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March 2011

South Australian Arid Lands Natural Resources Management Board

Climatic variability, fish and the role of refuge waterholes in the Neales River catchment: Lake Eyre Basin, South Australia

Dale McNeil, David Schmarr and Amanda Rosenberger

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Report to the South Australian Arid Lands Natural Resources Management Board

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This report may be cited as:

McNeil, D.G, Schmarr, D.W. and Rosenberger A. E. 2011. Climatic variability, fish and the role of refuge waterholes in the Neales River Catchment: Lake Eyre Basin, South Australia. Report by South Australian Research and Development Institute (Aquatic Sciences) to the South Australian Arid Lands NRM Board, Port Augusta.

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ACKNOWLEDGEMENTS

The authors would like to thank Henry Mancini from the SAALNRMB for supporting this project and managing its progress for the board. Qifeng Ye provided overall management of SARDI staff. Justin Costelloe from Melbourne University kindly provided access to hydrological data. Thanks to the School of Fisheries and Ocean Sciences at the University of Alaska, Fairbanks for supporting Asst. Professor Rosenberger's travel and involvement.

Katherine Cheshire (SARDI) provided a great deal of assistance in the field during the 2010 survey, as did Dean Hartwell and Neil Wellman (both SARDI) during the November 2009 survey. Janet Walton and Henry Mancini (SAALNRMB), Justin Costello (Melbourne Uni), Graham Tomlinson and Peter Richards, Gini Lee and Brooke Madill also helped out with fish surveys whilst in the field and are greatly appreciated contributors.

The authors would like to thank the landholders who assisted with the selection of sites, and provided access; without their co-operation, this work would not have been possible. In particular we would like to thank the owners, managers and staff from Allandale, Peake, Nilpinna and Todmorden stations. Also we offer a special thanks to Rodney Fullarton, who generously provided assistance, advice, directions, and stories which helped tremendously with field progress.

The community at Oodnadatta showed great support, especially the staff and students of the Oodnadatta aboriginal school and the senior citizens group who participated with great enthusiasm in the community day at Hookeys waterhole. The kids of Oodnadatta are fabulous and will be great ambassadors for native fish in the future. Thanks to Lynnie and Adam at the Pink Roadhouse for helping to organise and promote activities and supplying much needed showers (for the weak) and refreshments and to their eclectic and well dispersed staff for the same.

Collaborators: Gini Lee, Gresley Wakelin-King, Glen Scholz, Dave Deane, Justin Costello and Graeme Tomlinson.

EXECUTIVE SUMMARY AND RECOMMENDATIONS

This report presents the findings of a catchment scale investigation of the Neales River aquatic ecology and is one component of the South Australian Arid Lands Natural Resources Management Board (SAAL NRMB) project: "Understanding and managing critical refugia in the arid lands of central northern Australia" (The Critical Refugia Project). Funding was granted through the Australian Government Caring for our Country 2009/10 Program.

Previous monitoring programs have reported that fish populations in the Neales Catchment have been recovering for the last three years from severe drought that reduced available habitat for aquatic species. Surveys during this period indicated a radiation of species from Algebuckina Waterhole to waterholes upstream. However, the limited spatial sampling regime was unable to track the full extent of fish movement within the catchment or reveal which waterholes are critical for recovery and maintaining healthy populations. In addition, an established population of the introduced pest fish Gambusia was sampled consistently in the catchment, although the extent of this invasion and its impact on native fish species were unknown.

The aim of the study was to address two objectives: 1) identify and monitor critical refugia in the Neales Catchment and 2) map the extent and impact of Gambusia. Several other research groups collaborated in this project to identify and understand the hydrological, geomorphological, terrestrial ecological and landscape design processes contributing to the health of the Neales Catchment.

Two surveys were conducted – one in November 2009 and April 2010. The first survey included a reconnaissance flight over the entire system to evaluate the extent of permanent or semi-permanent waterholes and springs, which was followed by a fish survey at most of the sites identified. In the second survey, crews re-sampled as many of the sites as possible and added sites missed in the initial survey.

Key results from the study were:

1) Good flows in the Neales and Peake were accompanied by further radiation of most species throughout the catchment, including Gambusia, which had become widespread compared to past surveys. However, golden perch displayed a pattern of distribution and abundance contradictory to that of most other species.

2) Self-sustaining populations of most species had established at key waterholes including Hookeys, Cramps and Algebuckina.

3) Climatic extremes and hydrological variability was closely linked with fish communities and habitat use across the Neales Catchment.

4) Distinct groups of waterholes were identified, each with distinct fish community patterns. These groups support proposed classifications for aquatic drought refugia and can be used to optimise management of key waterhole types across the catchment.

5) Algebuckina waterhole was again highlighted as a critical Ark refuge for the Neales fish community during periods of drought and subsequent recovery.

6) Varying degrees of tolerance to climatic impacts (hypersalinity and hypoxia) were identified linking to the species habitat use and in particular utilisation of hypersaline Polo Club Refugia by extremely tolerant species.

7) At least three Gambusia “mega refugia” were identified at One Mile Bore, North Freeling Spring and Big Blyth Bore.

8) Trial events to stimulate community participation in NRM and research programs in the LEB were fruitful culminating in a successful community event at Hookeys waterhole attended by children and adults from the Oodnadatta community.

Key recommendations are:

1) Further surveys should be conducted to estimate the environmental impacts of Gambusia populations in the Neales Catchment;

2) ”Mega-refugia” (particularly bore drains) provide source populations for Gambusia and should be eliminated or managed;

3) The impact of Gambusia on native species in North Freeling Springs required further investigation to determine the risk of invasion of nearby Freeling Springs and thus the rest of the Neales Catchment;

4) Continue monitoring key waterholes in the Neales system to gain a more complete understanding of the processes underpinning the resilience of arid river fish species,

5) Consider collating tolerance data to inform on the resistance thresholds of fishes to climatic harshness,

6) Develop an empirical, data driven model to help manage arid river ecosystems such as the Neales River.

INTRODUCTION

Drought in the Neales Catchment

Recent monitoring of fish populations in the Neales River (McNeil *et al.* 2008, McNeil and Schmarr 2009) identified severe drying and habitat isolation following extreme drought conditions throughout 2006 (McNeil *et al.* 2008). Much of the catchment dried completely during the drought period, including many more permanent waterholes in the system.

Refilled by isolated tributary and reach flows during the 2006/07 wet season, dry waterholes refilled but remained fishless. A small number of critical refuge habitats persisted, where multiple fish species remained through extreme drought conditions (McNeil and Schmarr 2009).

The Algebuckina Waterhole, in particular, was identified as the key refuge habitat within which all species of fish present in the catchment were able to persist and survive throughout the drought (McNeil and Schmarr 2009). Such refugia, recently termed as 'Ark' type refuges (Robson *et al.* 2008), convey protection to a range of species under relatively benign environmental conditions. During the drought of 2006, Algebuckina Waterhole was the sole Ark refuge habitat in the Neales River catchment and is therefore identified as the most critical refuge for native fish biodiversity in the system (McNeil and Schmarr 2009).

Two other refuge waterholes were identified, but contained only subset (1-2) of fish species native to the Neales catchment at the peak of the drought (McNeil *et al.* 2008). Peake Waterhole was environmentally harsh - shallow and warm, with high levels of salinity (approximating seawater). Accordingly, Peake contained species tolerant to high salinities, namely, the desert goby (*Chlamydogobius eremius*) and Lake Eyre hardyhead (*Craterocephalus eyresii*), which have previously been recorded within highly saline waters of the Lake Eyre Basin (Wager and Unmack 2000). Termed 'Polo Club' refugia (Robson *et al.* 2008), harsh-but-permanent habitats such as Peake Waterhole are excellent refugia for a subset of the Neales fauna adapted to persist through disturbances and harsh conditions within these sites.

At the peak of the drought, Hookeys Waterhole at Oodnadatta possessed a single species, spangled perch (*Leiopotherapon unicolour*) (McNeil *et al. 2008*). Although the reason for the presence of this single species is unknown, it is understood to be highly mobile, relatively tolerant to water quality impacts related to climate (Gehrke and Fielder 1988, Allen *et al.* 2002), and may have recolonised from some other refuge such as a dam of a tributary pool that remains unidentified. In addition, early descriptions of the biology of this species point towards aestivation, enabling spangled perch to survive in mud through periods of drought (Lake 1971), but this is as yet unsubstantiated. This species is also the most popular for local recreational fishing and was perhaps re-introduced to this popular waterhole intentionally to supply food and recreation for the local community.

Post Drought Recovery

In post-drought surveys, we observed variable rates of recolonisation across species (McNeil and Schmarr 2009). Two species, spangled perch and bony herring (*Nematalosa erebi*) rapidly recolonised all sites directly after small, within channel flows resumed in the 2007/08 wet season, restoring hydrologic connectivity throughout the catchment. Other species, however, did not move to other sites, remaining restricted to the Algebuckina refuge and the saline Peake Waterhole. The following year, similar levels of within-channel connectivity were accompanied by recolonisation of desert rainbowfish (*Melanotaenia splendida splendida*) throughout the catchment. Golden perch (*Macquaria ambigua*) and Lake Eyre hardyhead recolonised to the mid catchment to Stewarts Waterhole. At Peake Waterhole, connecting freshwater inflows led to catches of spangled perch and introduced Gambusia (*Gambusia holbrooki*). The barred grunter (*Amniataba percoides*) did not recover and remained confined to the single refuge at Algebuckina Waterhole (McNeil and Schmarr 2009).

The monitoring of drought impact and subsequent recovery provided unique insights into the high level of variability that can exist in desert fish assemblages during harsh climatic periods. In particular, the important role that very few refuge waterholes play in protecting drainage diversity through drought highlighted the need to understand and protect key refuge sites in the Neales River. Any impacts that reduce the quality or permanence of localized refugia would have a disproportionate impact on the viability and persistence of native fish populations at the catchment scale.

In addition, variable recolonisation patterns observed across fish species highlighted the requirements of some species for extended periods of normal rainfall or specific circumstances (e.g. floods) before they can recolonise and recover following drought disturbance. The ability for some species (i.e. desert goby and barred grunter) to eventually recolonise the system following the drought remains undetermined. This pattern highlights the need to maintain ecological conditions that promote resilience within native fish populations to allow them to recover from serious climatic disturbances. Barriers to migration, extraction or diversion of flows or reduced inflows due to climate change may impact heavily on the resilience potential and survival of sensitive species in the Neales catchment.

Pest Fish in the Neales River

The restricted distribution and cryptic movements of the pest Gambusia suggest that this species may have trouble establishing self-sustaining populations in Neales River waterholes given their historical presence over the past fifty years (Costelloe *et al.* 2010). The natural disturbance conditions in the watershed may provide managers with an opportunity for controlling this pest by targeting small refuge populations of the species for removal, assuming control activities can be successfully implemented before broader colonisation of the catchment occurs.

The recent monitoring presented by McNeil *et al.* (2008) and McNeil and Schmarr (2009) revealed much about the ecology of fish populations in the Neales River. However, monitoring data were restricted to a small number of sites in the middle reaches of the catchment considered the most permanent waterholes in the system. A comprehensive survey of waterholes in the Neales/Peake system has not been conducted, and it is possible that other important refugia and fish habitats exist elsewhere in the catchment. To fully explore the role of critical refugia, in particular the Algebuckina Waterhole, for protecting native fish, a broader survey and assessment of waterholes is required.

With steadily improving rainfall and hydrologic conditions in the Lake Eyre Basin since 2006, such a survey would provide information about the distribution and recovery of fish populations during longer periods of connectivity, habitat and resource availability. With improved climatic and rainfall conditions, biological interactions, local habitat quality and complexity, resource availability and other biotic mechanisms are likely to play an increasingly important role in determining fish assemblage and abundance patterns as the impacts of harsh environmental controlling factors are lessened (Magoulick 2000, Jackson *et al.* 2001).

Project Aims

The current report outlines the most comprehensive assessment of aquatic habitats and fish ecology yet made within the Neales catchment. The purpose of the study is to identify important waterholes throughout the catchment, in particular, refugia that protect fish during drought and provide opportunities for fish populations to recover during intervening periods of improved hydrologic conditions.

In addition, the study aims to investigate some of the mechanisms that drive the response of fish species to drought conditions such as their tolerance of hypersalinity or hypoxia. These data may help explain the distribution of species across gradients of environmental harshness throughout the catchment.

Finally, we will establish the distribution of pest Gambusia to determine potential source populations that will feed dispersal into the broader catchment area. The ability of this species to dominate habitats across the neighbouring Murray-Darling Basin (Macdonald and Tonkin 2008) leads to great concern for the future of native fish in the Lake Eyre Basin. Any information that can assist with their control is of great value. Specifically, the aims of the study are:

- To map and survey significant fish habitats throughout the Neales catchment
- Estimate hydrologic connectivity across sites and determine key refugia, recolonisation pathways, and important habitats for native fish
- Assess fish assemblage structure, abundance, population structure and health of fish communities across the catchment
- Assess water quality and physical habitat characteristics that may influence fish ecology in Neales waterholes
- Assess the salinity and hypoxia tolerance of selected Neales River fishes
- Assess the distribution of pest fish (Gambusia) throughout the catchment
- Explore the importance of fish to local communities and tourists and undertake community consultation, extension and education activities

Survey data will be used to develop key management recommendations, specifically:

- Specify locales that should be prioritized by managers to protect native fish species and maintain resilient populations within the catchment
- Develop management strategies for the control or elimination of Gambusia populations throughout the Neales catchment
- Identify management actions to maximise recreational and indigenous connections to fisheries resources whilst protecting conservation and biodiversity values
- Identify communication and information pathways to maximise awareness of fish and related environmental issues within the Neales catchment.

FISH SURVEY

Sites

Fish surveys were undertaken at sites across the Neales Catchment (Neales River and Peake Creek) in the western Lake Eyre Basin in November-December 2009 and April/May 2010. Sites in the Neales River (16) included: Slate Hole, Afghan Waterhole, Angle Pole Waterhole, Shepherds Waterhole, Hookeys Waterhole, Mathiesons Waterhole, Stewarts Waterhole, South Stewarts Waterhole, Cramps Waterhole, Hann Creek, Ockenden Creek, Algebuckina Waterhole, Eaglehawk Dam, Cliff Waterhole, South Cliff Waterhole and an unnamed Waterhole near Tardetakarinna Waterhole (Figure 1). Sites in the Peake Creek (6) were: Peake Waterhole near the Oodnadatta Track, Baltacoodna Waterhole, Warrarawoona Waterhole, Lora and Arckaringa Creeks (Figure 1). Springs and bores (9) sampled were Old Nilpinna Station Spring, One Mile Bore, Freeling North Springs, Freeling Springs, Big Blyth Bore, Outside Spring, Fountain Spring, Hawker Spring and Milne Spring (Figure 1). Five of these sites; Hookeys, Stewarts, Mathiesons, Peake and Algebuckina Waterholes were surveyed previously in December 2007, May 2008, November 2008 and May 2009 and re-sampled during the current survey.

Survey Methodology

Fishing effort is summarised in Table 1. Fyke nets were used in most reaches and pools greater than 50m long. Fykes are effective in catching large numbers of both large and small-bodied fish (McNeil and Hammer 2007). Three types of fyke nets were used; two single-winged designs [small fykes (3 m leader, 2 m funnel) and large fykes (5 m leader, 3 m funnel)] and a double-wing design (2x 5m leaders, 3 m funnels). All types consisted of 4mm mesh fabric with an inlet arch of 650mm in diameter. All fyke nets were set overnight for approximately fifteen hours.

A small larval seine net was used to sample shallow pools. In some shallow springs and bore-drains, a larval dab-net was used to capture fish in addition to visual assessment in clear water. All fish were identified using keys (Allen *et al.* 2002; Wager & Unmack, 2000; J. Pritchard, unpublished data) and the total number of each taxon counted.

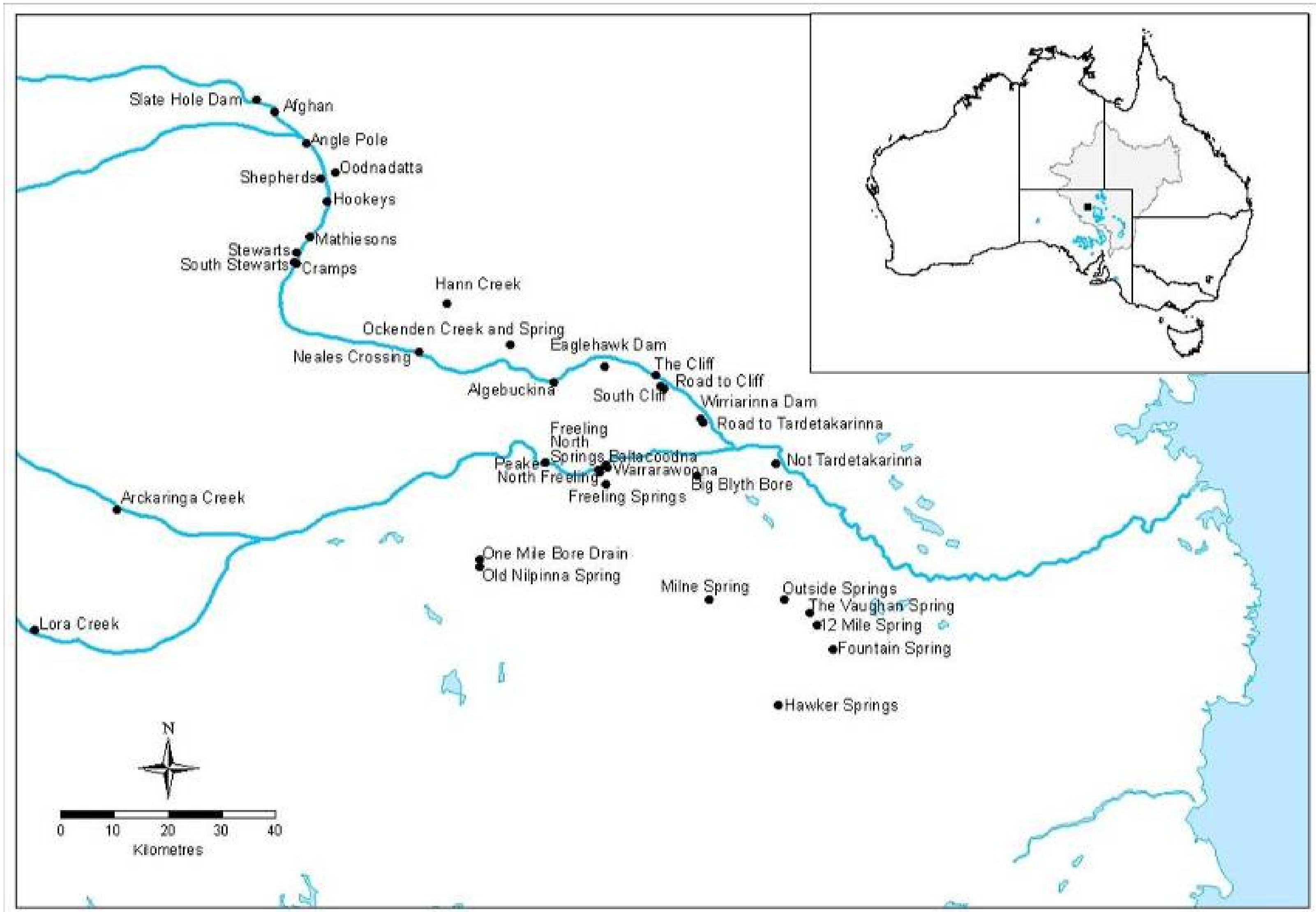

Figure 1. Sites sampled during the fish survey 2009/10 in the Neales River Catchment.

All captured fish were measured up to a maximum number of 100 individuals at each waterhole. Once 100 individuals were measured, the remainder of that species in that net was also measured to reduce the potential for any sub-sampling bias. As a result, more than 100 individuals may have been measured at sites where they were highly abundant. Measured fish were visually inspected for signs of disease and all were returned to the water at the point of capture.

Water quality parameters including temperature, dissolved oxygen, turbidity, pH and conductivity were measured at each site during each survey using a YSI 6920 water quality sonde. Water quality was measured at the surface and at 0.5 m depth intervals to detect stratification. Observations of the dominant substrate, in-stream macrophytes and riparian vegetation were also recorded at each site.

Table 1. Typical netting effort for common habitat types sampled in the Neales catchment.

Description	Netting Effort
Large Reach e.g. Algebuckina	6x small fykes 4x large fykes 6x double wing fykes
Large Waterhole e.g. Stewarts, Cliff, Angle-pole Waterholes	4x small fykes 2x large fykes 4x double wing fykes
Small or shallow waterhole e.g. Peake Railway Bridge, Eagle Hawk dam	4x small fykes 2x large fykes 2x double wing fykes 3m larval seine 10m tow
Bore drain or spring e.g. One Mile Bore, Hawker Springs	3m larval seine 10m tow or Visual observation or 30cm dab net 1-2m tow

Catch per unit effort (CPUE) was calculated by first determining the catchability index of each net type by dividing the total catch per net type by the total catch for all net types for both surveys at all sites. Total effort for each site was calculated by multiplying the number of each type of net used at a site by its catchability index then summing the effort from each net type. Finally, CPUE was calculated as the total catch divided by the total effort.

Survey Results

Overall 40,694 fish, representing 9 species, were captured in the 2009/10 surveys (Table 2). This included 18,141 fish at 24 sites in November 2009 and 22,553 fish at 22 sites in April 2010. Of these, only one was an alien species (Gambusia). Gambusia was in low abundance in waterholes of the Neales and Peake, but reached high abundance in some springs and bore drains. Details of Gambusia distribution, abundance and management will be addressed separately in a later section of this report.

Spawning (presence of ripe adults) and recent recruitment (the presence of small juvenile size classes) were recorded for all species in the November survey (Table 2). Whilst recruitment was still identified for all species except barred grunter in April 2010, fish in ripe or spawning condition were not identified for any species. It should also be noted that as Gambusia give live birth, recruitment data might indicate continuation of spawning into April.

All species except for golden perch moved into new waterholes over the wet season compared to past distributions (McNeil and Schmarr 2009) (Table 2). Golden perch showed the opposite pattern and disappeared from a large number of waterholes, where they were caught prior to the wet season. This was a contraction of their range following previously recorded recolonisation throughout the catchment (McNeil and Schmarr 2009).

Catch per unit effort (Figure 2) indicated that densities of fish were often highest in relatively shallow bore drains, springs and pools where pest Gambusia were extremely abundant (with the exception of Baltacoodna). This may be due to either higher densities of fish or lower sampling efficiency of nets in relatively larger systems.

Whilst the majority of sites appear to have increased CPUE during the period of connectivity (Nov – April), four sites showed a reduction in CPUE (Figure 2). These four 'losing' sites included both ends of Algebuckina Waterhole, Baltacoodna Waterhole and North Freeling Springs, three of the most permanent waterholes sampled. Such a loss of fish following connectivity is consistent with these pools being 'source' populations supplying fish to other "gaining" waterholes. This suggests that these three waterholes are important refuge waterholes for supporting fish species through harsh periods and providing colonists to new sites following reconnectivity.

Table 2. Total number of fish captured at sites in the South Australian portion of the Lake Eyre Basin in November 2009 (shaded) and April 2010. Sites not surveyed denoted by dash (-), M denotes migration to the site, S denotes spawning observed, R denotes recruitment of juveniles within the site, † denotes fish dead or dying in waterhole.

Scientific Nomenclature	*Amniataba percoides*		*Chlamydogobius eremius*		*Craterocephalus eyresii*		*Gambusia holbrooki*		*Leiopotherapon unicolor*		*Macquaria ambigua*		*Melanotaenia splendida*		*Nematalosa erebi*		*Scortum barcoo*
Common Name	**Barred Grunter**		**Desert Goby**		**Lake Eyre Hardyhead**		**Gambusia**		**Spangled Perch**		**Golden Perch**		**Desert Rainbowfish**		**Bony Herring**		**Barcoo Grunter**
Sampling Date	Nov-09	Apr-10	Nov-09	Apr-10	Nov-09	Apr-10	Nov-09	Apr-10	Nov-09	Apr-10	Nov-09	Apr-10	Nov-09	Apr-10	Nov-09	Apr-10	Apr-10
Neales River catchment																	
Slate Hole									11	168 R			1	22	253	367 R	
Afghan Waterhole						1 M		1 M	77 R	147 R	2 M		21	273 R	119	540 R	
Angle Pole Waterhole									70 S	284 R			29 S	101 R	35	629 R	
Shepherds Waterhole		-		-		-		-	53 S,R	-	2 M	-	13 S	-	29 S	-	-
Hookeys Waterhole								4 M	10 S	613 R	3 M		27	1638 R	129	367 R	
Mathiesons Waterhole						1 M			12 R	39 R	3 M		9 R	18	243 R	317	
Stewarts Waterhole		2 M							15 R	76 R				82 M	7 R	131 R	
Cramps/South Stewarts Waterhole					6 M			4 M	62 R	12 R			151 R	233	763 R	171 R	
Ockenden Creek	-		-		-		-		-	7 M	-		-	3 M	-	2 M	
Hann Creek	-		-		-		-		-		-		-		-	7 M	
Neales Crossing	-	3 M	-	1 M	-	37 M	-		-	39 R	-		-	625 R	-	56 R	
Algebuckina Waterhole	483 S,R	242	5		159 R		25	1	237 R	196 R	12	24 R	350 R	224	4109 R	1177 R	1 M?
Eaglehawk dam		-		-		-	1	-	11 S	-		-	20 S	-	445 R	-	-
Cliff Waterhole	4 S	-		-		-	29	-	67 S	-	92 S,R	-	5	-	212 R	-	-
South Cliff Waterhole		1 M	1				45 R		16 S	48	5 S	1 R	56 S	5	143 R	191	
Road to Cliff	-		-	1 M	-		-	1789 R	-	1 M	-		-		-	4 M	
Road to Tardetakarinna	-		-		-		-	10 M	-	1 M	-		-	1 M	-	30 M	
Not Tardetakarinna		-	4 †	-	77 †	-		-		-		-		-		-	-

Table 2 Continued….

