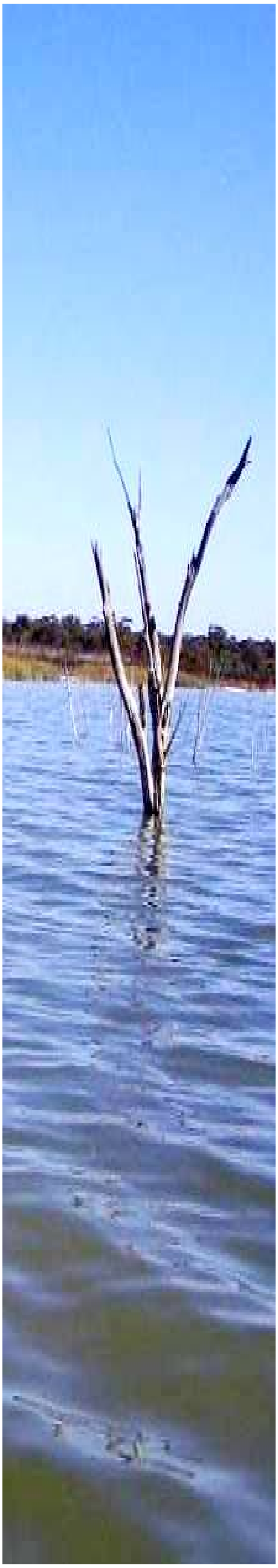
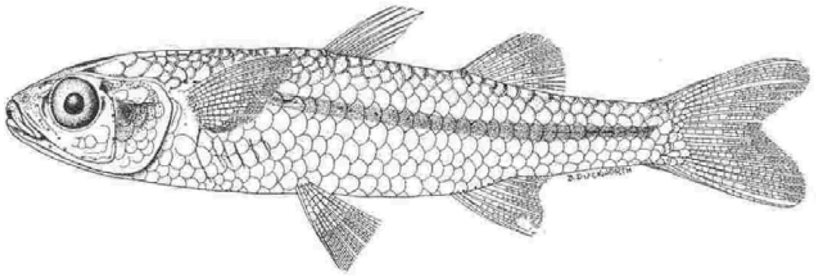


Ecology and breeding seasonality of the Murray hardyhead *Craterocephalus fluviatilis* (McCulloch), Family Atherinidae, in two lakes near Mildura, Victoria.



Prepared for the Mallee Catchment Management Authority.

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MDFRC LBL Report 5/2005

November 2005

The Murray-Darling Freshwater Research Centre

mallee catchment management authority

Ecology and breeding seasonality of the Murray hardyhead *Craterocephalus fluviatilis* (McCulloch), Family Atherinidae, in two lakes near Mildura, Victoria.

A report prepared for the Mallee Catchment Management Authority by the Murray-Darling Freshwater Research Centre.

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Acknowledgements

The project was funded by the Mallee Catchment Management Authority. The author wishes to thank those individuals who contributed information used in this report. These include staff at the Murray Darling Freshwater Research Centre, individuals from Arthur Rylah Institute for Environmental Research (Bill Dixon, Tarmo Raadik, and Jarod Lyon), Scotte Wedderburn (Adelaide University), Brendan Ebner (Environment ACT) and Michael Hammer (Adelaide University). Thanks are also extended to Oliver Scholz, Clayton Sharpe, Shaun Meredith, Scotte Wedderburn and Catherine Hall for reviewing drafts of the literature review and the final report, and MDFRC staff that assisted in field collection for this project. I also thank Shar Ramamurthy from the MCMA for assistance in managing this project. Ethics approval for the project was granted by the Charles Sturt University Animal Care and Ethics Committee, ref. no. 04/044.

Cover Images:

Picture by B. Duckworth, from Crowley and Ivantsoff 1990.

Table of contents.

Executive Summary 4
Introduction 5
Objectives 6

Section 1: Ecology of the Murray hardyhead *Craterocephalus fluviatilis* (McCulloch),Family Atherinidae. 7

1.1 Ecology 7
1.1.1 Species Description 7
1.1.2 Conservation Status 8
1.1.3 Taxonomic status 9
1.1.4 Distribution 11
1.1.5 Habitat 15
1.1.6 Salinity Tolerance 16
1.1.7 Diet 17
1.1.8 Reproduction 17

1.2 Conservation 19
1.2.1 Threats to the Murray hardyhead 19
1.2.2 Key knowledge gaps 23
1.2.3 Recovery Plan 25
1.2.4 Current Research 26

Section 2: Breeding seasonality of the Murray hardyhead within Lakes Hawthorn and Cardross. 27

2.1 Methods 27
2.1.1 Site Descriptions 27
2.1.2 Water Quality 29
2.1.3 Larval Fish 30
2.1.4 Adult Size Class Distribution 30
2.1.5 Reproductive Biology 31

2.2 Results 35
2.2.1 Hydrology 35
2.2.2 Water Quality 37
2.2.3 Larval Abundance 39
2.2.4 Adult Size Class Distribution 40
2.2.5 Reproduction 43

2.3 Discussion 48

2.4 Management Recommendations 53
Future Research 53

References 55

Executive Summary

The Murray hardyhead (*Craterocephalus fluviatilis*) is listed as threatened under the Victorian Flora and Fauna Guarantee Act 1988 and as vulnerable under the Commonwealth EPBC Act (1999). The species is critically endangered in Victoria (DSE 2003) and described as threatened, with a restricted distribution in the Murray-Darling Basin Commission's Native Fish Strategy 2003-2013. As such, there is a statutory requirement to manage the existing habitat of this species.

Within the Mallee Catchment Management Authority (Mallee CMA) region there are only two known Murray hardyhead populations – the Cardross Lakes and Lake Hawthorn near Mildura. Both these waterbodies are used for irrigation drainage disposal, and have experienced declining volumes as a result of improvements in the efficiency of drainage practices and/or altered land use. In the case of Cardross Lakes, management intervention has been necessary since 1996 (through the provision of environmental water from the State's Murray River Environmental Water Allocation (EWA)) to maintain water levels in order to support littoral habitat and reduce salinity levels. This intervention is considered necessary for supporting the Cardross Lakes unique faunal assemblage. In response to increasing salinity in Lake Hawthorn from below 4,000 to over 12,000$\mu S.cm^{-1}$ since 1999, the Mallee CMA successfully bid for a similar EWA for Lake Hawthorn in 2005 which has yet to be supplied to the lake.

The aims of this research were to determine the seasonality of breeding of the Murray hardyhead within Lake Hawthorn and the Cardross Lakes, and produce management guidelines that will help to maintain viable populations of this endangered fish. Our previously poor understanding of the breeding seasonality and requirements of the species had presented a risk that current management practices could contribute to the decline of existing Murray hardyhead populations.

Based on analysis of the last years field data (larval abundance and gonado-somatic Index) it appears Murray hardyhead populations in Lake Hawthorn and Cardross Basin 1 have a prolonged spawning season from September to as late as February. The species is a batch spawner, with females depositing clutches of eggs amongst submerged vegetation several times throughout the breeding season. Murray hardyhead appears to be a largely annual species with the population dominated by 0+ individuals. The number of larger mature adult fish captured declined at the end of the breeding season and through the cooler months, before recruitment through spring and summer contributed to higher abundances the following breeding season. Failed recruitment in a single year may therefore be catastrophic to a population of Murray hardyhead.

This project will continue through 2006 aiming to determine the maximum age of adult Murray hardyhead, and will also investigate the effect of increased salinity on the diet and food source availability in the two Mallee populations. The recent extinction of Murray hardyhead populations from Lake Elizabeth and Golf Course Lake near Kerang highlights the importance of such information to the management of this species.

Introduction

Murray hardyhead (*Craterocephalus fluviatilis*) are small native fish endemic to the lowland floodplains of the Murray and Murrumbidgee river systems. For a variety of reasons Murray hardyhead have suffered a reduction in range whilst remaining locally abundant, and are now thought to be limited to a few populations (NSW Fisheries 2002). Murray hardyhead are currently restricted to several lakes near Mildura, Kerang, Renmark, and the Lower Lakes of the Murray River. Murray hardyhead appear to be largely restricted to the more saline water of deflation basin lakes.

The Murray hardyhead is listed as threatened under the Victorian Flora and Fauna Guarantee Act 1988, and vulnerable under the Commonwealth EPBC Act (1999). The species is critically endangered in Victoria (DSE 2003) and is described as threatened, with a restricted distribution according to the Murray-Darling Basin Commission's Native Fish Strategy 2003-2013. As such, there is a statutory requirement to manage the existing habitat for this species.

Within the Mallee region there are only two known Murray hardyhead populations – Cardross Lakes and Lake Hawthorn. Both these water bodies, which have been and are currently used for irrigation drainage disposal, are experiencing declining water levels as a result of improved drainage practices and/or altered land use. In the case of Cardross Lakes, management intervention has been necessary since 1996 (through the provision of environmental water from the State's Murray River Environmental Water Allocation (EWA)) to maintain water levels, support littoral habitat and reduce salinity levels, considered necessary for supporting its unique faunal assemblage, which includes a Murray hardyhead population. Lake Hawthorn has experienced an increase in salinity since 1999 from below 4,000 to over 12,000$\mu S.cm^{-1}$. In 2005 the Mallee Catchment Management Authority (MCMA) successfully bid for 800 megalitres of water from the EWA for Lake Hawthorn for the same purposes as those described for the Cardross Lakes. This allocation had not been used at the time the report was submitted.

This project was conducted on behalf of the Mallee Catchment Management Authority to determine the seasonality of breeding of the Murray hardyhead within two systems (Cardross Lakes and Lake Hawthorn) near Mildura. We currently have poor understanding of the breeding biology, recruitment and age and structure of Murray hardyhead populations – information important in determining management responses for the conservation of the species. Until we better understand the breeding ecology of this species, there exists a risk that current management practices could contribute to the decline of existing Murray hardyhead populations. This is particularly topical, given the recent disappearance of the species from Lake Elizabeth near Kerang. The results from this project will assist management of existing populations to maximise successful recruitment, by ensuring the full range of environmental conditions likely to trigger successful spawning are covered.

Objectives

This project aims to determine the seasonality of breeding of the Murray Hardyhead within Lakes Hawthorn and Cardross. The timing of the appearance of larvae will be used in conjunction with measurements of the gonadosomatic index of adult fish to determine breeding seasonality. Management guidelines will be produced that will help to maintain viable populations of this endangered fish.

The first section of this report is adapted from a literature review of the Ecology of the Murray hardyhead conducted in 2005 by the author. The second section reports on the technical details and results of research conducted by the Lower Basin Laboratory to determine the seasonality of breeding of the Murray hardyhead within two systems, Cardross Lakes and Lake Hawthorn throughout 2004/2005.

Section 1: Ecology of the Murray hardyhead *Craterocephalus fluviatilis* (McCulloch),Family Atherinidae.

1.1 Ecology

1.1.1 Species Description

The Murray hardyhead (*Craterocephalus fluviatilis*) belongs to the Family Atherinidae, a group consisting of about 170 fish species, most of which are marine or estuarine (Ivantsoff *et al.* 1987). Twenty-six species are found in Australia, with 14 inhabiting primarily freshwater systems (Cadwallander and Backhouse 1983). Freshwater hardyheads evolved from marine ancestors which originally colonised inland saline waters and gradually adapted to freshwater habitats (Allen 2002).

Murray hardyheads (Figure 1) are small and highly mobile schooling fish, known to reach about 76 mm in length (Ellis personal observation, Ivantsoff and Crowley 1996, Ebner and Raadik 2001). They are moderately deep bodied with a small protrusible mouth (jaws project forward as a tube when open) and a single row of small teeth on the anterior part of both jaws (Crowley and Ivantsoff 1990). The colour varies from silver or silvery-green to dark golden dorsally, with a silvery-black (sometimes golden or reddish) mid-lateral stripe running along the body, and a pale abdomen with a silvery iridescent sheen (Crowley and Ivantsoff 1990). The fins may turn orange during the spawning period (Ebner and Raadik 2001).

Figure 1.1. Murray hardyhead (*Craterocephalus fluviatilis*).
Photo: Michael Hammer

The first or anterior dorsal fin in adult fish has 4 to 7 spines (IV–VII), originating behind the tips of the pectoral fins which contain one spine and 11 to 13 rays (I, i, 11–13). The second dorsal fin consists of one spine and 5 to 8 fin rays (I, i, 5-8), and originates above the origin of the anal fin (I, i, 6-9). There is a single large inter-orbital scale on top of the head reaching as far as the anterior margin of the eyes. The species has thin, almost circular, deciduous (easily detached) scales, with a mid lateral count of 31-35, and a transverse count of 10-12 rows (Ivantsoff and Crowley 1996).

1.1.2 Conservation Status

International

Listed as **Endangered** on the International Union for the Conservation of Nature Red List of Threatened Species (criteria A1c; B1+2ab – version 2.3, 1994) (IUCN 2003).
http://www.redlist.org

National

Listed as **Vulnerable** under the Commonwealth *Environment Protection and Biodiversity Conservation Act* 1999 (EPBC Act 2004)
http://www.deh.gov.au/cgi-bin/sprat/public/publicspecies.pl?taxon_id=56791

Considered **Endangered** by the Australian Society for Fish Biology (ASFB 2001)
http://www.asfb.org.au/research/tsp/tfp_mhhead.htm

Victoria

Listed as **Threatened** under the *Flora and Fauna Guarantee Act* 1988.
http://www.dse.vic.gov.au/dse/nrenpa.nsf/FID/-EADA0F1874AF9CF24A2567C1001020A3?OpenDocument

Considered Critically Endangered by the Department of Sustainability and Environment, *Advisory List of Threatened Fauna in Victoria* (DSE 2003).
http://www.dpi.vic.gov.au

New South Wales

Listed as **Endangered** under the New South Wales Department of Primary Industries *Fisheries Management Act* 1994 (DPI 2004).
http://www.fisheries.nsw.gov.au/threatened_species/threatened_species2/content/fn_murray_hardyhead.htm

South Australia

Listed as **Endangered** under the National Parks and Wildlife Act 1972, *Draft Threatened Species Schedules* (IUCN 2003, criteria B1 ab (I,ii,iv)).

1.1.3 Taxonomic status

The Murray hardyhead (*Craterocephalus fluviatilis*) was originally known as the Mitchellian hardyhead, and has historically been confused with a number of species from the same genus that are similar in appearance. These include the Lake Eyre hardyhead (*Craterocephalus eyresii*), the Darling River hardyhead (*Craterocephalus amniculus*) and the flyspecked (or unspecked) hardyhead (*Craterocephalus stercusmuscarum fulvus*).

Although the Murray hardyhead was first described by McCulloch (1913), its status in the genus *Craterocephalus* was reviewed by Ivantsoff (1987), with fish from south eastern Australia originally recognised as *C. fluviatilis* re-classified as two distinct species - *C. stercusmuscarum fulvus*, a southern subspecies of the more northerly occurring flyspecked hardyhead (*C. stercusmuscarum*), and *C. eyresii,* a junior synonym of the Lake Eyre hardyhead (Ivantsoff *et al.* 1987).

In light of electrophoretic and osteological work, Crowley and Ivantsoff (1990) have since revised these classifications again, concluding the junior synonym of *C. eyresii* from the Murray-Darling Basin in fact comprised two genetically separate species, the Murray hardyhead (*C. fluviatilis*) and the Darling River hardyhead (*C. amniculus*). The separation of the Murray-Darling and Lake Eyre drainage basins (pre-Pleistocene, or at least 10,000 years ago) allowed speciation to occur, resulting in *C. fluviatilis* in the lower reaches of the Murray-Darling basin, and *C. amniculus* in the higher reaches of the same basin (Crowley and Ivantsoff 1990).

