Adapting to climate change: A risk assessment and decision making framework for managing groundwater dependent ecosystems with declining water levels

Supporting Document 4: Environmental variables in the habitats of south-western Australian freshwater fishes: An approach for setting threshold indicator values

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NCCARF
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Cover image: Yarragadee intrusion zone of the Blackwood River, Western Australia © Stephen Beatty, Murdoch University, Freshwater Fish Group

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ABSTRACT

Freshwater fishes have been used as indicators of aquatic ecosystem health as many teleosts are sensitive to water quality and habitat decline. Developing field derived thresholds, or tolerance indicator values (TIVs), based on matching distributions with prevailing water quality and habitat variables is a common approach that allows modelling of population viabilities under changing environmental conditions such as declines in water quality or hydrology. The freshwater fish fauna of south-western Australian is depauperate but has the highest rate of endemism of any Australian Drainage Division (82%). It is highly imperilled due to existing anthropogenic stressors such as secondary salinisation and riparian degradation. Secondary salinisation in particular has caused considerable inland range reductions of stenohaline species and the distributions of most are now limited to the western and southern parts of the region. The importance of fresh groundwater in maintaining lentic and lotic refuge habitats during the naturally dry summer and autumn in the region has also recently been recognised. For example, groundwater discharge during baseflow in the NCCARF project study area in the Blackwood River maintains habitat connectivity for the largest freshwater fish of the region, *Tandanus bostocki* and provides refuge habitat for the nationally endangered *Nannatherina balstoni*. However, modelling future population viabilities under projected surface and groundwater level reduction scenarios has been limited by the lack of TIVs for most species. Therefore, the current study aimed to analyse an extensive database of fish distributions and water quality variables across south-western Australia (1098 sampling points) to develop TIVs for species that could then be used in a risk assessment and decision making framework for managing groundwater dependent ecosystems (GDEs) subjected to declining water levels in the Blackwood River.

There were significant differences between species in TIVs relating to water temperature and conductivity. For example, some of the species that occupied narrow ranges and are considered to be threatened, such as *Galaxiella munda* and *N. balstoni*, occupied the coolest environments, whereas the common and widespread introduced *Gambusia holbrooki* and native *Galaxias occidentalis* occupied warmer and more saline environments. The findings from the current supporting document were subsequently incorporated into the Bayesian Belief Networks (Supporting Document 6) that modelled the likelihood of population declines for species following groundwater level reductions in the Blackwood River during baseflow. The BBNs were then mapped (Supporting Document 7) to spatially represent likelihood of population declines in the Blackwood River study area. The TIVs developed here will also be extremely useful for modelling population declines in response to other projected environmental changes in south-western Australia, such as increasing temperature and salinity.

EXECUTIVE SUMMARY

Freshwater fishes are increasingly recognised globally as useful response indicators of aquatic ecosystem health and have been used to detect the impacts of stressors on those ecosystems. However, whilst macroinvertebrates have been routinely used in Australia as biotic indices of ecosystem health, the use of freshwater fish communities has been much less common. A major limitation in the use of freshwater fishes as indices, particularly in southern Australia, is the number of fish species available. A common approach in their use is to assign thresholds for individual species (tolerance indicator values, or TIVs) that allow classification of species into tolerance groups for subsequent use in assessing and monitoring the health of aquatic ecosystems, such as determining the relative influence of habitat disturbance and water quality decline. Determining the TIVs of fishes have, in the past, largely relied on expert judgement; however, increasingly, empirical approaches have been adopted that allow more robust estimates to be generated. These principally include the use of controlled bioassays to determine tolerance to water quality variables, or the use of field data to characterise the environments in which species exist.