Common Name	Barred Grunter		Desert Goby		Lake Eyre Hardyhead		Gambusia		Spangled Perch		Golden Perch		Desert Rainbowfish		Bony Herring		Barcoo Grunter
Sample Date	Nov-09	Apr-10	Nov-09	Apr-10	Nov-09	Apr-10	Nov-09	Apr-10	Nov-09	Apr-10	Nov-09	Apr-10	Nov-09	Apr-10	Nov-09	Apr-10	Apr-10
Peake Creek catchment																	
Arkaringa Creek	-		-		-	1 M	-		-	35 M	-		-	11 M	-	8 M	
Lora Creek	-		-		-		-		-	144 M, S	-		-		-	14 M	
Peake Creek Railway Bridge		19 M	37 †	122	45 †	99		30 M		48 M				60 M		2437 M	
Baltacoodna Waterhole		5 M	155 R	10 R	1801 R	28 R		81 M	53	151 M				102 M	872 R	1522 R	
Warrarawoona Waterhole	-		-		-		-	2	-	12	-		-	92	-	105 R	
Springs and Bores																	
Old Nilpinna Station Spring		-	11 R	-		-		-		-		-		-		-	-
One Mile Bore				89			1000	4940								1	
North Freeling Spring			2261	560	901	27	2082	812									
North Freeling Spring 2	-		-		-		-	21	-		-		-		-		
Freeling Spring 1		-	50	-		-		-		-		-		-		-	-
Freeling Spring 2		-	20	-		-		-		-		-		-		-	-
Freeling Spring 3		-	10	-		-		-		-		-		-		-	-
Hawker Spring		-	8	-		-		-		-		-		-		-	-
Fountain Spring		-	10	-		-		-		-		-		-		-	-
Outside Springs		-		-		-	58	-		-		-		-		-	-
Ockenden Spring	-		-	5	-		-		-		-		-		-		

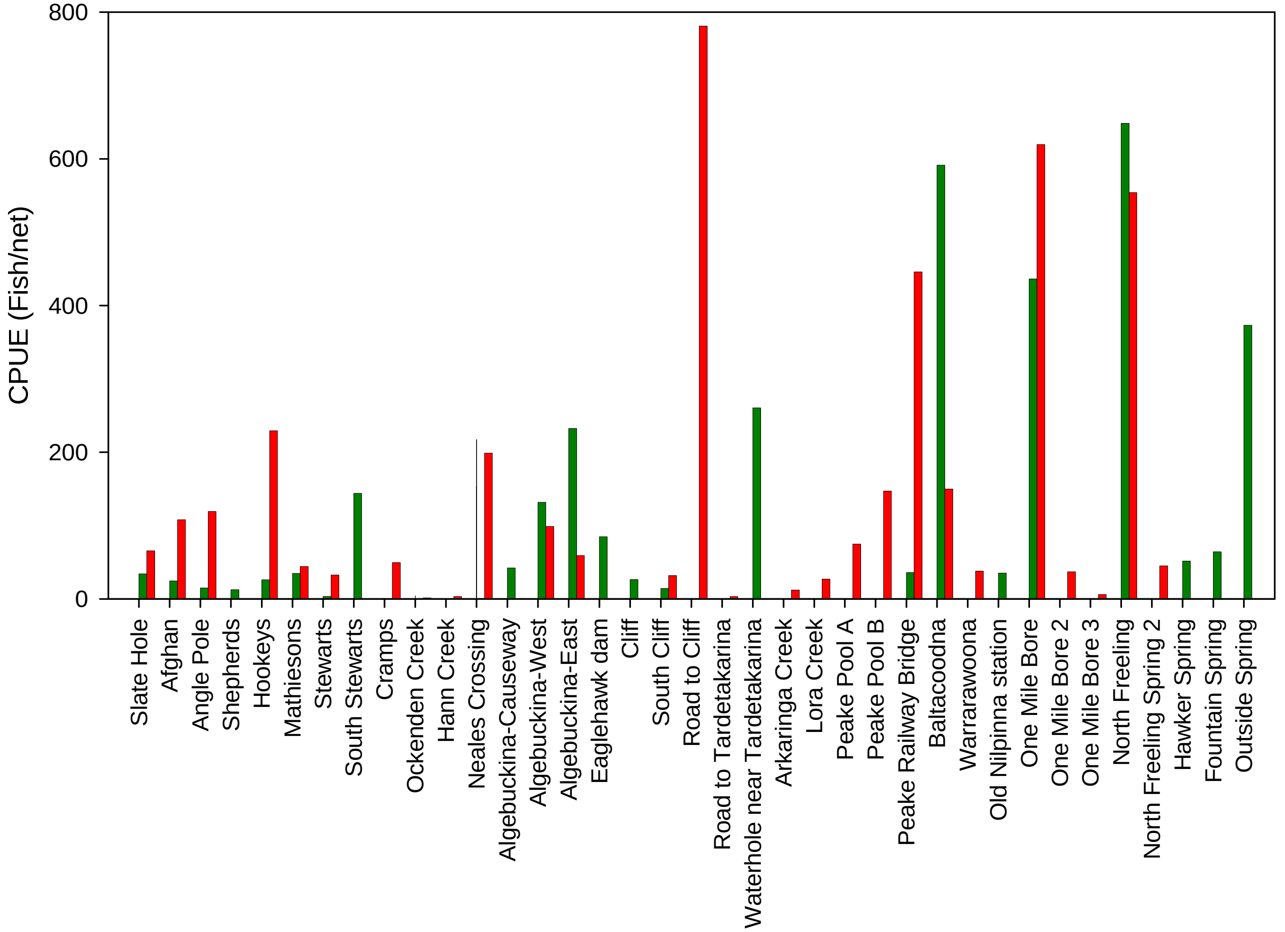

Figure 2. Catch per unit effort (CPUE) abundance data for waterholes in the Neales catchment. Green bars indicate Nov. 2009 survey and red bars April 2010. Not all sites were sampled in both seasons.

CATCHMENT-WIDE FISH SPECIES TRENDS

The following section provides demographic information for each of the fish species sampled. Patterns are described in relation to those observed in other studies (McNeil *et al.* 2008, McNeil and Schmarr 2009)

Barred Grunter

Prior to November 2009, the distribution of barred grunter was limited to Algebuckina waterhole. Following the return of flows to the Neales catchment, this species increased in numbers in a refuge waterhole by November 2009 and was subsequently found in higher numbers in new sites in April 2010 (Table 2). However, it was the slowest species to move into new habitats, with most other species reaching further upstream and earlier than barred grunter.

Desert Goby

Desert goby maintained stable populations in spring and saline habitats. The species did not expand its distribution throughout the survey period, instead persisting in high salinities in parts of the mid to lower Neales River and Peake Creek. Desert gobies may be most vulnerable to the impacts of Gambusia invasion. In sites where Gambusia were present, desert gobies were either absent or in very low abundance, but thriving in isolated spring habitats without Gambusia.

Lake Eyre Hardyhead

Much like desert gobies, Lake Eyre hardyhead were consistently found in stable spring and saline habitats, but small numbers also migrated upstream into the upper Neales River between the November and April surveys (Table 2). The population in North Freeling Spring also appeared to crash for an unknown reason, although the other species in that location also diminished in abundance.

Gambusia

Gambusia populations, previously confined to bore drains and springs in drier years, migrated throughout the entire catchment right up to the upper Neales River sites. They were also found in dense aggregations in isolated pools formed by floodwaters.

Spangled Perch

Spangled perch showed great resilience and moved to all suitable habitats since the end of the drought years. The only sites where they were not present were hypersaline, although; once those sites became fresh again, spangled perch were captured almost immediately.

Golden Perch

After the drought years, golden perch recruits were captured in the Neales River as far upstream as Afghan Waterhole. However, despite good flows, the population has receded back into Algebuckina Waterhole and the nearby South Cliff Waterhole (Table 2). Cliff Waterhole was inaccessible for sampling in April 2010, so it could not be confirmed whether the large number of golden perch captured in November 2009 persisted at this site in 2010.

Desert Rainbowfish

Along with spangled perch and bony herring, desert rainbowfish were amongst the most resilient species in this catchment. They were found throughout the Neales River in high abundances. Their strong resilience, high densities and ecological niche make them a likely competitor to pest Gambusia, and may be critical in preventing the spread of Gambusia throughout the catchment.

Bony Herring

Along with spangled perch, bony herring established populations throughout the Neales catchment prior to these surveys. They maintained those populations in the subsequent wetter period. Bony herring were the most abundant species at most sites, dominating overall fish biomass throughout the catchment.

Barcoo Grunter

The April 2010 survey was our first record of this species in the Neales catchment, although Barcoo grunter was collected at Algebuckina during AridFlo surveys (Costello *et al.* 2004). AridFlo surveys also noted the presence of a potential “hybrid” grunter species in Algebuckina waterhole (Costello *et al.* 2004), although the relationship between the current catches and the reported hybrid are not known. The Barcoo grunter appears to be extremely rare in the Neales catchment, although grunters are very common in other Lake Eyre catchments.

SITE SUMMARIES

Neales River

The total catch of all fish species in the Neales River is reported in Table 2. Spangled perch and bony herring were the most abundant species in the Neales River in November 2009 and April 2010, while desert rainbowfish appeared in large numbers in April 2010.

Algebuckina waterhole remains the epicentre of fish diversity, with species numbers declining with distance from this location both up and downstream. Downstream populations are dominated by salt tolerant species; desert gobies and Lake Eyre hardyhead. Upstream populations are dominated by bony herring, desert rainbowfish and spangled perch.

The discovery of a Barcoo grunter in Algebuckina in the April survey raises some questions about connectivity between the Neales catchment to the west of Lake Eyre and the Cooper and Warburton to the east. The grunter could also represent a hybrid species (Barcoo x Welch's grunter) – described in previous surveys in the Neales for the AridFlo project (Costello *et al.* 2004). More detailed anatomical and genetic data is required to clarify the existence of hybrid grunter species.

Slate Hole

Slate hole is a deep (>4m) dam carved out of a small anabranch on the floodplain and is believed to be permanent. Spangled perch, rainbowfish and bony herring were captured in both surveys at this site (Table 2). Size frequency analysis detected recruitment of spangled perch and bony herring before the April survey. The increase in rainbowfish numbers may have been due to migration from nearby waterholes.

Afghan Waterhole

Afghan Waterhole displayed an increase in richness of its fish assemblage between surveys. Despite the loss of one species (the golden perch, which had probably migrated upstream before the first survey), richness was offset by the gain of Lake Eyre hardyhead and Gambusia, which are likely to have migrated to the site between surveys (Table 2). Conditions between surveys had prompted recruitment of rainbowfish and bony herring, and the further recruitment of spangled perch.

Angle Pole Waterhole

Angle Pole Waterhole showed a pattern of spawning and recruitment for each species captured. Species spawned in November and recruited juveniles by the following April survey (Table 2).

Shepherds Waterhole

Shepherds Waterhole was only surveyed in November due to floodplain accessibility problems during the second survey. Nevertheless, each species sampled there showed signs of spawning except for golden perch, which was a recent arrival from downstream (Table 2).

Hookeys Waterhole

Hookeys Waterhole had low numbers of spangled perch, rainbowfish, bony herring, and golden perch for the November survey. By the April survey, large numbers of juveniles had recruited to the population, while golden perch had disappeared and small numbers of Gambusia had found their way to this upstream site (Table 2).

Mathiesons Waterhole

Mathiesons Waterhole bucked the trends of other waterholes, showing recruitment of spangled perch, rainbowfish and bony herring before the November survey, but only spangled perch had recruited new juveniles before the April survey (Table 2). As with other sites in the upper Neales River, golden perch disappeared between the two surveys after only just having moved into this reach prior to the November survey. One adult hardyhead was captured in April, likely a migrant from downstream

Stewarts Waterhole

Stewarts Waterhole was the upstream limit of barred grunter migration by April 2010. Spangled perch and bony herring showed steady recruitment throughout the survey period while rainbowfish reappeared in this waterhole in April after being absent in the November survey (Table 2). Golden perch and hardyhead were absent during the survey period after being captured in the May 2009 survey (McNeil and Schmarr 2009).

Cramps/South Stewarts Waterholes

This site was sampled at two separate but nearby waterholes. South Stewarts Waterhole – an anabranch off the main channel – was sampled in November due to inaccessibility across the floodplain, and Cramps waterhole was sampled in April. Spangled perch and bony herring both displayed recruitment preceding each survey, although changes in abundance cannot be attributed to migration or recruitment due to the different sites surveyed (Table 2). Nevertheless, the pattern of recent upstream Gambusia migration was repeated here (Table 2). Small numbers of adult hardyhead were captured during the November survey but not in April, while golden perch were not captured in either survey despite being captured in previous surveys (McNeil and Schmarr 2009).

Ockenden Creek

This site was only sampled in April. It contained adult spangled perch, rainbowfish and bony herring (Table 2). Given that this creek dries completely apart from the shallow Ockenden Spring, it is likely that these fish had migrated up-stream from the Neales with recent flows.

Hann Creek

This site, where the creek crosses the Oodnadatta track, was also only sampled in April and contained adult bony herring moving upstream against the flow (Table 2). Similar to Ockenden Creek, this creek dries quite quickly and the fish here had most likely migrated upstream from the Neales River. Vehicles caused some mortality as fish crossed the road in water rivulets and were run over or splashed out of water by the passing vehicles.

Neales Crossing

Again, this site was only sampled in April. It is an intermediate site between Algebuckina and the upper Neales sites and contained all the species present at the upper Neales sites in the April survey as well as low numbers of desert gobies (Table 2). Given that this site dries significantly to become a saline waterhole in drier times, apart from hardyhead and gobies, most of the fish here had probably recently migrated from either Algebuckina or the upper Neales sites.

Algebuckina Waterhole

The permanent freshwater refuge of Algebuckina Waterhole had the greatest diversity of species throughout the survey period. Most species showed signs of spawning and recruitment during the November survey, while only spangled perch, golden perch and bony herring had new recruits at the April survey (Table 2). In addition to the decreased reproductive activity, Algebuckina Waterhole was one of the few sites to record a drop in abundance between surveys, as indicated by the CPUE data (Figure 2). The April survey also recorded the first capture of Barcoo grunter after 4 years of sampling at this site (McNeil *et al.* 2008, McNeil and Schmarr 2009) although the species was recorded from Algebuckina in the past (Costelloe *et al.* 2004).

Eaglehawk Dam

Eaglehawk Dam is a shallow off-channel dam that receives input from high flows and a small tributary. It has no cover and very little habitat complexity. This site was only sampled in November and contained spangled perch, rainbowfish and bony herring, as well as a small number of Gambusia. All fish showed signs of spawning or recruitment.

Cliff Waterhole

Cliff Waterhole was only sampled in November due to floodplain inaccessibility in April. There was high diversity, with 6 species including Gambusia (Table 2). All native species except for rainbowfish showed signs of spawning and recent recruitment. Golden perch recruits were captured in high numbers here.

South Cliff Waterhole

Nearby to the Cliff Waterhole, South Cliff Waterhole is on the opposite side of the floodplain. Spangled perch, golden perch, rainbowfish and bony herring were captured in both surveys, showing signs of spawning and recruitment in November, while only golden perch had new recruits in April (Table 2). Low numbers of barred grunter had moved down to this site by April, while the low numbers of desert gobies from November were not encountered in April (Table 2). High numbers of Gambusia in November did not reappear in the April catch (Table 2).

Road to Cliff

This was an opportunistic site sampled while attempting to access Cliff Waterhole in April. Small numbers of migrating desert goby, spangled perch and bony herring had been trapped on the floodplain track downstream from Cliff and South Cliff waterholes (Table 2). In these shallow still pools, large numbers of juvenile Gambusia had built up very quickly.

Road to Tardetakerinna

Similar to the previous site, this survey was taken whilst attempting access to Tardetakerinna Waterhole in April. Small numbers of Gambusia, spangled perch, rainbowfish and bony herring had been trapped in pools on the track whilst moving during recent flows (Table 2).

Waterhole near Tardetakerinna

This waterhole was shallow and hypersaline (124ppt). The fish observed here were either all dead (gobies), or mostly dead or in poor condition (Lake Eyre hardyhead).

Peake Creek

Due to good rains over the 2009/2010 period, Peake Creek has experienced several flows resulting in the reestablishment of several species at sites in this area. The flows have provided longer-term freshwater conditions compared to the saline conditions normally observed in this system. As a result, more freshwater tolerant species such as spangled perch, bony herring, desert rainbowfish and Gambusia have exploded in numbers in addition to the resident populations of gobies and hardyhead. Baltacoodna and Warrarawoona Waterholes were identified as large refugia that may play important roles in the maintenance of healthy fish populations and allowing connectivity between the Neales and upper Peake sites.

Arkaringa and Lora Creek

These samples were opportunistically taken during the April survey. Both Arkaringa and Lora Creeks were flowing at the time from recent rains in the upper catchment. At Arkaringa Creek, small numbers of hardyhead, spangled perch, rainbowfish and bony herring were captured, while at Lora Creek, only spangled perch and bony herring were captured, but some of the spangled perch were observed in spawning condition (Table 2). Net data indicates that the fish were captured moving downstream, although it was unclear whether this was due to migration or fish seeking cover.

Peake Waterhole

We observed significant contrast between the November and April samples at this site. In November, this site was hypersaline (128ppt) with dead or dying desert gobies and hardyhead (Table 2). Flows prior to and during the April survey transformed the site into a freshwater habitat with a deep halocline. Barred grunter, Gambusia, spangled perch, rainbowfish and bony herring had all moved into this site joining the replenished populations of desert goby and hardyhead (Table 2). Bony herring were present in the highest density of any site observed during both surveys.

Baltacoodna Waterhole

In November, Baltacoodna Waterhole was slightly saline (ca. 5ppt) and contained large numbers of desert goby, Lake Eyre hardyhead, spangled perch and bony herring, with all except spangled perch showing recent recruitment (Table 2). The site was fresher in April and barred grunter, Gambusia, rainbowfish and large adult spangled perch had migrated into the site (Table 2). This was another site where the CPUE dropped between surveys (Figure 2) due to the movement of many of the newly recruited juveniles to other sites in the river.

Warrarawoona Waterhole

This freshwater site was only sampled in the April survey. Small numbers of Gambusia and spangled perch along with larger numbers of rainbowfish and bony herring were captured here. Only bony herring showed recent recruitment.

Springs and Bores

Several springs and open bore drains were sampled during these surveys. The most consistent observations were that where Gambusia were present in springs or bore drains, desert gobies were absent or in very low abundance and *vice versa*. It was also discovered that at least two bore drains – One Mile Bore and Big Blyth Bore – and the large spring complex at North Freeling are serving as “mega refugia” for Gambusia and possibly acting as source populations for the rest of the Neales catchment.

The fish assemblage at North Freeling did appear to have reached equilibrium between the three species present there, with stable numbers of Lake Eyre hardyhead, desert goby and Gambusia. However, other native species were not present despite the site being suitable habitat for species such as rainbowfish, spangled perch and bony herring. This spring complex is also less connected to the main channel of Peake Creek and may pose less of a risk as a source population of Gambusia than the open bore drains.

Springs

Old Nilpinna Springs, Freeling Springs, Hawker Springs, Fountain Spring and Outside Springs (above left), were all Great Artesian Basin (GAB) mound springs surveyed in November. Apart from Outside Springs, each of these mound springs was home to desert gobies and no other fish species.

Outside Springs only had Gambusia. North Freeling Springs (above right) were surveyed in November and again in April, and contained a large stable population of desert gobies, Lake Eyre hardyhead and Gambusia. North Freeling showed a decrease in total CPUE largely due to the decrease in hardyhead abundance (Table 2). Ockenden Spring was only sampled in April and contained a small population of desert gobies despite the nearby Ockenden Creek site having spangled perch, rainbowfish and bony herring (Table 2).

Bore Drains

One Mile Bore (above left) and Big Blyth Bore (above right) were both large open bore drains with stable shallow-water habitats and large amounts of emergent macrophyte cover. Both contained extremely large populations of Gambusia and relatively small numbers of desert gobies, bony herring (One Mile) and spangled perch (Big Blyth) (Table 2). A more detailed survey of Big Blyth Bore was conducted on a separate occasion in November 2010 following the visual observation of large Gambusia numbers in November 2009.

CLIMATIC DRIVERS OF FISH COMMUNITY

Background: Recovery from Drought

The results of the current study have continued to indicate the ongoing recovery of fish populations following the severe drought conditions of 2006/07, reported in McNeil *et al.* (2008) and McNeil and Schmarr (2009). These patterns are based on a limited number of sites that have been sampled continuously before and after wet seasons over the past three years, which constitute an important long-term data set. The distribution of species across the catchment has changed dramatically. In 2007, few waterholes contained more than one fish species, with only Algebuckina Waterhole containing more than two species and the majority of fish richness in the catchment. Since then, regular periods of seasonal connectivity have facilitated the expansion of fish species throughout the catchment with 4+ species present in all long term sites by April 2010 (Figure 3).

This can be seen in the steadily increasing species richness throughout the catchment over the long-term survey period. These patterns show the gradual recolonisation of the catchment, firstly by rapid recolonisers such as spangled perch and bony herring, followed by golden perch and rainbowfish, with slower colonisers, Lake Eyre hardyhead and barred grunter only just beginning to recolonise after three years of repeated in-channel connectivity. Of particular interest are the desert goby and the newly discovered Barcoo grunter, which remain around refuge habitats and have not recolonised following drought. Also of interest is golden perch which, although quick to recolonise the catchment, was not found outside of the Algebuckina refuge during the most recent survey, suggesting either large scale mortality, or contraction back to refugia.

The patterns at Peake Waterhole clearly demonstrate the changing nature of refugia in the Neales catchment with only desert goby and Lake Eyre hardyhead thriving in the saline conditions during drought with sporadic appearances of Gambusia and spangled perch following re-connectivity. After three years of connectivity, however, the most recent survey saw all but one species residing in the waterhole, matching the species richness of the Algebuckina refuge at that time. Species abundances at these sites also have generally increased over the same period (SARDI unpublished data).

In each successive survey, large numbers of new recruits were observed at most sites, indicating that recolonising fish established self-sustaining populations at those locations. A contradictory finding from these surveys was the disappearance of golden perch from the upstream waterholes that they had recolonised in the earlier stages of drought recovery. Golden perch now appear to be confined to Algebuckina Waterhole and the population consists mainly of large adults and not the smaller juveniles observed in 2008/09.

These results have provided an insight into the recovery of arid fish populations following harsh climatic episodes and the continued monitoring of sites with long term data is highly recommended to support the development of conceptual models that accurately capture the expanding and contracting “boom-bust’ nature of LEB rivers (Balcombe *et al.* 2007, Balcombe and Arthington 2009), driven by highly variable rainfall patterns (Armstrong 1990). This provides for informed and adaptive management strategies in maintaining the sustainability and viability of Lake Eyre Basin’s fish communities.

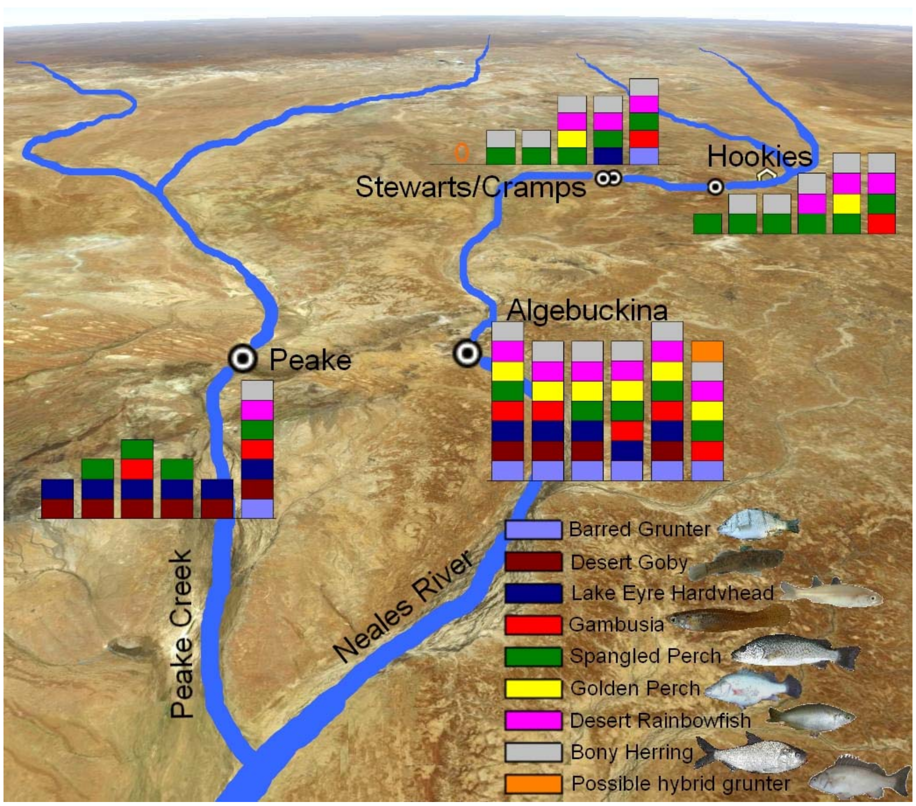

Figure 3. Catchment-scale recovery from drought. The distribution of fish species in the six surveys conducted in the Neales Catchment from December 2007 - April 2010. Species colours correspond to those in the legend. Surveys in order from left to right are Dec 2007, May 2008, Dec 2008, May 2009, Nov 2009 and April 2010.

Aquatic Refugia in the Neales catchment

Long term data collected from a small subset of waterholes has provided insight into the nature of fish communities in the Neales River, however, a key aim of the current project was to identify the location and role of various aquatic refuge waterholes throughout the Neales catchment. This study constitutes the most comprehensive survey of waterholes undertaken in the catchment simultaneously and provides a unique insight into the relationship between climate and fish community and catchment refugia. This is optimised when considered in conjunction with data from previous surveys under Aridflo, (Costelloe *et al.* 2004, Janet Pritchard unpublished data) LEBRA and SAAL NRM Board monitoring projects (McNeil *et al.* 2008, McNeil and Schmarr 2009).

Whilst permanent waterholes were considered rare in the arid Neales catchment (Costello *et al.* 2004) surveys undertaken during the Millennium drought in 2007/08 revealed the extreme value of very few refugia in protecting native fish biodiversity in the catchment. Surveys of the more permanent waterholes (Janet Pritchard pers. comm.) revealed Algebuckina to be the sole refuge in the Neales catchment for all but one of the local native fish species (McNeil *et al.* 2008). In the Peake Creek, saline waterholes persisted but contained only two species, the Lake Eyre hardyhead and desert Goby, both considered extremely tolerant of saline conditions (Wager and Unmack 2000).

Drought surveys, therefore, revealed at least two distinct refuge types (based on the classification of Robson *et al.* 2008) operating within the catchment. Algebuckina serves as the principal “Ark” type refuge, a habitat where catchment species are able to avoid the impacts of climatic disturbance during drought and from which they are able to rebuild catchment populations once disturbance eases. Local information (Travis Gotch, SAAL NRMB pers. comm.) also suggested Baltacoodna waterhole may also serve as an Ark refuge on the Peake creek, although this site was not included in any previous fish surveys. Peake and other saline waterholes, common to the far downstream reaches of the catchment, also serve as “Polo Club” refugia where only a select few species are able to persist due to their specialised tolerance to the hypersaline conditions prevalent in those refugia.

During recovery from drought, McNeil and Schmarr 2009 observed fish species recolonising the broader catchment from these refuges and rebuilding population structure following brief periods of seasonal within-channel connectivity. These authors suggest that these refuge waterholes serve as source populations for the recolonisation of the broader catchment during recovery from drought.