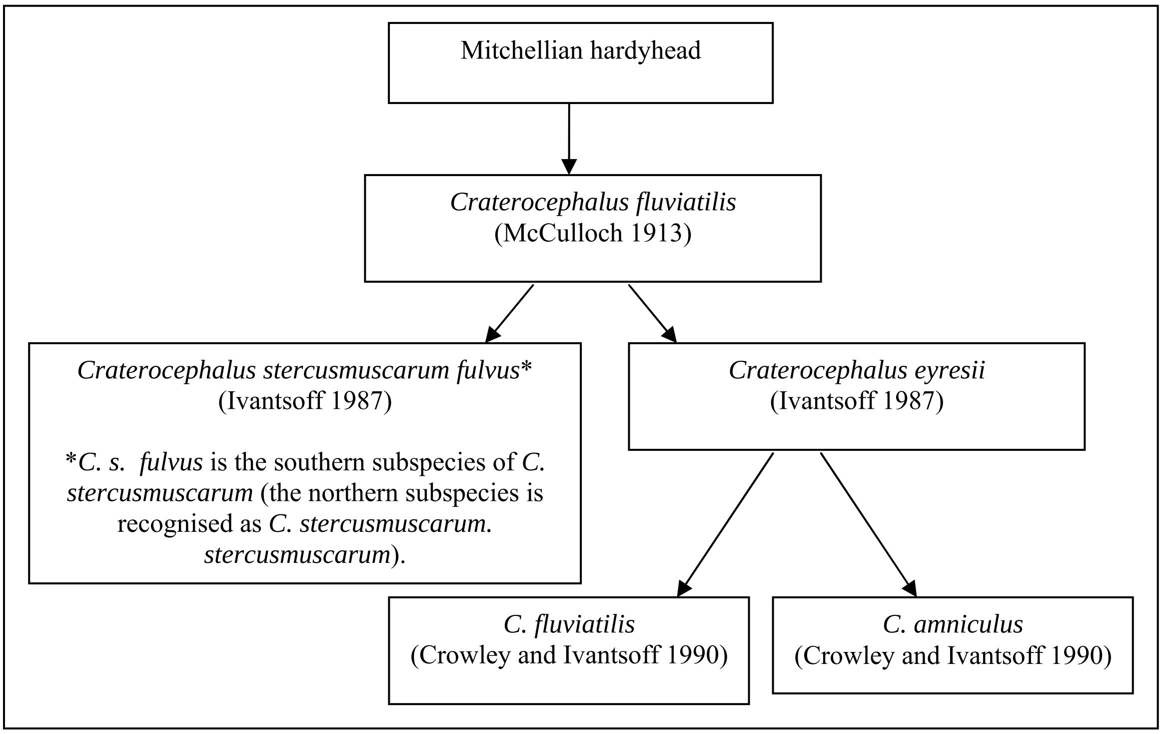

Figure 1.2. Flow chart illustrating the changes in the historical taxonomic classifications of Murray hardyhead.

The Darling River hardyhead (*C. amniculus*) is restricted to the upper tributaries of the Darling River system and is easily distinguished from other hardyheads by its large number (of up to 17) transverse scales (Ivantsoff and Crowley 1996). The Lake Eyre hardyhead (*C. eyresii*), although morphologically very similar to the Murray hardyhead, is endemic to the Lake Eyre Basin and there is no natural range overlap with the Murray hardyhead.

The only other Atherinid species co-inhabiting the Murray-Darling Basin is the small-mouthed hardyhead (*Atherinosoma microstoma*) and the southern form of the flyspecked hardyhead, or unspecked hardyhead *Craterocephalus stercusmuscarum fulvus* (Ivantsoff and Crowley 1996). The small-mouthed hardyhead inhabits coastal waters, and is restricted to the Lower Lakes region of the River Murray in South Australia where it has been recorded alongside both Murray hardyhead and unspecked hardyhead (Wedderburn and Hammer 2003, Ivantsoff and Crowley 1996). The unspecked hardyhead has been historically present throughout the Murray-Darling drainage system (Ivantsoff and Crowley 1996). Although their geographical distributions overlap, the unspecked hardyhead and Murray hardyhead are rarely sympatric (co-existing in the same water body), with Murray hardyhead most commonly found in slightly to highly saline waters, particularly ephemeral deflation basin lakes (Ebner and Raadik 2001).

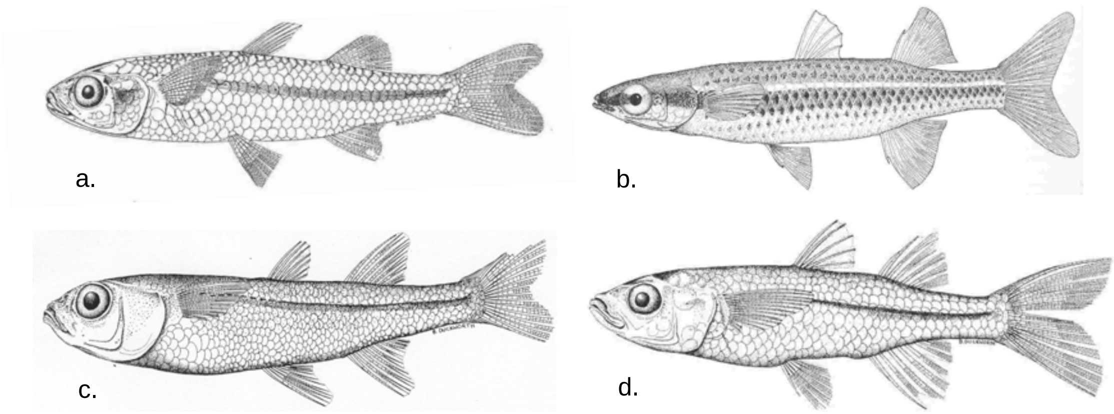

Figure 1.3. Sketches of (a) Murray hardyhead, (b) flyspecked hardyhead, (c) Darling River hardyhead and (d) Lake Eyre hardyhead.
(Sketches a., c. and d. by B. Duckworth from Crowley and Ivantsoff 1990. Sketch b. by B. Pusey from Pusey *et al.* 2004).

Murray and flyspecked hardyhead are distinguished by pigmentation on the lateral scales in the flyspecked hardyhead (giving rise to its name) and the lack of a distinct midlateral line across the snout in the Murray hardyhead (Figure 1.4). Coloration may be less vivid in some flyspecked (or unspecked) hardyhead in southern and inland areas (Pusey *et al.* 2004, Ivantsoff and Crowley 1996), and scales in smaller individuals are often difficult to see. Consequently identification can be problematic and it is recognised that some specimens from Victoria do not key out clearly as either species, especially smaller adults, juvenile and larval specimens (Raadik personal communication 2005).

Murray hardyhead have a transverse series scale count of 9-12 (including 4-8 above the mid lateral band), while flyspecked (unspsecked) hardyhead have 7–8 larger scales in transverse series (Ivantsoff and Crowley 1996). The scale count is made by counting the scale rows from beneath the first dorsal fin (downwards and forwards) to, and including, the either one or two scales on the lateral line. Then the scale rows from just above the pelvic fins, upwards and backwards, to just below the lateral line are counted. On adding these two counts Murray hardyhead should have 9 or more rows, and flyspecked hardyhead should have 7 to 8 rows (Raadik personal communication 2005).

Figure 1.4. Adult Murray hardyhead (a) and flyspecked hardyhead (b) both collected from Lake Hawthorn near Mildura, illustrating the similarity in their appearance. (N.B. The speckling of the southern form of flyspecked hardyhead, or unspecked hardyhead is often absent or less apparent). Photos: I. Ellis.

1.1.4 Distribution

Murray hardyhead are thought to be endemic to the lowland floodplains of the Murray and Murrumbidgee systems, having been collected as far upstream as Yarrawonga, and as far downstream as Lake Alexandrina at the mouth of the Murray (Ebner *et al.* 2003, Ivantsoff and Crowley 1996, Hammer *et al.* 2002). The species appears unique among fish of the Murray-Darling Basin in that it is largely restricted to the saline water of deflation basin lakes (Ebner *et al.* 2003).

The current distribution of Murray hardyhead is unclear due to confusion in identification with other hardyheads (Crowley and Ivantsoff, 1990). Wedderburn and Hammer (2003) stated that several sympatric populations of Murray and unspecked hardyhead are known in South Australia, while Cardross and Hawthorn Lakes near Mildura also contain populations of both Murray and unspecked hardyhead (Ellis, personal observation). Research currently incorporating field surveys and genetic identification of populations, may further clarify distribution of the Murray hardyhead (Wedderburn in prep. Lyon *et al.* in prep).

Although several populations of Murray hardyhead are currently found throughout the Murray-Darling Basin their distribution is patchy (Lloyd and Walker 1986). Once considered widespread and abundant in the Murray and Murrumbidgee river systems in southern NSW and northern Victoria, Murray hardyhead have suffered a reduction in range whilst remaining locally abundant, and is now thought to be limited to a few populations (NSW Fisheries 2002). In many localities from which the species was formally known and still persist, there are now large numbers of introduced carp and gambusia (Ivantsoff and Crowley 1996). Current known populations are outlined below and shown in Figure 3.

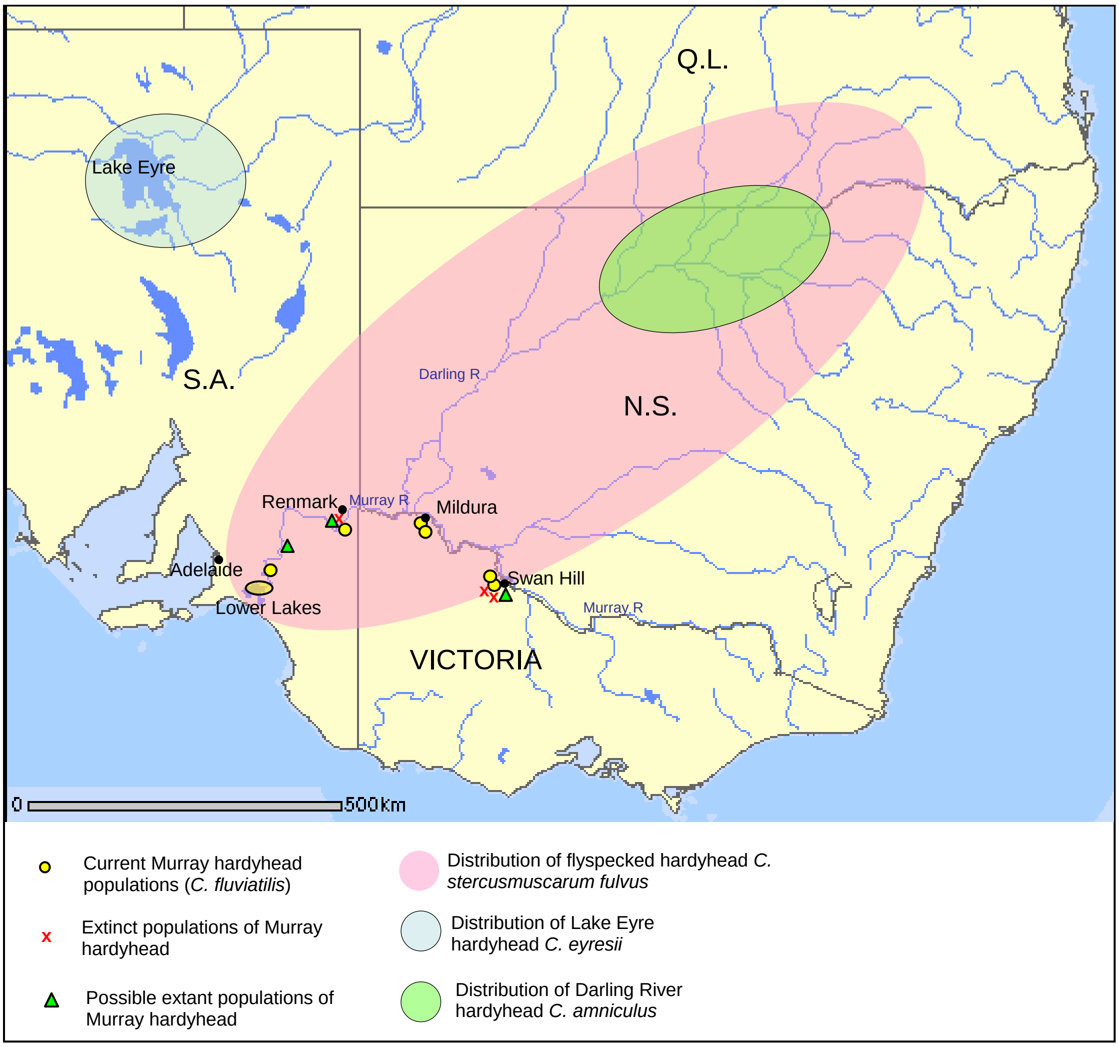

Figure 1.5. Current known and potential distribution of the Murray hardyhead, also showing distribution of Lake Eyre and Darling River hardyheads.(Template image from Department of Environment and Heritage, Environmental reporting tool).

Cardross Lakes and Lake Hawthorn (Mildura region, north-west Victoria)

The Murray hardyhead has been continuously recorded in the Cardross Lakes system (Basins 1, 2 and 3) near Mildura since 1995 (Raadik and Harrington 1996) and more recently in Basin E (Sharpe *et al.* 2003). The Cardross Lakes population is one of the largest known and consequently the Lakes are considered critical habitat for the persistence of the species (Raadik 1996). Murray hardyhead were first recorded in Lake Hawthorn in 1963 (Chessman and Williams 1974), and have been recorded since in surveys by the Murray Darling Freshwater Research Centre (Ebner in prep., Ho *et al.* 2004, Ellis 2005). In both Cardross and Hawthorn, the species co-exists alongside unspecked hardyhead (Raadik personal communication).

Kerang Lakes (Swan Hill area, northern Victoria)

Populations of hardyhead in several lakes near Swan Hill and Kerang were first documented in 1974 by Chessman and Williams, including Lake Elizabeth, Cullen's Lake, Lake Tutchewop and Long Lake. In 1989 Fleming recorded what was considered to be the Lake Eyre hardyhead in Golf Course Lake, North Woorinen Lake and Lake Elizabeth, although none were found in Lake Tutchewop, Cullens or Long Lake due most likely to increases in salinity over the previous 10 years (Lugg *et al.* 1989). The revision of hardyhead taxonomy in 1990 by Crowley and Ivantsoff implies these fish must have been either Murray hardyhead or unspecked hardyhead.

A survey of the fish fauna in the Kerang Lakes area was conducted by McGuckin (1999) after changed irrigation practices had resulted in increased salinities in some of the lakes. This survey found small numbers of Murray hardyhead in Golf Course Lake, and a 'good' population in North Woorinen Lake, suggesting the fish captured in these lakes by Fleming in 1989 may have been Murray hardyhead (McGuckin, 1999). McGuckin (1999) also recorded 'good numbers' of Murray hardyhead in Round Lake.

A later survey by Hardie in 2000 found 'good numbers' of Murray hardyhead in Round Lake and in Lake Elizabeth, but none in Golf Course Lake due most likely to lowered water level and corresponding high salinity. Hardy (2000) also reported that hardyhead had disappeared from Cullens Lake, Lake Tuchewop and Golf Course Lake.

Surveys in the Kerang and Swan Hill region by Arthur Rylah Institute for Environmental Research (ARI) in February and April 2002 have confirmed Murray hardyhead populations survived in North Woorinen and Round Lakes (Lyon *et al.* 2002). These two populations still existed in September 2004, although the lack of vegetative habitat compared to previous surveys in Round Lake was cause for concern (Lyon, personal communication 2005). Golf Course Lake had been completely dry prior to the 2002 survey by ARI, and it was assumed the population had become extinct (Lyon *et al.* 2002).

The 2002 survey by ARI demonstrated that the Lake Elizabeth population had survived and may have successfully recruited for several years in elevated salinities up to 50,000 $\mu S.cm^{-1}$, (Lyon *et al.* 2002). However, the Lake Elizabeth population was not recorded in a September 2004 survey by ARI and is assumed to have become extinct (Lyon, personal communication). There is a suggestion that increasing salinity coupled with a possible acid sulfate event in the lake caused the extinction of the population, with tests currently underway to explore this possibility (Lyon personal communication).

South Australia

Although initially mis-identified as Lake Eyre hardyhead, the Murray hardyhead has for some time been regarded as rare in the River Murray in South Australia, occurring in small populations and either restricted in range or scattered over a broad area (Lloyd and Walker, 1986). Collection in the River Murray south of Renmark and in Lake Bonney prior to 1990 by Crowley and Ivantsoff (1990) failed to yield any specimens, where previously this species had been reported (Lloyd and Walker 1986). Several discreet isolated populations have more recently been reported in River Murray wetlands in South Australia including, Disher Creek Evaporation Basin and Barry Evaporation Basin (near Berri), and Riverglades Wetland near Murray Bridge (Scotte Wedderburn personal communication 2005). A previously recorded population in Lake Littra may have become extinct in recent years due drying of the Lake (Scotte Wedderburn personal communication 2005).

Populations of Murray hardyhead currently exist in the Lower Lakes of the River Murray mouth region, as recorded by Wedderburn and Hammer (2003). Most are located in broad proximity to Hindmarsh Island (particularly in interconnecting drainage channels), with outlier populations scattered nearby (Dunn's Lagoon, Mud Island, Loveday Bay and Jacobs Bight).

New South Wales and Queensland

Previous reports of Murray hardyhead in the northern tributaries of the Darling River (McCulloch 1913) are now considered to have referred to the Darling River hardyhead (Ivantsoff and Crowley 1996). Reports of Murray hardyhead in the Murrumbidgee River today are also considered doubtful (DEH 2005).