The freshwater fishes of south-western Australia are depauperate (11 species) yet highly endemic (~82%) and have experienced major population and range declines primarily due to the anthropogenic stressors of secondary salinisation, riparian degradation and surface flow regulation, along with the impact of introduced freshwater fishes. However, recently the importance of fresh groundwater in maintaining refuge habitats for these species has been recognised, and projected severe surface flow and groundwater level reductions is predicted to exacerbate other stressors. Whilst considerable information exists on the distribution and biology of these freshwater fishes, only limited research has been undertaken to determine their environmental tolerances, and no comprehensive characterisation of the water quality of their habitats has been undertaken. This has restricted the understanding of the relative contribution of stressors to past species range declines. Furthermore, determining TIVs for freshwater fishes is crucial to undertake modelling of population viabilities under future environmental conditions such as reduced surface flow and groundwater levels; the latter being the focus for the Blackwood River study area in the current NCCARF project.

The aim of the current study was to characterise the water quality of sites of occupancy of six endemic freshwater fishes and one introduced species in order to set TIVs that can be used in running the risk assessment and decision making framework for managing groundwater dependent ecosystems (GDEs) with declining water levels in the Blackwood River. Specifically, developing TIVs would allow Bayesian Belief Networks (BBNs, Supporting Document 6, Speldwinde *et al.* 2013) to be parameterised and GIS spatial mapping (Supporting Document 7, Neville 2013) of the likelihood of population declines under groundwater reduction scenarios in the Blackwood River groundwater risk assessment study area.

The study reviewed the distribution and water quality at sites of occupancy throughout the range of the six native and one introduced freshwater fish species that were known to be present in the Blackwood River groundwater study area, i.e. *Galaxias*

occidentalis, *Galaxiella munda*, *Nannoperca vittata*, *Nannatherina balstoni*, *Bostockia porosa*, *Tandandus bostocki*, and *Gambusia holbrooki*. This review included published papers, unpublished reports, and the unpublished raw data of the authors. From this review, sampling points (n = 1098) were extracted that had recorded both the presence of a relevant species and water quality variables that included temperature, pH, conductivity, dissolved oxygen, turbidity, total nitrogen and total phosphorous. Whilst some information on minimum pool and riffle passage depth requirements of several species existed, expert opinion was also used to set TIVs for those variables for the various species. For each species, the proportion of sites of occupancy, and the median, mean, maximum, minimum, 5th, 25th, 75th and 95th percentiles of environmental variables were calculated across their range.

The analyses revealed significant differences in several environmental variables between the sites occupied by the seven freshwater fishes. Temperature and conductivity varied significantly among species, with the introduced *G. holbrooki* occupying significantly warmer habitats than all other species. The more common native species *G. occidentalis*, *N. vittata* and *B. porosa* occupied sites with similar conductivities, temperatures and pH. The two threatened endemic fishes, i.e. *G. munda* and *N. balstoni*, occupied the coolest habitats with the former species also occupying the sites with lowest conductivity. The difference between the two galaxiids was notable, with *G. occidentalis* occupying sites that were on average 2.7°C warmer and ~1784 µS.cm^{-1} higher in conductivity than *G. munda*. Difference also existed amongst the percichthyids with the temperature at sites occupied by *N. balstoni* being >~1.1 °C cooler than *N. vittata* or *B. porosa*. The largest native species of the region (*T. bostocki*) occupied sites that were of relatively high conductivity compared to other native species. Dissolved oxygen, turbidity, TN and TP at occupancy sites were not significantly different between species; probably a result of the relatively low number of data points examined for these variables.

The study represents the first comprehensive analysis of the water quality of the habitats of freshwater fishes in south-western Australia and, as far as we are aware, the first of its kind that has examined the majority of species within any drainage division of Australia. Whilst limitations exist in using these data in setting precise TIVs, particularly with regard to sampling bias and the use of presence/absence data, they nonetheless provide much more robust estimates than expert judgement alone. Using these data, BBNs were successfully developed that modelled likelihood of population declines in the Blackwood River groundwater study area (see Speldewinde 2013) and spatial mapping of those likelihoods (see Neville 2013) demonstrated how projected groundwater level reductions may impact population viabilities. These data will also enable the modelling of future population viabilities in response to water quality decline due to a range of anthropogenic stressors across south-western Australia.