The role of climatic refugia, however, were proposed to vary over time and space depending on the predominant climatic conditions being more important for the persistence of fish species during periods of climatic harshness, and potentially less important for survival during more benign climatic conditions. An interesting parallel can be drawn with overwintering habitat in Arctic areas, where available habitat can shrink to a mere 5% of what is available during summer months (Reynolds 1997). Although isolated overwintering habitats (usually in areas of upwelling) are disproportionately important to the overall persistence of fish species in these relatively harsh environs, they are unproductive during the milder summer months (Reynolds 1997).

Survival or “resistance” to drought must be complemented with recovery during favourable climatic periods to enable rebuilding of populations and resilience of species assemblages to future disturbances (McNeil *et al.* 2011). Post-drought surveys revealed distinct differences among species in recovery or resilience building. Whilst spangled perch and bony herring rapidly recolonised the catchment and restored multi-generational populations across the catchment following in-channel connectivity, other species such as desert goby and barred grunter remained in refuge habitats and, although resistant enough to survive drought, appeared less resilient once drought eased (McNeil and Schmarr 2009). The importance of drought refugia was maintained for those less tolerant species, even though other waterholes had already become equally productive habitats for highly resilient species.

In arid zones, therefore, the temporal importance of refugia can vary among species due to differential resistance and resilience traits. The conditions within refugia rely on stochastic factors such as localised rainfall and isolated tributary flows, highly characteristic of the storm-driven and patchy arid rainfall system. Therefore, the temporal nature of waterholes in providing adequate habitat values varies greatly.

The current survey, spanning the 2009/10 wet season, therefore, provided an opportunity to examine the role that refugia play during increasing water availability and connectivity, and to track potential expansion of slow colonising species such as barred grunter, desert goby and Gambusia. Whilst the nature of refugia, survival and recolonisation has been informed by recent surveys, these have focussed on relatively few sites within the Neales catchment (McNeil and Schmarr 2009). Due to limitations of budget and access, previous surveys targeted the most permanent and accessible habitats that were likely to provide the best long-term monitoring data for the LEBRA fish trajectory and monitoring program (Balcombe and McNeil 2008, Humphries *et al.* 2007), based largely on Aridflo sites.

This limitation affected the reliability of assumptions regarding the importance of Algebuckina Waterhole as the sole Ark refuge for fish biodiversity in the Neales catchment. Although Ark refugia like Algebuckina Waterhole may not have been present elsewhere in the catchment, the presence of other permanent waterholes downstream of Algebuckina seemed likely.

This notion is supported by the sporadic appearance and disappearance of Gambusia from Peake and Algebuckina waterholes from season to season, suggesting a nearby source for recolonisation. Identifying waterholes throughout the entire catchment was believed essential for establishing a sound understanding of the ecology of fish populations in the catchment, particularly if the presence of permanent refugia existed outside the initial survey areas.

The presence of several known bore drains, GAB mound springs and saline waterholes downstream of Algebuckina also suggested that refugia were likely to exist outside of the initial survey sites, at least for some species such as desert goby, known to inhabit GAB springs and saline pools (along with Lake Eyre hardyhead) and Gambusia, which have an affinity for shallow fresh habitats such as bore drains.

The current project was designed to survey as many waterholes as possible throughout the Neales and Peake catchments and to capture as broad a range of habitats as possible, including GAB springs, bore drains, constructed dams, permanent and temporary pools and tributaries. This comprehensive spatial survey of the Neales catchment will provide a more detailed assessment of the distribution and role of aquatic refugia in the catchment and enable a more reliable assessment of fish community data collected during more spatially limited surveys in the past.

Waterhole Classifications: Refuge Typologies

An important aspect of this study was to pre-classify waterholes into distinct refuge types following the protocols of Robson (2008) and McNeil *et al.* (2011). As many of the waterholes were surveyed for the first time during this study, classifications were largely based on multi-disciplinary expert opinion incorporating geomorphic (Dr Gresley Wakelin-King) Landscape (Professor Gini Lee – Melbourne University), Hydrological (Dr Justin Costelloe – Melbourne University) expertise incorporating physical habitat (e.g. depth, size, water quality) and historical data for the Neales catchment (Dr Dale McNeil, David Schmarr – SARDI). In the following section, the various refuge typologies identified will be discussed in relation to determining factors and fish survey data outlined in the previous section. The various classifications used and waterholes to which they were applied were:

ARK refugia: Permanent waterholes that provide refuge for all species throughout severe climatic disturbances.

Algebuckina Waterhole remains the sole proven Ark refuge where all species are likely to survive extreme drought. The current study therefore supports the role of Algebuckina as the principal waterhole for fish biodiversity in the Neales catchment. However, in Peake Creek, Baltacoodna Waterhole also possessed a large suite of species during both sampling times. Its large size and similar geomorphic location to that of Algebuckina Waterhole in Neales River (i.e. directly downstream of the dissection of the Peake Denison Range), indicate that this waterhole may serve as an important Ark type refuge. The absence of historical data adds caution to its classification as an Ark refuge, however, and further investigation should be directed towards assessing its permanence and significance to biodiversity in the catchment. For the current study, however, Baltacoodna has been classified as an Ark refuge.

Figure 4. Algebuckina Waterhole from the air looking upstream from the end of the permanent reach towards the Old Ghan rail bridge.

Figure 5. Algebuckina Waterhole in 2007 with a strong growth of flowering Myriophyllum. At the time, this was the sole refuge waterhole for all but one fish species in the Neales River.

Saline Polo Clubs: Environmentally harsh habitats (often permanent) that are intolerable to all but a few highly adapted species

Saline refugia (Figures 6a & b) are largely defined by the geomorphic and hydrological characteristics of the catchment and are present throughout a number of reaches where saline water appears to accumulate catchment salts back into the river channel as surface water (Armstrong 1990, Williams 1990, Costello *et al.* 2004, Costello 2010).

As a result, saline reaches exist between Algebuckina and Stewarts/Cramps Waterholes proximal to the junction of Ockenden Springs, downstream of the junction of the Neales River and Peake Creek and in the Peake Creek upstream of the bisection of the Peake-Denison Ranges (see Figure 1). The full downstream extent of saline refugia were not possible to survey due to weather-related access problems, but aerial surveys revealed numerous saline waterholes (Figure 7) that extend as far downstream as the Neales Mouth at Lake Eyre.

Figure 6. Saline Polo Club Refugia such as Peake Crossing (A) protect a select few species (desert goby and Lake Eyre hardyhead) that are able to tolerate the hypersaline conditions during harsh times. If salinity concentrates too high as in this waterhole near Tardetakarinna (B) not only single species (Lake Eyre hardyhead), but only small size classes can survive (120ppt) during 2009/10. Note salt scalds on banks.

Desert gobies and Lake Eyre hardyhead are the permanent beneficiaries of saline refuge habitats in the Neales catchment and both maintain high abundances in salinities of <80ppt. Hardyhead persist in salinities of ~120ppt, beyond which these habitats become fishless (the clear and shallow nature of hypersaline habitats made the assessment of fish presence possible through visual means). Spangled perch and bony herring moved into saline habitats during periods of flow in relatively low numbers. Once conditions improved, Gambusia moved into these refugia, but were not present during harsh times.

Figure 7. Saline Waterholes extending throughout the lower Channel of the Neales towards Lake Eyre. Waterholes under ~120ppt could contain Lake Eyre hardyhead, whilst those <70ppt could also possess desert gobies.

Great Artesian Basin (GAB) Springs: Permanent spring fed wetlands receiving groundwater inputs from the GAB.

GAB springs (Figures 8A, B & C) are permanent but shallow habitats that are infrequently connected to the main river channels during large flood events. Historically, desert gobies were the sole refuge species in these springs; however, in recent decades Gambusia have been introduced either intentionally or during periods of connection to riverine surface waters. Gambusia now dominate all low lying GAB springs where connectivity is likely, whilst gobies have disappeared from these habitats, persisting in slightly elevated spring groups such as Hawker, Freeling, Ockenden and Nilpinna.

As such, GAB springs form separate refugia for Gambusia (low-lying) or desert goby (elevated). The exception is the North Freeling Spring (Figure 9), which, although maintained by a permanent inflow of GAB water, lies in the valley floor and is regularly inundated by high channel flows. Whilst still dominated by Gambusia, this spring also possessed large populations of desert goby and Lake Eyre hardyhead, most likely as a function of higher than normal habitat complexity and diversity and greater depth than other GAB springs.

Figure 8. Great Artesian Basin (GAB) springs in the Neales catchment provide aquatic refugia in a vast arid landscape (A) and provide shallow water refuge habitats (B & C) for desert goby and pest Gambusia.

Figure 9. North Freeling Spring against the Peake-Denison Range, a unique permanent spring fed pool close to the channel floor.

Bore Drains: Permanent, spring fed aquatic habitats derived from drilling into the Great Artesian Basin Aquifers.

Constructed bore drains (Figure 10) are present throughout the Neales catchment, with major drains sampled at 1 Mile Bore (Nilpinna) and Big Blyth (Peake). Flowing bore drains were either fishless (Milne Spring) or were dominated by Gambusia, with a small number of spangled perch observed around the vent of Big Blyth Bore.

Otherwise, bore drains contained extremely high densities of the pest Gambusia for which these shallow, permanent and stable habitats appear to be the key refuge type in the Neales catchment. Consequently, bore drains are extremely poor habitats for native fish and therefore, capping of flowing bores is likely to have net benefits in terms of controlling pest fish with minimal impact on native fish ecology.

Figure 10. Big Blyth bore drain showing shallow habitat (>15mm) dominated by very large densities of pest Gambusia.

Disco Refugia: Non-permanent waterholes that operate as permanent aquatic habitats during wetter seasons, but dry up completely during harsh climatic periods.

The highly variable nature of the climate and hydrology of the Neales catchment gives rise to a very high degree of spatial and temporal variability in the distribution and nature of aquatic habitats, including refugia. As a result, a large number of significant refuge waterholes (Figure 11) throughout the Neales River catchments are too permanent to be considered "stepping stone" refuges (Robson *et al.* 2008) and can persist for many years, depending on climatic and rainfall conditions. Locals often refer to these waterholes in terms of their persistence without rainfall, e.g., an eight month waterhole will persist for that long but in periods of significant rainfall or flow, may remain full for many years, or dry up completely.

Figure 11. Disco waterholes such as these are high quality aquatic habitats but dry up after extended periods without flow. They are critical for fish to rebuilt resilient populations following dry periods.

During the current project, the term *Disco* refugia was adopted for these waterholes, as they are most certainly significant refugia at local reach scales and provide refuge for aquatic biota throughout the dry season when interconnecting reaches become completely dry. During the drought of 2006/07, all Disco waterholes sampled were either dry or were fishless and filled by non-connecting local rainfall (McNeil *et al*. 2009).

The term Disco is derived from their role during wetter climatic phases where fish surviving in Ark refugia are able to recolonise formerly dry catchment areas. During less severe, seasonal drought, disco waterholes become important refugia in which a range of species is able to access resources and begin the task of re-building populations to pre-disturbance levels. Disco refugia are therefore critically important habitats for the building of population resilience following drought disturbance.

The current project has found that more rapidly recolonising species, in particular, spangled perch, bony herring, golden perch and desert rainbowfish were abundant throughout disco refuge sites during the pre-flow survey in November 2009. Length frequency data show these sites predominantly occupied by smaller size classes of fish and, therefore, represent an important platform for recruitment, a key aspect of re-building resilience following disturbance. These habitats are somewhat fleeting in nature - popping up for a few years at a time - and provide an excellent place for fish to rear, breed, and rebuild population levels while the good times last - somewhat analogous to the role of discos for young singles.

The temporal nature of the importance of Disco refugia is therefore distinct from that of Ark refugia. Arks become critical during peak disturbance levels to facilitate the survival (and, therefore, convey *Resistance* following Holling 1973 and Wu and Loucks 1995) to fish species. Disco habitats, however, are generally dry during these periods and instead become important following periods of drought as places where fish are able to rebuild populations that are able to survive future disturbances such as drought. Therefore, the number of disco refugia functioning within a catchment will increase following drought, and decline once the climatic cycle moves towards drought conditions, a common and repeated cyclical occurrence in arid zone rivers such as the Neales. The distribution of the various refuge types across the Neales catchment is mapped for all sites in Figure 13.

Stepping Stones: Temporary habitats that serve as important pathways for colonisation during flows.

Stepping stone habitats were predominantly surveyed where ephemeral tributaries or river sections were transected by roads and tracks and as a result were sampled opportunistically. As periodically inundated waterways, they provide fish passage but are not of value as long term refuge habitats. For example, sampling at Ockenden creek revealed three species of rapid colonists (bony herring, spangled perch and desert rainbowfish) when flowing, but a repeat survey a week later, after flow had ceased, failed to locate any fish whatsoever within isolated remnant pools. This highlights the temporary and transient nature of these sites as fish habitats.

The primary stepping stone sites sampled were in Lora and Arkaringa Creeks and on tracks crossing the Peake and Neales on the way to Tardetakarinna and Cliff waterholes. There were only very low numbers of species captured at these sites, representing local migrational patterns. Catches in the larger tributaries at Lora, Arckaringa, Hann and Ockenden creeks consisted of some or all of the rapid colonisers, bony herring, spangled perch and desert rainbowfish. The road crossing near Tardetakarinna was unique in possessing large numbers of Gambusia, which could represent migrating colonists from permanent refuge habitats at Big Blyth bore and other strongholds for this species around the lower Peake and Neales junction. Opportunities for colonisation are few in these arid catchments and stepping stone habitats form a significant function in fish movement and migration.

There is absolutely no research conducted on species movements in the entire Lake Eyre Basin and this field of research deserves significant attention. It is likely that during periods of inundation, the characteristics and patterns of connectivity across stepping stone habitats will be found to be a key aspect of species persistence in the arid part of the Lake Eyre Basin.

The identification of pathways between stable refuges for Gambusia in the Peake area and critical refuges for native fish such as Algebuckina upstream in the Neales is important for Gambusia control management, as the prevention of colonisation between refuge and main channel habitats during connectivity may be a key aspect of Gambusia control. These sites are therefore important hydrologically to understand the conditions under which pest colonisation movements may occur successfully, or be prevented.

Farm Dams: Constructed waterholes, usually harvesting floodplain or channel flows. Varying degrees of permanence.

Dams (Figure 12) were generally created on the edge of main riverbeds, where natural channels had been engineered to capture and hold standing water over clay substrates. The resulting structures generally have higher permanence than natural waterholes on the adjacent channel area and are predominantly fresh, even when nearby river channels are largely saline; they are recharged during high flows only and not by saline catchment seepages. The fauna of farm dams sampled are representative of natural Disco-type refugia throughout the catchment and can be considered as human constructed Disco-type refugia.

Figure 12. Eaglehawk Dam on the edge of the highly braided Neales Channel is fed by a small channel in the middle-left of the picture.

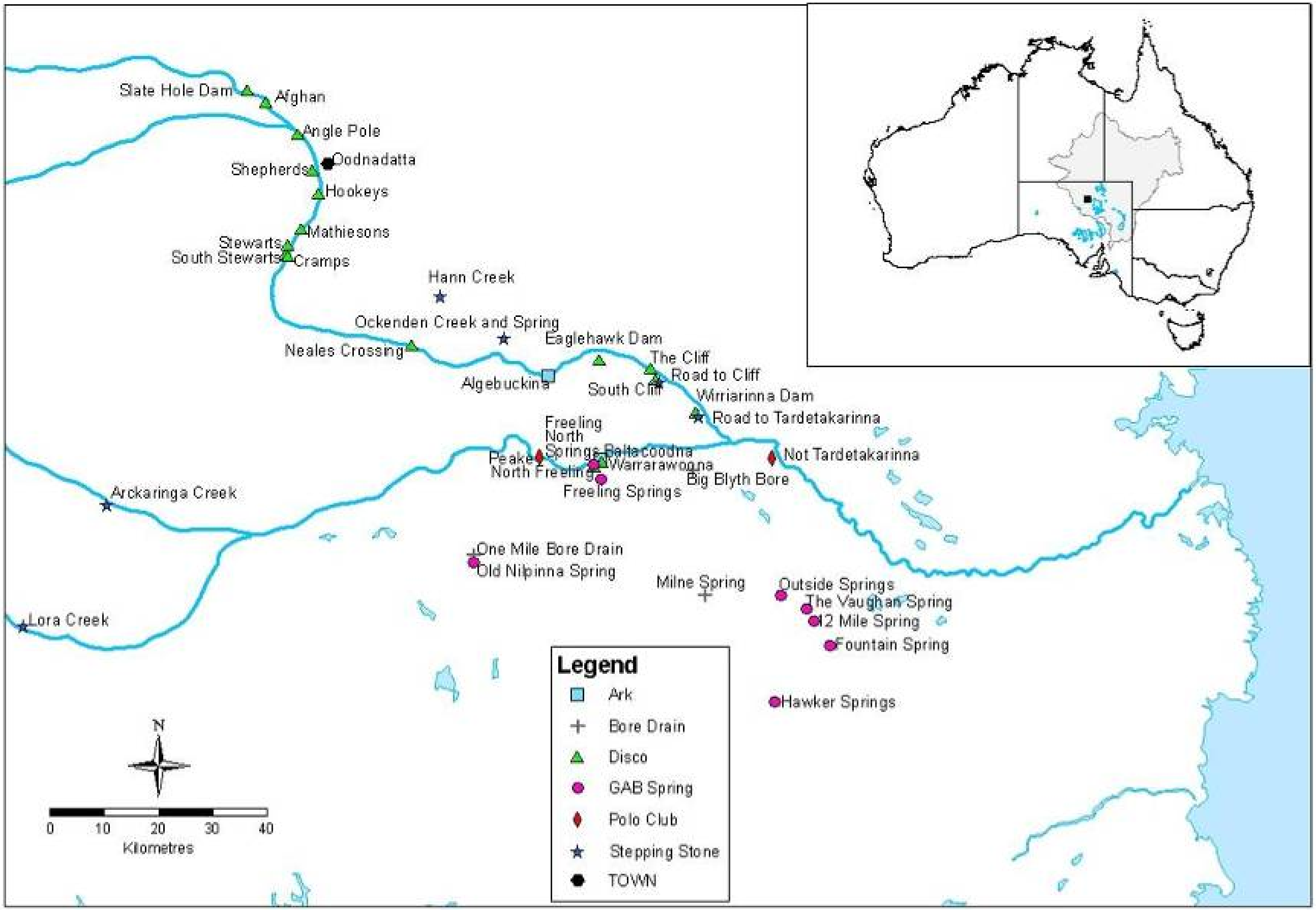

Figure 13. Distribution of refuge types throughout the Neales catchment.

TESTING REFUGE CLASSIFICATIONS

Classification of habitats into refuge types were made at the beginning of surveys to ensure that a broad range of waterholes were sampled, and that the refuge classifications of Robson *et al.* (2008) could be applied in relation to fish habitats in the Neales river. A formal appraisal of the usefulness of this classification system has not yet been conducted, but is potentially useful in systems such as the Neales where climatic driven variability is highly influential in driving ecosystem dynamics. Classifications were made based on landscape, geomorphic, and hydrological variables. Classifications were made by a multi-disciplinary "expert panel" consisting of hydrological, geomorphic and biological scientists within the broader project team using available published and unpublished data as well as expert opinion – based on the details of the Robson *et al.* typology.

The distinct hypotheses that can be tested regarding these classifications include

- Ark refuges should possess almost complete suites of species for the catchment, although migrations out of these sites following recent connectivity may negate this.
- Disco refuges should contain rapidly colonizing species such as bony herring, spangled perch and desert rainbowfish, but also an increasing number of additional species as slower colonizers (Barred grunter, Lake Eyre hardyhead, Gambusia, desert goby) are able to spread throughout the catchment (under extended periods of connectivity)
- Climatic drying should lead to the disappearance of Disco refuges and the contraction of available habitat back to ark refuges during increasing drought intensity (not likely to be observed for a number of years given the currently wet climatic regime).
- Polo Club refuges are likely to show increasing species diversity but only under scenarios of improved water quality. Sites that maintain hypersaline or other harsh habitat characteristics should continue to be inhabited by only a few highly tolerant species (i.e. Lake Eyre hardy head and desert goby).

- Recent increases in rainfall and flow should provide an opportunity to sample stepping stone habitats where it is predicted that rapid colonists will dominate the transient fish communities at these hydrologically flashy sites.
- Predictions that can be made around bores and springs are more difficult as these sites may contain a range of species tolerant of shallow still water conditions although historical data suggests that these will be dominated by desert goby and Gambusia (Morton *et al.* 1995).
- As increasing connectivity and flow continues following recent drought disturbance, it is predicted that waterholes across the catchment should exhibit increasingly homogenous assemblages of fish species as resilience building processes of colonisation and population growth/expansion continue. In this way, pre-determined estimates of refuge typologies based on drought patterns may deteriorate during current conditions but are predicted to re-appear again following future declines in catchment rainfall and flow.

Methods

Fish abundance matrices were created for each species and site for all sites sampled. Hierarchical Cluster Analysis was carried out using Bray-Curtis similarity scores from Fourth root transformed (to reduce the influence of highly abundant species on subsequent analyses) catch per unit effort (CPUE) data. Cluster analysis was performed separately for each of the two sampling trips (spring 2009 and autumn 2010) to identify the relationship between fish community data and refuge classification. Cluster groups were identified using SIMPER analysis at a 5% significance level. Refuge classifications for *Ark*, *Disco*, *Polo Club*, and *Stepping Stone* were used for riverine sites, whilst *springs* and *Bore Drain* definitions were used for GAB-fed habitats. PERMANOVA analyses were conducted for each season to determine whether statistically significant differences existed between the community patterns across refuge types. A separate Hierarchical Cluster Analysis was carried out for the combined data using the same methodology as the seasonal analyses to investigate the temporal aspects of refuge types. This combined matrix was also used to perform a Principal Components Analysis (PCA) based on Euclidean Distance (to allow the expression of shared 0 or absence data to influence similarity). PCA was restricted to 2 principal components. All analyses were carried out using PRIMER analysis software.

Results

Spring 2009

Cluster and SIMPROF Analysis of spring 2009 data (Figure 14) revealed a statistically significant distinction of fish assemblage and abundance structure across pre-determined refuge types (PERMANOVA (df=4) F=15.439, P<0.001).

Pairwise comparisons across refuge groups identified that significant differences (at the 0.05 significance level) existed between most refuge types with the exception of Ark/Polo Club and Spring/Polo Club (Table 3).

Table 3. Pairwise Permanova results for refuge types in spring 2009. Blue values (bottom left half of matrix) are average similarity scores whilst bold black values (upper right) are probability scores. Significantly different comparisons are marked with *.

Refuge Types	Disco	Ark	Polo Club	Spring	Bore
Disco	73.635	**0.002***	**0.014***	**0.001***	**0.001***
Ark	47.749	56.419	**0.063**	**0.008***	**0.028***
Polo Club	15.206	37.169	87.542	**0.19**	**0.004***
Spring	16.635	22.456	54.265	63.184	**0.013***
Bore	16.485	12.14	15.198	29.309	88.17

Simper Analysis (Appendix 1) revealed that these non-significant refuge types were characterised by similar species; with Ark/Polo Club both influenced strongly by Lake Eyre hardyhead and desert goby and Spring/Polo Club both influenced strongly by desert goby. Bony herring and Spangled perch were an important community component for Ark and Disco habitats, however, Disco refuges were strongly characterised by rainbowfish and Arks by Lake Eyre hardyhead and desert goby. For both Disco and Ark refuges, Golden perch were also species that differentiated these from other refuge communities. Polo Clubs were also characterised by Lake Eyre hardyhead and desert goby but also by a lack of influence from other species. Springs were characterised principally by desert goby and bores by Gambusia.

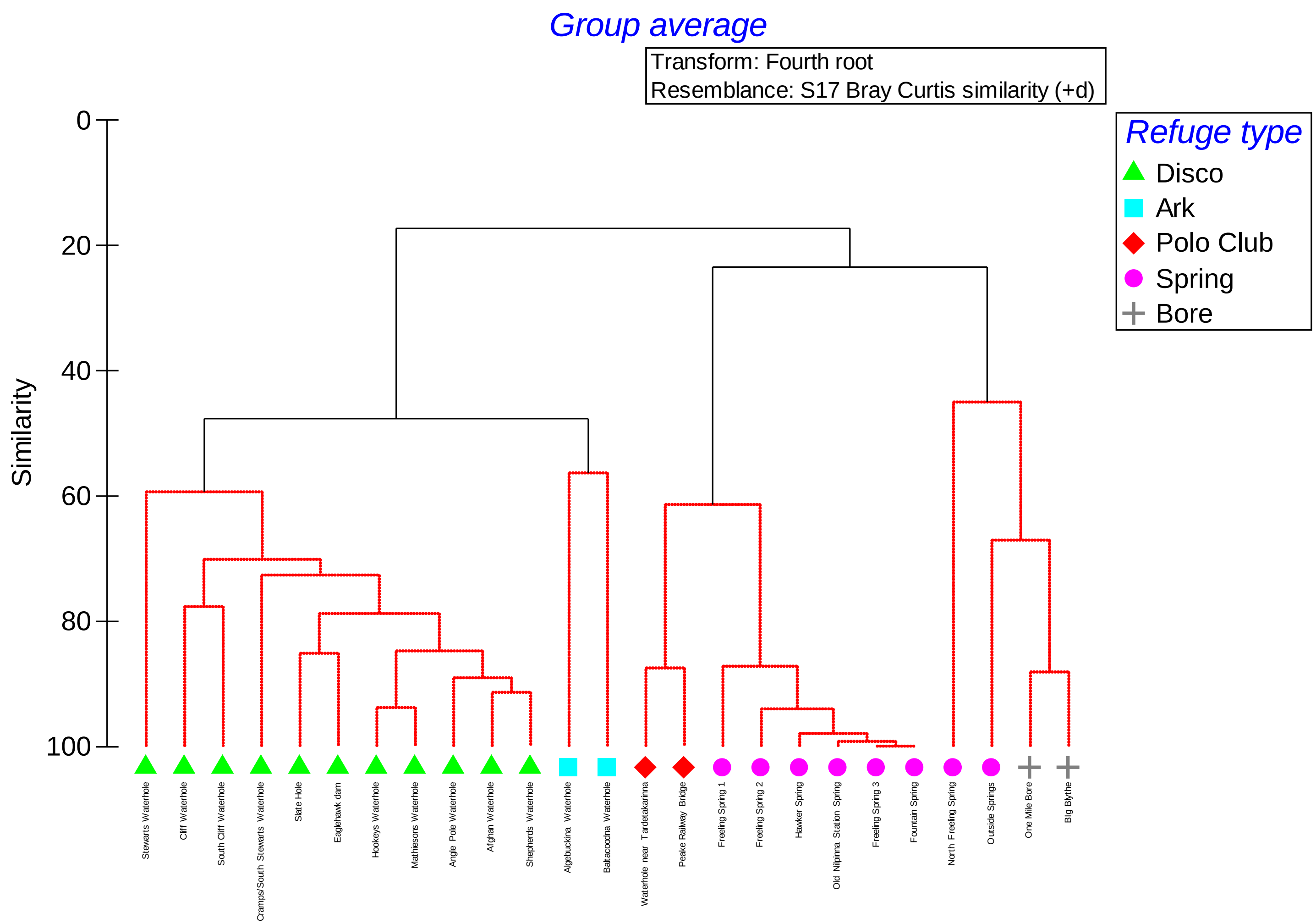

Figure 14. Cluster Analysis of fish community patterns (fourth root transformed abundance data) from spring 2009 showing distinct clustering of data into distinct groups matching pre-determined refuge classifications. Red lines show independent clusters identified using SIMPROF analysis, although PERMANOVA analysis found some closer similarities between cluster groups.