1.1.5 Habitat

Murray hardyhead are typically found in lakes, billabongs and still or slow flowing areas of creeks and rivers, usually amongst aquatic vegetation (Cadwallader and Backhouse 1983, Lloyd and Walker 1986, Crowley and Ivantsoff, 1990, Allen *et al.* 2002). The species may exhibit a preference for inland waters with elevated salinities (Cadwallader and Backhouse 1983, Backhouse *et al.* 2004), being most commonly found in slightly saline to highly saline waters in deflation basin lake systems (Ebner and Raadik 2001). In such systems Murray hardyhead inhabits open water, often over shallow sandy flats and deeper, well vegetated areas (Ebner and Raadik 2001).

Murray hardyhead specimens in Cardross and Hawthorn Lakes near Mildura have been observed schooling within distinct size classes (cohorts), with schools of larval/early juvenile fish often observed around sparsely spread stands of emergent Cumbungi. Schools of larger fish are often observed in shallow open water, over sand or mud substrate close to the lake edge. Larger specimens are also often observed in deeper water, although this may be related to the greater density of submerged vegetation (*Ruppia* sp.) several meters from the edge at these deeper sites, into which schools of larger fish flee rapidly when disturbed (Ellis, personal observation). Larger fish in Round Lake near Swan Hill may be associated with patches of *Ruppia* sp. located in deeper water towards the middle of the lake, providing shelter and a foraging source (Lyon personal observation).

Plant species typically associated with Murray hardyhead include the fringing emergent rushes *Typha* sp. and *Juncus* sp. The submerged macrophyte *Ruppia* sp. in particular appears to be a key aquatic plant species in saline lakes where Murray hardyhead occur (Backhouse *et al.* 2004) providing shelter, a food source and a substrate for spawning. Current research into habitat and vegetation association for the Murray hardyhead by Wedderburn (in progress) may provide a better understanding of the specific habitat preferences of the species.

Assessment of the range and relative abundance of small freshwater fish species in the Murray River in South Australia by Lloyd and Walker (1986) found Murray hardyhead (misidentified at the time as Lake Eyre hardyhead) in river-edge and backwater habitats only. In the Lower Lakes of the River Murray in South Australia, the species seems to prefer areas with localised flow-driven disturbance (such as flow driven channel networks on Hindmarsh Island), and was most abundant in areas with dense aquatic vegetation (Wedderburn and Hammer, 2003). Murray hardyhead were also found to be in higher abundances in more saline and open areas, possibly taking advantage of conditions or vacant resources not suitable to other species (Hammer *et al.* 2002).

1.1.6 Salinity Tolerance

Adult Murray hardyhead have a broad salinity tolerance and are found in both fresh and saline waters. Wedderburn and Hammer (2003) collected Murray hardyhead from populations in the Lower Lakes of the River Murray from salinity of 2700µS.cm^{-1}, while specimens were in Riverglades Wetland near Murray Bridge inhabit relatively fresh water with salinity as low as 749µS.cm^{-1} (Wedderburn, personal observation). Murray hardyhead currently inhabit and are recruiting in the Cardross Lakes and Lake Hawthorn near Mildura in salinities of up to 6800µS.cm^{-1} and 12900µS.cm^{-1} respectively (Ellis personal observation).

In 1974 hardyhead were collected by Chessman and Williams in Long Lake near Swan Hill in salinities up to 48000µS.cm^{-1}. At the time they were mistakenly classified as C. *eyresii*, although a survey by McGuckin in 1999 which recorded Murray hardyhead (and no unspecked hardyhead) in Round, Golf Course and Woorinen North Lakes (near Kerang) suggested the individuals recorded earlier by Chessman and Williams were probably Murray hardyhead (McGuckin 1999). In that report McGuckin proposed 67500µS.cm^{-1} as the upper tolerance level for survival of adult Murray hardyhead in Golf Course Lake near Kerang; that being the concentration at which adult specimens were recorded in 1999. It should be noted that only two adult fish were sampled at this high salinity.

Murray hardyhead have been reported in Lake Elizabeth in salinities up to 48000µS.cm^{-1} (Hardy 2000), and adult fish may have survived increases in salinity up to 60000µS.cm^{-1}. However, little is known of the salinity tolerance of eggs and larvae of the Murray hardyhead, and successful recruitment and survival or early life stages at the high levels recorded in Lake Elizabeth is unlikely (Lyon personal communication). A report by Cadwallader and Backhouse (1983) of *C. fluviatilis* captured in salinities up to 156000µS.cm^{-1} actually pertains to the Lake Eyre hardyhead which was mistakenly identified as Murray hardyhead at the time. Consequently the extremely high salinity tolerance level of 156000µS.cm^{-1} cannot be applied to the Murray hardyhead.

Recommended upper limits for salinity in lakes from the Kerang Lakes region where populations of Murray hardyhead could survive have previously been proposed at 25000µS.cm^{-1} (Lugg *et al.* 1989, McGuckin 1999). Lyon *et. al.* (2000) recommended that Woorinen North Lake near Kerang be maintained at 18000µS.cm^{-1} or below during the summer breeding season, while the remainder of the year have an average not higher than 20000µS.cm^{-1} with a maximum of 25000µS.cm^{-1}.

More recently, Dixon *et al* (2005) investigated the salinity tolerance of Murray hardhead from Lake Elizabeth, Round Lake and Woorinen North Lake (near Kerang). This study found that the salinity tolerance of the fish was *not independent* of the population or lake salinity regime, meaning that the different populations have different sensitivities to salinity. These authors found that there was no specific '*species tolerance*' to salinity, and that it was possible that historical regimes were having an effect on the populations even though these regimes had been survived. Furthermore, the recent decline and possible loss of the Lake Elizabeth population indicates that long term survival and recruitment of the species is not possible at levels of salinity that may be acutely toxic (Dixon, personal communication). At present there is no information on the chronic effects of elevated salinity on Murray hardyhead (such as the effect on reproduction or recruitment), and the short life span of adults means that failure to recruit in any given year can lead to catastrophic population effects.

1.1.7 Diet

The diet of the Murray hardyhead has not been rigorously investigated though is known to consist primarily of micro-crustaceans (Cadwallader and Backhouse 1983, Hardie 2000, Ebner in prep.). Allen *et al.* (2002) described the species as omnivorous, feeding on algae, aquatic insects and micro-crustacea. No information on the diet of larvae is available, although larvae of other *Craterocephalus* species have been recorded feeding on rotifers and micro-crustaceans. Researchers from MDFRC will investigate the diet of the Cardross Lake and Lake Hawthorn populations in 2005-06.

1.1.8 Reproduction

Murray hardyhead lay eggs with adhesive filaments amongst aquatic vegetation (Ivantsoff and Crowley 1996). The aquatic macrophyte *Ruppia* sp. appears to be particularly suitable for the deposition of eggs, with adults in spawning condition captured in stands of the plant, and eggs (presumed to be Murray hardyhead) removed from *Ruppia* collected in Lake Hawthorn (Ellis personal observation). Section 2 of this document investigates and describes the breeding season of the species. Adult fish may display orange fins briefly when very ripe or spawning (Ebner and Raadik, 2001, Ellis personal observation). The stimulus for spawning is unknown but may like most fish corresponds with increasing water temperature and photoperiod. The account of the spawning and development of Murray hardyhead by Llewellyn in 1979 actually refers to the flyspecked (unspecked) hardyhead.

Adult male flyspecked hardyhead have been observed nudging and nipping females, adjacent to aquatic macrophytes before sperm and eggs are simultaneously shed into the macrophyte, all the while touching along their lateral lines (Pusey *et al.* 2004). Murray hardyhead may perform a similar ritual amongst macrophyte beds whilst spawning. Length at maturity is around 25 to 30mm standard length and may be reached within 4-5 months for fish spawned early in the breeding season (see results in Section 2 of this report). For comparison, hatching takes 4 to 10 days after fertilisation in flyspecked hardyhead in waters between 25 and 29°C (Pusey *et al.* 2004).

In the Cardross Lakes the species appears to have a prolonged spawning season from September to April/May (Sharpe *et al.* 2003) and adults with mature gonads have been observed from October to February. Small individuals have been observed in the Cardross Lakes and Lake Hawthorn in summer and winter (Ebner 2003, Raadik and Fairbrother 1999, Raadik and O’Connor 1996).

Populations appear to fluctuate in abundance both within a year, and between years. Raadik and Fairbrother (1999) noted that in Cardross Lakes the species can be plentiful during summer to autumn, with the population appearing to decline over the cooler month. In an October 1996 survey of Cardross Lakes near Mildura, it was noticed the abundance of Murray hardyhead was much lower then the previous February's survey, (at which time large schools had been visible). No schools were noticed in the October survey, although there was still a size range of fish present indicating a range of age classes within the population, with some individuals running ripe (Raadik and O'Connor 1996).

Several more surveys conducted in the following years confirmed the presence of a range of size classes, the authors noting that the species was often plentiful during summer to autumn, declining over the cooler months, with low abundances observed from spring to early summer (Raadik and Fairbrother 1999). The continued presence of small, most likely aged 0+ fish, suggested that Murray hardyhead have a prolonged spawning season from late spring to mid-autumn, or April-May, in water temperatures above 15°C (Raadik and Fairbrother 1999). Section 2 of this report describes more thorough investigation of breeding seasonality of the species.

Small individuals (11-17 mm standard length) captured in Cardross Lakes in April 2003 were approximately 48 – 61 days old, implying reproduction occurred in late January/early February (Sharpe *et al.* 2003). A second cohort of slightly larger individuals captured at the same time (18 – 28mm standard length) was thought to be spawned several months earlier (September/October 2002). This second cohort of 0+ fish dominated the population several months later (February/March) and larger 1+ fish had declined in abundance, causing a contraction of average size in the population (Sharpe *et al.* 2003). Consequently Murray hardyhead are considered a largely annual species having a lifespan of approximately 1 year, with populations dominated by 0+ fish (Sharpe *et al.* 2003). Upcoming research by the MDFRC will attempt to verify the maximum age of the species.

It should be noted, however, that the larvae and small fish recorded in autumn/winter in previous surveys of Lakes Hawthorn and Cardross could be unspecked hardyhead, given the co-habitation of the two species in these lakes. As mentioned earlier, there is considerable difficulty in distinguishing between small and larval fish of the two species. Section 2 of this report describes more thorough investigation of trends in size class frequency of the species

The species is a batch spawner, with eggs at various stages of development apparent in mature ovaries during the breeding season (Ellis personal observation). It has previously been unclear whether the species spawns a discreet number of times during the spawning period or are serial spawners, spawning as many times as conditions allow (Raadik and Fairbrother 1999). Section 2 of this report discusses more precisely the results of investigation into the breeding biology of Murray hardyhead, verifying the species is a batch spawner.

1.2 Conservation

1.2.1 Threats to the Murray hardyhead

The following section has been derived largely from in the Draft Recovery Plan for Murray hardyhead 2005 – 2009 (Backhouse *et al.* 2004).

Salinity increases.

Salt diversion into lowland lakes occupied by Murray hardyhead threatens several Victorian populations (Ebner 2003), including those near Swan Hill (Kerang Lakes) and Mildura (Cardross Lakes and Lake Hawthorn). Improved irrigation practices (both on farm and through water delivery) have led to a decrease in the supply of water to some basins, which has in turn led to increasing salinity and a reduction in surface area and appropriate habitat (Raadik and Harrington 1996, Lyon *et al.* 2002). Without intervention, salinity in some basins could increase to lethal levels, despite the relatively high tolerance of the species for saline conditions (Backhouse *et al.* 2004).

Reduced water levels

Reduced water levels in wetlands such as those occupied by Murray hardyhead near Mildura and Swan Hill not only impact on the fish communities through increases in salinity, but also through a loss of habitat. Even small reductions in water depth can expose significant areas of submerged macrophytes and woody debris that may be vital substrate for the recruitment of the species. Drops in water level could also increase intra- and inter-specific competition for resources already present in the lake.

Some non-riverine lakes inhabited by Murray hardyhead have for some time been used as deflation basins for excess water run-off from irrigated land, with water levels kept higher than would naturally occur. As mentioned above, in some cases, there has been a decrease in disposal water due to increased efficiency in water use by irrigators, which may pose a threat to the long-term viability of Murray hardyhead populations in these lakes (Backhouse *et al.* 2004). For example, in February 2002 dropping water levels in Woorinen North Lake led to 30% of submerged vegetation habitat becoming unavailable to hardyhead. A further drop in water level by 0.2 m would have at the time have increased this figure for unusable habitat to 60% (Lyon et al. 2002).

In the case of the Cardross Lakes, management intervention has been necessary since 1996 (through the provision of environmental water from the State's Murray River Environmental Water Allocation) to maintain water levels, supporting littoral habitat and reducing salinity levels to support its unique faunal assemblage which includes the Murray hardyhead population. In 2005 a similar allocation was successfully acquired for Lake Hawthorn due to recent escalation of salinity and isolation of significant areas of macrophytes.

River regulation

Removed or altered connectivity between floodplain lakes and the Murray River may pose a significant threat to the species (Ebner 2003). Reduced and altered flooding and run-off can lead to the drying out of lakes, facilitate evaporative saline increases, and create barriers to migration and gene mixing between isolated populations of Murray hardyhead (Backhouse *et al.* 2004). Spinal deformities of fish observed in Cardross Lakes (Ebner and Raadik 2001) and Lake Hawthorn (Ellis personal observation) may be a symptom of genetic isolation.

Barriers to movement

Although Murray hardyhead are able to complete its life cycle in isolated lakes, barriers to migration may restrict re-colonisation after extinction of local populations, (Backhouse *et al.* 2004), such as those recently lost from Lake Elizabeth and Golf Course Lake. Installation of regulators and more water efficient irrigation systems such as closed pipelines, may also reduce dispersal of the species which may have formerly utilised older systems such as open irrigation channels (Backhouse *et al.* 2004), or the flow through drain networks of Hindmarsh Island in the River Murray Lower Lakes of South Australia (Wedderburn and Hammer 2003).

Alien species

Alien fish pose a threat to native fish species and their habitats in the Murray-Darling River system, through predation, competition, disease transmission and other effects such as habitat degradation (MDBC 2003 – Native Fish Strategy 2004). The precise impact of alien fish on Murray hardyhead is not known, but its small size, pelagic habit and requirement for aquatic vegetation in which to spawn renders it susceptible to predation and habitat degradation (Backhouse *et al.* 2004).

In many of the former localities where the Murray hardyhead was known, there are now large numbers of carp (*Cyprinus carpio*) and gambusia (*Gambusia holbrooki*) (Ivantsoff and Crowley 1996). These two species currently occur alongside Murray hardyhead in many of its remaining localities including the Cardross Lakes and Lake Hawthorn (Ellis personal observation), Lake Albert and the Hindmarsh Island area of the Murray River Lower Lakes (Wedderburn and Hammer 2003).

Gambusia is currently the only other fish species occurring alongside Murray hardyhead in North Woorinen Lake near Swan Hill (Lyon personal communication). Goldfish (*Carassius auratus)* have been recorded coexisting with Murray hardyhead in Cardross Basin 1 and 3 (Raadik and Harrington 1996, Raadik and O’Conner 2996, Raadik *et al.* 1999, Raadik 2000, Raadik *et al.* 2001, Sharpe *et al.* 2003), while goldfish and redfin perch (*Perca fluviatilis*) have been recorded in the Hindmarsh Island area of the Lower Lakes alongside Murray hardyhead (Wedderburn and Hammer 2003).

Stocking of predators

As its small size makes it susceptible to predation, the Murray hardyhead is potentially at risk from stocking of larger native species such as Murray cod (*Maccullochella peelii peelii*) and golden perch (*Macquaria ambigua*) for recreational angling (Backhouse *et al.* 2004).

Acid sulphates sediments

Acid sulfate sediments are a potential cause for the local extinction of Murray hardyhead populations in recent years in several saline lakes in northern Victoria. Recent investigations into the formation of sulfidic materials in sediment, such as pyrite (FeS_2) and monosulfides (FeS) have highlighted the potential for their development in inland saline waters. When oxidised, elevated levels of sulfidic materials can cause a number of water quality problems, such as acid formation (lowered pH) and low dissolved oxygen levels. This has the potential to cause serious harm to aquatic organisms, as was observed at Bottlebend Lagoon (NSW) in 2002, where a large fish kill occurred, cause by acid sulfate sediments. Drying of the large areas of sediment in the highly salinised wetland (up to 33,100 µS.cm-1) is thought to have led to the oxygenation of reduced sulphur compounds and release of hydrogen ions that acidified the surface water upon later inundation (McCarthy *et al.* 2003)

Although it is not known if extant Murray hardyhead habitats contain sulfidic materials, preliminary studies indicate are that at least some populations occurring in saline lakes may be at risk from this phenomenon. The extinction of the Lake Elizabeth population may have been due to such an event, with pH recorded as low as 4.83 in September 2004 during a survey which failed to capture any Murray hardyhead (Lyon personal communication). Tests are currently underway to determine if an acidification event did occur, contributing to the loss of Murray hardyhead (Lyon personal communication).