1. INTRODUCTION

1.1 Tolerance indicator values of freshwater fishes

Environmental indicators include chemical, physical and biological measures that are further separated into stressor indicators (quantitative measures of activities that cause degradation to aquatic systems), exposure indicators (measures of the realised effects of stressors such as concentrations of chemicals in the environment, and bioassays to determine levels of lethal effects), and response indicators that are composite measures of the cumulative effect of the stressor and exposure indicators (e.g. biological indices such as those based on communities or populations of faunal groups) (Yoder and Smith 1999). It is important to not substitute the use of biotic response indices with pressure and exposure indices, as this can result in positive overestimation of the condition of surface waters (Yoder and Smith 1999).

Assigning tolerance values to aquatic taxa in assessing aquatic ecosystem health has a long history (e.g. Chutter 1972). The use of freshwater fish as response indicators of water quality and aquatic ecosystem health is well recognised and they can be used to assess and monitor both the direct and indirect stressors on aquatic ecosystems (Fausch *et al.* 1990; Simon 1999; Meador *et al.* 2008; Angradi *et al.* 2009). For example, they were an important component of the development of the Index of Biological Integrity (IBI) (Karr 1981; Fausch *et al.* 1990). However, as opposed to macroinvertebrate communities (Simpson and Norris 2000), freshwater fish communities have not been widely used in assessing aquatic ecosystem health in Australia (although see Kennard *et al.* 2006). There are several limitations relating to the use of freshwater fish communities in assessing ecosystem health, including the often relatively small number of species (that increases bias in multivariate analyses), adequately determining reference conditions, and detecting species relationships with environmental conditions, particularly in highly variable systems such as found in Mediterranean Europe or Australia (Kennard *et al.* 2006; Ferreira *et al.* 2007).

Traditionally, assessment and classification of the tolerance of fish species to environmental disturbance has relied mainly on professional judgement (Meador and Carlisle 2007). Species are usually classified into the broad categories of intolerant, intermediate and tolerant or freshwater versus generalist species (Karr 1981). Relying solely on professional judgement, however, has obvious limitations and biases such as level of experience of the experts, and assumptions of tolerance levels of species based on naturally restricted distributions. Nonetheless, particularly at a local scale, incorporating expert knowledge on fish species ecology in developing indices for bioassessments can be very effective (Ferreira *et al.* 2007).

Determining freshwater fish tolerance indicator values (TIVs) (Meador and Carlisle 2007) of major water quality exposure indicators such as salinity, temperature, and dissolved oxygen has also been achieved by employing controlled bioassay trials to calculate lethal concentrations (e.g. Altinok *et al.* 1998; Landman *et al.* 2005; Beatty *et al.* 2011). However, relying solely on bioassays to set environmental thresholds or TIVs also has limitations such as the influence of fishes being housed and trialled in

artificial environments, and the lack of assessment of the interactions between environmental variables.

Using large databases of fish distributions and environmental variables is also increasingly being used to develop field-based estimates of TIVs (e.g. Meador and Carlisle 2007). However, there are also a number of limitations in threshold assessments based on field data; a major being the influence of natural variability and co-variability amongst environmental variables (Sergurado *et al.* 2011). Accounting for co-variability between variables to determine those that most influence the classifications can be achieved using Principal Components Analysis (Meador and Carlisle 2007). Natural variability can be exacerbated by historical factors. For example, a historically restricted distribution of a fish species to less disturbed sites may result in classifying it as sensitive to human pressures due to it being absent from more disturbed sites; which may or may not necessarily be the case (Sergurado *et al.* 2011). Another major limitation is sampling bias driven by non-random sampling design (e.g. sampling sites with similar environmental conditions more likely to house fishes). There is also often only a limited number of physical and chemical water quality components used in these analyses. Nonetheless, using analyses of field data can at least partially overcome the problems of relying solely on judgement-based threshold assessments and comparing these approaches can also result in reasonable agreement (Sergurado *et al.* 2011).