Autumn 2010

By autumn 2010, Cluster Analysis no longer grouped fish community groups into refuge types (Figure 15) although PERMANOVA still identified significant differences across pre-determined refuge types (PERMANOVA (df= 5) F=7.933, P<0.001).

Pairwise analysis however, identified significant difference existed between Disco refuges and all other types, including Ark habitats, which also differed significantly from Stepping Stone and Bore refuges (Table 4). These differences largely support the further splitting of the right hand cluster (Figure 15) to separate the Disco, Ark, Polo Club sites from stepping stone sites that were lumped together under SIMPROF analysis.

Table 4. Pairwise Permanova results for refuge types in autumn 2010. Blue values (bottom left half of matrix) are average similarity scores whilst bold black values (upper right) are probability scores. Significantly different comparisons are marked with *.

Refuge Types	Disco	Stepping Stone	Ark	Polo Club	Bore	Spring
Disco	76.504	**0.002***	**0.023***	**0.019***	**0.001***	**0.001***
Stepping Stone	50.554	50.703	**0.042***	**0.165**	**0.057**	**0.063**
Ark	66.502	37.382	71.068	**0.568**	**0.037***	**0.138**
Polo Club	60.603	36.077	76.618	0	**0.147**	**0.458**
Bore	21.094	35.358	26.102	32.468	73.511	**0.428**
Spring	16.943	28.703	23.97	35.067	53.298	37.788

SIMPER Analysis (Appendix 1) revealed that the similarity across Disco, Ark and Stepping Stone waterholes is due to the strong influence of bony herring, spangled perch and desert rainbowfish forming each group. However, Ark refuges differed from both groups through the influence of barred grunter, and the addition of rarer species including Gambusia, Lake Eyre hardyhead, desert goby and golden perch and a reduced importance of spangled perch.

Differences between disco, stepping stone and ARK refugia was also related to the differing influence of abundances despite the similar species structure with desert rainbowfish more influential in Disco habitats than Ark or Stepping Stones. Therefore, Disco refugia may be regarded as sites particularly important for desert rainbowfish during this time.

The sole polo club refuge sampled in autumn 2010 (Peake) showed a very different pattern to that observed in the previous spring with a number of additional species (most importantly the rare barred grunter) beginning to influence the community structure. This change has implications for the change in polo club structure, with more species influencing the community as conditions become wetter.

SIMPER analysis also revealed both springs and bores to be characterised by Gambusia, however, it must be noted that not all sites were sampled during both seasons and differences are not wholly due to changes in fish community structure within sites over time, but also reflect the community structure of new sites, particularly for springs, bores and stepping stone habitats which were not inundated during the spring 2009 survey. In addition, the spring habitats sampled in 2010 were dominated by Gambusia (and a range of species in the case of North Freeling) whilst those in 2009 were largely desert goby habitats, thus influencing the similarity between springs and other refuge types.

The individual analysis of seasonal fish community data has revealed changes in the fish community structure across refuge types, showing that over a period of rainfall, catchment flow and broad catchment connectivity, the distinct refuge communities present at the end of the 2009 dry season (which was preceded by an extended period of drought) began to change and converge as species moved into or expanded populations across the catchment. These results are somewhat complicated by the fact that seasonal surveys did not necessarily represent a repeat sampling of identical sites, but included omissions and new sites between seasons.

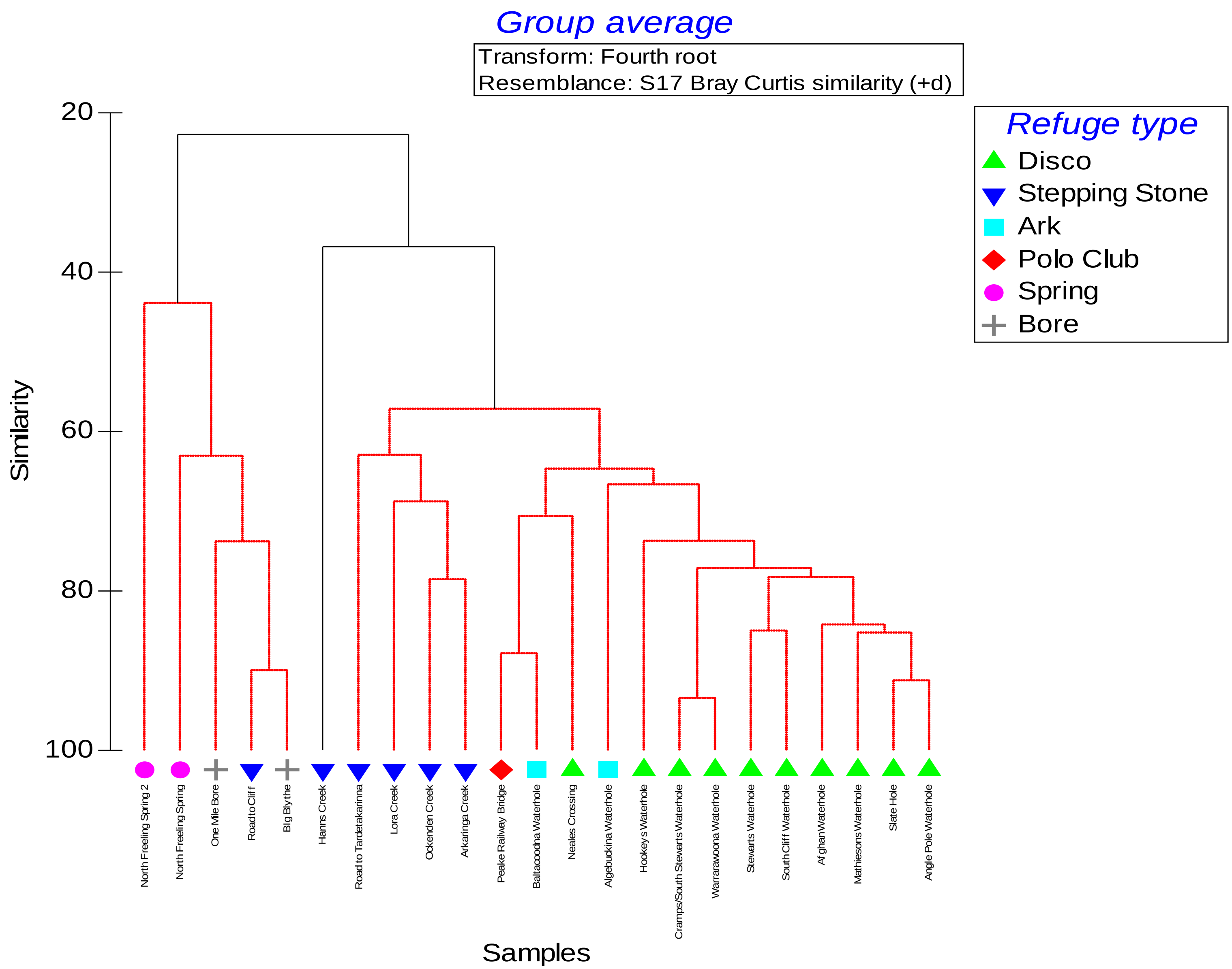

Figure 15. Cluster Analysis of fish community patterns (fourth root transformed abundance data) from autumn 2010 showing two distinct clusters identified using SIMPROF analysis (red linkages), although PERMANOVA analysis found some significant differences between some cluster groups.

Combined data

By combining the fish community data collected across both seasons, it is hoped that a broader assessment of the relationship between fish community and refuge type can be made. Cluster analysis of all sites combined reveals that, regardless of season, strong groupings of fish community can be observed (Figure 16.). SIMPROF analysis indicates four distinct clusters in the data (with one outlier) which broadly represent a group of Disco and Stepping stone habitats, a cluster of Ark refuges with a single polo club and Disco site included, a group of springs and polo clubs and a group of bores and springs (with a single stepping stone site).

Analysis of the refuge type data indicates that significant differences in fish community structure still exist across pre determined refuge types (PERMANOVA (df=5) F= 15.526, P<0.001). Pairwise comparisons (Table 5) reveal strong differences exist across pre-determined refuge types with the exception of Polo Clubs, which did not differ significantly from springs or Ark refuge types when considered over the entire study.

Table 5. Pairwise Permanova results for refuge types with all sites and seasons combined. Blue values (bottom left half of matrix) are average similarity scores whilst bold black values (upper right) are probability scores. Significantly different comparisons are marked with *.

Refuge Types	Disco	Stepping Stone	Ark	Polo Club	Bore	Spring
disco	72.523	0.002*	0.001*	0.001*	0.001*	0.001*
Stepping Stones	51.725	46.366	0.009*	0.027*	0.011*	0.004*
Ark	55.168	34.755	65.644	0.067	0.001*	0.003*
Polo Club	29.156	27.008	44.809	54.519	0.004*	0.113
Bore	19.028	35.603	20.289	21.278	80.263	0.007*
Spring	16.103	27.174	20.592	43.017	36.336	54.711

SIMPER Analysis of combined data (Table 6) reveals that the lack of distinction between Polo Club habitats against ARK and springs is likely to be due to the influence of desert goby which contributes strongly to the community distinctiveness for all three groups, but not other refuge types.

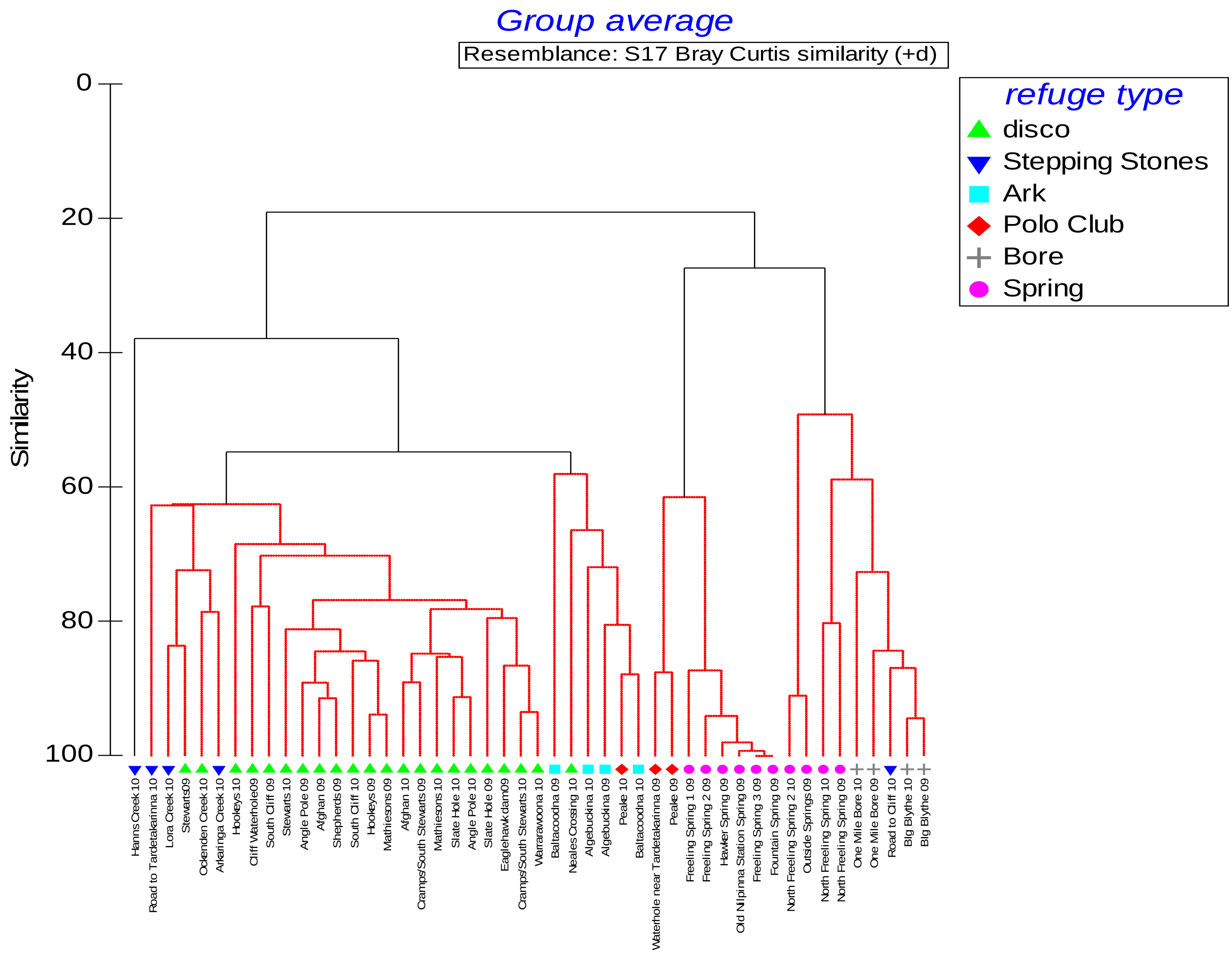

Figure 16. Cluster Analysis of all sites combined indicating refuge classification. Significant clusters by SIMPROF analysis are indicated by red linkages. Re-sampled sites are indicated by season (09 = spring 2009 or 10 = autumn 2010).

Table 6. Key Fish Community drivers for various pre-determined refuge groups identified through SIMPER analysis of combined data from spring 2009 and autumn 2010 across the Neales catchment.

Refuge Type	Average Similarity	Key Species	Average Abundance	Average Similarity	% Contribution	Cumulative % Contribution
Disco	69.7	Bony Herring	3.58	28.91	41.47	41.47
		Spangled Perch	2.62	21.41	30.72	72.19
		Desert Rainbowfish	2.58	17.05	24.46	96.65
Stepping Stone	36.57	Bony Herring	1.52	21.29	58.22	58.22
		Spangled Perch	1.58	11.31	30.94	89.16
		Mosquitofish	1.66	2.43	6.65	95.81
Ark	64.08	Bony Herring	6.39	25.21	39.34	39.34
		Spangled Perch	3.47	13.78	21.5	60.84
		Desert Rainbowfish	2.84	7	10.93	71.77
		Lake Eyre Hardyhead	3.09	5.87	9.16	80.93
		Barred Grunter	2.53	4.66	7.28	88.21
		Desert Goby	1.7	3.49	5.45	93.66
Polo Club	50.69	Lake Eyre Hardyhead	2.9	31.51	62.15	62.15
		Desert Goby	2.4	19.18	37.85	100
Bore	77.99	Gambusia	7.2	77.99	100	100
Spring	42.49	Desert Goby	2.36	37.01	87.11	87.11
		Mosquitofish	1.7	5.16	12.13	99.25

Bony herring and spangled perch and desert rainbowfish are important components of Ark, Disco and Stepping Stone habitats; however, other species contribute strongly to the differences in fish community across these refuge types. In particular, desert rainbowfish remain extremely important to Disco refuge communities, whilst Gambusia are important in stepping stones (an important result given that these sites represent migration pathways) and Ark sites have strong representations of a range of species including desert rainbowfish, Lake Eyre hardyhead, Barred grunter and desert goby. Interestingly, the golden perch that were important to Ark refuge community structure in autumn 2010 was not as strong when considered over the combined data.

Polo Clubs were defined by two species, the Lake Eyre hardyhead and desert goby, whilst springs were characterised by desert goby and Gambusia. Bores were characterised solely by Gambusia, which dominated the fish community in these artificial habitats.

The analysis of the combined dataset provided the first field based test of refuge classifications developed for drought refugia by Robson *et al.* (2008) with some additions proposed for freshwater fish by McNeil *et al.* (2011). Generally, the survey of waterholes in the Neales River between spring 2009 and autumn 2010 revealed distinct fish community structures across refuge types.

Seasonal data emphasized, however, that the nature of fish community structure can vary seasonally and that distinct refuge type communities that may be present at the end of the dry season, or following severe drought, may break down somewhat through the addition of new species (through migration) or changes in the relative abundance of species within sites as a result of ecological processes such as recruitment, differential mortality, competition, predation etc.

Summary

This section has revealed the close linkages between fish species distributions and the physical characteristics of waterholes across the Neales catchment. Species are non-randomly distributed and instead appear to be structured in an increasingly homogeneous pattern across the catchment compared to the more heterogeneous distributions present in historical surveys and earlier data from the present survey.

There was, however, very clear assemblage structure across habitats that suggest the classification of refuge typologies, developed from Robson *et al*. (2008) and McNeil *et al.* (2011) are relevant to the drivers of fish community structure over the long term. The climatic context of this project focuses on a period of recovery from drought. Refuge habitats are still strong drivers of fish assemblage. A key step in understanding the role of climate in influencing this catchment-wide assemblage structure is to explore the interaction of species traits that influence the resistance and resilience of fishes to climatic disturbance and long-term climatic cycles and variability. The following sections therefore explore in further detail, the resistance and resilience traits of fishes with the aim of developing conceptual models to integrate climate, catchment and biotic structure in the Neales River catchment.

RESISTANCE TO CLIMATE IMPACTS: ASSESSING TOLERANCE THRESHOLDS

Introduction

The previous sections have focussed on broad scale patterns of fish community structure and habitat structure across the Neales catchment. The following sections will focus instead on the individual traits and ecological characteristics of fish from the Neales catchment to determine how resistance and resilience factors might be integrated with these catchment scale processes. This section deals specifically with some of the key tolerance traits of fishes for dealing with salinity and hypoxia, key impacts related to the climatic variability and drought disturbance that drive the boom and bust cycles to which fish are subjected continually in the Lake Eyre Basin.

Species adapted to the resistance (following Hollings 1973, Wu and Loucks 1995) of climatic impacts exhibit specialized adaptations that allow them to tolerate a harsh environment, including high salinities, extreme temperatures and hypoxia (low oxygen). At a landscape level, the possession of traits maximising tolerance increases the number of potential drought refugia for these species that can then persist and use precious resources released within waterholes as other species are extirpated (Ostrand and Wilde 2001).

We hypothesize that salinity is the primary environmental filter shaping fish community structure in this particular drought-prone environment. After salinity, hypoxia and high temperatures may be important sources of mortality of individuals that remain. Our goal for this portion of the study was to relate specific distributional features of fish within the Neales River basin to their tolerance to increasing salinities and declining oxygen levels (hypoxia) to support proposed mechanisms of persistence through periodic or chronic drought conditions.

Salinity Tolerance

Although observations of fish in highly saline waters are common for the Lake Eyre Basin (Glover 1973, Ruello 1976, Glover 1990, Thomson 1990), little or no data are available from laboratory experiments using physiological or behaviourally derived indicators and measurements of tolerance thresholds. A major impediment is the remoteness and poor accessibility of the Neales River and harsh conditions under which fish must be transported to research laboratories (the nearest is over 1000km away). To circumvent these issues, a makeshift field laboratory was designed and constructed to determine salinity tolerance thresholds in the field using standard laboratory methods (McNeil *et al.* 2010b & c).

Derived salinity tolerance thresholds, if proven reliable or comparable, provide an indication of the salinity levels under which various species can persist. Native fish species avoid hypersaline areas and their movement in south Australian waterbodies can be dictated by hypersaline masses (McNeil *et al.* 2010d). Following disconnection and isolation, waterholes that maintain salinity levels below threshold values are available as refugia, whilst harsh waterholes can only be inhabited by species that display specialized adaptations for salinity tolerance.

A pilot salinity tolerance trial was conducted in the field at Algebuckina Waterhole to explore the feasibility of field tolerance testing. Derived tolerance thresholds can subsequently be compared to field distribution data to investigate the relationship between field based threshold values and the actual relationship between species distributions and salinity levels throughout the Neales River catchment. In particular, tolerance thresholds may explain the absence of species from saline waterholes and provide a baseline for prediction of faunal attenuation as droughts persist. As an example, Algebuckina waterhole is understood to be periodically highly saline, and, as the most critical refuge in the Neales catchment, increasing salinity levels may threaten the persistence of less tolerant species, potentially leading to the failure of the Ark refuge and catchment-wide extirpation of salinity sensitive species.

Methods

Trials for salinity tolerance were conducted in a makeshift laboratory constructed in an anabranch of the Algebuckina Waterhole during April 2010 (Figure 17). Such remote field trials are rarely attempted because they are subjected to a wide range of climatic and uncontrolled factors that hinder investigation. However, experience with past studies using remote tolerance field trials (Rosenberger and Chapman 2000, McNeil 2004) was of maximum advantage in setting up the field laboratory, and non-experimental factors were controlled to the highest degree possible.

Salinity tolerance trials were conducted for five species of Neales River fish: desert rainbowfish, desert goby, barred grunter, spangled perch and bony herring. All fish were collected from the Neales River Basin and transferred into aerated holding containers submerged in the anabranch to maintain ambient temperatures. Test fish were not fed prior to experimentation; in all cases, experiments were initiated within 24 hours of fish capture. Following trials, live fish were returned to the point of capture.

Salinity tolerance trials were conducted within two types of experimental containers. Smaller bodied species, rainbowfish and desert goby, were tested within 1 litre square container, partially immersed in water and supported by a polystyrene frame. Containers were covered with 1mm plastic mesh to prevent fish escape and each aerated using a solar-powered air pump and individual bubblers. An aquarium ammonia capture product was used to maintain water quality within the containers, which were checked regularly for ammonia levels. Larger bodied species - spangled perch, barred grunter and bony herring - were tested within 20L plastic lidded buckets, partially immersed in water, and buoyed by a polystyrene floating bed. Lids were fitted to each container through which a bubbler was inserted. Access panels were cut into the bucket lids to allow fish and salt to be placed within buckets with minimal disturbance to fish already within test containers. These experimental pontoons were shaded using tarpaulins and the shelters were moved throughout the day to compensate for changes in the angle and direction of sunlight.

Treatment salinities were based on the estimated tolerance range of species obtained from field observations, ranging from freshwater to levels anticipated well beyond the range of tolerance for those species. Salinity was gradually increased from freshwater to the highest salinity level designated for each species in a reverse-logarithmic scale over six treatments (Table 7). Three trials were carried out for each of the six salinity treatments set prior to trial initiation by adding sea salt. The ionic composition of Lake Eyre salts is extremely close in composition to sea salt with ~95% NaCl content (Williams 1990), making sea salt the closest, readily available alternative to naturally occurring catchment salts. Once salinity gradients were set, fish were placed randomly into each container to begin the trial. The number of surviving and deceased fish within each container was counted at regular intervals (at approximately 0, 3, 6, 12, 24, 36, and 48h).

Trials were conducted following direct transfer methods of McNeil *et al.* (2010c); however, the estimation of Lethal Concentration (LC) values using probit analysis was unsuccessful for this data-set, predominantly due to the poor survival of 0 PPT treatments compared to higher survival under slightly higher salinity concentrations. This pattern is likely to be better suited to non-linear estimates of Lethal Concentration, which are not available to us at this time. As a result, LC_{50} estimates have been directly implied from the intersection point of experimental percent survival curves.

Table 7. Information on treatment levels, including sample size and size range of fish (mm) for salinity trials conducted on four Neales River fish species. Note that treatment levels differ for each species based on predicted tolerance.

		Size range (mm) of test fish		
Species	Salinity (ppt)	Trial 1	Trial 2	Trial 3
Barred grunter	0.2	59-116	46-120	62-120
N = 6 per treatment	4.9	50-87	59-70	50-82
	7.8	63-110	65-100	35-103
	12.8	62-80	60-127	66-95
	21.2	67-79	47-86	44-91
	35	73-117	57-80	59-78
Spangled Perch	0.1	48-122	47-61	59-86
N = 6 per treatment	9.4	54-112	23-58	57-68
	15.1	52-132	46-56	49-59
	25.1	53-95	43-57	57-77
	41.7	61-108	44-68	60-73
	69.3	57-105	39-56	55-67
Rainbowfish	0.2	41-61	25-67	46-70
N = 5 per treatment	4.6	45-69	42-70	42-70
	7.6	45-65	45-67	40-68
	12.4	39-77	30-53	38-66
	21.1	42-55	43-54	46-70
	35.1	50-67	51-63	45-74
Desert goby	0	27-47	21-32	23-41
N = 6 per treatment	15.9	29-49	18-35	23-48
	26	32-46	24-37	37-47
	43.2	25-47	29-42	25-43
	71.6	32-47	24-40	25-47
	120.5	29-38	22-38	24-39

Although trials were attempted for five species, we present results for only four. Attempted trials on the bony herring were considered unusable. This pelagic and surprisingly sensitive species maintained constant swimming activity within test containers and unidirectional swimming around the edge of containers resulting in abrasion along the side of fish as they swam against the wall surface. Injuries led to mortality unrelated to salinity conditions within the test containers.

Figure 17. A) Makeshift field laboratory for salinity trials allowing three trials of six salinity treatment levels. Buckets (20L) for larger fish species and (B) 1L containers for smaller species (C) floating on polystyrene pontoons partially immersed in water at Algebuckina Waterhole and aerated using a solar powered pump.

Results

The response of fish to increasing salinity levels varied widely among Neales River fish species, with rainbow fish demonstrating the lowest tolerance thresholds (LC_{50} 10 ppt, Figure 18) and desert goby the highest (LC_{50} 52 ppt). Barred grunter and spangled perch displayed comparable tolerance thresholds (LC_{50} at 15 and 21, respectively). Confidence intervals around mortality estimates at different salinity levels suggest the need for a larger data set to conclusively set LC_{50} in a manner comparable to literature values for other species. With the exception of rainbowfish, the highest survival rates were observed not at the lowest salinity levels, but rather treatments that were slightly saline (<10ppt, or 10-20% of seawater).