Dissolved Oxygen

Dissolved oxygen depletion can result from a variety of factors. Throughout diurnal cycles, all aerobic organisms consume oxygen through respiration. During the daytime, algae and aquatic plants contribute dissolved oxygen to the system during photosynthesis. The night-time combination of oxygen depletion through respiration and reduced photosynthetic re-oxygenation means that dissolved oxygen concentrations can fall, and are often lowest at dawn.

Microbial breakdown of organic matter can also lead to oxygen depletion. In some locations inhabited by Murray hardyhead such as Cardross Lakes and Woorinen North Lake, the high density of aquatic macrophytes, most notable *Ruppia* sp., could pose a threat to the species through reduction in dissolved oxygen as decaying plant matter breaks down, and the plants themselves utilise dissolved oxygen over night (Lyon personal communication 2005). This type of oxygen depletion would be unlikely without extraneous circumstances such as flash flooding of the surrounding floodplain or algal blooms, but deserves consideration.

Sedimentation

Increased sedimentation from soil erosion, agricultural run-off and riparian or forest clearing can block channels, divert flows and reduce the depth of wetlands, thus reducing available habitat for many freshwater fish species (Backhouse *et al.* 2004). Sedimentation can create barriers to migration and reduce or eliminate gene mixing between isolated populations, and may also disrupt reproductive processes by rendering substrate unfit for egg deposition and by blocking development through settling on eggs and reducing larval feeding efficiency (Backhouse *et al.* 2004). The elevated salinities (apparent many of the locations inhabited by Murray hardyheads) can potentially create a flocculating effect on suspended particles, resulting in reduced turbidity of the water (Grace *et al.* 1985). This lower turbidity may be one factor in the Murray hardyheads apparent preference for saline water bodies (Ellis personal observation). High salinity may also contribute to a lower abundance of sediment disturbing fish species such as carp. Additionally, saline tolerant macrophytes like *Ruppia* sp. may stabilise the bottom sediment in many hardyhead inhabited localities, also contributing to reduced turbidity. If increases in sedimentation also lead to an increase in turbidity, the feeding capabilities of visual pelagic feeding species such as Murray hardyhead may be impaired.

Nutrient levels

Nutrient enrichment (primarily nitrogen and phosphorus) from urban and agricultural run-off or from biological source such as large numbers of waterbirds, can lead to excessive growth of phytoplankton (algal blooms). Algal blooms can interfere with light transmission at the air-water interface which in turn can lead to macrophyte decline. Large algal blooms have high night time respiration rates, and can significantly reduce dissolved oxygen in the water column to concentrations stressful and even lethal to many fish species (Codd 1995). Round Lake near Swan Hill supports a population of Murray hardyhead, however the overall condition of the waterbody for fish is considered moderate to poor, due to the combination of low water level, high turbidity and suspected high nutrient levels (Lyon *et al.* 2002).

Environmental contaminants

Environmental contaminants such as petrochemicals and pesticides are utilised in urban and agricultural areas, and can accumulate in terminal lakes through storm water run-off, spray drift and transport through the irrigation system (Backouse *et al.* 2005). Even low concentrations of many agro-chemicals can have an effect on aquatic organisms (Wightwick personal communication). Many of the lakes currently supporting Murray hardyhead populations are at risk of contamination from such sources given their proximity to farmland and the use of the wetlands for the disposal of excess water run-off from irrigated land. Spinal deformities have been observed in Murray hardyheads in Lake Hawthorn and Cardross Lakes (Ebner and Raadik 2001, Ellis personal observation), the cause of which is not known, but may be due to an environmental contaminant (Backhouse *et al.* 2005).

1.2.2 Key knowledge gaps

Reproductive biology

Until recently there has been little specific information on the breeding biology, recruitment, or spawning requirements of the Murray hardyhead. Section 2 of this report will discuss recent investigations into the timing of the species breeding season in Cardross Lakes and Lake Hawthorn. This information is key to determining population trends and ultimately to the preservation and ongoing viability of other populations, and will be important in determining management strategies for the conservation of the species. It is necessary to determining spawning requirements is necessary to ensure suitable conditions are available (or can be created) in lakes supporting isolated populations, and also at potential reintroduction sites to maximise chances of successful population establishment and recruitment (Backhouse *et al.* 2004).

There has also been limited information on age at length, maximum age and fecundity (Raadik and Fairbrother 1999). Sharpe *et al.* (2003) made a preliminary assessment of age at length for the population in the Cardross lakes through otolith examination. Small individuals of 11-17mm standard length were determined to be approximately 48 – 61 days old. Raadik and Fairbrother (1999) reported large gonads and assumed reproductive capacity for most individuals larger than approximately 45mm in Cardross Lakes, although minimum size at maturity was not validated. Section 2 of this report outlines preliminary investigation by the MDFRC into fecundity and minimum size at maturity, and suggests minimum age at maturity. Age at length information for larger fish does not currently exist, and will be explored by the MDFRC during 2006.

Salinity tolerance of early life stages

While adult Murray hardyhead can tolerate low to quite high salinity, early life stages, particular eggs and larvae, may be more sensitive to high salinity levels. Currently researchers at Arthur Rylah Institute for Environmental Research are investigating salinity tolerance of various life stages of Murray hardyhead as part of their assessment of the biological risks of increasing salinity in Victoria (Dixon personal communication). Results demonstrate that salinity tolerances vary between different populations of Murray hardyhead, and consequently tolerance limits for one population should not be used as a management guide for maintaining salinities in the habitat of other populations (Dixon *et al.* 2005). Tolerance levels therefore need to be determined for individual populations to be able to develop management recommendations for managing salinity levels in isolated water bodies where the species occurs (Backhouse *et al.* 2004).

Confusion in identification

The southern subspecies of flyspecked or unspecked hardyhead, (*C. s. fulvus*) is often less vividly coloured then the more northern *C. s. stercusmuscarum*, (Pusey *et al.* 2004, Ivantsoff and Crowley 1996) with the lateral speckling pattern absent or less apparent (resulting in the alternative name of unspecked hardyhead). Murray hardyhead (especially larval, juvenile and small adult specimens) often resemble specimens of the unspecked hardyhead, with which it overlaps in range (see Figure 4). The two species occur together in the Cardross Lakes and in Lake Hawthorn (Ellis personal observation 2005) and in the Lower Lakes area of the River Murray in South Australia (Wedderburn and Hammer 2003). An understanding of the biology of the Murray hardyhead within these systems, including spawning behaviour/requirements, food sources and habitat use, will remain incomplete until genetic scrutiny of hardyhead populations in each locality is made. Currently, an examination of the distribution of the species involving genetic analysis of fish from each of its current locations is in progress (Wedderburn in prep.).

Habitat requirements

As the Murray hardyhead is found in both lakes and rivers, it is likely to occur in areas with different habitat characteristics. Identification of those characters that could provide important habitat, especially for breeding and recruitment, is important for subsequent use in environmental restoration programs such as provision of environmental water allocations and riparian revegetation projects (Backhouse *et al.* 2004).

It is also likely there are distinct habitat preferences for different size ranges of Murray hardyhead, which also need to be considered for the preservation of existing populations, and site selection for re-establishment of extinct populations. For example, fish in Cardross and Hawthorn Lakes appear to school within distinct size classes (Ellis personal observation). Schools of larval/early juvenile fish are often observed amongst stands of emergent Cumbungi (*Typha* sp.) on the fringes of the lakes. Small adults are often observed schooling in this Cumbungi, but also in shallow open water over sandy or muddy substrate closer to the lake edge where Cumbungi beds are often absent. Larger specimens are observed in both habitats, but appear more often to be associated with deeper water containing denser vegetation (thickets of *Ruppia* sp.). This preference for deeper well vegetated water is most obvious on hot still days with direct sunlight and little cloud cover, and could indicate an avoidance of heat, direct sunlight or predators by larger individuals (Ellis personal observation). Larger fish in Round Lake near Swan Hill have also been observed displaying a possible association with patches of *Ruppia* sp. located in deeper water in the middle of the lake (Lyon, personal observation)

Response to extremes in hydrology

Response of populations of Murray hardyhead to the wetting and drying regimes in lakes needs investigating, as several populations exist in regions of extensive irrigation development where large variations in water level may result (Ebner and Raadik 2001).

Specific diet

The Murray hardyhead is omnivorous, feeding on algae, insects and micro-crustacea (Allen *et al.* 2002, Ebner in prep.). Examination of the specific food sources available to each surviving population would assist in our understanding of the requirements necessary for the ongoing viability of each population. This information is especially important for consideration of the re-establishment of extinct populations outlined in the Draft Recovery Plan for Murray hardyhead 2005-2009 (Backhouse *et al.* 2004) to ensure re-established fish have a continued and abundant food source. The MDFRC will examine temporal variation in diet and food sources of the Cardross Lakes and Lake Hawthorn populations in 2006.

1.2.3 Recovery Plan

A strategy for the conservation and recovery of the Murray hardyhead is currently being formulated by Backhouse *et al.* (2004), focusing on the protection and management of locations where the species currently occurs, investigation of key biological and ecological attributes, and determination and distribution of critical habitat.

As described in the draft recovery plan for Murray hardyhead (Backhouse *et al.* 2004) the overall objective of recovery is to minimise the probability of extinction of the Murray hardyhead in the wild, and to increase the probability of important populations becoming self-sustaining in the long term. Within the life span of this Recovery Plan (2005-2009), the specific objectives of recovery of the Murray hardyhead are to:

1. Identify important populations of Murray hardyhead.
2. Identify critical, common and potential habitat.
3. Investigate important life history attributes to acquire targeted information for management.
4. Ensure that all populations and their habitat are protected.
5. Investigate and manage threats to populations and habitats.
6. Investigate the potential for reintroduction of Murray hardyhead to the wild.
7. Increase awareness of Murray hardyhead conservation with resource managers and the public.
8. Manage Recovery Plan implementation.

1.2.4 Current Research

Research projects are currently underway addressing several of the objectives above. This report examines the seasonality of breeding of the species within two systems (Cardross Lakes and Lake Hawthorn near Mildura) providing management recommendations to maximise spawning and recruitment success. Scotte Wedderburn from Adelaide University is conducting a PhD examining the distribution of Murray hardyhead and its associated fish assemblages and habitat, and will also examine the genetic structure of Murray hardyhead throughout its current range.

Researchers from the Arthur Rylah Institute for Environmental Research (ARI) are currently assessing the salinity tolerance of small adult and juvenile Murray hardyhead from lakes in the Kerang region as part of their assessment of the biological risks of increasing salinity in Victoria. ARI are also attempting to validate age at length of the same fish populations and are monitoring Lake Elizabeth to confirm the extinction of the Murray hardyhead population within the lake. An ongoing project by the Murray Darling Freshwater Research Centre (MDFRC), will investigate food source availability and diet of Murray hardyhead populations in Cardross Lakes and Lake Hawthorn near Mildura in 2006 to determine whether elevated salinities are limiting food availability within Lake Hawthorn. The project will also investigate maximum age and length at age, to determine whether a proportion of the population survive more then one year, thus contributing to consecutive breeding seasons.

Section 2: Breeding seasonality of the Murray hardyhead within Lakes Hawthorn and Cardross.

This project aims to produce management guidelines that will help to maintain viable populations of Murray hardyhead by conducting intensive seasonal field surveys targeting a variety of life stages. The timing of the appearance of larvae will be used in conjunction with measurements of the gonadosomatic index of adult fish to determine breeding seasonality, and infer key information regarding the size class distribution and population dynamics of the species.

2.1 Methods

2.1.1 Site Descriptions

The project studied populations of Murray hardyhead from two locations, the Cardross Lakes and Lake Hawthorn, both near Mildura in north west Victoria. Four sites were chosen within each of Cardross Basin 1 and Lake Hawthorn, incorporating a variety of habitats including open water, woody debris, submerged vegetation (predominantly *Ruppia* sp.) and emergent vegetation (*Juncus* sp. and *Typha* sp.). Sampling was conducted on a three-weekly basis to ensure spawning events were not missed (it is unlikely a spawning event could occur with larval fish maturing to juvenile stages within a single three week period).

Cardross Lakes

The Cardross Lakes (Figure 2.1) are a series of inter-connected drainage basins created from excess and sub-surface irrigation drainage water from the Sunraysia district irrigation industry. The main lakes of the system (Basins 1, 3, E, D and C) host one of Victoria's most diverse assemblage of freshwater fish (Raadik 2001). This assemblage consists of up to 14 species including ten native (Murray hardyhead, Australian smelt, carp gudgeon, flathead gudgeon, bony bream, catfish, golden perch, Murray cod, unspecked hardyhead and southern purple-spotted gudgeon) and four introduced species (carp, gambusia, goldfish and redfin). Five of the native species are listed at the state and two at the federal level, although one species (the southern purple-spotted gudgeon) is now considered extinct from the system (Raadik 2001, Ryan *et al*. 2003). The Murray hardyhead has been recorded in the Cardross Lakes system (Basins 1, 2 and 3) near Mildura since 1995 (Raadik and Harrington 1996) and more recently in Basin E (Sharpe *et al.* 2003). The Cardross lakes populations are considered one of the largest known and consequently the Cardross Lakes are considered critical habitat for the persistence of Murray hardyhead (Raadik 1996).

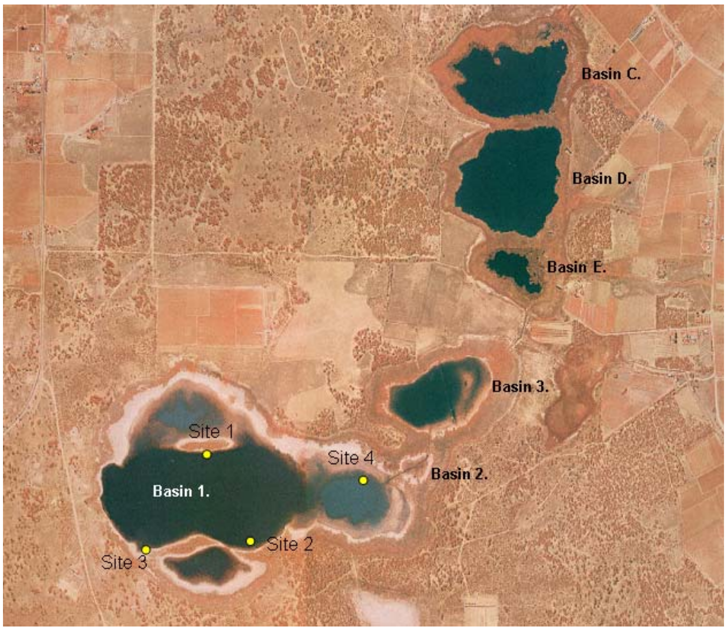

Figure 2.1. Aerial view of the Cardross Lakes with sampling sites in Basin 1.

This survey concentrated on the largest and terminal basin in the main system (Basin 1, shown in Figure 2.1). Of the four sites selected at which to collect both larval and adult fish, three correspond closely to Sites 1, 2 and 3 used by Sharpe *et al.*(2003). The fourth site was selected close to the inlet channel of Basin 1, where water enters the lake after passing through Basin 2 (Figure 2.1).

Lake Hawthorn

Lake Hawthorn (Figure 2.2) is also an irrigation drainage basin managed by Goulburn Murray Water, although its location close to the Murray River and the existence of an interconnecting channel indicates it would have naturally received inflows directly from the river during periods of high flow. Levee banks currently prevent connection of the lake to the river channel and irrigation drainage inflows to Lake Hawthorn enter through pipes located on the eastern perimeter of the lake. Stormwater outfalls are currently under construction on the north east perimeter of the lake. Lake Hawthorn is located adjacent to Lake Ranfurly (a super-saline drainage disposal basin), being separated by a levee and culvert across the inter-connecting channel.

The western and southern perimeters of Lake Hawthorn are utilised by both old and new housing developments, with irrigated horticulture developments operating on the northern edge of the lake. The north-east and eastern perimeter have been grazed for some time, and the Lake is also used by recreational water sports clubs including sailing and water skiing.