1.2   Freshwater fishes of south-western Australia

South-western Australia has the highest rate of endemism in freshwater fishes of any Australian Drainage Division (~82%) but they have undergone major range reductions and are highly imperilled through a number of stressors such as salinisation, riparian degradation, instream barriers, introduced fishes, flow and groundwater reductions, and climate change (e.g. Morgan *et al.* 1998, 2003, 2004; Morgan and Beatty 2006; Beatty *et al.* 2007, 2010, 2011; Morrongiello *et al.* 2011; Beatty and Morgan 2013). Of the 11 native freshwater fishes found in south-western Australia, two are listed as threatened under the Federal EPBC Act 1999 and those species, plus an additional two, are listed under the State Wildlife Conservation Act 1950 (Morgan *et al.* 2011).

Much is known of the reproductive and population biology of the freshwater fishes in south-western Australia (e.g. Pen *et al.* 1991; Morgan *et al.* 1998), as well as the distribution and range shifts that have been mostly associated with inland salinisation (Morgan *et al.* 2003; Beatty *et al.* 2011). However, specific tolerances and field or laboratory derived thresholds to key physical and chemical environmental exposure indicators have only recently begun to be determined. Beatty *et al.* (2011) determined acute salinity tolerances of three species and found the threatened (listed as *Vulnerable* under the EPBC Act 1999) *Nannatherina balstoni*, which has a restricted distribution, had a tolerance level far lower (i.e. 8.2 *cf* 14.6 ppt) than the more common and widespread *Galaxias occidentalis* and *Nannoperca vittata*. The general lack of information on TIVs of most species has prevented modelling their population viabilities under projected environmental change scenarios such as those expected from reductions in groundwater levels and surface flow.

1.3   Aim of the study

The aim of the current study is to quantify several environmental variables associated with the habitats of freshwater fishes in south-western Australia so they may be used as TIVs. These TIVs can then be used to parameterise the Bayesian Belief Networks (BBNs) that will model the likelihood of population declines in the Blackwood River groundwater study area as part of the current NCCARF project: *Adapting to climate change: a risk assessment and decision framework for managing groundwater dependent ecosystems with declining water levels.*

2. METHODS

2.1 Study area and databases used

Data included to characterise the environmental variables at sites of occupancy for freshwater fishes of south-western Australia was sourced from: 1) Freshwater Fish Group and Fish Health Unit (FFGFHU, Murdoch University) database (981 sites) which included published and unpublished resources (>50) collected between 1995-2012 (full publication list see http://www.freshwaterfishgroup.com), 2) Department of Water, Government of Western Australia (DoW) data (117 sites) gathered as part of the trial of the Framework for the Assessment of Wetland and River Health (Fig. 1). The FFGFHU at Murdoch University maintains an active, verified database of freshwater fish distributions at >2000 sites throughout Western Australia; approximately half of these have suitable complementary environmental variable data. The DoW has undertaken recent sampling of freshwater fishes along with a broad sweep of environmental variables in a wide range of systems throughout south-western Australia, including sites in the following Surface Water Management Areas: Moore-Hill, Albany, Collie, Harvey, Preston, Busselton Coast, Shannon, and Denmark (Fig. 1).

Given the broad range of habitats in which these studies have been conducted, and the broad range of objectives of the various studies (including simple baseline distributional studies, determining the biology of a species, patterns in fish communities, and migration patterns), sampling approaches varied but principally included the use of one or more of the following gears: seine nets (5, 10, 21 m, of 2 or 3 mm mesh), back-pack electrofishers (Smith Root 12A, LR20), boat mounted electrofisher (Smith Root VVP), fyke nets (each net 11.2 m width, 1.2 m wide mouth, 0.8 m depth, 5 m length, 2-mm woven mesh), box-traps baited with poultry pellets.