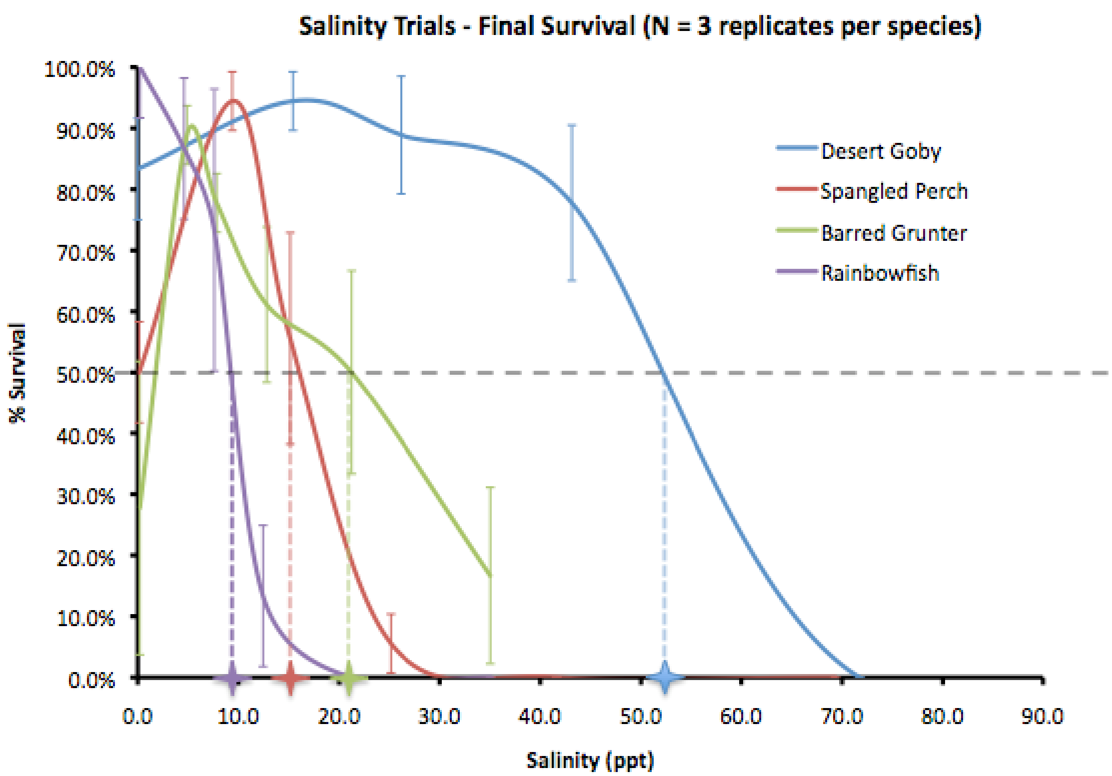

Figure 18. Final survival of four species of Neales River fish under increasing salinity levels. Error bars represent standard deviations of % survival and dotted lines indicate 50% mortality intercepts.

Our data provide the first baseline data on relative-estimates of salinity tolerance for the four Neales River species examined. Rainbowfish were least tolerant, while the desert gobies could withstand salinities twice that of seawater. This high tolerance is among the many characters of desert goby that could be associated with a strategy of drought resistance. Species with rapid dispersal potential and a strategy of resilience were comparatively low in tolerance levels (spangled perch and rainbowfish). The barred grunter - a species that remains restricted in its distribution and has been slow to disperse from Ark refugia - is intermediate in tolerance levels. The desert goby showed extreme tolerance to hypersaline conditions. It is an extremely slow coloniser that remains within shrinking refuge pools under conditions of declining water quality. The goby possesses a collection of traits categorised as *resistant* rather than *resilient*, (McNeil and Schmarr 2010). There was relatively high survival under slightly saline conditions (10% that of seawater) compared to fresh-water (control) treatments for all but the rainbowfish. This result is potentially indicative of osmoregulatory preferences but also may be an artefact of possibly stressful conditions within treatment containers. Further field experimentation is warranted to determine the accuracy and transferability of values derived in remote field locations.

Hypoxia Tolerance

Second to salinity, low oxygen conditions, or hypoxia, may be the most important determinant of what species remain in remnant pools and refugia as drought conditions increase in severity. Therefore the high salinity tolerance observed in Neales River species may correspond with equally high tolerance to low oxygen conditions.

Water-breathing fish posses a wide range of evolutionary responses to hypoxia, including both instantaneous behavioural responses (e.g., avoidance) and physiological responses (e.g., increased haemoglobin for oxygen transfer) (Perry and McDonald 1993, Timmerman and Chapman 2004). An integrative approach to examining the relative differences in hypoxia tolerance among species is to observe behavioural response to gradual reductions in oxygen in a controlled environment, which incorporates both physiological and behavioural adaptations to low oxygen levels.

Many water-breathing fish are known to use aquatic surface respiration (ASR, Kramer and Mehegan 1981) in response to hypoxia, ventilating their gills with water from the air-water interface where diffusion produces a very thin layer of well-oxygenated water (Kramer and McClure 1982; Chapman *et al.* 1995). In addition, some species hold bubbles in their buccal cavities, which may increase the oxygen content of water passing over the gills or increase buoyancy at the water surface. This is often viewed as a form of air-breathing behaviour, accompanied frequently by the presence of highly-vascularised buccal cavities or intestine (Graham 1997). Gobioid fishes, in particular, are known to use both surface breathing and bubble holding to compensate for low oxygen conditions (Gee and Gee 1991).

Our purpose in this portion of the study was to examine the presence of adaptive behaviour of Neales River species to declining oxygen conditions. Remote field conditions and distance from laboratory facilities made sophisticated, long-term behavioural experiments impossible. However, our experiments are the first known attempt at observing adaptations to lowering oxygen conditions for these fish species and will provide further insight to the resistance of these species to drought conditions and mechanisms for faunal attenuation as drought progresses in severity over seasons and through years.

Methods

Captured fish were held in large plastic containers continuously aerated with a solar-powered bubbler. To minimize interdependence among trials, new individuals were used for each trial run. Individuals were transferred to a Plexiglas aquarium (29 x 20 x 18cm) and acclimated for one hour at ambient temperatures (Table 8). After the acclimation period, oxygen was lowered with the addition of small amounts of sodium sulphite (following Chapman and Liem 1995; Olowo and Chapman 1996), and then held at trace oxygen levels until the fish lost equilibrium or survived these conditions overnight. The remoteness of the field site precluded the use of nitrogen gas to lower dissolved oxygen levels. However, Lewis (1970) found no observable differences in the behavioural responses of fishes to water freed of oxygen with sodium sulphite and water freed of oxygen by bubbling with nitrogen gas.

Table 8. Experimental conditions for hypoxia trials for Neales River fish, including baseline water quality, sample size (N), and range of fish lengths (TL = total length).

Species/ Trial	Size range (mm)	N	Temperature (°C)	pH	Salinity (ppm)
Desert goby					
Trial 1	37 - 22	5	25.8	7.8	0.1
Trial 2	31 - 18	5	25.9	7.9	0.15
Trial 3	48 - 34	6	27.5	7.3	0.2
Spangled Perch					
Trial 1	121 - 51	5	19.8	7.7	0.1
Trial 2	122 - 52	5	21	7.1	0.1
Trial 3	67 - 52	5	26.7	7.5	0.1
Rainbowfish					
Trial 1	67 - 46	5	26.2	7.7	0.18
Trial 2	65 - 36	5	21.6	7.9	0.2
Trial 3	65 - 49	5	31	7.5	0.15

Every 15 minutes, we recorded the following parameters from behind the blind: gill ventilation rate (number of ventilations in a 15-sec period recorded for each fish), number of fish using ASR (recorded every 10 sec for 100 sec), aggressive interactions (recorded every 10 sec for 100 sec), number of individuals using buccal bubble holding, and speed of movement at the surface during ASR (distance moved in 10-sec for each of the eight fish). Gill ventilations were recorded when deep enough to be clearly visible. The outline of buccal bubbles could be clearly observed when an individual's mouth was extended to obtain air at the surface. Aggressive interactions usually involved fish pursuing other individuals, sometimes nipping at fins or tails. The edge of the experimental aquarium was marked at regular individuals in order to gauge movement of fishes at the surface and time spent in the bottom, middle, and top of the tank. If any individual lost equilibrium, it was quickly removed from the experimental tank and placed in well-oxygenated water to recover.

The level of oxygen at which 90% of the fish performed ASR (ASR_{90}) was estimated by fitting curves to plots of oxygen levels and percent ASR. Percent ASR was calculated as the number of fish in a group using ASR divided by the total number of fish, averaged over the 10 observations in a given sample. In addition, we made observations of any unusual behaviours such as jumping (flight attempts), beaching, or ramp breathing, particularly for goboid fishes, known to use such behaviours (Gee and Gee 1991).

Results

Neales River fish varied in their behavioural traits and responses to hypoxia, demonstrating a wide range of adaptations to the presence of low oxygen. Summary results for each test species are presented separately in the following section.

Desert Goby

Desert gobies initiated surface respiration at oxygen levels of 5ppm; as oxygen levels declined, the percentage of individuals near the tank surface increased dramatically (Figure 19) and aquatic surface respiration increased exponentially (Figure 20). Aquatic surface respiration was accompanied by buccal bubble holding (Figure 24). Desert goby were not uniform in their surface behaviours; they not only performed surface respiration, but also either rested on this surface of the waters or used the ramp provided (with fins supporting arching position to extend mouth above the surface of the water) associated with air-breathing and surface respiration.

These observations are not novel for Gobioid fishes; Gee and Gee (1991) also observed similar behaviours (Figure 21). The effectiveness of the surface respiration for this species is apparent via observation of gill ventilation rates (Figure 22). Differences among trials in baseline levels of gill ventilation are not surprising or meaningful due to different size of experimental individuals (Table 6). However, for each of these trials, initiation of ASR apparently alleviated respiratory stress, as indicated by a drop in gill ventilation rates; an alternative explanation is that the fish were using deeper, slower gill ventilations to maximize water uptake.

A novel behaviour we observed was complete beaching by fish; occasionally, an individual would jump from the water to the ramp or the lip of the aquarium. These individuals would remain in those areas, motionless and with no apparent gill ventilation. If touched, beached individuals returned to the water of their own volition, apparently unaffected. Desert goby did not lose equilibrium at any point in these trials. No fish perished when left at trace levels of oxygen overnight and were observed in the same breathing positions after extended exposure to only trace levels of oxygen.

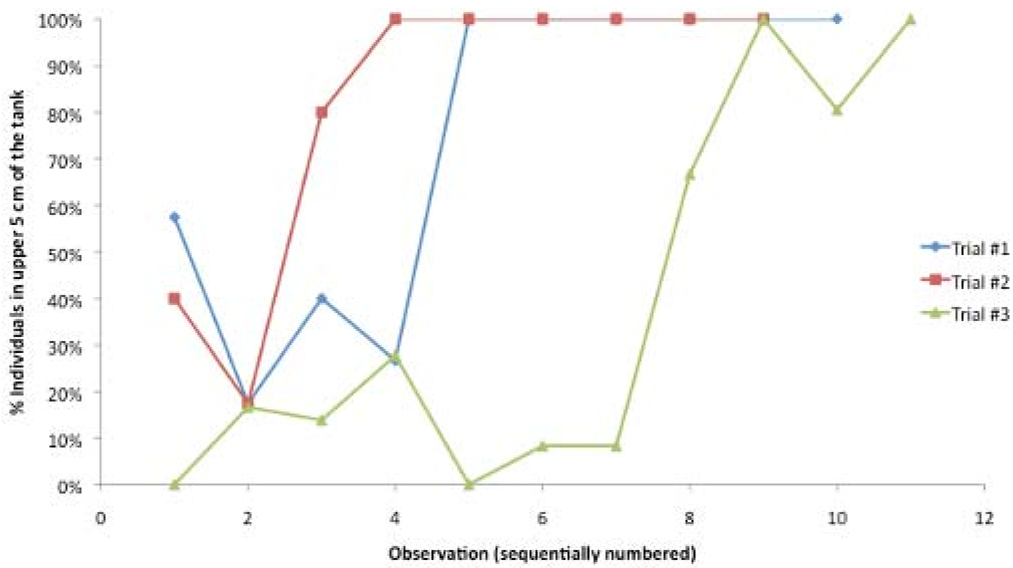

Figure 19. Percent individuals of desert goby in three trials using the upper 5 cm of the experimental tanks for sequential behavioural observations, indicating an increase number of individuals to the surface in response to a decrease in oxygen levels.

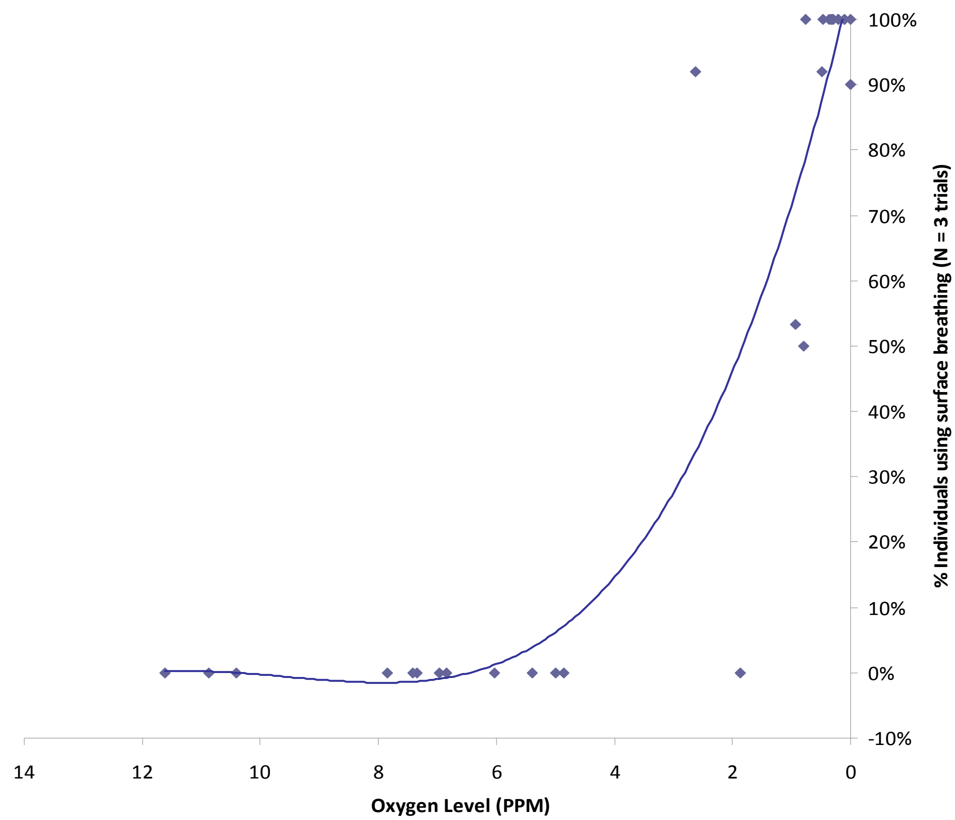

Figure 20. Use of surface breathing in response to gradual hypoxia in the desert goby.

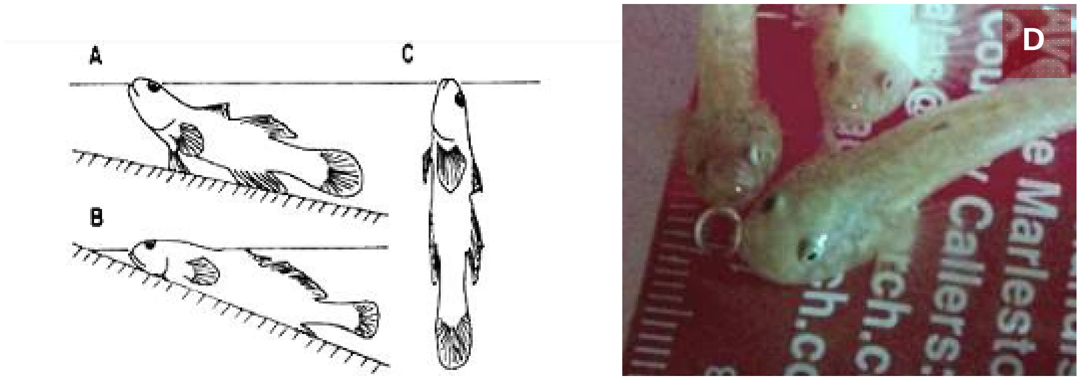

Figure 21. Positions used by benthic Eleotrids and gobies to perform ASR. A – arching, B-emersed, and C-vertical or attached (from Gee and Gee 1991). Both A and B were observed in desert gobies but not behavior C; however, we did note "beaching" of individuals on provided ramps or the lip of the observation tank. Note bubble shine inside mouths of emersed gobies (D).

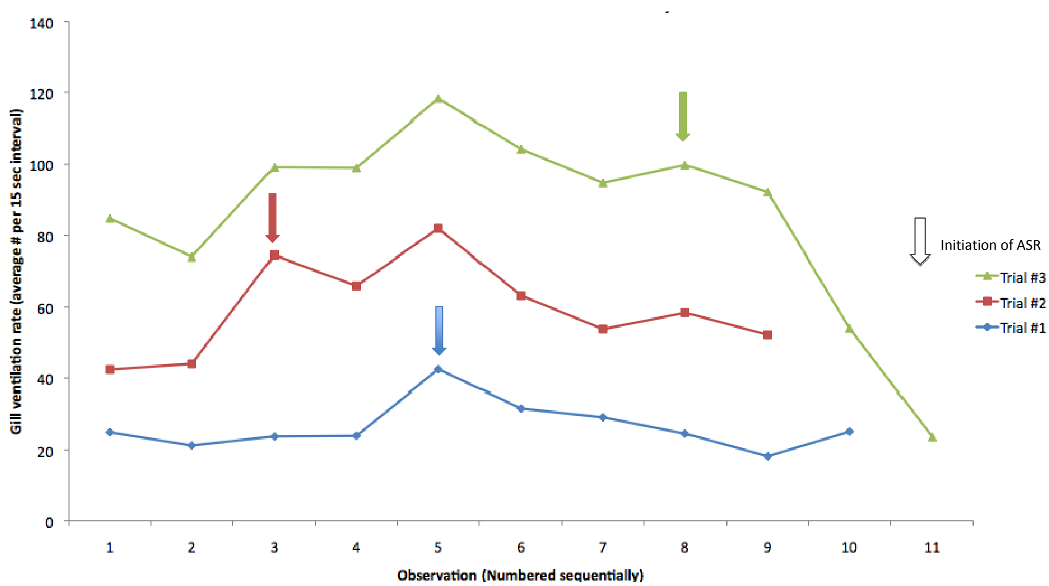

Figure 22. Gill ventilation rates in response to gradual hypoxia and the initiation of aquatic surface respiration (ASR) for three desert goby trials.

Spangled Perch

Unlike desert goby, spangled perch are a demersal species, displaying, in general, greater levels of activity and variability in location within experimental tanks. However, as with the desert goby, this species rose to the water surface over progression of the trials and a decrease in oxygen levels (Figure 23).

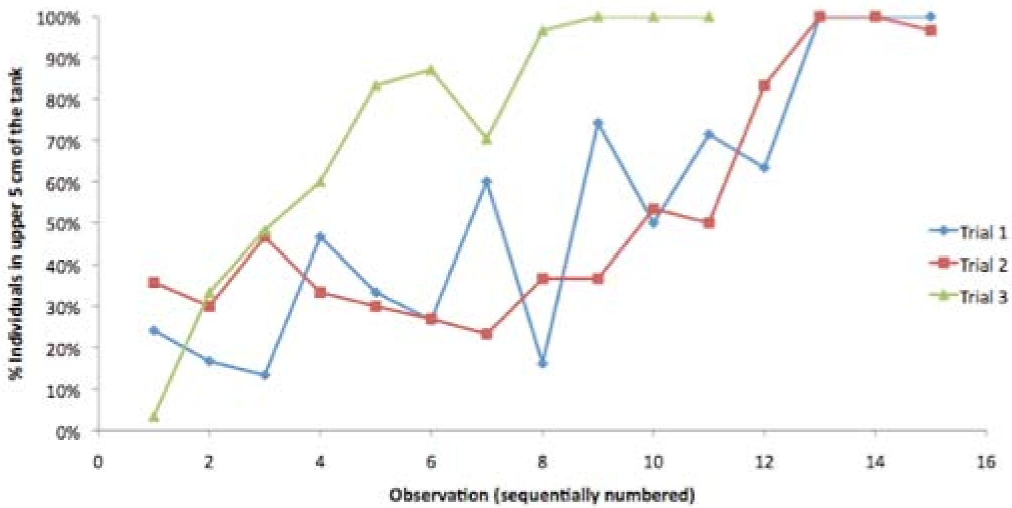

Figure 23. Percent individuals of spangled perch in three trials using the upper 5 cm of the experimental tanks for sequential behavioural observations, indicating an increase in number of individuals to the surface in response to a gradual decrease in oxygen levels over the duration of the experiment.

Spangled perch were more inclined to swim at the surface, even when oxygen levels were relatively high (Figure 24). However, at ASR levels of <50%, individuals only spent a few moments at the surface. Surface swimming was often accompanied by escape attempts from the experimental tank. We observed aggression between spangled perch as oxygen levels dropped – usually a nip of a tail or defence of an area of the tank, often near the surface. Individuals that displayed aggression often did so before attempting escape from the tank. Once ASR increased to 90%, these escape attempts were abandoned in favour of constant surface respiration.

Unlike the desert goby, ASR did not increase exponentially in spangled perch; instead, the species gradually increased the rate of aquatic surface respiration as oxygen levels dropped (Figure 25), reflecting the tendency of the species to swim at the surface, even under normoxia. This may reflect the foraging behaviour of spangled perch for terrestrial insects, exploratory behaviour prior to an escape attempt, or a response to the stressful situation of confined conditions. However, these behaviours were consistently observed, even after long holding periods in experimental tanks (unpublished data). We would expect if surface breathing at normoxia was a sign of stress, incidence of this behaviour would decline with acclimation. ASR dominated spangled perch behaviour once oxygen levels decreased past 3ppm (Figure 27); ASR_{90} for this species is at approximately 1ppm.

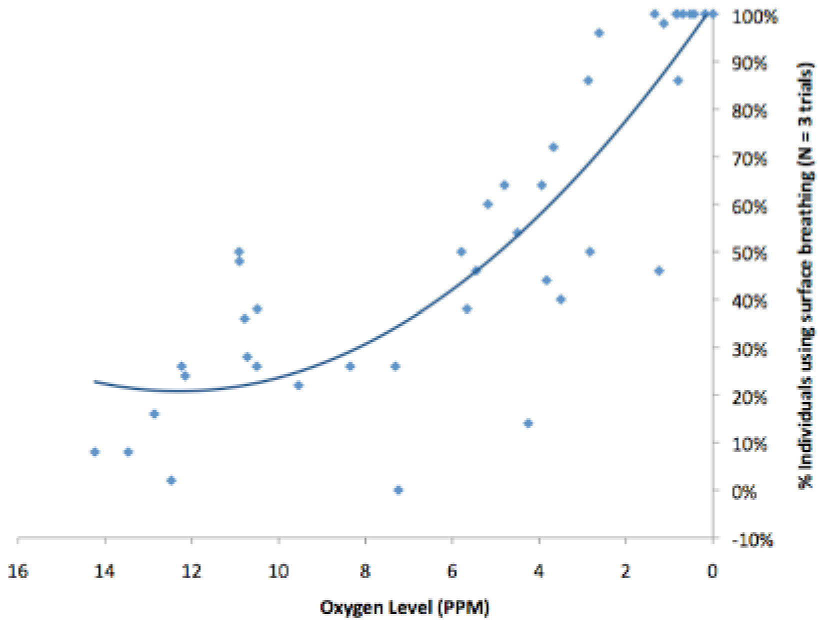

Figure 24. Utilisation of surface breathing in response to gradual hypoxia in the spangled perch.

Once surface respiration dominated experimental fishes' behaviour, gill ventilations dropped (Figure 25), demonstrating the effectiveness of this behaviour for alleviating respiratory stress and the tendency of fish to widen gill ventilations under hypoxic conditions. Unlike the desert goby, spangled perch lost equilibrium after approximately one hour (4 behavioural observations) at trace levels of oxygen in the water column. This indicates that ASR is effective for short-term exposure to anoxic conditions.

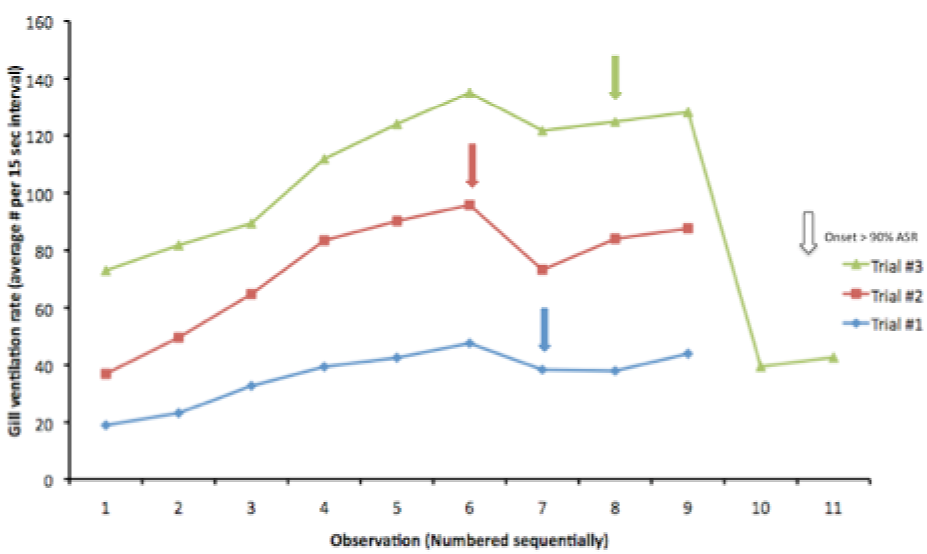

Figure 25. Gill ventilation rates in response to gradual hypoxia and the initiation of 90% aquatic surface respiration (ASR) for three spangled perch trials.

Desert Rainbowfish

Of all of our behavioural hypoxia trials, data from the desert rainbowfish could be considered most suspect due to the rapidity of hypoxia drop and the relatively short duration of the trials (Figure 26). Unfortunately, it is difficult to control the gradual decrease of oxygen using sodium sulphite. However, we present these data as the first known attempt to quantify desert rainbowfish to a gradual reduction in oxygen.

As in the other two species, movement to the surface took place progressively with a decrease in oxygen (Figure 26). Rainbowfish initiated ASR at oxygen levels of around 2.5 ppm; ASR_{90} was observed at 1ppm (Figure 27). However this is based on only 3 data points; rainbowfish tended to lose equilibrium quickly once ASR dominated their behaviour. This may be a reflection of the ineffectiveness of this behaviour for relieving respiratory stress although a slight decrease in gill ventilation rate was observed once ASR was being utilised (Figure 28). Alternatively, this may simply be a response to the rather severe drop in DO they experienced during these trials. Like the spangled perch, loss of equilibrium that resulted in a termination of the experiment, usually occurred after only 45 minutes (3 behavioural observations) at trace levels of oxygen.