Murray hardyhead were first recorded in Lake Hawthorn in 1963 (Chessman and Williams 1974). In a survey by the Murray-Darling Freshwater Research Centre (Ho *et al.* 2004), only 4 native fish species (Murray hardyhead, bony bream, flathead gudgeon and carp gudgeon sp.) were recorded in Lake Hawthorn, along with 2 introduced species (carp and gambusia). Unspecked hardyhead are also present in Lake Hawthorn (Ellis personal observation) and may have been overlooked in the

2004 survey through confusion in identification. Three species of turtle were also recorded in the same survey, including the Victorian Flora and Fauna Guarantee-listed listed Broad Shelled Turtle, Murray River Turtle and Eastern Long-necked Turtle). Four sites were chosen throughout the lake incorporating a variety of habitats. As with Cardross Basin 1, Site 4 was located near the natural inlet channel to the lake (Figure 2.2).

Figure 2.2. Aerial view of Lake Hawthorn with sample sites shown.

2.1.2 Water Quality

Water quality was measured at each site within Cardross Basin 1 and Lake Hawthorn every three weeks, beginning the week of September 20, 2004, through until the week of October 3, 2005. Electrical conductivity standardized to 25 ºC ($\mu S.cm^{-1}$), pH, dissolved oxygen ($mg\ l^{-1}$), turbidity (NTU) and temperature (ºC) were determined *in situ* at a depth of 0.25 m below the water surface using a U-10 multi-probe (HORIBA Ltd., Australia). These parameters were monitored through out the year due as changes may indicate the presence of relevant threats to the Murray hardyhead (listed in Section1.2.1), as well as upcoming spawning events. Salinity may be a direct threat to certain life history stages of the species, drops in pH could indicate an acid sulfate event, low dissolved oxygen levels can cause fish kills and increases in turbidity can be used to indicate sedimentation, which may reduce recruitment and foraging efficiency of the species. Changes in temperature may be used as cues for development and spawning by the species.

In Lake Hawthorn, water quality was only measured at sites 1, 2 and 3 for the first 12 sampling events, after which time the fourth site near the natural inlet channel was also included. In Cardross Basin 1, water quality was only recorded at sites 1, 2 and 3 on the first, third, sixth and eighth sampling occasions with Site 4 also recorded on other sampling occasions.

2.1.3 Larval Fish

Modified quatrefoil light traps were constructed from perspex and steel, with a removable 250µm mesh sieve attached such that larvae could be easily retrieved. Light traps were set during the afternoon by placing a yellow Cyalume light stick in the core perspex tube of each light trap. Three traps were placed on the lake bed amongst woody debris, emergent and submerged vegetation in the littoral zone of each of Sites 1, 2 and 3 in both Cardross Basin 1 and Lake Hawthorn. Traps were left overnight and harvested in the morning. To ameliorate the effect of predation on larvae within light traps, 3mm mesh was wrapped around the light traps to limit the size of the fish entering the traps. However, the mesh failed to exclude access by several macroinvertebrate taxa (mayfly and dragonfly nymphs, and Corixiid and Notonectid adults), which at times were quite numerous in the catch of individual traps (especially in Cardross Basin 1 during spring and summer). Adult Corixid and Notonectids are predatory and feed on small larvae (Hawking, MDFRC personal communication), potentially leading to underestimations of the larvae caught in seasons when these taxa were considerably numerous. Despite this, a general trend in larval abundance through time was derived.

Total abundance was recorded to observe first appearance and changes in larval abundance through time. The standard length and development stage of each larva was also recorded according to Serafini and Humphries (2004). The different developmental stages of larvae can be used to estimate timing of spawning events.

Given the presence of both Murray hardyhead (*C. fluviatilis*) and unspecked hardyhead (*C. s. fulfus*) in Cardross Basin 1 and Lake Hawthorn, identification of some smaller larvae in the light traps was at times problematic. Accurate identification between the two species was uncertain until lateral scales could be distinguished on the sides of larvae which does not occur until a standard length of 14-20mm had been reached. Consequently overall abundance of larvae from both Hardyhead species, as well as data which separates the larval abundance into three categories, *C.fluviatilis*, *C. s. fulfus* and uncertain, are presented in this report.

2.1.4 Adult Size Class Distribution

Throughout the survey period adult fish were captured using both seine netting and small fyke nets. Adult fish were collected on a three weekly basis by dragging a 5m long 3mm mesh beach seine net through the edge habitat at Sites 1 and 3 in Cardross Basin 1, and Sites 1 and 3 in Lake Hawthorn. Seine hauls were repeated each sampling occasion until at least 30 adults of breeding size were collected. Breeding size was designated at 30mm standard length, based on preliminary laboratory analysis of gonad maturity condition (Ellis unpublished data).

On each sampling occasion from September 2004 to May 2005, a single small fyke net was set overnight at Site 3 in each lake to supplement the seine catch in the case where 30 adult fish could not be captured. From 30 May until 12 September 2005, four small fyke nets were set at sites 1 to 4 in each lake on each sampling occasion to estimate variation in adult Murray hardyhead abundance between the two systems. Small fyke nets had dual wings (each 2.5 m x 1.2 m), with a first supporting hoop (∅ = 0.4 m) fitted with a square entry (0.15 m x 0.15 m) covered by a plastic grid with rigid square openings (0.05 m x 0.05 m). Each small fyke net had a stretched mesh size of 2mm and was set amongst woody debris, fringing and submerged vegetation. Fish not kept for gonad analysis had their standard length recorded before being released back to the water at the site from which they were captured.

2.1.5 Reproductive Biology

The spawning condition of adult Murray hardyhead is described based on analysis of maturity stage and gonad size of adult the fish collected from September 2004 through until October 2005. Batch sizes of seven ‘ripe’ ovaries from fish collected in October 2005 were also determined. Captured fish were euthanized in an ice slurry before transferral to a 4% Formaldehyde solution for at least one week solution to ‘fix’ the fish. The fish were later transferred to a 95% ethanol solution and stored until dissection of the gonad (ethanol is a less hazardous chemical).

To dissect the gonad, fish were removed from the ethanol solution, and their standard length was recorded to the nearest millimetre. Standard length (SL) refers to the distance from the anterior margin of the mouth to the tip of the hypural joint. The fish were then blotted dry before being individually weighed after a ten second interval to allow evaporation of residual ethanol (±0.00005g). Fine cuts were then made along the ventral apex of body, before the body wall was peeled outward to expose the abdominal cavity including internal organs. The gonad was located and removed by carefully unwinding intestine and internal organs (Figure 2.3). The extracted gonad was submerged in 95% ethanol and excess tissue was removed before examination under a stereomicroscope.

Maturity stage

Adult *C. fluviatilis* were assigned to one of seven maturity stages (Table 2.1) by assessing gonad colour, extent of swelling and the presence of ovulated (translucent) eggs. Stages were derived and modified from those developed for the flyspecked (unspecked) hardyhead *C. stercusmuscarum* by Milton and Arthington (1983).

Table 2.1. Classification of maturity stages in Murray hardyhead (based on the classification for *C. stercusmascarum* in Milton and Arthington (1983).

Stage	Description	
	Male	Female
1 - juvenile	Gonad not visible or small, thin and strap like. Not swollen at all, appear empty.	Gonad not visible or small, thin and strap like. Not swollen at all, appear empty.
11 - inactive	Testes elongate, thin whitish sac, just starting to swell.	Ovary pale orange, with a few tiny opaque oocytes, visible through the ovary wall at x 20 mag.
111 - Early developing	Testes grey-white or cream coloured, slightly swollen and starting to branch/fold.	Ovary pale orange, starting to swell, eggs opaque and range in size, larger eggs just visible to naked eye,
IV – Late developing	Testes opaque, white to pale cream/white, swollen to at least half body cavity and wrapped around gut.	Ovaries swollen and orange, eggs at various stages of development clearly visible, mostly opaque, with a few larger eggs turning translucent.
V – Gravid	Teste may distend body cavity on external examination. Testes large, white-cream; swollen such that gut tightly wrapped around teste.	Ovary distends body cavity on external examination. Ovaries yellow-orange with many large translucent round eggs amongst smaller opaque eggs at varying stages of development.
V1 – Spawning	Body swollen on external examination. Testes large, white-cream; swollen such that gut tightly wrapped around teste. Live specimens exude milt without pressure (noted in field).	Body swollen on external examination. Ovaries with large numbers of round, yellow translucent ovulated eggs (chorion developed, and adhesive filaments visible). Some eggs apparent in gonopore, or extruded with slight pressure in live specimens.
V11 - spent	Gonads reduced in size, irregularly shaped. Testes thin, flaccid and virtually translucent.	Gonads reduced in size, irregularly shaped. Ovary reduced, small and flaccid, some enlarged oocytes remain but are irregularly distributed in ovary.

Examples of female ovaries and male testes at various stages of development are shown in Figure 2.3. Figures a(i) and b(i) show immature female and male gonads respectively. Neither example is swollen, with oocytes barely visible in the female ovary, and the teste consisting of a thin sack. Ripe gonads from fish in spawning condition when captured (sperm and eggs extruded with minimal abdominal pressure) are shown in figures a(ii) and b(ii). Large translucent oocytes are seen through the wall of the swollen ovary and the teste is large, swollen with milt and folded to fill the body cavity. Figure a(iii) shows a batch of eggs extruded from a female in 'running ripe' condition (Stage VI in Table 2.1), with transparent chorion and adhesive filaments (for attaching eggs to aquatic vegetation) clearly visible. Figure b(iii) shows a completely spent teste from a large male collected at the same time as the ripe female.

a. b.

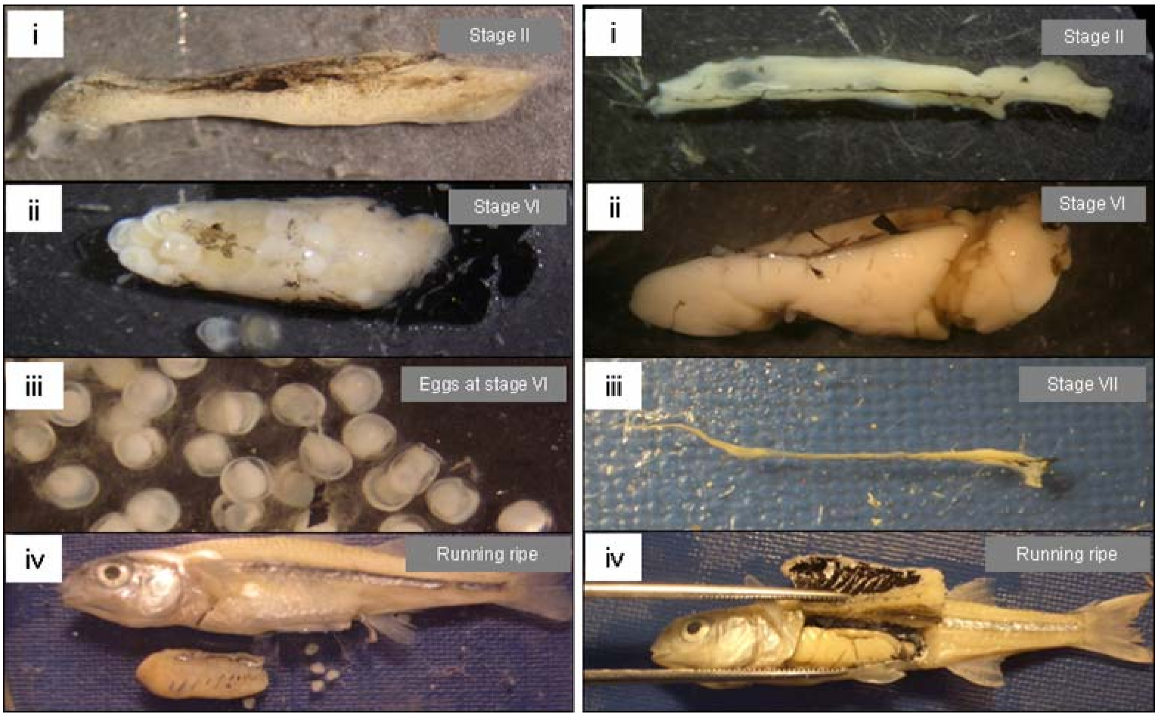

Figure 2.3. Ovaries (a) and Testes (b) at various stages of development.

Gonadosomatic Index

After extraction each dissected gonad was removed from ethanol, dabbed dry on paper towelling and gonad weight was recorded. The Gonadosomatic Index (GSI) for each adult fish was calculated using the formula below.

$$\textbf{GSI} = \frac{\textbf{Gonad weight}}{\textbf{Total weight}} \times \textbf{100}$$

Mean GSI for male and female fish from each lake on each sampling occasion was calculated to describe changes in breeding condition of adult fish within each population. Preliminary analysis showed minimum standard length of male and female fish from both populations at maturity Stage V was between 28 and 30 mm. Consequently to overcome size-dependant bias from immature fish this report only presents mean GSI data for adult fish 30mm or greater in standard length (and thus large enough to attain spawning condition) (West 1990). On several sampling occasions in January and March 2004 gaps appear in the GSI data where abundances of adult Murray hardyhead were so low that insufficient numbers of adult fish greater than 30mm standard length were able to be captured.

Batch size

Batch size was estimated from counts of enlarged translucent oocytes present within the ovaries of seven female fish at Stage VI from each population (captured during a period of high mean GSI on 4 October 2005).

2.2 Results

2.2.1 Hydrology

Cardross Basin 1

In addition to water from irrigation drainage disposal, the Cardross Lakes receive targeted environmental flows through the State's Murray River Environmental Water Allocation (EWA). Water supplied to the lakes as an environmental flow first enters Basin E, of which around 50% flows through to Basins D and C, with the other 50% flowing through to Basins 3, 2, 1, and 4 (Sharpe *et al.* 2003). Water level (AHD) and inflow (ML/d) data for Cardross 1 between February 1996 and October 2005 are shown in Figure 2.4.

From September 2004 to July 2005, a total of 1702 ML of environmental inflow entered the Cardross Lakes, with an additional 1344ML delivered between June 2005 and October 2005 (Figure 2.4). The surrounding littoral fringe of Basin 1 was consequently kept submerged, or connected with the water body, for most of the survey period.

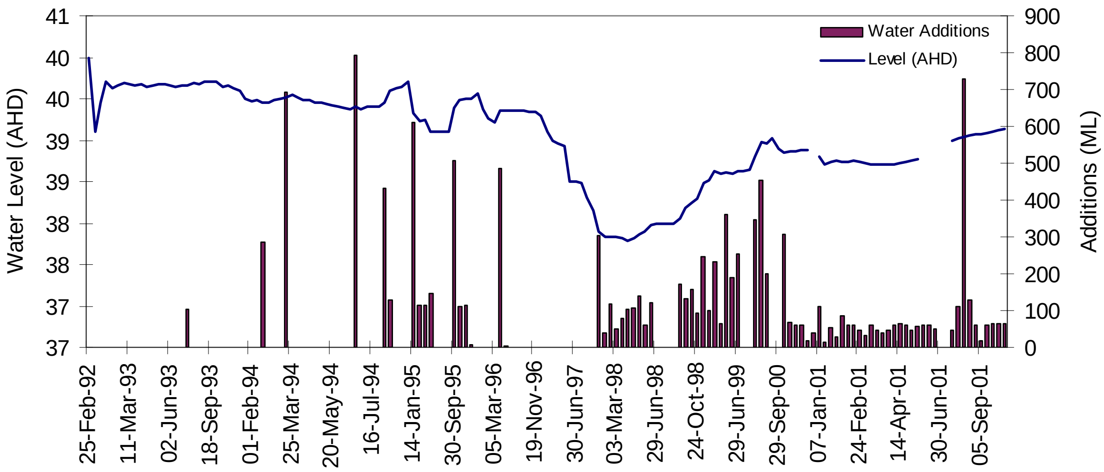

Figure 2.4. Water levels and additions to Cardross Basin 1 (source G. Sayers Lower Murray Water).

Lake Hawthorn

Lake Hawthorn receives irrigation drainage disposal water from First Mildura Irrigation Trust (FMIT), whilst operation of outflows is managed by Goulbourn Murray Water (GMW). Water level (AHD) and salinity data for Lake Hawthorn between July 1998 and October 2005 are shown in Figure 2.5. GMW diverts water from Lake Hawthorn to the Wargan Basins near Mildura when the lake level reaches 34.85mAHD. Diversions to the River Murray can also be made when specific operating criteria are met pertaining to salt levels in Lake Hawthorn, and river flow and salt levels downstream of Lake Hawthorn (T. Coulpern GMW, personal communication). Consequently water levels in Lake Hawthorn have traditionally fluctuated substantially on a year by year basis, with fresh annual inflows maintaining salinity levels within the lake between 2000 and 6000µS.cm^{-1} (Figure 2.5). Since 2000 however, inflows into Lake Hawthorn have been substantially reduced due to increased efficiency in water usage by irrigators and drought conditions. As a consequence water levels have not fluctuated in the magnitudes recorded prior to 2000, with levels maintained between 34.5 and 35mAHD. This reduction in fresh inflows and water level fluctuation has resulted in salinity levels within Lake Hawthorn increasing steadily since the year 2000 to current levels of almost 13000µS.cm^{-1}.