The fishes included in this assessment were the native species *Galaxias occidentalis*, *Galaxiella munda*, *Nannoperca vittata*, *Nannatherina balstoni*, *Bostockia porosa* and *Tandanus bostocki*, and the introduced *Gambusia holbrooki* (Fig. 2), as these are all known to be present in the Blackwood River groundwater study area as revealed by the studies of Morgan *et al.* (2003), Morgan and Beatty (2005), Beatty *et al.* (2006, 2009, 2010). While many of the studies included standardised quantitative assessment of species abundances (usually determining the density of each species at each), a large number did not. However, the studies invariably aimed to collect the full range of freshwater fishes present and therefore the collated database allowed an analysis based on presence absence data which is commonly used in field based metadata threshold analyses (e.g. Segurado *et al.* 2011).

2.2 Data analyses

The analysis of the environmental variables at the 1098 sampling points was conducted for those that housed (i.e. presence sites) one or more of the seven target species. Environmental variables included in the current analysis included temperature, conductivity, pH, dissolved oxygen and turbidity. We also included a review of other historical studies that have recorded those species and environmental variables to ensure that maximum extreme ranges of the variables were identified. We

also reviewed the limited published and unpublished information on habitat depths and passage requirements of the various species to set threshold limits for refuges and aquatic passage. It should be stressed that the passage and depth requirements are to some degree subjective, as for most species, habitat requirements in terms of pool refuge and riffle depths remains to be determined.

For each species, the proportion of sites of occupancy, median, mean, maximum, minimum, 5th, 25th, 75th and 95th percentiles were calculated for all presence sites in south-western Australia. Differences in mean water quality variables between species at their presence sites were tested using analysis of variance. Data were tested for normality and heterogeneity of variance and log-transformed. If transformation did not correct heterogeneity of variance, significance level for analyses was reduced from 0.05 to 0.01, to reduce the chances of Type I error. Either Tukeys or Tamhane's post hoc tests were employed assuming either equal or unequal variances, respectively, to determine pairwise differences between each species for each environmental variable.

Figure 1: Sites used in the analyses of environmental variables for south-western Australian freshwater fishes. N.B. FFGFHU = Freshwater Fish Group and Fish Health Unit, Murdoch University database, DoW = Department of Water, Government of Western Australia database.

Figure 2: Freshwater fishes analysed to determine Tolerance Indicator Values of water quality variables based on their sites of occupancy in south-western Australia. (left to right from top left) Galaxias occidentalis, Galaxiella munda, Nannoperca vittata, Nannatherina balstoni, Bostockia porosa, Tandanus bostocki and Gambusia holbrooki (introduced) (Photos: S. Beatty, D. Morgan and M. Allen).

3. RESULTS

There were significant differences between several freshwater fishes for temperature, conductivity and pH within their sites of occupancy in south-western Australia (Table 1). Below is a summary of the similarities and differences of environmental variables for six native and one introduced freshwater fish species known from the Blackwood River groundwater study area.

Table 1: Significant levels of post hoc tests for pairwise comparisons between freshwater fishes for each of the five water quality variables in south-western Australia. Significant contrasts (p<0.05) are highlighted in bold. Species codes: Go=Galaxias occidentalis, Gm=Galaxiella munda, Nv=Nannoperca vittata, Nb=Nannatherina balstoni, Bp=Bostockia porosa, Tb=Tandanus bostocki, Gh=Gambusia holbrooki.