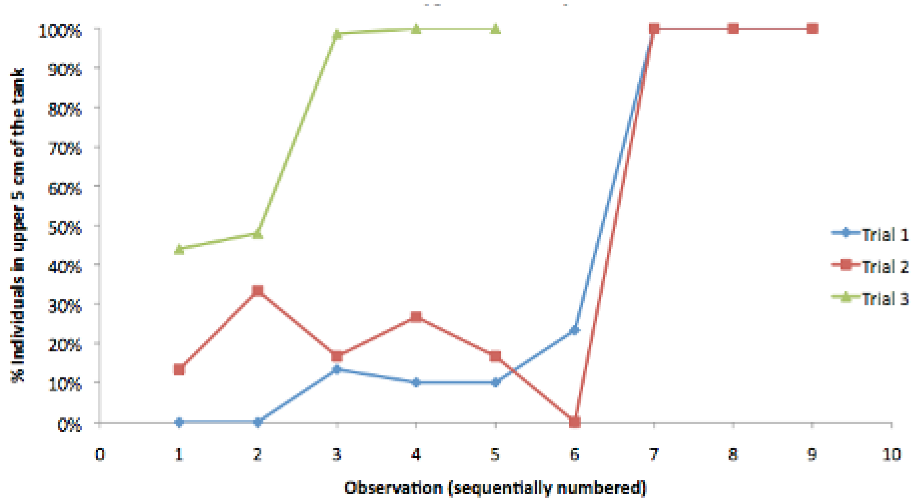

Figure 26. Percent individuals of desert rainbowfish in three trials using the upper 5 cm of the experimental tanks for sequential behavioural observations, indicating an increase in number of individuals to the surface in response to a gradual decrease in oxygen levels over the duration of the experiment.

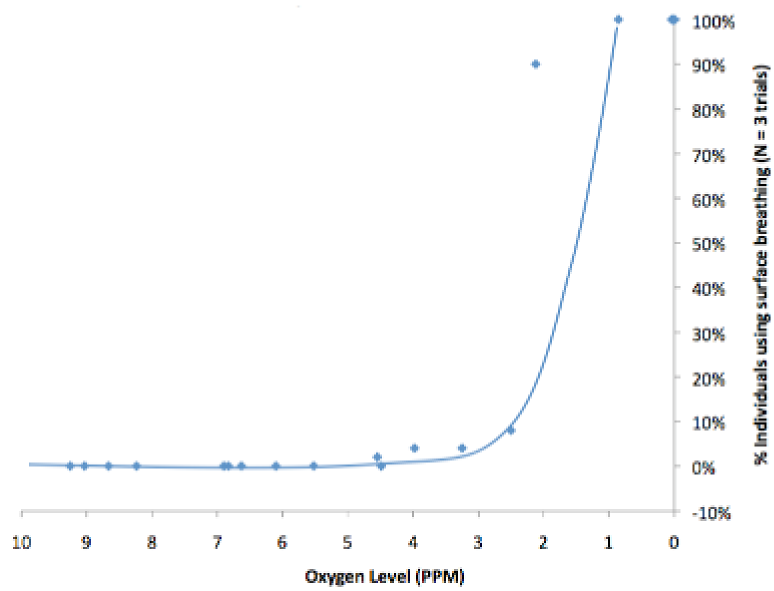

Figure 27. Utilisation of surface breathing in response to gradual hypoxia in the desert rainbowfish.

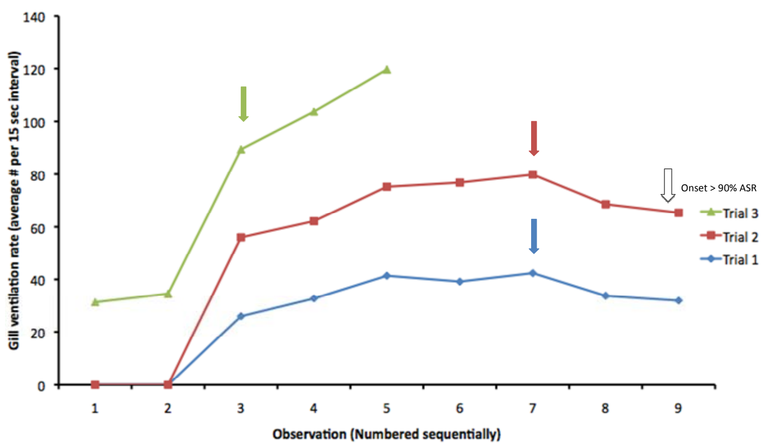

Figure 28. Gill ventilation rates in response to gradual hypoxia and the initiation of aquatic surface respiration (ASR) for three desert rainbowfish trials.

Discussion

All species of Neales River fish examined for tolerance to low oxygen conditions demonstrated adaptive responses, including ASR (all three species) and surface breathing (desert goby). However, species differed both at the levels at which they initiated surface breathing, the duration of tolerance prior to losing equilibrium, and the speed to which they ascended to the surface once oxygen levels within the tank declined below the tolerance levels (Figure 29). Both the spangled perch and the rainbowfish appear capable of tolerating harsh conditions typical of high-quality waterhole refugia, while desert goby display extraordinarily high tolerances that allow them to persist in the most severe remnant peripheral habitats during drought.

The desert goby proved again to be the most tolerant of the three species, showing adaptations to prolonged exposure to low oxygen conditions. In addition, this species appeared to have a variety of behavioural responses, including both surface breathing and outright beaching. We hypothesize that beaching may be an approach for a fish to persist in mud during stressful periods, camouflaged from terrestrial and aerial predators seeking fish crowded into the smaller waterholes.

Both spangled perch and rainbowfish displayed adaptations to temporary hypoxia. Although displaying 'typical' ASR, they also displayed aggression (in the case of spangled perch) and attempts to escape (both spangled perch and rainbowfish) from the confined conditions of the experimental tank, regardless of acclimation period.

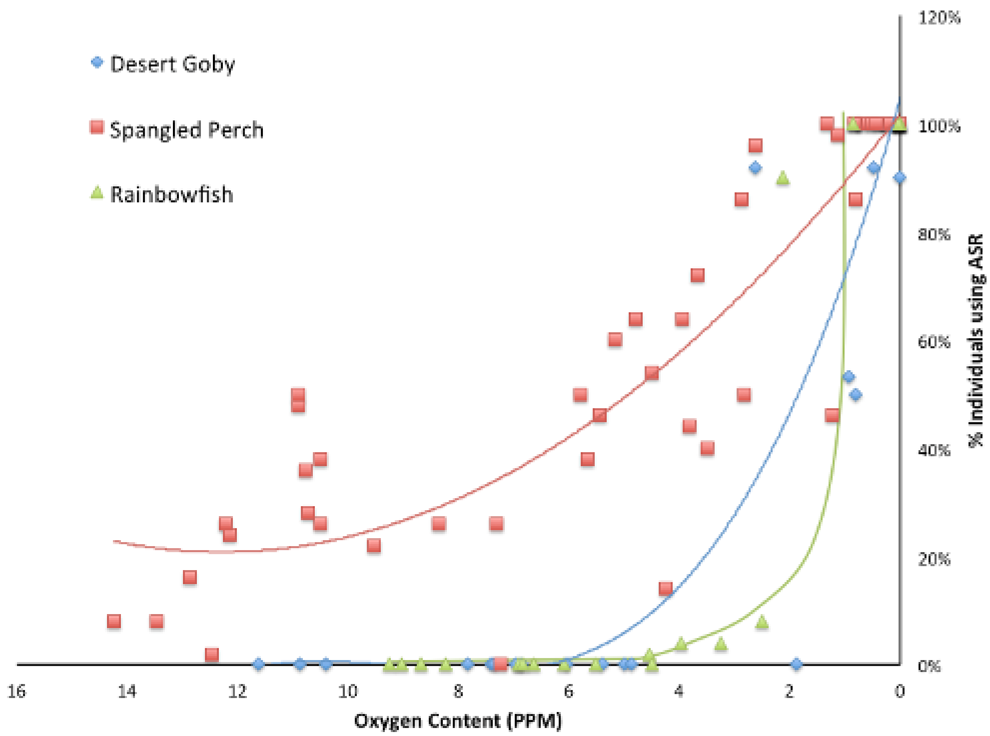

Figure 29. Comparative surface breathing (% individuals using ASR) in response to gradual hypoxia in three species from the Lake Eyre Basin, the desert goby, the spangled perch, and the rainbowfish.

This may represent an attempt at dispersal away from the inclement and confined conditions of the experimental tank, a strategy that may benefit a species with a 'resilience' strategy that relies on dispersal ability. Jumping and dispersal through shallow waters was observed by the authors during periods of high water availability subsequent to rainfall events during our sampling period in April - indicating that attempts to escape are not behaviours unique to our experimental set up.

Finally, we noted that most fish that we characterized as "resilient" (Table 3) frequently attempted escape from confined spaces (holding buckets and tanks) and were difficult to keep in confinement. This suggests that high levels of active response to confinement may allow some species to use naturally adapted traits (such as high velocity swimming) to respond to disturbance and stress.

INTEGRATING FISH SPECIES TRAITS, COMMUNITY STRUCTURE AND REFUGE CLASSIFICATIONS

Species within the Neales catchment fish community have shown distinct associations with different waterholes across the catchment, closely linked with refuge classifications. Given that refuge classifications have been developed to describe waterholes with different degrees of permanence and quality of habitat, or their potential role during periods of climatic disturbance, it is of great interest to explore the relationship between these refuge communities and the various ecological and life history traits that may drive ecological patterns in various fish species.

This is of particular relevance given the recent drought in the Neales catchment and the recent pattern of improving rainfall, flows and connectivity across the catchment. With all species presumably restricted to very few key refugia over the drought period, the current situation allows us to explore whether particular species traits may be implicated in the current patterns of distribution across the catchment and in particular, the interaction of species traits with the various refuge communities identified in the previous section.

Furthermore, we have explored a few of the primary climatic resistance drivers and relative species tolerances for key fish species which show that variable resistance tolerances exist, even across the relatively hardy fishes from the Neales River. A critical component of our study is to explore the way that the Neales River fish assemblage interacts with the various habitat types across the Neales catchment, and finally, how these interactions are associated or driven by variability in climatic harshness.

To explore these questions, an exploration of relevant literature was conducted to compile a table of species traits that are likely to relate strongly with the persistence of freshwater fishes through periods of climatic harshness, or in habitats such as the Neales catchment, where extremes of drought and flood present an ongoing cycle of challenges and opportunities for fish populations.

A number of species traits and ecological variables relating to climatic variability for each species from the Neales catchment were collated in Table 2. These characteristics were subsequently used to propose simplified strategies that Neales fish species have adapted to, persisting through the harsh climatic variability within the Neales catchment (Table 9). In particular these characteristics were used to identify broad strategies of *resistance* or *resilience* that have recently been proposed as key drivers for Australian fish communities in persisting through periods of severe climatic disturbances such as drought (Crook *et al.* 2010, McNeil *et al.* 2011). Of particular interest is whether the relative permanence or quality of waterholes (implicit in refuge type classifications) can be related to different life history strategies for persisting in such a climatically harsh and variable system as the Lake Eyre Basin (Puckridge *et al.* 2000).

Consideration of species characteristics reveals some distinct classifications of species as either resistance or resilience strategists. Considered in relation to refuge fish communities, a pattern emerges of highly tolerant *resistance* strategist, in particular desert goby and Lake Eyre hardyhead, dominating the fish community in springs and Polo Clubs, which present especially shallow, warm and potentially saline water bodies. These resistance strategists are necessarily tolerant and the current data suggests that this tolerance and resistance strategy is driving their dominance of harsher, shallow and saline waterholes within the Neales catchment.

Alternatively, spangled perch, bony herring and desert rainbowfish are identified as resilience strategists which possess rapid migration and population growth strategies consistent with their domination of Disco and stepping stone habitats which once again became available following the resumption of flows following the recent drought.

Table 9. Species traits and ecological distribution patterns that relate to Resistance, Resilience and the persistence of fish under extreme climatic variability in the Neales Catchment.

	Species traits and ecological factors that relate to Climatic Resistance and Resilience										
Species	**Dispersal Ability**	**Distribution (Neales)**	**Distribution (LEB)**	**Distribution (Australia)**	**Reproductive Ecology**	**Etho-ecological reproductive guild**	**Fecundity**	**Longevity**	**Juvenile Mortality**	**Growth to maturity**	**Local abundance in drought conditions**
Barred grunter	LOW	Common	Uncommon/ patchy	Widespread	Non-adhesive eggs (0.6-.85 mm)	Non-guarder, Lithophil	40,000-77,000	MOD (3-5y)	Unknown	RAPID (<1 y)	MOD
Spangled perch	HIGH	Common	Common	Widespread	Sinking non-adhesive eggs (0.60-0.85mm). Facultative flow trigger.	Non-guarder, Lithophil	100,000+	MOD	Unknown	RAPID	MOD
Golden perch	MOD	Uncommon/ patchy	Common	Widespread	Semi-buoyant eggs (3.9mm) Flow trigger	Non-guarder, Pelagophil	500000+	HIGH (5y+)	Unknown	Likely SLOW (2+ y)	LOW
Bony herring	HIGH	Common	Common	Widespread	Buoyant eggs (1mm). Independent of flow. Spring and summer spawning.	Non-guarder, Pelagophil	30,000-800,000	MOD	Unknown	MOD (1 y)	MOD
Desert rainbowfish	MOD	Common	Common	Widespread	Adhesive eggs (0.8-0.95mm).	Non-guarder, Phytophil	20-100	LOW (1-3y)	Unknown	RAPID	LOW
Desert goby	LOW	Common	Moderate	Indigenous	Eggs 3mm. Nov-march spawning under rocks.	Guarder, Substrate Chooser, Lithophil	50-300.	LOW	Unknown	RAPID	HIGH
Lake Eyre hardyhead	MOD	Common	Uncommon/ patchy	Indigenous	Unknown, likely few adhesive eggs and protracted spawning.	Non-guarder, Phytophil	50-800 (~220 mean)*	LOW	Unknown	RAPID*	HIGH
Gambusia	LOW	Common	Uncommon/ patchy	Widespread	Live young. Protracted spawning.	Internal Bearers, Ovoviparity	<375	LOW	Unknown	RAPID	LOW
Barcoo grunter	HIGH	Uncommon/ patchy	Moderate	Indigenous	Buoyant, non-adhesive. Likely flow spawners.	Non-guarder, Pelagophil	100,000	MOD.	Unknown	Unknown	LOW
Source:	(McNeil and Schmarr 2009)	Current Survey + (Allen *et al.* 2002, McNeil *et al.* 2008, Balcombe and Kerszy 2008, McNeil and Schmarr 2009)		(Allen *et al.* 2002)	(Wager and Unmack 2000)	(Winemiller and Rose 1992, Wager and Unmack 2000, Pusey *et al.* 2004)	(Wager and Unmack 2000, Allen *et al.* 2002, Pusey *et al.* 2004)	(Wager and Unmack 2000, Pusey *et al.* 2004)		(Allen *et al.* 2002, Pusey *et al.* 2004)	(McNeil *et al.* 2008)

Table 10. Various predicted strategies used by fish species in the Neales catchment to maintain resilient populations and for resisting the impacts of drought.

	Hypothesized strategies for persisting during drought (*Resistance* & *Resilience*)				
Species	**Reproductive strategy**	**Tolerance**	**Genetic differentiation**	**Phenotypic diversity**	**Strategy for continued persistence during drought**
Barred grunter	Opportunistic	Low/ mod	High	Low	Unknown/ vulnerable
Spangled perch	Opportunistic/ periodic	High	Low	High	Resilience
Golden perch	Periodic	Low/ mod	High	Low	Unknown/ vulnerable
Bony herring	Opportunistic/ periodic	High	Mod	High	Resilience
Desert rainbowfish	Opportunistic	Mod	Low	High	Resilience
Desert goby	Equilibrium	High	High	Low	Resistance
Lake Eyre Hardyhead	Opportunistic	High	Mod	High	Resistance
Gambusia	Opportunistic	Mod	Low	Low	Unknown/ vulnerable
Barcoo grunter	Opportunistic/ periodic	Mod	Low	Low	Unknown/ vulnerable

Of interest are the barred grunter and Barcoo grunter, both of which were found only in the Ark refuge of Algebuckina, with neither species showing any migration out of this refuge during connecting flows subsequent to the end of the drought. This pattern, along with their absence from extremely harsh waterholes, suggests that early expansion of catchment populations is not the key mechanism of resilience building in these species, nor is a strategy of resistance in harsh environments. Whilst Barcoo grunter were too rare to allow any hypotheses to be developed, the current study, when considered in line with past data from the catchment has identified two factors that may help us to understand the specific strategy of barred grunter for dealing with climatic variability. Initially, catches of barred grunter in Algebuckina waterhole were extremely rare (McNeil *et al*. 2008), but have steadily increased at the site during successive seasons (McNeil and Schmarr 2010).

This suggests that the species may be particularly sensitive to the impacts of drought and climatic harshness, even within refuge habitats. It also suggests that barred grunter appear to be re-building population size and structure within the refuge waterhole, rather than rapidly expanding in range and exploding catchment wide populations as is the case for bony herring, spangled perch and desert rainbowfish. The final indication of barred grunters' potential strategy is the discovery of the species during the autumn 2010 survey at two sites, Peake and Neales Crossing both of which are directly upstream neighbours of the Ark refuges. This suggests that after re-building refuge population structure, this species is now undertaking a range expansion.

As such it is hypothesised that future surveys should detect this species from a wider range of habitats and that the species may eventually spread across the catchment to compete with the highly mobile species that already dominate Disco habitats across the catchment. This pattern identifies the barred grunter as a particularly important species for monitoring the broader ecological condition of the Neales River. Natural conditions must allow for the gradual rebuilding and recolonisation by this species over several successive seasons of good flow conditions. Impacts such as water extraction or climate change that might change flow regimes are likely to be expressed in the failure of this species to gradually rebuild refuge populations or to recolonise catchments.

In the current study, Golden perch were common across the catchment in spring 2009 associated with Disco and Ark waterholes, and were found to be in spawning condition and to have recruited strongly with a large number of 0-1+ age fish caught. However, in autumn 2010 the species was caught only at Algebuckina (and 1 individual from South Cliff) and was no longer associated with disco habitats. However, the majority of the autumn 2010 catch were recent recruits suggesting that whatever the reason for the contraction in range, the species continues to spawn and recruit strongly within the Algebuckina refuge. This pattern suggests a resilience strategy, whereby the species responded to improved flow and catchment connectivity through migration and reproduction, but has curiously contracted in range and shown no signs of integrating the recruits from 2009 into stable adult populations across the catchment. An untested hypothesis that may explain this pattern is the presence of extremely high levels of disease in this species following high flows in other catchments of Lake Eyre (Balcombe and McNeil 2006, McNeil *et al.* 2009). Alternatively, recent observations that this species spawns regularly under low flow conditions in the Cooper Creek (Balcombe and Kereszy 2009, Kereszy *et al.* 2011) may outline a sub-optimal spawning strategy and large scale flooding and long term connectivity may be required for this species to become dominant across the Neales catchment. It should also be noted that large bodied perch have previously been found to select only larger, deeper and more permanent waterholes to reside in following general floodplain inundation (McNeil 2004) and that Golden perch may utilise Disco habitats fleetingly for spawning and early development before returning to the security of larger refuge waterholes.

CONCEPTUAL MODELS FOR THE ECOLOGY OF FISH IN THE NEALES CATCHMENT

When considered in combination with patterns, from previous fish surveys, the data informs the development of conceptual models that begin to capture and represent the interactions between climatic cycles, hydrological connectivity, the presence and distribution of waterholes and the key drivers of fish population dynamics in the Neales River and perhaps other arid zone rivers.

The conceptual model consists of a number of interrelated components all of which occur over complex temporal and spatial scales. To simplify, each component will be introduced and outlined separately before being brought together as a single conceptual model. Finally, the utility and applications for the model will be outlined and discussed.

Component Factors

Climatic Cycles

The predominant driving force of fish ecology in the Neales River is the extreme climatic variation between wet and dry periods. Cycles occur at two scales: seasonal, annual cycles of wet and dry periods, and longer term multi-annual climatic cycles that range between very wet and very dry periods. Both annual and multi-annual cycles are highly variable, yet somewhat predictable. Annual rainfall often occurs in relation to Northern Australian Monsoonal rainfall with wet seasons more likely between December and March each year. Summer rainfall averages ~450-500mm whilst winter rainfall is ~90-100mm (Allen 1990). However, the strong influence of the El-Niño Southern Oscillation (ENSO) results in some of the most temporally variable rainfall patterns in Australia (Allen 1990) leading to one of the most variable hydrological regimes of any catchment in the world (Puckridge 2000). In general however, climatic and hydrological variability occur at two scales (Figure 30). Seasonal climate cycles are variable, but generally cycle annually between wet and dry seasons. Supra-seasonal drought cycles form a ramping level of disturbance that magnifies the impact of seasonal dry periods within annual cycles (Lake 2003, McNeil *et al.* 2010a).

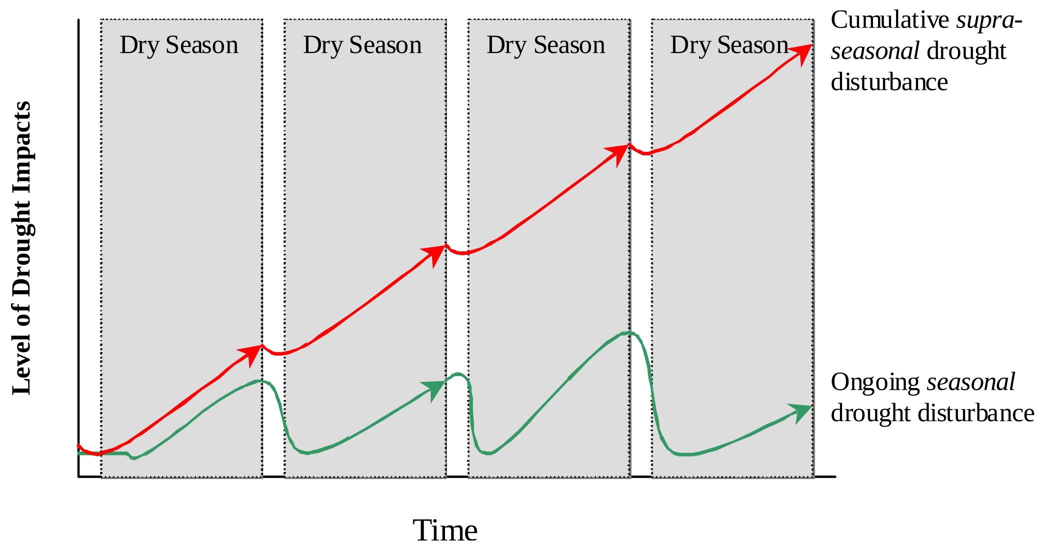

Figure 30. Nature of seasonal climatic cycles during relatively wet periods and during ramping disturbance of supra-seasonal drought (from McNeil *et al.* 2010a)

Whilst these summaries account for increasing drought impacts the pattern is repeated in reverse as drought impacts decline and wetter climatic conditions again prevail, thus forming a supra-seasonal climatic cycle that reflects the wave-like pattern of seasonal climatic cycles (Figure 31). Water regularly reaches Lake Eyre through the Diamantina/Warburton system, while the Cooper Creek flows to the terminal wetland much less regularly. High flows from both of these key tributaries are required to create significant lake filling events (Armstrong 1990).

A reliable indicator to climatically wetter periods is the operation of the Lake Hope fishery (Turner 1994), which relies on significant levels of flows reaching the bottom end of Cooper Creek before it is activated. Over the past 30 years, this fishery has become operational in ten-year cycles (PIRSA in prep). The ten-year cycle fits both with the timing of ENSO cycles and with modelled predictions of a high likelihood of Lake Eyre filling by over three meters (Armstrong 1990). In the hypothetical model, we assume an underlying supra-seasonal climatic cycle of ~10 years, although it is acknowledged that actual climatic phases may vary significantly (Figure 32).

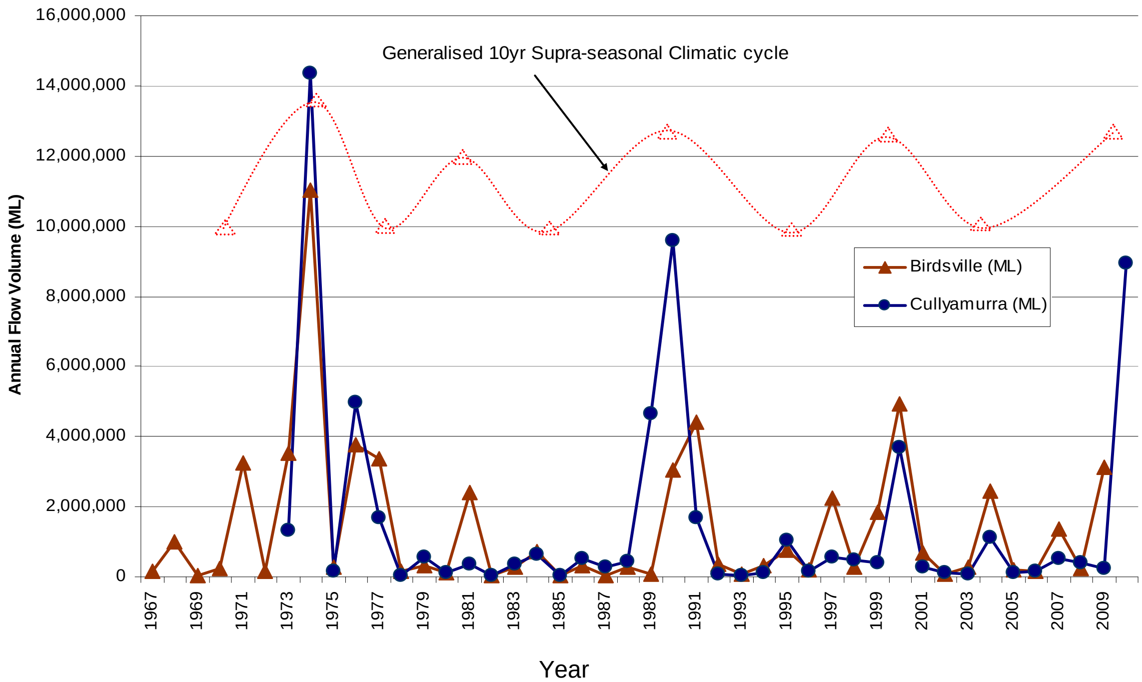

Figure 31. Annual flow rates in the lower Diamantina River and Cooper Creek showing the fluctuating supra-seasonal climatic cycle of wet and dry phases covering 10-15 cycles.