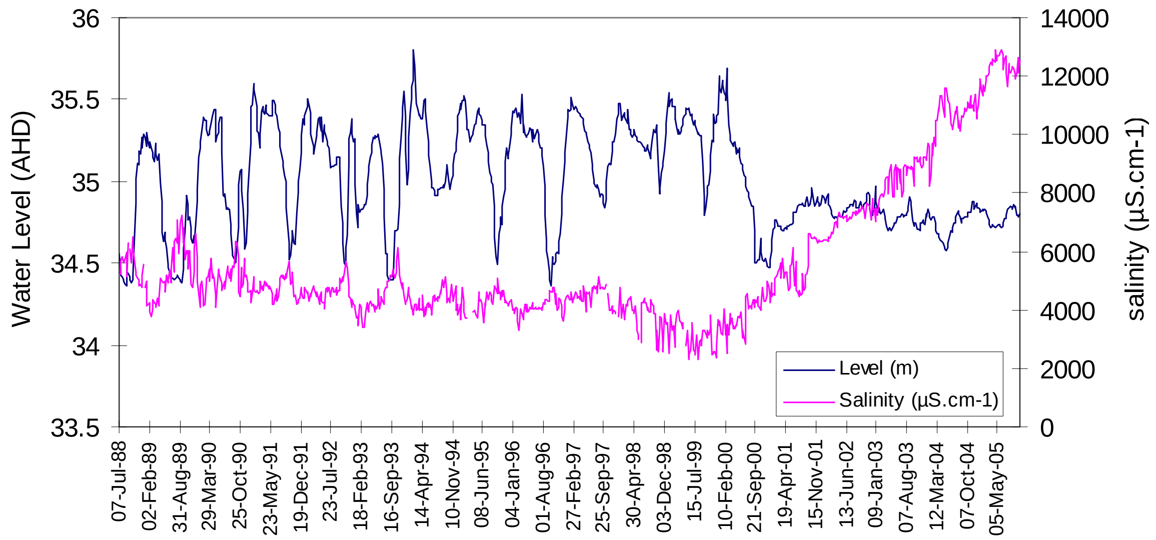

Figure 2.5. Water levels and salinity in Lake Hawthorn (source Tony Hetherington, Goulburn Murray Water).

2.2.2 Water Quality

Cardross Basin 1

Seasonal variation in mean water quality parameters for the duration of this survey in Cardross Basin 1 are shown in Figure 2.6(a). Over the course of the year from September 2004 to September 2005, salinity within the basin was kept relatively stable between 5200 and 6700 $\mu S.cm^{-1}$. After initial increases in the early summer of 2004, salinity dropped from a high of 6690 EC to 5370 $\mu S.cm^{-1}$ by the following October, 2005. This decrease in salinity was due to a combination of comparatively (to recent years) high rainfall throughout 2005 and EWA water added to the Cardross Lakes throughout 2004-05 (Figure 2.6). Water temperature fluctuated in line with seasonal variation in ambient temperature and turbidity remained low (less than 40 NTU) for most of the sampling period. Instances of higher turbidity are likely due to wind driven wave action in shallower exposed sites on certain sampling occasions. The pH of the basin was consistently high (between 8.5 and 10), as were dissolved oxygen concentrations (9 to 16 mg.l) due to the high density of vegetation, especially *Ruppia* sp., within the lake.

Lake Hawthorn

Seasonal variation in mean water quality parameters for the duration of this survey in Lake Hawthorn are shown in Figure 2.6 (b). From September 2004 to October 2005, salinity within the lake increased from around 10100 to above 12850$\mu S.cm^{-1}$. A decrease in salinity from approximately 12950 to 12500$\mu S.cm^{-1}$ in July and August 2005 may have been due to due to a combination of comparatively (to recent years) high rainfall and inflows to the Lake Hawthorn from FMIT excess flows (Figure 2.5). Water temperature fluctuated in-line with seasonal variation in ambient temperature, while turbidity remained low (less then 20 NTU). The pH of the basin was consistently high (between 8 and 10) throughout the survey period, as were dissolved oxygen concentrations (9 to 16 mg.l) due to the high density of vegetation, especially *Ruppia* sp., within the lake.

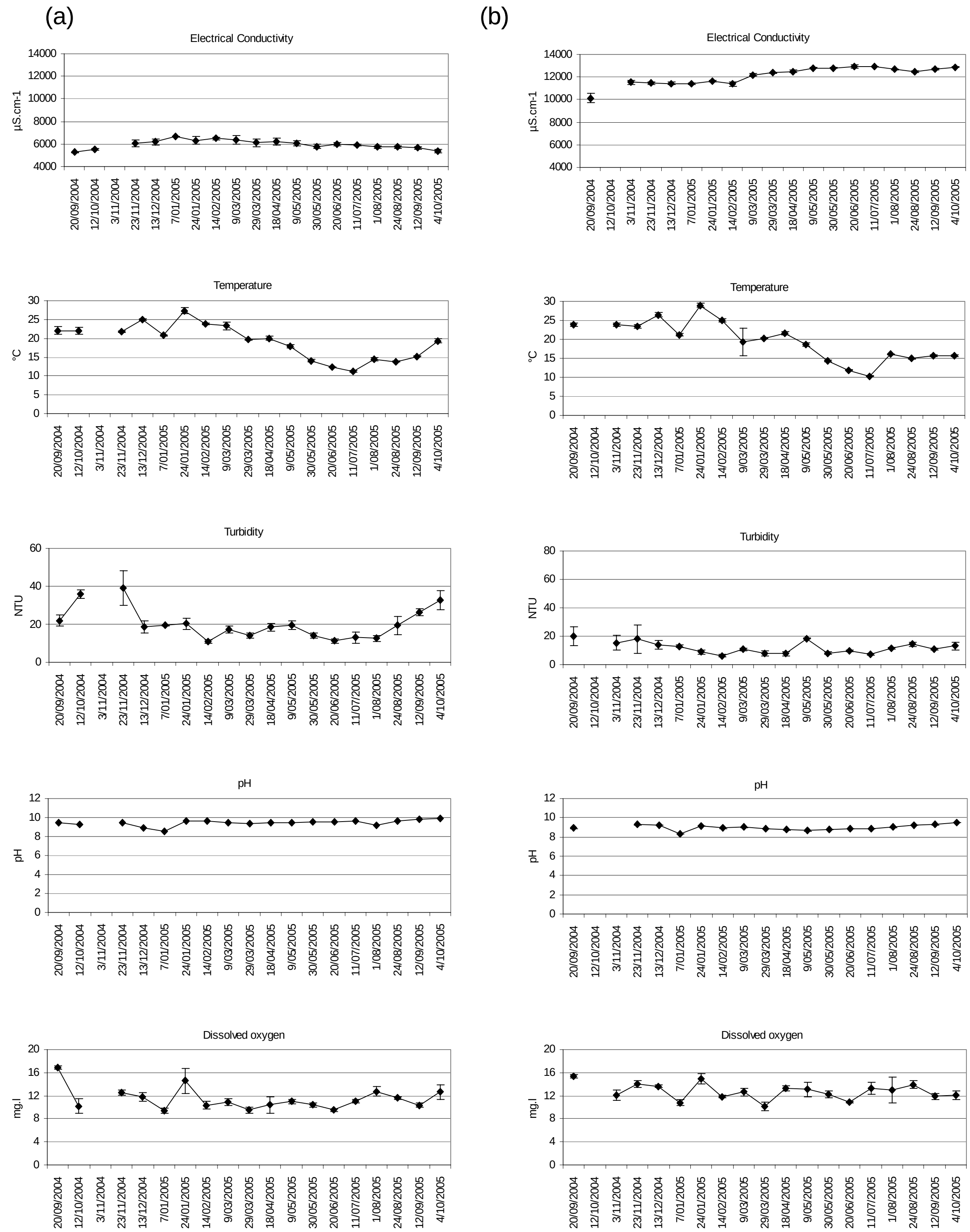

Figure 2.6. Temporal fluctuations in water quality in Cardross Basin 1 (a) and Lake Hawthorn (b) throughout this survey.

2.2.3 Larval Abundance

Temporal variation in larval hardyhead abundance in Cardross Basin 1 and Lake Hawthorn are shown in Figure 2.7. Due to confusion in the identification of small larvae, the total hardyhead catch is separated into three categories (Murray and unspecked hardyhead, and hardyhead spp.).

Temporal trends in total larval abundance were similar between the two lakes with a clear peak in abundance in early November 2004. In both wetlands total larval abundance fell from late November 2004 through until February 2005, with smaller peaks in abundance appearing in March and June 2005. Larval abundances in both lakes remained low until the following October. This temporal trend in larval presence/absence was similar in both wetlands irrespective of differences in salinity and water levels.

Total larval abundances were consistently higher in Lake Hawthorn than in Cardross Basin 1, although this is more than likely a product of increased predation within the light traps set in Cardross Basin 1. Predatory macroinvertebrates and small adult fish were more numerous in the catch of light traps in Cardross Basin 1 despite the exclusion mesh on the traps (Ellis personal observation).

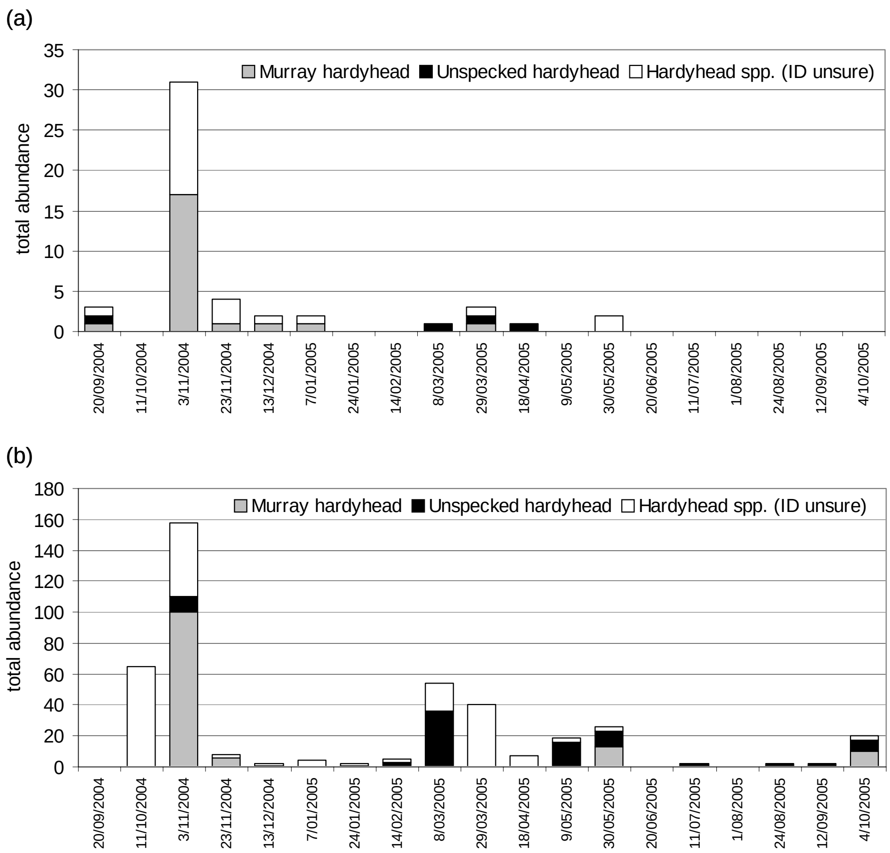

Figure 2.7. Temporal variation in the abundance of Murray hardyhead, unspecked hardyhead and unidentified hardyhead in (a) Cardross Basin 1 and (b) Lake Hawthorn (for 9 light traps, 3 set at each of 3 sites per lake).

Unspecked hardyhead eggs hatch to protolarva around 7 days after fertilisation (Pusey *et al*. 2004). Assuming larval Murray hardyhead have a similar incubation period of days to weeks, Figure 2.7 indicates spawning of Murray hardyhead began in Cardross Basin 1 in early September 2004 (potentially even late August) and continued through until early January 2005. The absence of larvae in mid October 2005 may merely be due to heavy predation by macroinvertebrates within the light traps as discussed earlier. Spawning of Murray hardyhead in Lake Hawthorn appears to have begun in late September 2004 and ceased in early December the same year (earlier than in Cardross).

Despite the number of larvae for which identification was uncertain, the peak in Murray hardyhead abundance in early November 2004 is clear. Murray hardyhead dominate the larval catch at this time and it is therefore likely that many of the smaller unidentified larvae recorded in Lake Hawthorn in October 2004 were also Murray hardyhead. These fish would have contributed to the peak in Murray hardyhead abundance several weeks later in early November by which time they had attained a size at which distinguishing characteristics were evident.

Similarly, the unidentified larvae in Cardross Basin 1 in the months after the November peak are likely to be Murray hardyhead given the absence of unspecked hardyhead during this period. Furthermore, due to the appearance of unspecked hardyhead larvae in the March and April 2005 catch in Cardross Bain 1 and February through until June 2005 in Lake Hawthorn, it is likely most of the unidentified larvae in each lake during these smaller abundance peaks were unspecked hardyhead.

2.2.4 Adult Size Class Distribution

Temporal variations in size class frequency of Murray hardyhead captured throughout this survey in Cardross Basin 1 using larval fyke nets and seine netting are shown in Figure 2.8. From September to early November 2004 the adult population was dominated by a cohort of fish from 30 to 50mm standard length (SL). By late November, a second cohort of smaller fish was observed, consisting of recruits from the September-October spawning period documented in the larval fish section of the results (above). Figure 2.8 shows that over the next few months these smaller fish gradually became the dominant cohort in the Cardross Basin 1 population as the larger cohort diminishes in the catch. Modal size class of the smaller cohort gradually increased for the remainder of the survey by which time a similar size class distribution was evident within the population than that of September 2004.

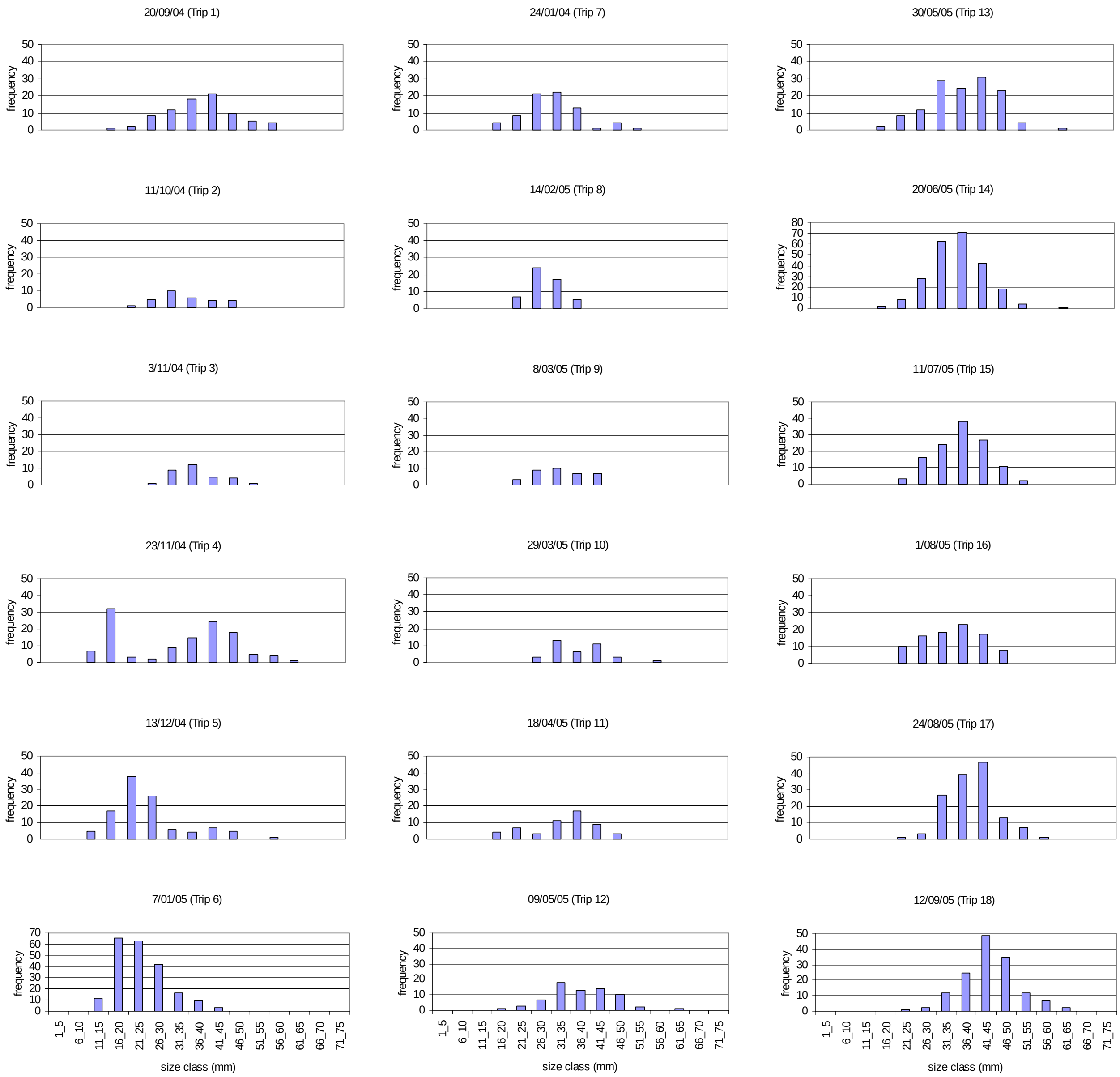

Figure 2.8. Temporal variation in size class distribution for adult and juvenile fish captured in fyke and seine netting within Cardross Basin 1.