Variable	Species	Species					
		Go	*Gm*	*Nv*	*Nb*	*Bp*	*Tb*
Temperature	*Go*						
	Gm	**0.000**					
	Nv	0.999	**0.000**				
	Nb	0.010	0.842	**0.034**			
	Bp	0.299	**0.011**	0.654	0.416		
	Tb	0.754	**0.013**	0.936	0.379	1.000	
	Gh	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
Conductivity	*Go*						
	Gm	**0.000**					
	Nv	**0.007**	**0.000**				
	Nb	1.000	**0.000**	0.648			
	Bp	0.461	**0.000**	0.992	**0.995**		
	Tb	**0.000**	**0.000**	**0.000**	**0.009**	**0.000**	
	Gh	**0.012**	**0.000**	**0.000**	0.552	**0.000**	0.497
Dissolved oxygen	*Go*						
	Gm	0.915					
	Nv	0.994	0.762				
	Nb	1.000	0.972	0.998			
	Bp	0.892	0.582	0.999	0.973		
	Tb	1.000	0.960	0.993	1.000	0.918	
	Gh	0.517	0.318	0.881	0.776	0.983	0.598
pH	*Go*						
	Gm	**0.000**					
	Nv	**0.039**	**0.000**				
	Nb	**0.000**	**0.003**	**0.009**			
	Bp	**0.001**	**0.000**	1.000	0.092		
	Tb	1.000	**0.000**	0.094	**0.000**	**0.004**	
	Gh	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
Turbidity	*Go*						
	Gm	1.000					
	Nv	0.999	1.000				
	Nb	0.415	0.979	0.671			
	Bp	1.000	0.999	0.998	0.415		
	Tb	0.412	0.997	0.758	0.999	0.427	
	Gh	0.998	0.993	0.963	0.298	0.999	0.307

3.1 Temperature

Temperature at sites of occupancy was significantly different among species (p=0.000). Mean (±S.E.) temperature at sites occupied by *G. holbrooki* (21.94 °C ±0.29) was significantly different to all other species (Tables 1 and 2, Fig. 3). *Galaxias occidentalis* occupied the next warmest sites (mean = 18.46 °C ±0.22) and were significantly different to the endemic *G. munda* and *N. balstoni* (Tables 1 and 2). *Galaxiella munda* occupied sites that were significantly cooler than all other species aside from *N. balstoni*, which occupied the second coolest habitats (Table 1 and 2, Fig. 3).

Table 2: The mean, range and percentiles of water temperature (°C) in the sites of occupancy of six endemic and one introduced freshwater fish in south-western Australia. Species codes: *Go=Galaxias occidentalis, Gm=Galaxiella munda, Nv=Nannoperca vittata, Nb=Nannatherina balstoni, Bp=Bostockia porosa, Tb=Tandanus bostocki, Gh=Gambusia holbrooki.*

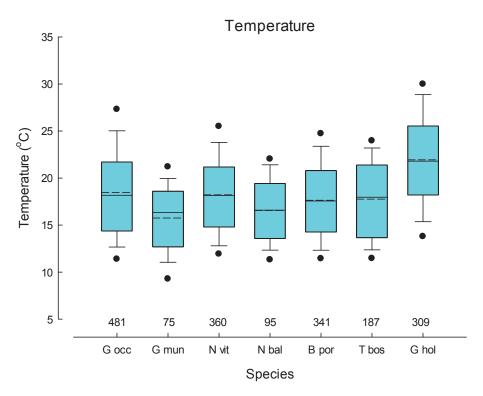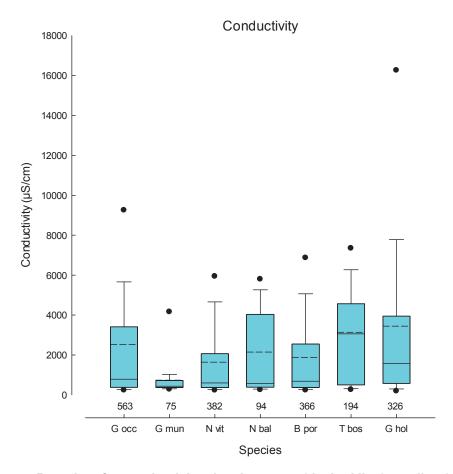|		Go	Gm	Nv	Nb	Bp	Tb	Gh
Number of sites		481	75	360	95	341	187	309
Mean		18.46	15.76	18.22	16.56	17.65	17.76	21.94
Std. Error of Mean		0.22	0.41	0.24	0.37	0.23	0.31	0.29
Median		18.17	16.33	18.15	16.60	17.57	17.97	21.80
Range		24.47	14.10	26.50	16.40	20.97	17.37	26.43
Minimum		8.33	8.40	8.33	8.33	8.33	8.33	8.40
Maximum		32.80	22.50	34.83	24.73	29.30	25.70	34.83
Percentile	5	11.38	9.27	11.91	11.30	11.43	11.46	13.78
	25	14.39	12.70	14.80	13.57	14.26	13.67	18.20
	50	18.17	16.33	18.15	16.60	17.57	17.97	21.80
	75	21.72	18.60	21.18	19.43	20.80	21.40	25.55
	95	27.30	21.19	25.49	22.03	24.73	23.96	29.98
Extreme known value of variable if it exceeds range of current analysis		33.9	33.9	NA	33.9	33.9	NA	NA

