the measured environmental variables ($\rho_w = 0.418$). A combination of mean depth (m), wetted width (m), dissolved oxygen content (ppm) and the presence of redfin perch best matched the recorded fish assemblages. All four environmental variables appear related to decreased Lake levels but were not investigated any further given the poor correlation.

The lack in strength of correlation between fish assemblages and environmental conditions in the current project when compared to previous work (i.e. Bice and Ye 2006; Bice and Ye 2007) is likely due to the rapidly diminishing habitat quality within the Lower Lakes. There are likely to be various effects of extreme water level recession including increased competition and predation, changes in environmental parameters (i.e. water temperatures, dissolved oxygen content, salinity) and increases in disease transmission due to higher densities of animals in aquatic habitats. Consequently, many direct and indirect factors are likely to be affecting the species composition and abundance of fish assemblages in the Lower Lakes in a synergistic manner.

4.5 CONCLUSION

This study has provided data on the 'condition' of Murray hardyhead, southern pygmy perch and Yarra pygmy perch populations in the Lower Lakes in spring 2007 and summer 2008. This data will provide a basis for comparison upon the advent of an 'intervention' and assist in meeting the targets of; '*improved spawning and recruitment success in the Lower Lakes for endangered fish species, including Murray hardyhead and Pygmy perch'* and to *'maintain and enhance habitat for native fish',* as pertained in the LLCMM EMP.

Murray hardyhead, southern pygmy perch and Yarra pygmy perch were collected in low to very low abundances, within restricted distributions, in spring 2007 and summer 2008, displaying a continued decline from summer 2007 (Bice and Ye 2007). Declines in abundance can be attributed to the rapid decline in habitat area and quality in the Lower Lakes. Similarly, the extreme drought conditions currently being experienced have likely resulted in the diminished recruitment of Murray hardyhead and southern pygmy perch, and potentially, absence of recruitment for Yarra pygmy perch in 2008.

The effect of rising salinities on threatened species is an issue of concern. Murray hardyhead are considered to have a high salinity tolerance (Wedderburn *et al.* 2007) often found in saline wetlands and evaporation basins (Lyon and Ryan 2005), however, the salinity tolerance of southern pygmy perch is unknown, whilst preliminary data suggest Yarra pygmy perch larvae are unable to tolerate salinities greater than 17000 uS/cm (McNeil and Westergaard in prep.). With the current elevated salinity in the Lower Lakes and the likelihood of further increases in salinity, this may represent a severe threat to these populations and should be further investigated.

Forecast conditions mean that the current status of these populations is dire, with a high risk of local extinctions. As short-lived species, consecutive years of poor or no recruitment represents an extreme threat to these populations. At the time of writing the condition of the Lower Lakes is worse than at the time of sampling for this project. Hence, further declines are likely to have occurred.

Ex-situ conservation measures for Yarra pygmy perch (i.e. safeguarding populations and genetic integrity in aquaria) are currently being undertaken by Native Fish SA and SARDI Aquatic Sciences. Whilst these are ongoing the potential for in-situ conservation without inflows into the system is low. However, the possibility that extreme low-water refuge sites for these species do remain in the Lower Lakes should be investigated and potentially management actions undertaken, where possible, to conserve these sites. Secondly, sites for the re-introduction of aquarium held fish should be monitored to assess their suitability upon the re-establishment of favourable Lake levels. A drought management plan for threatened fish species in South Australia is currently being compiled by the Department of Environment and Heritage (DEH) and Primary Industries and Resources South Australia (PIRSA) to assist in these management decisions.

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APPENDIX 1. Presence/absence table for fish sampled at each of the sites in the Lower Lakes region in spring 2007 and summer 2008. Dashes indicate sites not sampled in a given season.

Site No	Year	Murray hardyhead	Southern pygmy perch	Yarra pygmy perch	Tamar River goby	Bridled goby	Western blue-spot goby	Lagoon goby	Small-mouthed hardyhead	Unspecked hardyhead	Common galaxias	Carp gudgeon	Flat-headed gudgeon	Dwarf flat-headed gudgeon	Sandy sprat	Congolli	Australian smelt	Bony herring	Eastern gambusia	Redfin per ch	Carp
1	07	P		P		P	P	P	P	P	P		P	P			P	P		P	P
	08		P	P				P	P		P	P	P					P	P	P	
2	07				P		P	P	P		P		P	P			P	P			
	08						P	P	P		P		P			P	P	P	P	P	
3	07				P		P	P	P		P		P				P				
	08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	07	P			P		P		P		P		P			P	P	P		P	P
	08	P			P		P	P	P		P		P				P	P		P	
5	07				P				P		P		P				P				
	08	P			P	P		P	P		P		P			P	P	P	P	P	P
6	07	P			P			P	P		P		P				P	P		P	
	08				P			P	P		P		P			P	P			P	
7	07	P	P			P	P	P	P		P		P				P	P	P	P	
	08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	07	P	P			P			P		P	P	P	P			P	P	P	P	
	08	P			P	P		P	P	P	P		P				P		P	P	
9	07	P							P		P	P	P				P	P		P	
	08									P	P		P						P	P	
10	07	P					P	P	P	P	P		P	P		P	P	P			
	08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	07	P						P	P	P	P		P	P			P	P			
	08	P				P	P	P	P	P	P		P	P			P	P	P	P	
12	07		P		P		P	P			P	P	P				P	P		P	P
	08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	07				P		P	P	P		P		P		P		P	P		P	
	08						P	P	P	P	P		P	P					P		
14	07				P			P	P	P	P		P	P	P	P	P	P			P
	08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	07		P				P	P	P		P		P	P			P	P	P	P	P
	08								P		P						P	P		P	
16	07	P					P	P		P	P		P				P	P		P	
	08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	07			P			P	P		P	P	P	P	P			P	P	P	P	
	08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	07				P			P	P	P	P		P	P			P	P		P	P
	08						P	P	P		P		P						P		
19	07						P				P								P	P	P
	08	P			P		P	P	P	P	P		P				P	P	P	P	
20	07				P		P	P		P	P		P				P	P		P	P
	08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	08										P	P	P						P		
22	07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	08		P								P	P	P						P		