A similar pattern in size class distribution is evident within the Lake Hawthorn population (Figure 2.9), albeit with consistently lower abundances of adult fish caught throughout most of the survey. The larger size class disappeared from the catch earlier in Lake Hawthorn (late November) then it did in Cardross Basin 1 (early January).

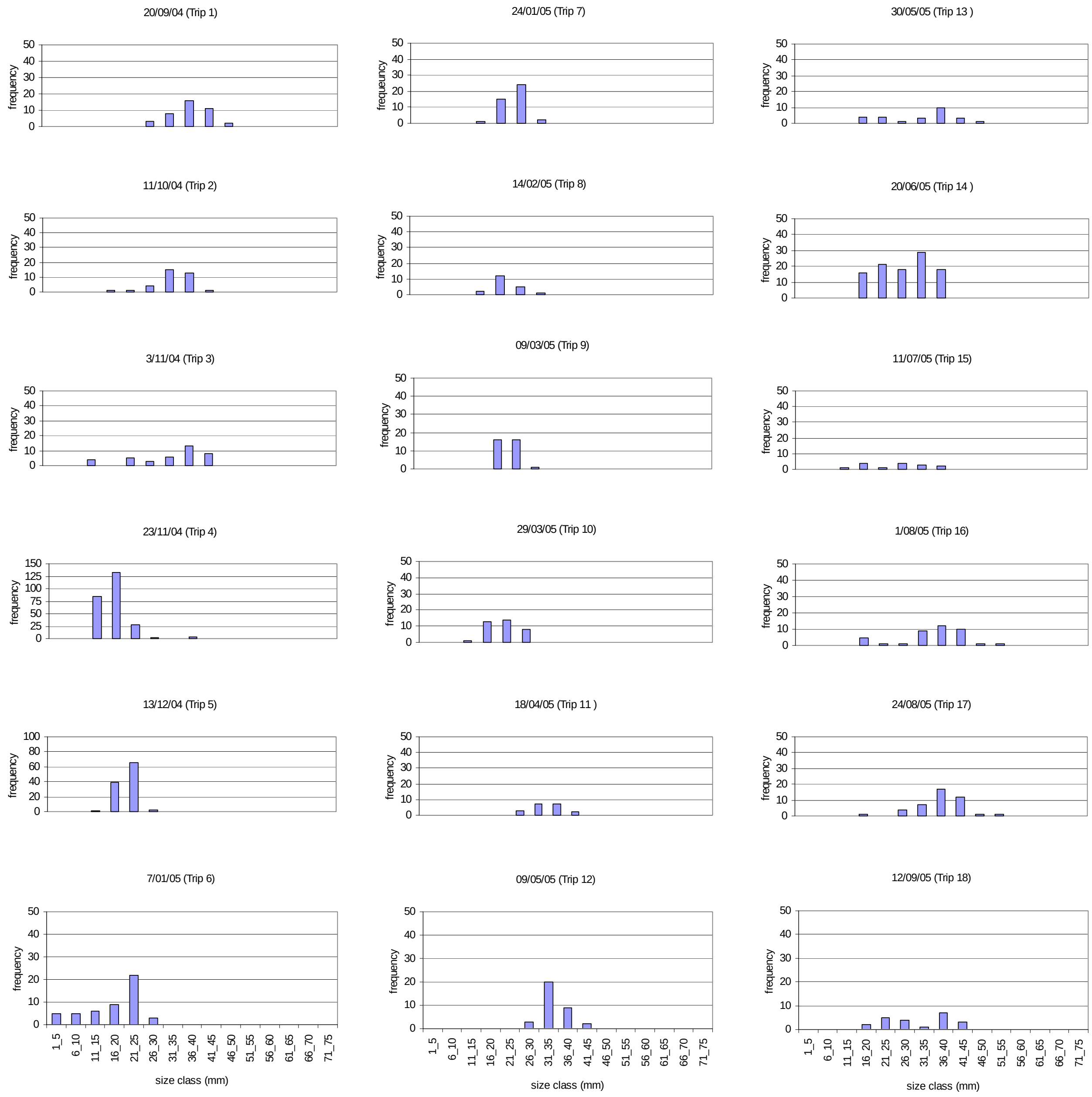

Figure 2.9. Temporal variation in size class distribution for adult and juvenile fish captured in fyke and seine netting within Lake Hawthorn.

Total catches of Murray hardyhead were significantly lower in Lake Hawthorn than in Cardross Basin 1 (paired t-test: P= 0.0033, n=6) (Figure 2.10). This lower abundance implies weaker recruitment from larvae to adult size classes in Lake Hawthorn, despite the lower densities of larval predators noted in the light trap catch than in Cardross Basin 1.

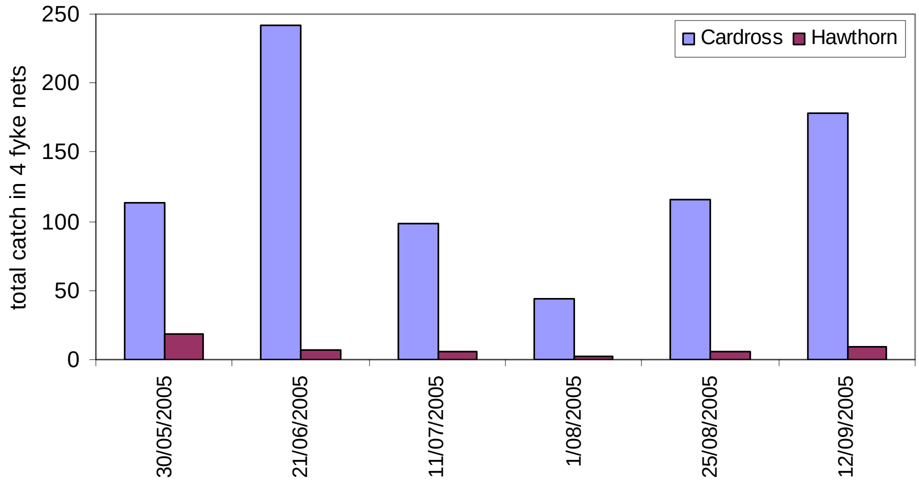

Figure 2.10. Variation in total fyke net catch (four nets set in each lake per sampling occasion) in Cardross Basin 1 and Lake Hawthorn.

2.2.5 Reproduction

<u>Gonad stage</u>

A seasonal pattern in gonad maturation was apparent for both populations of Murray hardyhead (Figure 2.11). Mature reproductive condition in both lakes (indicated by stages V and VI) was already pronounced within a large proportion of the adult populations of Cardross Basin 1 and Lake Hawthorn when sampling began in September 2004. Ripe Murray hardyhead were collected from September 2004 to January 2001 in Cardross Basin 1, and from September to mid December 2005 in Lake Hawthorn.

Mature females continued to make up a large proportion of the population until early January in Cardross Basin 1, and until mid December in Lake Hawthorn. Mature and late devolving males (which may be mature males that had recently partially spawned) dominated the adult male population in Cardross Basin 1 from September through until early January in Cardross Basin 1. A similar trend was observed in Lake Hawthorn, however, mature and late developing males were absent from catches by early January. A large proportion of each population remained reproductively inactive (stages I, II and III in Table 2.1) for much of the sampling period between January and early August 2005 in Cardross Basin 1, and from mid December until late August in Lake Hawthorn. Male and female fish in Cardross Basin 1 reached advanced reproductive condition (stage IV in Table 2.1) earlier in the year (May to September) then in Lake Hawthorn (August).

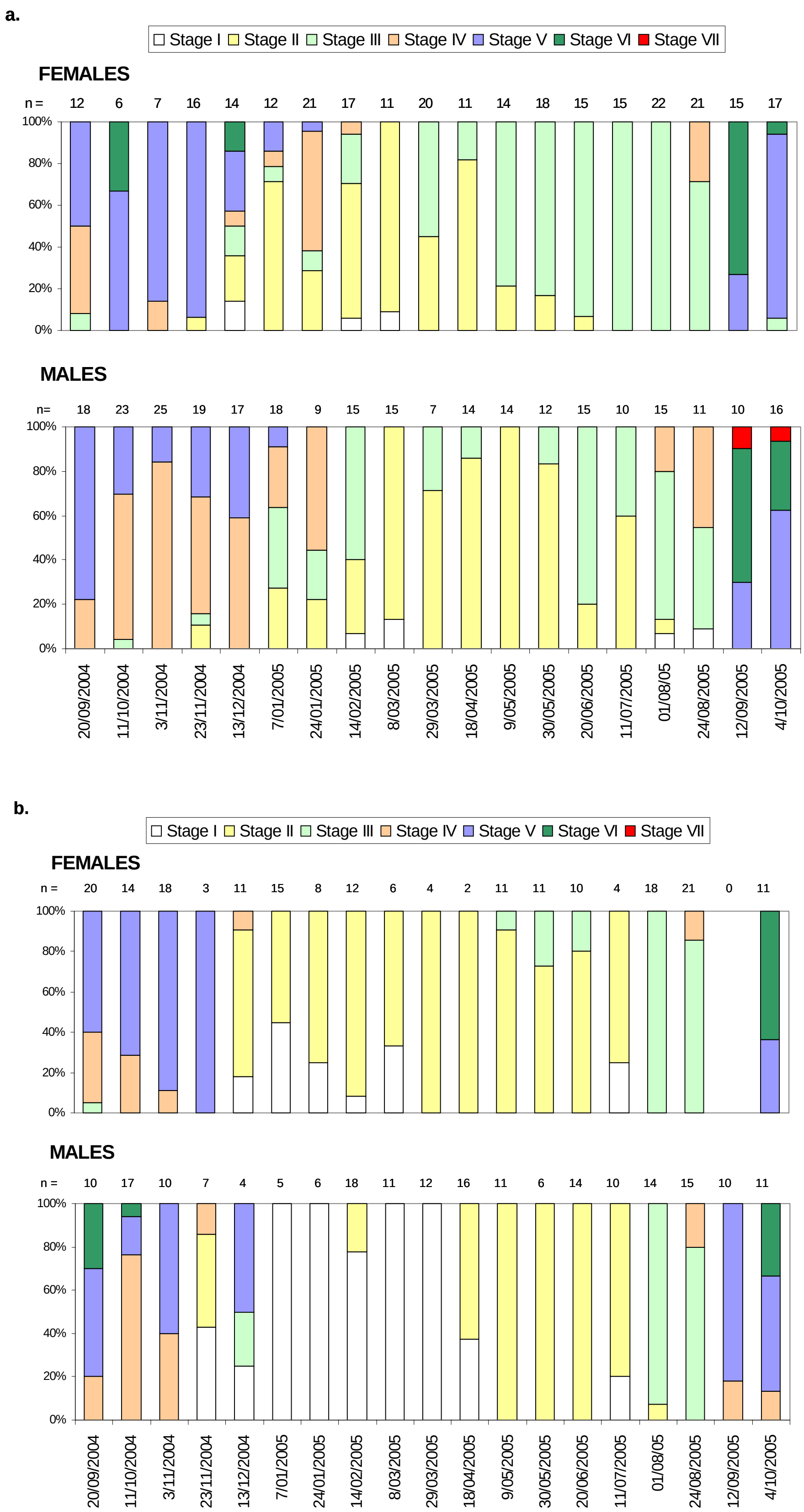

Figure 2.11. Temporal changes in the reproductive stages of Murray hardyhead populations in (a) Cardross Basin and (b)Lake Hawthorn. Sample sizes for each occasion are above each figure.

Gonad Somatic Index

The seasonal pattern in maturation described by trends in gonad stage was also indicated by changes in Gonad Somatic Index (GSI) for adult fish from both lakes (Figure 2.12). Only those adult fish over 30mm standard length (the minimum length at which individual fish at maturity Stage V were encountered) are included in this data set. Data points are missing on sampling occasions when insufficient numbers of fish greater than 30mm SL were captured.

Mean GSI for fish in both lakes was already elevated in September 2004 (when sampling began), remaining high through until mid December 2004. During this period, mean GSI fluctuated between 6.9 and 9.2 % in males and 8.1 – 12.1% in females from Cardross Basin 1, and between 7.6 – 11.4% in males and 7.3 – 11.5% in females from Lake Hawthorn. Mean GSI for both populations then decreased gradually between late December 2004 and February 2005, with reduced GSI (below 2%) for both male and female fish attained early in March and maintained until early July. The following breeding season maturation of both males and females gonads began in late July, with GSI over 8% attained by early to mid September 2005. In both lakes higher GSI values were measured for females in October 2005 then at any occasion during the previous breeding season.

GSI began to increase in both lakes during July 2005, as day length and water temperature began to increase, indicating one or both of these parameters cue gonad development. The decrease in GSI apparent the previous January also corresponds with reduction in both day length and water temperature.

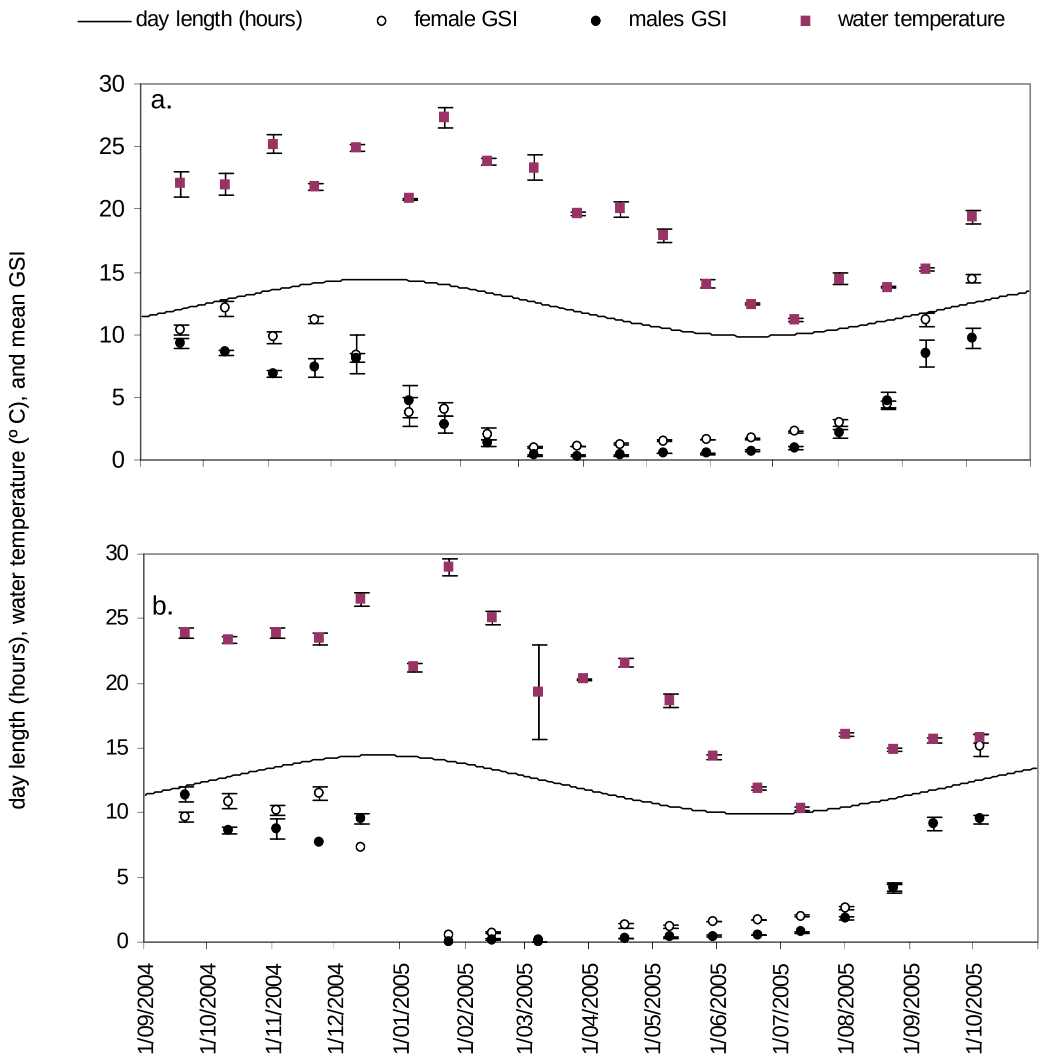

Figure 2.13. Mean GSI for adult Murray hardyhead (fish ≥ 30mm SL), day length and water temperature in Cardross Basin 1 and Lake Hawthorn.