Figure 3: Box plots for temperature showing mean (dashed line), median (solid line in boxes), 25th, 75th (lower and upper box lines), 10th and 90th (bars), and 5th and 95th (dots) percentiles for the temperatures at sites of occupancy for six native and one introduced freshwater fish in south-western Australia. N.B. number of presence sites also shown above species names. Species codes: Gocc=*Galaxias occidentalis*, Gmun=*Galaxiella munda*, Nvit=*Nannoperca vittata*, Nbal=*Nannatherina balstoni*, Bpor=*Bostockia porosa*, Tbos=*Tandanus bostocki*, Ghol=*Gambusia holbrooki*.

3.2   Conductivity

Conductivities at the sites of occupancy were significantly different among species (p=0.000) with *G. munda* most notably occupying sites significantly fresher than all other species (mean = 739 µS.cm^{-1} ±109) (Table 3, Fig. 4). *Nannoperca vittata*, *N. balstoni* and *B. porosa* occupied sites that were not significantly different in conductivity (Table 1). *Gambusia holbrooki* occupied sites that had significantly different mean conductivity than all other species aside from *N. balstoni* and *T. bostocki* with the latter having the second highest mean and highest median conductivity at its occupancy sites (Tables 1 and 3, Fig. 4).

Table 3: The mean, range and percentiles of conductivity (µS.cm^{-1}) at sites of occupancy of six endemic and one introduced freshwater fish in south-western Australia. Species codes: *Go=Galaxias occidentalis*, *Gm=Galaxiella munda*, *Nv=Nannoperca vittata*, *Nb=Nannatherina balstoni*, *Bp=Bostockia porosa*, *Tb=Tandanus bostocki*, *Gh=Gambusia holbrooki*.
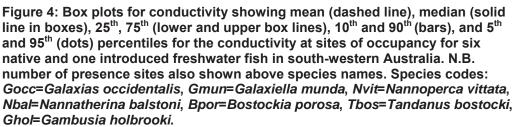
		Go	Gm	Nv	Nb	Bp	Tb	Gh
Number of sites		563	75	382	94	366	194	326
Mean		2522.5	738.6	1641.6	2146.0	1882.1	3135.9	3445.3
Std. Error of Mean		178.4	109.4	112.5	220.6	143.8	182.8	309.8
Median		787.3	432.3	600.0	576.1	684.9	3062.0	1573.0
Range		40992.1	4543. 7	17364.0	8510.0	29332.0	17314.7	36992.1
Minimum		107.9	261.0	136.0	190.0	168.0	185.3	107.9
Maximum		41100.0	4804. 7	17500.0	8700.0	29500.0	17500.0	37100.0
Percentile	5	233.3	282.6	230.7	256.6	236.8	261.6	189.4
	25	378.7	370.0	363.2	385.0	373.4	508.68	580.0
	50	787.3	432.3	600.0	576.1	684.9	3062.0	1573.0
	75	3413.3	720.0	2066.3	4038. 7	2542.6	4566.9	3945.4
	95	9254.4	4159.5	5940.2	5796.1	6871.9	7349.2	16262.0
Extreme known value of variable if it exceeds range in current analysis		Acute: 14.6 ppt (~26500 µS.cm^{-1}) Beatty *et al.* (2011)	5202	Acute: 14.6 ppt (~26500 µS.cm^{-1}) Beatty *et al.* (2011)	Acute: 8.2 (~15000 µS.cm^{-1}) ppt Beatty *et al.* (2011)	NA	NA	81 ppt (~ 145000 µS.cm^{-1})