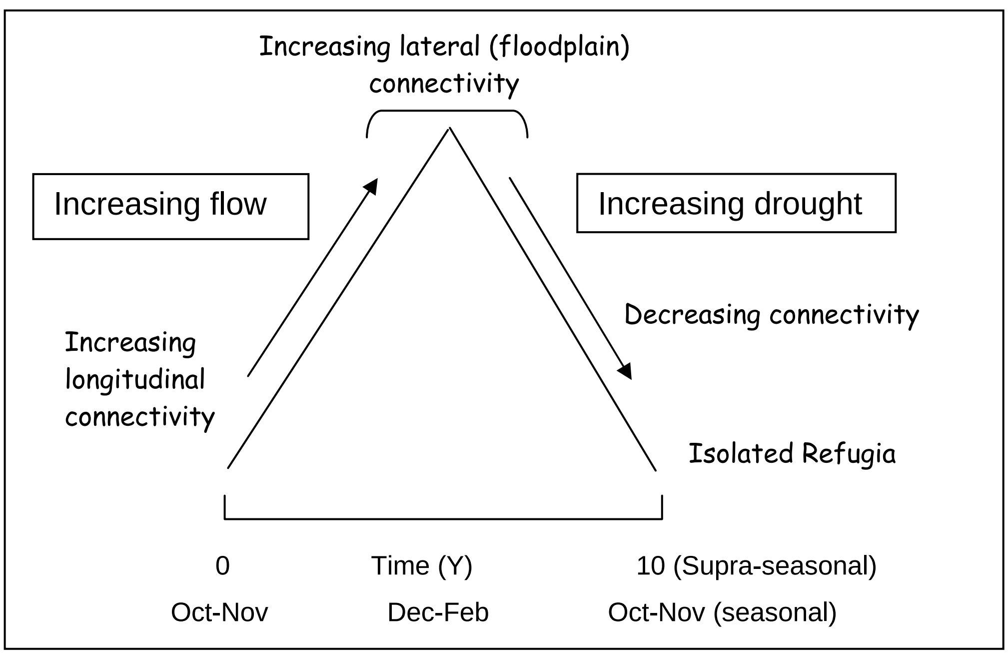

Figure 32. Cyclical hydrology and aquatic connectivity varying with climatic cycles (seasonal and supra-seasonal)

Resistance and Resilience Factors

It has become widely accepted in the scientific literature that arid zone river systems including those in Lake Eyre Basin (LEB), are driven by 'boom and bust' cycles, based on the overwhelming influence of climatic drivers of flooding and drought (Puckridge *et al.* 2000, Arthington *et al.* 2005, Balcombe *et al.* 2007, Balcombe and Arthington 2009). Boom and bust cycles of fish populations in LEB underpin two extremely important concepts, essential for aquatic biota to survive in such heavily disturbed arid environments. Biotic *Resistance* describes the ability for species to tolerate the impacts of the climatic disturbance whilst the concept of *Resilience* describes the ability for species to rebuild viable populations once the pressure of disturbance has eased (McNeil *et al.* 2010a).

Recent drought in the Murray Darling Basin has lead to an increased awareness of the role of *Resistance* and *Resilience* in determining how freshwater fish survive and recover after drought periods (McNeil *et al.* 2010a). These concepts can equally be applied to the Lake Eyre Basin as they underpin the response of biota to the climatic cycles outlined above and are added to the conceptual model (Figure 33).

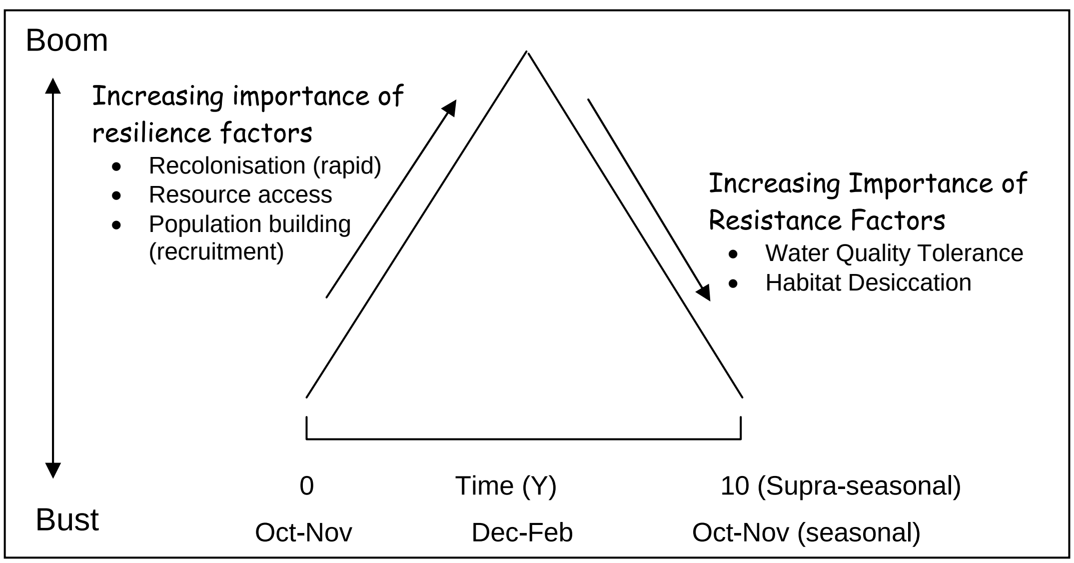

Figure 33. Boom and Bust cycles and the varying role of Resistance and Resilience factors in maintaining viable fish populations.

Resistance occurs at small, local scales such as individual waterholes, where species must tolerate the local conditions to survive. In the first instance, this may relate to the permanence of water as no LEB fishes are adapted to surviving without water for any length of time. As catchments dry, water quality rapidly deteriorates in many waterholes and, in the Neales, this is predominantly the concentration of salts that rapidly reach lethal levels for freshwater fishes (Figure 34). In fresh habitats, high temperatures and low dissolved oxygen levels can be equally lethal. Two species in the Neales stand out as having exceptionally high *Resistance Potential*; they are the desert goby and Lake Eyre hardyhead.

This process of tolerance and resistance underpins the Polo Club refugia concept, where species with low tolerance are knocked out of harsh habitats leaving highly tolerant species to survive. Fish must pass the Resistance phase to survive through drought. This requires that refuge habitats *must* persist in the isolated Neales catchment, where all catchment species can survive local conditions (Ark refuge). In the 2006/07 drought, the sole documented Ark refuge was at Algebuckina.

Once species have managed to survive the local and immediate impacts of seasonal or supra-seasonal drought, they must begin the task of rebuilding resilient populations, that is, populations that are able to regain former distribution, abundance and genetic structure sufficient to survive future disturbances intact. Resilience therefore relies on factors such as recolonisation ability, spawning and recruitment requirements and access to appropriate resources (Figure 33).

Recent studies in the Neales have revealed that the resilience potential differs greatly among fish species. Species such as spangled perch and bony herring have extremely high *Resilience Potential* and can recolonise rapidly, spawn and recruit easily and build new populations across the catchment after very short periods of in-channel flow following extreme drought (McNeil *et al..* 2008, McNeil and Schmarr 2009).

Other species have moderate *Resilience Potential* and are slower to recolonise (desert rainbowfish, Gambusia, barred grunter) or are able to recolonise quickly, but require a large flood before they can spawn and recruit in high numbers across the catchment (golden perch). Others appear to be focussed on remaining within refuge habitats, particularly Polo Clubs where the exclusively tolerable conditions exclude predators and competitors and facilitate the establishment of dense, localised populations (Lake Eyre hardyhead and desert goby). The rates of recolonisation and population building as conditions improve, and conversely, the rates of spatial decline due to climatic impacts as drought conditions prevail, will vary based on species resilience and resistance characteristics. The variable *Resistance and Resilience Potentials* are added to the conceptual model (Figure 34). Traits and ecological factors that define *Resistance* and *Resilience* Potentials for Neales River fishes are given in Table 9. These traits and factors can be used to predict the various strategies that fishes in the Neales use to deal with the highly variable climatic cycles of wetting and drying in the Neales catchment, a summary of which are presented in Table 10.

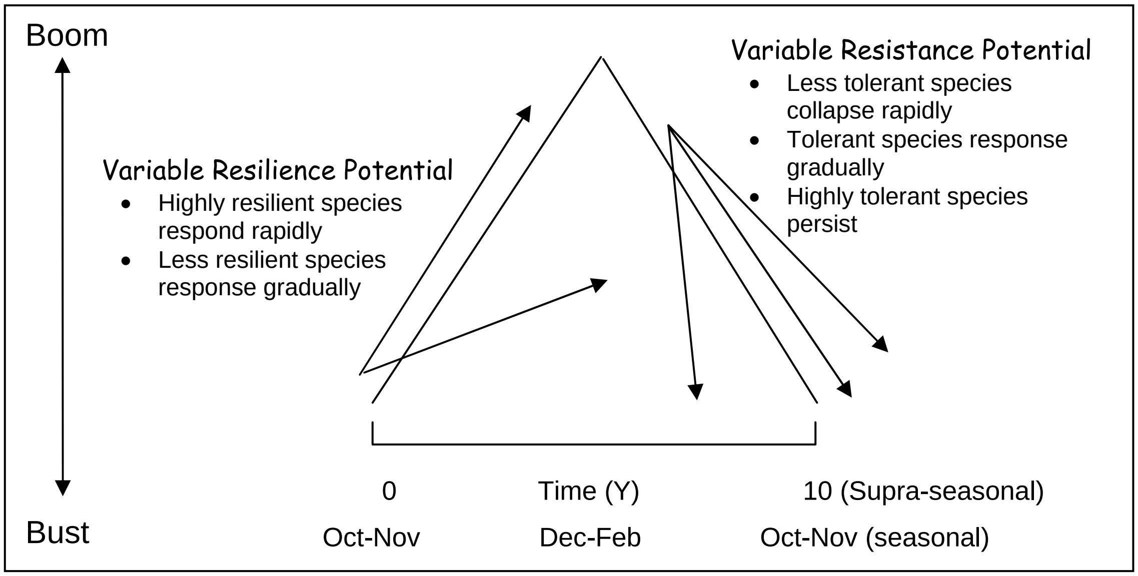

Figure 34. The role of variable *Resistance and Resilience Potentials* in determining the impact of climatic disturbance on fish populations and subsequent recovery.

Resistance and *Resilience* not only work differently across different spatial scales (local versus catchment wide) and across species (different *Resistance and Resilience Potentials*) but also work at different time scales, again related to the overlying climatic cycle. *Resistance* becomes important during the extremes of seasonal or supra-seasonal drought where conditions are at their worst (Bust periods).

Resilience becomes important once conditions improve and during the benign periods between droughts (Boom periods). Aspects of resistance and resilience become key factors at different times and for different species. For example, recolonisation is important soon after drought breaks, whilst access to resources and population rebuilding is important later once conditions improve and fish have access to resources and reproductive habitats across the catchment. Recolonisation is believed to be particularly important for biota in the Lake Eyre Basin, as the evolutionary history of the basin appears to favour widespread, poorly differentiated species rather than local endemism (Williams 1990).

Interactions of Climate, Refugia and Fish Assemblage

Finally, the conceptual model brings together the interactions between climate, habitat availability and refuge type across the catchment landscape (Figure 35). Whilst boom and bust cycles occur over seasonal and supra-seasonal timeframes, the roles of various refuge-types also vary. During drought (bust periods), Ark refugia are critical for the persistence of all species, whilst Polo Clubs allow highly tolerant taxa to maintain communities in harsh waterholes that can be source populations once drought conditions are alleviated. As wetter conditions develop, hydrological connectivity improves and the number of waterholes increases, switching on a large number of Disco waterholes where recolonising species rebuild their numbers and access food resources that also boom in formerly dry waterholes. Finally, when climatic conditions have provided flows and connectivity for an extended time, even slow colonisers are able to move throughout the catchment and as a result, formerly heterogeneous catchment assemblage patterns converge, with most species found in most waterholes. During wetter periods, a large number of waterholes effectively function as Ark refugia and enable all species to persist through dry seasons across the landscape. After several years, drought conditions will return and the environmental impact of disturbance will once again lead to the loss of species from harsh habitats. As fewer waterholes persist through dry seasons, the importance of Ark and Polo Club refugia will become increasingly important for the survival of fish populations.

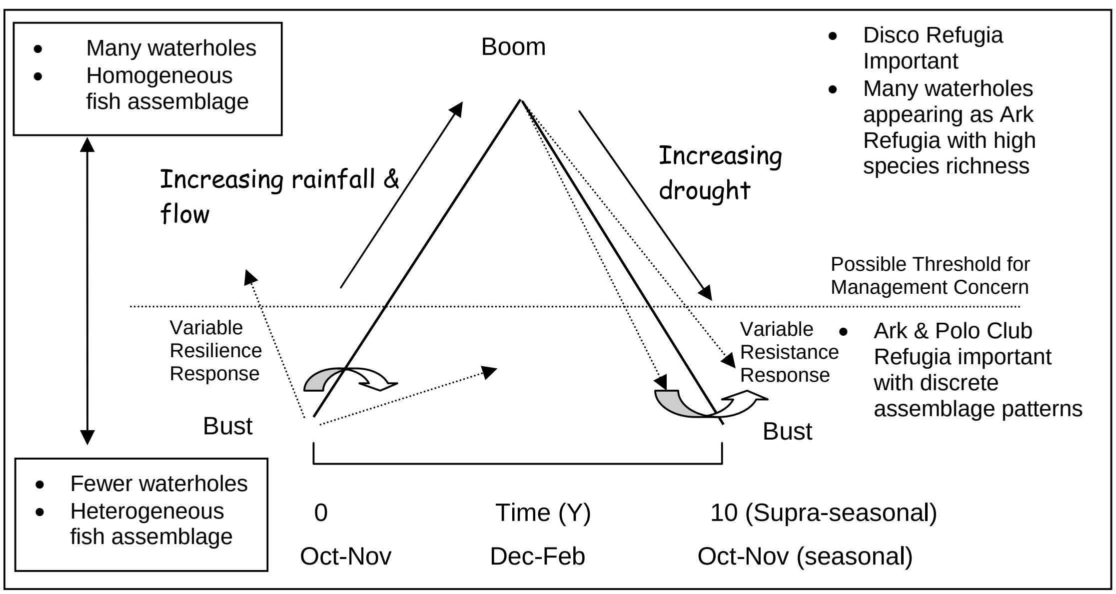

Figure 35. Conceptual model for the interaction of climatic cycles, refuge types and fish assemblage structure across the Neales catchment, integrating key points from Figures 35, 36 and 37.

STATUS AND CONTROL OF PEST FISH

Gambusia in the Neales Catchment

A single introduced species, the *plague minnow* Gambusia has been recorded from the Neales catchment. This fish was distributed intentionally throughout the region for the purpose of mosquito control, for which it was ineffective (Wager and Unmack 2000). Whilst the spread has been far and wide throughout the Lake Eyre Basin, populations of Gambusia are patchy and the species has not become as ubiquitous or dominant as it has in the Murray Darling basin (McNeil and Schmarr 2009).

Gambusia were widespread throughout the Neales and Peake catchments during the April 2010 survey despite its presence in a few isolated locations around the mid-catchment since 2006. This suggests that wetter conditions that developed in the area over the 2009/10 wet season were adequate for providing recolonisation pathways for this pest species. However, it remains to be seen whether these populations in recently colonized waterholes will persist. Data from previous Neales River surveys indicate that Gambusia often appear in riverine waterholes, only to disappear again in the following season (Figure 3 in McNeil and Schmarr 2009). However, the data show that while the pest species is a relatively slow colonist compared to most of the native fish species, it is certainly able to access habitats across the catchment under appropriate conditions.

As a result, the observed pattern that Gambusia have do not dominate Lake Eyre Basin catchments as they have in the Murray-Darling is unlikely to result from an inability to colonize new habitats, but more likely associated with an inability to establish and build viable long term, populations within particular waterholes. A number of possible explanations exist for this pattern, as invasive fishes face a number of barriers to establishing new and robust populations within new areas following translocation (Moyle and Light 1996). In particular, the intact native fish fauna and natural flow regime have been presented as explanations for the inability of Gambusia to dominate Lake Eyre Basin (Puckridge *et al.* 2000, Pritchard *et al.* 2004, Costelloe *et al.* 2010).

Areas that maintain a robust and diverse native fish assemblage are more likely to be resistant to new invasions, particularly where various trophic niches are already fully occupied by existing species. The Neales River possesses a relatively intact assemblage of native fish species that is unlikely to have been severely disturbed by European colonization. This is in contrast to the Murray-Darling Basin where native fish have become severely impacted by land use changes, water resource development, infrastructure and water abstraction, and a range of other impacts such as historical exploitation of fish as a resource and the introduction of many invasive fish species and diseases from overseas (MDBA 2009). This has lead to the loss of ~80% of native fish abundance and widespread localized extinctions and range contractions with the survival of many South Australian species becoming highly threatened (Hammer 2009).

In contrast, the relatively intact riverine and catchment environment, including native fish fauna, of the Lake Eyre Basin is likely to be more resistant to the easy establishment of Gambusia populations. Under this scenario, the maintenance of diverse and abundant native fish fauna in the Neales River is paramount to preventing Gambusia and other potential invaders such as carp from overtaking and dominating aquatic habitats (Moyle and Light 1996, Koehn2004).

Hydrological variability has also been implicated in the establishment and abundance of Gambusia in Australian systems. Floods have been shown to greatly reduce Gambusia abundances; however, diminished populations often recover during subsequent dry phases (McNeil 2004, Chapman and Warburton 2006). The very high degree of hydrological variability in Lake Eyre Basin Rivers has historically been suggested as a possible reason for Gambusia's patchiness across the Basin (Pritchard *et al.* 2004, Costello *et al.* 2010). Recently published data from the Lake Eyre Basin suggests that hydrographically variable habitats in the Cooper Creek system are more likely to have Gambusia populations than those that are relatively hydrographically stable (Costelloe *et al.* 2010).

The distribution and relative abundance of Gambusia collected in the current study are mapped (Figure 36), with the relative abundance of Gambusia reflected by the relative size of the Gambusia image for each site. In most of the sites surveyed in the present study, Gambusia numbers were relatively low and made up a small proportion of the total fish population (see Table 2). However, a few locations contained exceptionally large populations of the pest fish. In Big Blyth Bore, One Mile Bore, Freeling North Spring and the Outside Spring group, Gambusia were captured in very high numbers with the species either dominating or making up a significant proportion of the fish catch. Whilst catch rates at these sites numbered in the thousands, visual observations estimated actual numbers to be in the hundreds of thousands to millions.

The survey data shows that these sites are largely clustered around the lower Peake Creek proximal to the Denison Ranges and the Junction with the Neales River (Figure 36). All of these sites are either bore drains or springs fed by Great Artesian basin upwellings and therefore possess hydrologically stable environments. This supports the hypothesis provided by Costelloe (2010) that stable waterbodies characterized by very stable water levels and flow conditions serve as key refugia for introduced pests (Gambusia and goldfish) in the LEB.

Furthermore, the very shallow and stable nature of these springs correlates to the natural habitat preferences for this species (MacDowall 1996). In such sites, Gambusia attain a competitive edge against native fish species not available to them in deeper riverine waterholes where high levels of flow variability and large populations of native fishes detract from the competitive stature of the species. Indeed this hypothesis supports the patterns observed here, where riverine populations have remained absent or small under the recent post drought recovery period.

Figure 36. Distribution of Gambusia in the Neales catchment surveyed in November 2009 and April 2010. Size of fish is indicative of relative abundance.

This data also supports the hypothesis of Chapman and Warburton (2006) that control activities for Gambusia are best focused on periods directly following drought or flood disturbances when populations will be at their least abundant and possibly least resilient. The recolonisation observed in the most recent survey suggests that the opportunity may have been missed for the current drought period as the species seems to be recovering from the impacts of drought that peaked four years ago in 2006. These sites are connected to the main channel only in large flows where broad areas of the floodplain are inundated (Costelloe in prep).

However, the nature of the Gambusia population, being concentrated highly around a small number of largely isolated spring habitats suggests that these sites serve as ‘hot spots’ for this species and are probably acting as key refugia in the Neales catchment. At these spring habitats, Gambusia can build up large populations within stable and suitable conditions and continually re-invade the catchment during connecting flows.

As a slow colonizer (Chapman and Warburton 2004) it may take several high-flow seasons for Gambusia to successfully recolonise catchments after catastrophic drought and flood events. The long term data collected from a subset of sites since 2006 show that Gambusia has only just colonized upstream waterholes such as Stewarts and Hookeys in 2010. Prior to this study, they showed up at Algebuckina and Peake waterholes from time to time since 2006 but were never caught at upstream sites.

This result is a significant finding for the Neales study. Whilst recent research has indicated that hydrological variability may be a key factor that prevents Gambusia from becoming as dominant in the LEB as in the more unnatural and stable hydrological regime of the Murray Darling Basin (MDB) (Chapman and Warburton 2004, Costello *et al.* 2010). However, the evidence for this assertion is made from observations that large floods reduce the distribution and abundance of Gambusia in the LEB. Our results indicate that in addition to this, severe drought is also associated with the loss of Gambusia from many habitats and that several seasons of improved flow are necessary for the species to begin decolonizing and re-building populations. This is likely to work through similar mechanisms to naturally inundated floodplains within the MDB, where smaller and shallower habitats where Gambusia can dominate native fishes, often dry out under harsh climatic conditions, leaving larger habitats where native fish can outcompete Gambusia to seed catchments following natural, climate driven re-inundation (McNeil 2004). The role of hydrological variability in controlling Gambusia is therefore a two-pronged control with both large flooding and extreme drying contributing to the large scale control of Gambusia populations that persist largely within extremely stable refuge habitat where hydrological extremes do not occur.

The continuation of monitoring riverine sites is highly recommended as a means of understanding whether or not the pest species is establishing populations in these upstream sites or whether future conditions might see a natural contraction back to downstream refugia. If the later scenario occurs then control activities may be initiated to impact on or eradicate downstream remnants in refuge habitats.

Accordingly, these sites provide an ideal focus for any Gambusia control activities. Strategies that can eradicate or reduce the abundance of Gambusia at these sites may convey a disproportionately large impact on the overall population by removing the strongholds from which repeated colonization attempts can be based. Just as the risk of local extinction for native fish becomes very high if the refuge at Algebuckina Waterhole is taken away, so too the risk of Gambusia extinction will be increased if the Peake Spring refugia can be taken away or the Gambusia population there can be removed.

Gambusia in GAB Springs and Bore Drains

GAB springs in South Australia (with the exception of Dalhousie) are historically the sole domain of a single native fish, the desert goby. However, throughout the latter half of the twentieth century, records show Gambusia invading a number of springs throughout the Lake Eyre Basin.

GAB springs are isolated from the main channel habitats most of the time but can potentially become connected during floods allowing the migration of Gambusia into and out of lower lying springs. The data from current surveys suggests that low-lying springs closer to the channel bed are more likely to be dominated by Gambusia. Desert gobies have now been completely lost from all GAB springs that have Gambusia. This process has occurred gradually over the past fifty years or so with gobies and Gambusia co-existing for some time in these springs prior to Gambusia overrunning the sites to the exclusion of the native spring goby (Glover 1990, Wager and Unmack 200). Springs that occur at higher elevation tended to possess healthy populations of desert goby and were free of invasive Gambusia. Thus, the Hawker Springs remain dominated by gobies whilst the lower lying Outside Springs are dominated by Gambusia; Freeling springs possess gobies whilst the lower lying North Freeling spring is dominated by Gambusia. Similarly at Nilpinna, the Nilpinna Spring has gobies whilst only 100m away, the lower One Mile Bore had Gambusia.

This pattern suggests that Gambusia have colonized springs in the region via the river system and associated waterbodies and they may be able to invade new springs if connecting flows are significant enough to open up colonization pathways. If this occurs, resident desert gobies will almost certainly be lost within a decade. It is therefore recommended that management efforts be undertaken to prevent the further spread of Gambusia throughout GAB spring systems and eradicate them from existing spring sites. These efforts should incorporate the return of desert gobies sourced from neighboring springs.

The physical characteristics of GAB spring render them ideal for fish-control programs using rotenone, a proven ichthyocide that is natural and rapidly breaks down following application (McNeil *et al.* 2010e). However, extreme caution and care MUST be taken if considering such control methods that are lethal to a wide range of aquatic fauna and would result in the loss of endemic vertebrate species from control sites. Rotenone control programs often incorporate the removal, storage and return of invertebrates, turtles, frogs and other desirable aquatic fauna once treatment is complete. It must be noted that the use of rotenone is controlled and permits and approvals are required for its application as an ichthyocide.

The highly endemic nature of many GAB spring species requires that exceptional care must be taken to protect and restore GAB spring fauna either by temporary removal (<24hours) or transfer of new fauna from neighboring and genetically similar populations. Whilst the impacts of Gambusia are possibly disastrous for GAB spring ecosystems, great care must be taken to minimize the impact of control actions on those ecosystems. Control programs may also include netting and fencing to prevent recolonisation of Gambusia back into springs following removal. Relatively cheap fencing options are potentially available but care must be taken to seal off any aquatic pathways of colonization (Kerezsy 2009).

Recommendations for Gambusia control

A number of recommendations are suggested as the basis of a management plan for Gambusia in the Neales catchment:

1. As a priority, close off bore flows that support the Big Blyth and One Mile (Nilpinna) bore springs, which serve as the primary refugia for Gambusia in the Neales catchment. Native fish are unlikely to be a major issue at these sites.
2. Investigate control options for isolated GAB springs, in particular the outside spring group. Endemic invertebrate species are likely to be an issue at these sites.
3. Develop a targeted netting and removal program for Gambusia in Algebuckina waterhole and North Freeling spring to reduce Gambusia populations without impacting on native fish. This may be accomplished by expanding ongoing monitoring effort at these sites, e.g. additional netting efforts added to LEBRA monitoring program.

4. Continue to monitor long term data sites and recently discovered hot spots to inform on the invasion status of Gambusia throughout the catchment. New ‘hot spot’ sites may emerge during wetter climatic periods.

5. Investigate the potential to identify nursery wetland signatures from fish otoliths as a means for identifying the actual source of riverine colonists and identify key control habitats.

6. Construct barriers to prevent Gambusia moving upstream into new habitats, particularly GAB springs that still contain desert gobies.

7. Respond to any new GAB spring invasions immediately to prevent establishment of new Gambusia populations and the loss of more desert goby populations.

MANAGEMENT IMPLICATIONS

A number of key aspects of this study have specific implications for the ongoing management of aquatic habitats in the Neales catchment as well as the broader LEB. The identification of distinct habitat (refuge) types with distinct fish communities, serves as a lens through which improved management of ecosystem processes and aquatic health may be viewed.

The impact of various threats such as feral animal and land use impacts may impact most strongly on smaller springs and therefore upon spring populations of desert goby. During periods of drought however, critical refuge habitats will also become focal points for watering activities. During these periods, the potential for high density feral animal impacts to have disastrous impacts on native fish populations is very high. Fencing and other exclusion or control activities should be targeted towards springs and Ark refuges (i.e. most permanent waterholes) in the first instance. It should also be noted that permanent exclusion from spring habitats from grazing pressure leads to choking of open water fish habitats by emergent macrophytes (Kodric-Brown and Brown 2007) and must be managed to maintain micro-habitat heterogeneity. Failure to do so may lead to the loss of native fishes dependant on those micro-habitats (Kodric-Brown and Brown 1993). All management activities should be aimed at maximising a diversity of micro-habitat types within arid waterholes and to avoid any activities that lead to shifts in habitat diversity or relative abundance of habitat types. This is consistent with the intermediate disturbance hypothesis (Connell 1978) for maximising biodiversity and preventing ecological succession to a primary stage dominated by a single habitat type.