Batch size

Examination of gonads from Murray hardyhead indicates that this species is a batch spawner, with eggs at various stages of development apparent in mature ovaries during the breeding season. Mean fecundity or egg batch size for females collected on 4 October 2005 (Figure 2.11) was significantly higher for females in Cardross Basin 1 than those in Lake Hawthorn (two tailed t-test: P= 0.042, n=7), and ranged from 43-87 and 30–74 for ripe females in each lake respectively.

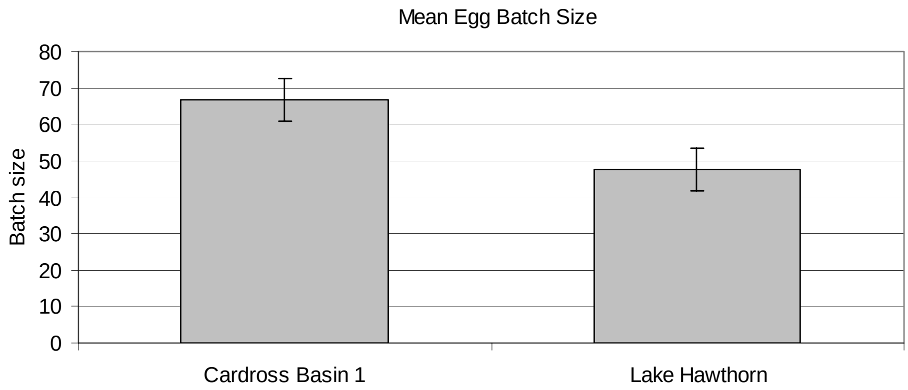

Figure 2.11. Mean egg batch size for females collected in spawning condition (gonad maturity stage VI) on 4 October 2005. Sample sizes in both cases were 7.

2.3 Discussion

Hydrology and water quality

Murray hardyhead abundances throughout this survey were consistently greater in Cardross Basin 1 than in Lake Hawthorn. Differences in abundance between the two systems might be attributed to differences in food availability, reduced breeding success, physicochemistry (specifically salinity) and/or hydrology imposed upon one or all life stages of Murray hardyhead in either population.

All water quality parameters monitored, (with the exception of salinity), remained consistently stable in both lakes throughout this survey, and were considered to pose no threat to the survival of either Murray hardyhead population. Turbidity in both lakes was low (< 20 NTU). Although high compared to other local wetlands (Ho *et al.*), pH remained constant throughout the survey period in both lakes. Significant drops in pH, should they occur in the future, could indicate sulfate acidification, potentially threatening the Murray hardyhead populations examined in this study.

Water temperature in each lake fluctuated with ambient temperature and at no stage was considered threatening to Murray hardyhead populations in either wetland. Water temperature would only be of concern if water levels in summer dropped sufficiently to allow the water body to heat above tolerance limits for Murray hardyhead (currently unknown) or their food sources. Elevated water temperatures may also decrease concentrations of dissolved oxygen in the water column, indirectly stressing fish and other organisms. Instantaneous daytime dissolved oxygen (DO) levels in both lakes were found to be high throughout this, possibly survey due to large amounts of vegetation in both lakes (especially *Rupia* sp.) which produce DO through photosynthesis, or wave action leading to high rates of diffusion of atmospheric oxygen into the water bodies.

Various upper salinity thresholds for adult Murray hardyhead have been reported as, 25000µS.cm^{-1} in lakes in the Kerang district (Lugg *et al.* 1989), 67500µS.cm^{-1} in Golf Course Lake (McGuckin 1999), and up to 48000µS.cm^{-1} in Lake Elizabeth (Hardie 2000). Research by Arthur Rylah Institute for Environmental Research (ARI) demonstrated that salinity tolerances vary between different populations of Murray hardyhead, and consequently tolerance limits for one population should not be used as a management guide for maintaining salinities in the habitat of other populations (Dixon *et al* 2005). Tolerance levels therefore need to be determined for individual populations to be able to develop management recommendations for managing salinity levels in isolated water bodies where the species occurs.

For this reason, despite salinity in both Cardross Basin 1 and Lake Hawthorn throughout this survey remaining below the reported thresholds for adult Murray hardyhead from other populations, increases in salinity in Lake Hawthorn over the last 5 years are of concern. Given findings of other studies and the potential for chronic/recruitment effects to occur at levels much lower than those that are acutely toxic, there is no evidence that the Lake Hawthorn populations will be sustainable at current salinities (Dixon personal communication). There is at present no information on the chronic effects of elevated salinity on Murray hardyhead such as the salinity tolerance of eggs and larvae.

Populations of annual species (which Murray hardyhead are postulated here to be) may be expected to fluctuate in abundance from year to year. This survey spanning one annual cycle is not sufficient to conclude that the observed difference in Murray hardyhead population abundance between the two study lakes is constant. Increasing salinity may, however, play an increasingly important structuring role in the population dynamics of Murray hardyhead in Lake Hawthorn if the current rate of salinisation continues.

Current levels of salinity in Lake Hawthorn (although they do not appear to directly prevent recruitment of Murray hardyhead), may indirectly limit recruitment by negatively impacting on food resources. Lake Hawthorn appears to maintain lower densities of macroinvertebrate taxa such as Mayflies and Dragonflies than does the Cardross Lakes system (Ellis personal observation), which may reflect lower abundances of zooplankton. Recent aquatic surveys have also shown that Lake Hawthorn also supports a less diverse fish community than the Cardross Lakes, with only four native and two exotic species as opposed to up to eight native and four exotic species in Cardross Basin 1 (Ho *et al*. 2004, Sharpe *et al*. 2004). The paucity in native fish species diversity in Lake Hawthorn may be indicative of reduced ecosystem functioning as a result of increasing salinity.

Reduced fluctuation in water levels in Lake Hawthorn since 2000 (which has caused a periodic loss of connectivity between the main water body and emergent macrophyte beds) may have also had a negative impact on habitat availability and/or recruitment success of Murray hardyhead. Water level variability in naturally ephemeral systems promotes an array of beneficial trophic processes such as nutrient remineralisation, and a boom in zooplankton abundance (Scholz and Gawne 2004, Scholz *et al.* 2005). It is conceivable that lower Murray hardyhead abundances in Lake Hawthorn than those in Cardross Lakes are due to a combination of higher salinities, lower habitat availability and reduced variability in water level.

<u>Larval Murray hardyhead</u>

Analysis of larval Murray hardyhead abundance in both lakes showed that spawning occurs in late spring/early summer with a clear peak in larval abundance in November 2004. With a delay of days to weeks between spawning and the appearance of newly hatched larvae, this peak in larval abundance indicated that spawning had taken place throughout October 2004 (potentially beginning earlier in September 2004), and continued through until January 2005. Aging of larvae would provide more precise estimation of the timing of spawning events, however. Examination of age at length relationships (including age at maturity and maximum age) and temporal variation in age structure for Murray hardyhead in both populations would address this issue, and could highlight differences in growth rates and hence food availability between the lakes.

Gonad analysis

Analysis of seasonal variation in mean gonadosomatic index (GSI) provided a second line of evidence in determining the breeding season of Murray hardyhead spanning from September through to February (in the Cardross Lakes and Lake Hawthorn populations). Elevated GSI values were apparent when this survey began in late September 2004, peaking again by mid-September the following year (2005). In the 2004/05 breeding season high GSI values were maintained through until December, fluctuating as successive spawning (deposition of egg 'batches') events occurred. A gradual decrease in mean GSI was apparent from mid-December 2004 with minimum GSI values reached by February/March 2005. The decrease in GSI corresponds with a decrease in the abundance of larger adult fish, and the transition to populations dominated by developing young of the year with inactive or immature gonads.

A small peak in larval hardyhead abundance was observed in both lakes from March through until May 2005. This peak was assumed to consist predominantly of unspecked hardyhead based upon the dominance of unspecked hardyhead in the identified larval catch at that time. The absence of any increases in adult Murray hardyhead GSI during this period supports this observation.

In July 2005 increases in the mean GSI of Murray hardyhead in both lakes coincided with increases in day length and water temperature. Most Murray-Darling Basin fishes spawn during spring and summer when day length and temperatures increase (Koehn and O'Conner 1990, Meredith *et al.* 2002). Environmental conditions at this time allow eggs and juveniles to maximise growth rates through increased metabolism supported by increased food availability. A decrease in GSI for Murray hardyhead during January 2004 corresponds with a reduction in both day length and water temperature.

In both lakes higher GSI values were measured in October 2005 than at any time during the previous breeding season. This may indicate more favourable conditions for breeding during the 2005 development period than for the same period during 2004 (thus allowing the achievement of larger ovaries). Alternatively the higher GSI in October 2005 could be a demonstration of GSI fluctuation within a breeding season as spawning events occur, a feature of small freshwater fishes which exhibit protracted reproduction.

The functional class of the species ovaries indicates that Murray hardyhead are batch spawners, with intra-ovarian eggs at various stages of development. For batch spawning species, discreet groups of eggs develop in concert and are simultaneously ovulated and oviposited in a batch (Pusey *et al.* 2004) after which another batch of immature eggs then begins to develop. Ovaries from female Murray hardyhead would be considered 'asynchronous', in which oocytes at all stages of development are present at the same time. Oocyte size-frequency distribution within the ovary is continuos except in ripe ovaries where there is a clear separation between ripe and yolked oocytes (West 1990).

During October 2005 females may have been captured immediately prior to or during a spawning event (and hence at their greatest GSI), while females fish collected during the 2004 breeding season may have just shed egg batches and were developing a new batch. Mean batch size was significantly higher for mature females in Cardross Basin 1 than in Lake Hawthorn in October 2005 ranging from 43-87 in Cardross Basin 1 and 30-74 Lake Hawthorn (in the absence of direct observation of spawning females, however, it is difficult to predict the number of spawning events and the number of eggs spawned on each occasion). This result implies ecological conditions (such as food sources and water quality) might be more suitable for reproductive development in Cardross Basin 1 then in Lake Hawthorn. Alternatively the difference between the two populations may be simply due to genetic differences given their geographical isolation, or could be attributed to differences in the abundance of large fish between the populations.

<u>Size class distribution</u>

Seasonal variation in size class distribution for each population of Murray hardyhead supported the seasonal breeding pattern for Murray hardyhead described above. The larval peak from early November 2004 appears as a cohort of small fish (juveniles and small immature adults) in fyke and seine catches in late November 2004 in both systems. The trend in size class distribution also indicates that 'young of the year' fish make up the majority of each population. Few if any of the larger fish present during the breeding season (September through to December in 2004) appear to survive a full year to spawn the following breeding season. The abundance of adult fish decreases from December to March indicating that they either perish at the end of the breeding season, as for many small species (Mathews 1998), or retreat to deeper water where sampling equipment was not set.

However, given fish as small as 28mm standard length were recorded with gonads at maturity stage V (Table 2.1) (Ellis personal observation), larvae hatched early in the breeding season (September) could potentially spawn at the end of the same season (January four months later) should conditions allow rapid maturation over the spring/summer period. Sharpe *et al.* (2003) suggests a cohort of 0+ fish captured in April 2003 that had attained a size of up to 28mm standard length, were potentially spawned in September the previous year. The maximum age of Murray hardyhead represents a key knowledge gap. Filling this knowledge gap by otolith examination will help to determine whether a small proportion of the adult population do survive longer then a year to participate in successive breeding seasons.

The patterns in larval abundance, GSI and size class distribution detailed above implies that Murray hardyhead in both Cardross Basin 1 and Lake Hawthorn are heavily dependant on successful recruitment each year to maintain a critical population size. This means that even short term changes in ecological parameters which may negatively impact on fish at any stage of their life history, such as salinisation or the isolation of potentially critical habitat through reduction in water levels, could infer serious consequences for the ongoing viability and conservation of Murray hardyhead populations in Cardross Basin 1 and Lake Hawthorn.

This study demonstrates that the breeding/recruitment season of Murray hardyhead spans September through until March, at which time water levels should ideally be high enough ensure submergence of *Ruppia* beds and connectivity with areas of emergent vegetation (predominantly *Typha* and *Juncus* sp.). It is currently unclear whether submerged root systems and stems of emergent macrophytes provides critical habitat for any of the life stages of Murray hardyhead, or their food sources. This issue should be addressed in future research projects. Until this knowledge gap is filled, ensuring that water levels are sufficiently high to permit access by all size classes of Murray hardyhead to potentially critical aquatic habitat is seen as a primary management objective for the ongoing viability of this species.

The lower salinity of Cardross Lakes may support a more abundant and diverse community of zooplankton and macroinveretebrates food sources for Murray hardyhead. A greater supply of these would not only support a larger population of adult fish, but may fuel more rapid growth of fish (allowing development of higher GSI). Future research into food source availability and dietary analysis of the two Murray hardyhead populations by the MDFRC in 2006 will determine whether elevated salinities are limiting food availability within Lake Hawthorn.

2.4 Management Recommendations

1. Maintain salinities in the Cardross Lakes and Lake Hawthorn at current levels to ensure tolerance limits of egg, larval and juvenile Murray hardyhead are not breached, given successful recruitment does occur at salinities levels currently over 12000µS.cm^{-1} in Lake Hawthorn. Differences in adult abundance between the two systems suggest recruitment success was greater in the Cardross Lakes during 2004/05, where salinities have been maintained below 6000µS.cm^{-1}. Ideally, a reduction in the existing salinity of Lake Hawthorn to levels as close as possible to those recorded before 2000 (below 6000µS.cm^{-1}) through the addition of environmental water allocations and salt diversion projects is suggested to maximise recruitment success.

2. Water levels within the Cardross Lakes and Lake Hawthorn should be maintained at levels sufficient to provide connectivity between the main body of each wetland and fringing emergent and submerged vegetation during the breeding/recruitment season of Murray hardyhead (September through until March), thus maximising habitat availability for each life history stage.

3. Re-introduce variability to Lake Hawthorn, timing high water levels to coincide with the breeding season of Murray hardyhead. This may maintain salinities below thresholds for all life history stages, and promote over-arching ecological benefits for the species through the provision of greater food sources. Water level variability has been shown to promote a range of beneficial trophic processes (Scholz and Gawne 2004, Scholz *et al.* 2005) such as nutrient cycling and completion of planktonic life cycles).

Future Research

Addressing key knowledge gaps will ultimately assist in the conservation of all Murray hardyhead populations. Recommendations for future research include:

1. Determining the maximum age of adult Murray hardyhead through otolith interpretation, to ascertain whether a small proportion of the adult population survive longer then a year, thus participating in successive breeding seasons. This knowledge gap will be addressed in the next phase of the Mallee CMA's current research program by the MDFRC.

2. Investigate the diet and food source availability of the two Murray hardyhead populations, to determine whether elevated salinities are limiting food availability within Lake Hawthorn. This knowledge gap will be addressed in the next phase of the current research program by the MDFRC.

3. Investigation of age at length relationships for the species should identify whether food availability or higher salinities limit growth rates of Murray hardyhead in Lake Hawthorn. Age/length relationships will also determine whether a proportion of the young spawned early in a breeding season attain

sexual maturity in the same season, thus contributing to at least two successive breeding seasons.

4. Monitor the abundance of Murray hardyhead in both populations over successive years to enhance our understanding of the population dynamics of the species, and allow more precise determination of factors that may inhibit or promote recruitment success. The abundance of Murray hardyhead within the Cardross Lakes has been documented to fluctuate from year to year, and within single years. No data currently exists which can be used to assess any population decline that may have occurred in Lake Hawthorn as a result of salinity increase since 2000.

5. Examination of ontogenetic habitat shifts (relationship between various life stages and specific habitats) would improve our understanding of the overall ecology of the species, and allow more targeted conservation strategies. In particular, an understanding of whether the submergence of fringing emergent macrophytes is critical to one or all life stages would allow timing of water level fluctuations to support Murray hardyhead recruitment, and would also promote beneficial ecological processes.

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