Processes such as salinisation, vegetation clearance, changes in geomorphic processes and climate shifts are likely to impact strongly on permanent Ark refugia and Disco refugia which are critical for resilience building between drought periods. The direct extraction of water resources from Ark or Disco habitats during periods of isolation for stock watering, road construction, resource industry applications or other uses, should be prohibited as these refugia are especially important for the resistance and resilience of fish species.

More broadly, any water resource development that impacts on the patterns of hydrological connectivity, within channels, across floodplains, and in tributary creeks is likely to impact on the resilience of fish species dependant on rapid periods of colonisation and population expansion. In addition, loss of connectivity through channelization and arroyo formation, construction of berms or levees, roads, culverts and bridges are likely to have a strong negative population scale impact on resilience strategists such as spangled perch, bony herring, desert rainbowfish and Golden perch.

Ark refugia and significant Disco waterholes are also the key sites for visitation by tourists and are often close to major tracks and thoroughfares, possibly due to their long historical significance for horseback travel (Gosse 1874), water for trains and stock routes. Historically of course, this reflects the high dependence of the indigenous community, on accessing water resources as they travelled throughout the region (Gosse 1874). However, observations made during the current study is that tourist access to waterholes and their impact on soils, vegetation and possibly native fish resources warrants very close management consideration before any significant impacts become evident.

The investigation of polo club refugia has re-emphasised the importance of the highly saline regions in maintaining the dynamic equilibrium of the Neales catchment. Large populations of Lake Eyre hardyhead and desert goby exist within saline waterholes, largely to the exclusion of competition and predation from other species. Saline waterholes are often considered to be extremely ‘low value’ ecologically but are extremely important to the maintenance of fish community structure in the Neales catchment. Although not presently believed to be at risk, any factors that influence flow regime including flow regulation and climate change impacts will present key threats to these habitats. The key recommendation is that saline waterholes must be considered as critical components of aquatic desert ecosystems and any processes identified that might impact on their maintenance and protection should be strongly considered by management groups.

The issue of pest Gambusia has largely been addressed as a key management threat. Although eradication is considered impractical, this study has revealed that the pest fish largely dominates stable, shallow waterbodies, in particular bore drains and springs. It is recommended that water troughs be considered as alternatives to remove Gambusia dominated habitats whilst maintaining pastoral requirements for bore water. GAB springs represent a more difficult threat that is of high conservation concern due to the identified process whereby Gambusia eliminate spring populations of desert goby in springs wherever periodic inundation and isolation occurs (e.g. in lower elevation springs closer to river channels). It is recommended that a Gambusia control plan be considered, to prioritise feasible management interventions that may reduce the distribution and impact of this pest fish.

SUMMARIES AND RECOMMENDATIONS:

Fish Community:

- The fish survey has revealed that recovery from drought continues across the Neales catchment with rapid recolonisers such as spangled perch and bony herring now joined by desert rainbowfish and pest Gambusia throughout the entire catchment.
- Golden perch were observed to spread throughout the catchment during 2009 but have curiously been caught only from Algebuckina Waterhole in the final survey in April 2010.
- Barred grunter and desert goby have begun to recolonise the catchment following regular periods of in-channel connectivity since 2007, but continue to be largely restricted to the mid-Neales and mid-Peake areas.
- The newly rediscovered Barcoo grunter was found at Algebuckina Waterhole only and appears to be restricted to this critical refuge waterhole.

Waterholes as Climatic Refugia:

- The study found strong linkages between climatic refuge typologies and fish community structure across the catchment. Ark, Polo Club, Disco and stepping stone refuges, as well as springs are all critical habitat types that each needs to be preserved.
    - It is recommended that management plans be drawn specifically to preserve the unique values of each refuge type.
- The survey has re-affirmed the status of Algebuckina Waterhole as the critical refuge waterhole (Ark) in the Neales catchment. The survey also suggests that Baltacoodna in the Peake Creek may be similarly important, although the surveys lack the extreme drought data that was available for Algebuckina to affirm this.
    - It is recommended that Ark refuges and significant Disco habitats be targeted for specific management planning and protection activities to optimise investment returns in the protection of biodiversity and waterhole habitat.

- The study revealed the critical importance that numerous non-permanent but significant (Disco) refugia have in the recovery of fish populations following the resumption of rainfall and flow. Colonisers are able to use these waterholes to rebuild populations across the catchment. It is anticipated that the species assemblage of disco waterholes should continue to grow under relatively wet climatic periods and decline drastically under a return to drought.
    - The importance of ephemeral and non-permanent waterholes was highlighted through this study and activities such as tourism, pastoral uses, water resource development and construction of roads, causeways and bridges - even in ephemeral watercourses and floodplains - should be subject to management approvals to maintain connectivity and habitat values during periods of inundation.
- GAB springs are important permanent refugia for desert goby but are under threat from invasion by Gambusia which has now excluded goby from all springs where the pest fish is present. This pattern is closely linked to hydrological connectivity and elevation of bores across the landscape.
- Bore drains have been found to serve as refugia for huge populations of Gambusia possibly due to the stable shallow conditions in these habitats. Native species are very low in abundance in these habitats.
    - It is recommended that all bore drains be converted to specific watering points (troughs) to increase flows to GAB springs and to prevent Gambusia hot-spots developing in the shallow stable bore drains.
- Saline refugia (Polo Clubs) are important in maintaining large populations of desert goby and Lake Eyre hardyhead. During wetter seasons, other species have migrated into these waterholes and the abundance of goby and hardyheads has declined.
- A conceptual model outlining the ecology of fish populations and the role of refuge waterholes under variable climatic and hydrological conditions in the Neales catchment was developed. Conceptual models developed using existing data can be used for predicative or climate scenario modelling to assist with the management of flows, water resources and other developments and impacts in the Neales catchment and other arid river systems. These models also set a basis for monitoring of arid river health and condition using fish as an indicator species.

- The authors seek feedback for the development of these conceptual models and in particular how they can be populated with empirical data collected during the current study (including geomorphic, hydrological, land use and landscape data) for application to specific management tasks.

Site Based Trends:

- At disco refugia, spawning was mostly observed during the November survey, while recruitment was observed at the April survey.
- Golden perch populations contracted back into Algebuckina despite flows.
- At Ark refugia (Algebuckina and possibly Baltacoodna), the assemblages were stable, but the abundance decreased in response to flows.
    - It is recommended that some level of monitoring continues to target these important refuge habitats. Linkages with programs such as the Lake Eyre Basin Rivers Assessment (LEBRA) may provide key collaboration and support for this monitoring.
- At Polo Club and stepping stone refugia, populations of salinity resistant species were joined by resilient species after flow events.
- At bores or springs where Gambusia were present, there were either no other species or a few other species at very low abundance. The exception was North Freeling where deeper habitat may partition the interactions between Gambusia, gobies and hardyhead.

Climatic Tolerance:

- Field trials to assess the tolerance of Neales fishes to salinity and hypoxia were conducted at Algebuckina Waterhole. Tolerance data revealed thresholds for salinity and hypoxia at which species are likely to be impacted.

- Field trials found that desert gobies were extremely tolerant to both threats and were able to hold buccal air bubbles and move to the edge of waterbodies to improve respiration under low oxygen conditions.

- Spangled perch, desert rainbowfish and barred grunter possessed lower tolerance to climate related water quality impacts, although tolerance levels measured were still relatively high for Australian freshwater fish species (SKM 2003).

- Results of the field trials largely matched the distribution of species responding to the field salinity gradients.
- These pilot trials revealed the difficulties in collecting comprehensive and accurate tolerance data in remote field conditions. However with modifications of experimental techniques to reduce experimental and holding stress, accurate and repeatable data can be collected in the field.
    - It is recommended that thresholds for survival under extreme climatic conditions be considered for a range of aquatic species. In particular, reductions in rainfall or increased water resource development could lead to increased environmental harshness. Knowledge of species thresholds will be vital for predicting potential species or community collapses.

Gambusia Control

- Gambusia were found to have expanded their range across the Neales catchment into upper and lower reaches but maintained relatively low abundances within riverine waterholes.
- High densities of Gambusia were found in GAB springs and bore drains where stable and shallow water conditions are present in permanent water bodies.
    - Control of Gambusia are best directed towards eradication in these refuge habitats either through a) closing off bore flows, b) eradicating Gambusia in GAB springs using rotenone or other control methods and re-stocking with native desert goby and other indigenous fauna and c) netting in North Freeling Spring to reduce populations in this regularly connected pest refuge.
    - Control efforts should be linked to monitoring to determine the relationship between pest refuge “hot spots” and riverine waterhole populations and to determine whether abundant native populations and variable hydrology in riverine waterholes are effective in preventing the short term spread and dominance of this pest species in the Neales catchment.

- Exclusion barriers may be considered to prevent the spread of Gambusia into nearby GAB springs where desert goby are still present (e.g. Hawker Springs, Freeling Springs, Ockenden Spring and Nilpinna Spring).

Community engagement

- Ongoing connection and interaction with all sectors of the community was a major attribute of the current project approach. Numerous conversations with landholders, local business owners, residents, indigenous groups and visitors to the region lead to a holistic approach to investigating fish and waterhole ecology as a part of managing the Neales catchment for all stakeholders.
- Engagement with indigenous groups requires time and persistence but was fruitful in terms of education, knowledge acquisition and improvements to the study approach, methodology and site selection.
- The indigenous community engagement session held at Hookeys waterhole was extremely successful and the format of that session should be further developed to serve as a template for utilising fish to connect with regional communities around waterhole management.
    - The program is an example of the close integration of indigenous community into arid river programs. As such it should be implemented elsewhere as it is an excellent tool for use in other arid river management programs such as LEBRA and Lake Eyre Fisheries Management Plan.
- This close community engagement made the research team feel welcome in the region and assisted in gaining access to waterholes and properties, and building lasting relationships with all aspects of the community. All community members engaged were crucial to the success of the study.
    - Engagement identified significant indigenous fisheries for spangled perch and to a lesser extent golden perch. This fishery should be acknowledged and information developed for inclusion into the draft Lake Eyre Basin Fisheries Management Plan.

CONCLUSIONS

This project has provided the most comprehensive 'whole of catchment' appraisal of fish and waterhole ecology in the Lake Eyre Basin to date. Linked with other disciplines such as landscape design, geomorphology, riparian vegetation, land use and hydrology, the study provides an excellent snapshot of one of Australia's most arid permanent catchments.

Fish have proved to be a useful indicator of catchment health, revealing a great deal about the natural cyclical processes happening in arid zone catchments such as the Neales. Furthermore, fish have proved to be an iconic and popular aspect of the Neales river system, providing unique opportunities to engage, educate and learn from the local and indigenous community in the western Lake Eyre region. The integration of community values and priorities throughout this project has contributed to our ability to integrate the needs and concerns of the local community into future natural resources and fisheries management activities.

Overall the Neales critical refugia project serves as an example of multi-disciplinary, collaborative research that is linked to management practices, providing the knowledge and perspective essential for effective catchment management into the future. The assessment of fish traits and ecological patterns, the typology and role of refuge waterholes and the assessment of pest fish populations will contribute to our ability to develop effective and comprehensive management plans and tools. The project outputs will assist in the delivery of tailored on-ground solutions to protect and maintain native fish and riverine ecosystems and address the key threats and processes that may lead to the deterioration of values important to the health and condition of the Neales catchment.

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APPENDICES

Appendix 1 SIMPER Analysis Outputs

SIMPER Outputs spring 2009

Group Disco
Average similarity: 70.68

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Bony Herring	3.46	30.34	4.29	42.92	42.92
Spangled Perch	2.32	22.31	3.95	31.56	74.48
Desert Rainbowfish	1.93	14.33	1.76	20.27	94.75

Group Ark
Average similarity: 54.61

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Bony Herring	6.72	22.52	~	41.23	41.23
Lake Eyre Hardyhead	5.03	14.72	~	26.95	68.18
Spangled Perch	3.31	11.18	~	20.47	88.65
Desert Goby	2.51	6.2	~	11.35	100

Group Polo Club
Average similarity: 84.90

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Lake Eyre Hardyhead	2.78	54.92	~	64.68	64.68
Desert Goby	1.94	29.98	~	35.32	100

Group Spring
Average similarity: 53.45

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Desert Goby	2.34	52.55	1.24	98.32	98.32

Group Bore
Average similarity: 86.35

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Mosquitofish	6.51	86.35	~	100	100

Groups Disco & Ark
Average dissimilarity = 55.54

	Group Disco	Group Ark				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Lake Eyre Hardyhead	0.14	5.03	16.06	2	28.92	28.92
Bony Herring	3.46	6.72	9.59	2.1	17.27	46.19
Desert Goby	0.09	2.51	8.12	1.66	14.62	60.81
Desert Rainbowfish	1.93	2.16	6.51	2.27	11.73	72.53
Barred Grunter	0.13	2.34	6.01	1.01	10.83	83.36
Mosquitofish	0.54	1.12	3.17	1.04	5.71	89.07
Golden Perch	0.86	0.93	3.04	1.23	5.47	94.54

Groups Disco & Polo Club
Average dissimilarity = 97.39

	Group Disco	Group Polo Club				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Bony Herring	3.46	0	24.53	4.22	25.19	25.19

Lake Eyre Hardyhead	0.14	2.78	19.89	2.92	20.42	45.61
Spangled Perch	2.32	0	16.92	3.8	17.37	62.98
Desert Goby	0.09	1.94	13.82	2.41	14.19	77.17
Desert Rainbowfish	1.93	0	13.13	2.35	13.49	90.66

Groups Ark & Polo Club

Average dissimilarity = 67.25

	Group Ark	Group Polo Club				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Bony Herring	6.72	0	23.38	42.85	34.76	34.76
Spangled Perch	3.31	0	11.53	33.75	17.15	51.9
Lake Eyre Hardyhead	5.03	2.78	9.27	1.14	13.79	65.69
Barred Grunter	2.34	0	6.74	0.87	10.02	75.71
Desert Rainbowfish	2.16	0	6.21	0.87	9.24	84.95
Desert Goby	2.51	1.94	4.24	1.1	6.3	91.25

Groups Disco & Spring

Average dissimilarity = 97.66

	Group Disco	Group Spring				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Bony Herring	3.46	0	28.28	3.05	28.96	28.96
Spangled Perch	2.32	0	19.71	2.57	20.18	49.13
Desert Goby	0.09	2.34	16.67	1.82	17.07	66.21
Desert Rainbowfish	1.93	0	14.93	2.09	15.29	81.49
Mosquitofish	0.54	1.19	8.08	0.77	8.27	89.76
Golden Perch	0.86	0	6.1	0.98	6.25	96.01

Groups Ark & Spring

Average dissimilarity = 83.46

	Group Ark	Group Spring				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Bony Herring	6.72	0	24.56	6.59	29.42	29.42
Lake Eyre Hardyhead	5.03	0.68	19.32	1.61	23.15	52.57
Spangled Perch	3.31	0	12.12	6.51	14.52	67.09
Barred Grunter	2.34	0	6.97	0.95	8.35	75.44
Desert Rainbowfish	2.16	0	6.43	0.95	7.71	83.15
Desert Goby	2.51	2.34	6.05	1.31	7.25	90.4

Groups Polo Club & Spring

Average dissimilarity = 57.69

	Group Polo Club	Group Spring				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Lake Eyre Hardyhead	2.78	0.68	37.47	3.27	64.95	64.95
Desert Goby	1.94	2.34	12.05	1.51	20.89	85.85
Mosquitofish	0	1.19	8.17	0.55	14.15	100

Groups Disco & Bore

Average dissimilarity = 94.51

	Group Disco	Group Bore				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Mosquitofish	0.54	6.51	39.51	3.04	41.8	41.8
Bony Herring	3.46	0	21.73	4.09	22.99	64.79
Spangled Perch	2.32	0	14.92	3.96	15.78	80.57
Desert Rainbowfish	1.93	0	11.7	2.33	12.38	92.96

Groups Ark & Bore

Average dissimilarity = 93.89

	Group Ark	Group Bore				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Bony Herring	6.72	0	21.96	27.99	23.39	23.39

Mosquitofish	1.12	6.51	18.96	2.13	20.19	43.59
Lake Eyre Hardyhead	5.03	0	18.07	1.87	19.24	62.83
Spangled Perch	3.31	0	10.84	26.73	11.54	74.37
Desert Goby	2.51	0	9.2	1.55	9.8	84.17
Barred Grunter	2.34	0	6.41	0.87	6.83	90.99

Groups Polo Club & Bore
Average dissimilarity = 100.00

	Group Polo Club	Group Bore				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Mosquitofish	0	6.51	57.78	13.27	57.78	57.78
Lake Eyre Hardyhead	2.78	0	24.95	6.86	24.95	82.74
Desert Goby	1.94	0	17.26	3.39	17.26	100

Groups Spring & Bore
Average dissimilarity = 86.47

	Group Spring	Group Bore				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Mosquitofish	1.19	6.51	62.89	2.36	72.73	72.73
Desert Goby	2.34	0	20.91	2.34	24.18	96.91

SIMPER Outputs for autumn 2010

Group Disco

Average similarity: 74.53

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Bony Herring	3.95	29.96	4.99	40.2	40.2
Desert Rainbowfish	3.43	21.48	3.73	28.82	69.03
Spangled Perch	3.05	20.77	4.6	27.87	96.9

Group Stepping Stone

Average similarity: 41.26

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Bony Herring	1.46	21.59	1.24	52.34	52.34
Spangled Perch	1.59	14.13	1.1	34.26	86.6
Desert Rainbowfish	0.69	3.91	0.47	9.47	96.07

Group Ark

Average similarity: 69.73

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Bony Herring	6.05	27.16	~	38.95	38.95
Spangled Perch	3.62	16.26	~	23.31	62.27
Desert Rainbowfish	3.52	14.74	~	21.14	83.4
Barred Grunter	2.72	6.93	~	9.95	93.35

Group Polo Club

Less than 2 samples in group

Group Bore

Average similarity: 70.97

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Mosquitofish	7.89	70.97	~	100	100

Group Spring

Average similarity: 29.28

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Mosquitofish	3.74	29.28	~	100	100

Groups Disco & Stepping Stone

Average dissimilarity = 55.31

	Group Disco	Group Stepping Stone				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Desert Rainbowfish	3.43	0.69	15.62	1.99	28.24	28.24
Bony Herring	3.95	1.46	14.54	2.6	26.28	54.52
Spangled Perch	3.05	1.59	10.23	1.39	18.49	73
Mosquitofish	0.5	1.38	8.21	0.76	14.85	87.85
Lake Eyre Hardyhead	0.45	0.17	2.79	0.7	5.05	92.9

Groups Disco & Ark

Average dissimilarity = 35.52

	Group Disco	Group Ark				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Barred Grunter	0.35	2.72	7.11	1.71	20.02	20.02
Bony Herring	3.95	6.05	6.35	2.76	17.89	37.91
Mosquitofish	0.5	2	4.84	1.48	13.63	51.53
Lake Eyre Hardyhead	0.45	1.15	3.5	1.09	9.86	61.39
Desert Rainbowfish	3.43	3.52	3.35	1.34	9.43	70.82
Golden Perch	0.1	1.11	3.32	1.03	9.34	80.16
Spangled Perch	3.05	3.62	2.87	1.57	8.09	88.25
Desert Goby	0.1	0.89	2.67	1.01	7.53	95.78

Groups Stepping Stone & Ark

Average dissimilarity = 67.32

	Group Stepping Stone	Group Ark				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Bony Herring	1.46	6.05	17.03	6.31	25.29	25.29
Desert Rainbowfish	0.69	3.52	10.61	3.07	15.76	41.05
Barred Grunter	0	2.72	10.13	2.09	15.04	56.09
Mosquitofish	1.38	2	8.24	1.63	12.25	68.33
Spangled Perch	1.59	3.62	7.73	1.58	11.48	79.81
Lake Eyre Hardyhead	0.17	1.15	4.29	1.03	6.38	86.19
Golden Perch	0	1.11	4.11	0.95	6.11	92.31

Groups Disco & Polo Club

Average dissimilarity = 41.65

	Group Disco	Group Polo Club				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Desert Goby	0.1	3.32	9.21	7.26	22.11	22.11
Bony Herring	3.95	7.03	8.8	3.93	21.14	43.24
Lake Eyre Hardyhead	0.45	3.15	7.78	3.1	18.67	61.92
Mosquitofish	0.5	2.34	5.28	2.64	12.67	74.58
Barred Grunter	0.35	2.09	4.95	2.98	11.89	86.48
Desert Rainbowfish	3.43	2.78	3.19	1.26	7.67	94.14

Groups Stepping Stone & Polo Club

Average dissimilarity = 68.42

	Group Stepping Stone	Group Polo Club				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Bony Herring	1.46	7.03	19.36	7.48	28.3	28.3
Desert Goby	0.17	3.32	11.09	5.46	16.2	44.5
Lake Eyre Hardyhead	0.17	3.15	10.47	5.58	15.3	59.8
Mosquitofish	1.38	2.34	8.12	2.25	11.87	71.67
Desert Rainbowfish	0.69	2.78	7.36	2.45	10.75	82.42
Barred Grunter	0	2.09	7.29	11.59	10.66	93.08

Groups Ark & Polo Club

Average dissimilarity = 24.42

	Group Ark	Group Polo Club				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Desert Goby	0.89	3.32	5.42	1.94	22.18	22.18
Lake Eyre Hardyhead	1.15	3.15	4.46	1.23	18.26	40.44
Barred Grunter	2.72	2.09	2.72	1.37	11.16	51.6
Golden Perch	1.11	0	2.46	0.71	10.08	61.67
Mosquitofish	2	2.34	2.23	2.08	9.11	70.78
Spangled Perch	3.62	2.63	2.21	6	9.04	79.82
Bony Herring	6.05	7.03	2.17	3.57	8.88	88.7
Desert Rainbowfish	3.52	2.78	1.65	1.52	6.74	95.45

Groups Disco & Bore

Average dissimilarity = 86.10

	Group Disco	Group Bore				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Mosquitofish	0.5	7.89	33.64	6.35	39.07	39.07
Bony Herring	3.95	0.5	15.88	3.15	18.45	57.51
Desert Rainbowfish	3.43	0	15.08	3.1	17.52	75.03
Spangled Perch	3.05	0.5	11.13	2.92	12.93	87.96
Desert Goby	0.1	1.54	6.39	1.01	7.43	95.39

Groups Stepping Stone & Bore

Average dissimilarity = 73.35

Species	Group Stepping Stone Av.Abund	Group Bore Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Mosquitofish	1.38	7.89	43.63	2.2	59.49	59.49
Desert Goby	0.17	1.54	8.81	1.01	12.01	71.5
Spangled Perch	1.59	0.5	7.84	1.19	10.69	82.19
Bony Herring	1.46	0.5	7.76	1.39	10.58	92.77

Groups Ark & Bore

Average dissimilarity = 78.54

Species	Group Ark Av.Abund	Group Bore Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Mosquitofish	2	7.89	18.39	5.09	23.41	23.41
Bony Herring	6.05	0.5	17.52	5.52	22.31	45.72
Desert Rainbowfish	3.52	0	11.06	7.51	14.08	59.8
Spangled Perch	3.62	0.5	9.7	8.31	12.35	72.16
Barred Grunter	2.72	0	8.53	1.91	10.86	83.01
Desert Goby	0.89	1.54	4.69	1.24	5.97	88.99
Lake Eyre Hardyhead	1.15	0	3.62	0.86	4.6	93.59

Groups Polo Club & Bore

Average dissimilarity = 71.57

Species	Group Polo Club Av.Abund	Group Bore Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Bony Herring	7.03	0.5	19.48	5.2	27.22	27.22
Mosquitofish	2.34	7.89	16.41	24.69	22.93	50.15
Lake Eyre Hardyhead	3.15	0	9.37	11.78	13.1	63.24
Desert Rainbowfish	2.78	0	8.27	11.78	11.56	74.8
Spangled Perch	2.63	0.5	6.25	4	8.73	83.53
Barred Grunter	2.09	0	6.2	11.78	8.67	92.2

Groups Disco & Spring

Average dissimilarity = 92.52

Species	Group Disco Av.Abund	Group Spring Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Bony Herring	3.95	0	22.49	2.84	24.31	24.31
Desert Rainbowfish	3.43	0	18.71	2.51	20.22	44.54
Spangled Perch	3.05	0	16.99	2.65	18.36	62.9
Mosquitofish	0.5	3.74	16.15	2.63	17.45	80.35
Desert Goby	0.1	2.43	10.18	1.01	11	91.35

Groups Stepping Stone & Spring

Average dissimilarity = 85.57

Species	Group Stepping Stone Av.Abund	Group Spring Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Mosquitofish	1.38	3.74	28.36	1.96	33.14	33.14
Bony Herring	1.46	0	15.45	1.24	18.06	51.2
Spangled Perch	1.59	0	14.33	1.08	16.75	67.95
Desert Goby	0.17	2.43	14.17	1.02	16.55	84.5
Lake Eyre Hardyhead	0.17	1.14	6.95	1.07	8.12	92.62

Groups Ark & Spring

Average dissimilarity = 81.52

Species	Group Ark Av.Abund	Group Spring Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Bony Herring	6.05	0	21.65	4.75	26.56	26.56
Spangled Perch	3.62	0	12.96	4.77	15.9	42.46
Desert Rainbowfish	3.52	0	12.6	4.25	15.46	57.92
Barred Grunter	2.72	0	9.72	1.77	11.92	69.85
Desert Goby	0.89	2.43	7.72	1.31	9.47	79.32
Mosquitofish	2	3.74	7.01	1.74	8.6	87.91
Lake Eyre Hardyhead	1.15	1.14	4.12	0.84	5.05	92.96

Groups Polo Club & Spring

Average dissimilarity = 69.43

Species	Group Polo Club Av.Abund	Group Spring Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Bony Herring	7.03	0	23.59	4.19	33.97	33.97
Desert Rainbowfish	2.78	0	9.34	4.19	13.46	47.43
Spangled Perch	2.63	0	8.84	4.19	12.73	60.16
Desert Goby	3.32	2.43	8.67	1.4	12.49	72.65
Lake Eyre Hardyhead	3.15	1.14	7.41	1.05	10.67	83.32
Barred Grunter	2.09	0	7.01	4.19	10.1	93.41

Groups Bore & Spring

Average dissimilarity = 52.87

Species	Group Bore Av.Abund	Group Spring Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Mosquitofish	7.89	3.74	28.69	1.39	54.27	54.27
Desert Goby	1.54	2.43	12.88	1.15	24.37	78.63
Lake Eyre Hardyhead	0	1.14	5.01	0.86	9.48	88.11
Spangled Perch	0.5	0	3.57	0.79	6.75	94.86