Figure 4: Box plots for conductivity showing mean (dashed line), median (solid line in boxes), 25th, 75th (lower and upper box lines), 10th and 90th (bars), and 5th and 95th (dots) percentiles for the conductivity at sites of occupancy for six native and one introduced freshwater fish in south-western Australia. N.B. number of presence sites also shown above species names. Species codes: *Gocc=Galaxias occidentalis, Gmun=Galaxiella munda, Nvit=Nannoperca vittata, Nbal=Nannatherina balstoni, Bpor=Bostockia porosa, Tbos=Tandanus bostocki, Ghol=Gambusia holbrooki.*
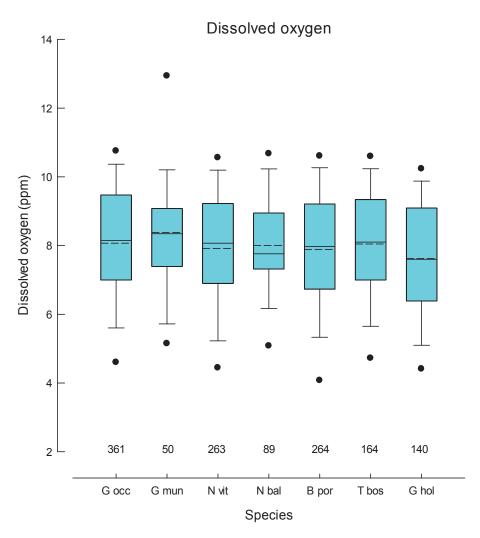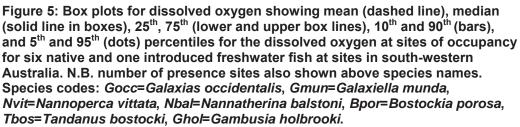
3.3 Dissolved oxygen

Mean dissolved oxygen at occupancy sites ranged from 7.62 mg.l^{-1} (*G. holbrooki*) to 8.38 mg.l^{-1} (*G. munda*) (Table 4). Dissolved oxygen was not significantly different among species (p=0.286) with the 5th and 25th percentiles of all species being greater than ~4.1 mg.l^{-1}, and ~6.4 mg.l^{-1}, respectively (Tables 1 and 4, Fig. 5).

Table 4: The mean, range and percentiles of dissolved oxygen (mg.l^{-1}) at sites of occupancy of six endemic and one introduced freshwater fish in south-western Australia. Species codes: *Go=Galaxias occidentalis*, *Gm=Galaxiella munda*, *Nv=Nannoperca vittata*, *Nb=Nannatherina balstoni*, *Bp=Bostockia porosa*, *Tb=Tandanus bostocki*, *Gh=Gambusia holbrooki*.

		Go	Gm	Nv	Nb	Bp	Tb	Gh
Number of sites		361	50	263	89	264	164	140
Mean		8.07	8.38	7.92	8.00	7.88	8.05	7.62
Std. Error of Mean		0.11	0.29	0.12	0.18	0.13	0.15	0.16
Median		8.15	8.35	8.07	7.76	7.97	8.10	7.60
Range		17.17	11.39	14.47	11.20	17.17	14.03	10.28
Minimum		0.30	4.65	1.57	3.44	0.30	3.44	3.30
Maximum		17.47	16.04	16.04	14.63	17.47	17.47	13.58
Percentile	5	4.60	5.15	4.44	5.08	4.08	4.72	4.41
	25	7.00	7.39	6.90	7.32	6.73	7.00	6.39
	50	8.15	8.35	8.07	7.76	7.97	8.10	7.60
	75	9.47	9.08	9.22	8.95	9.21	9.34	9.09
	95	10.75	12.94	10.56	10.68	10.61	10.59	10.23
Extreme known value of variable if it exceeds range in current analysis		NA	3.04	NA	2.90	NA	NA	NA

Figure 5: Box plots for dissolved oxygen showing mean (dashed line), median (solid line in boxes), 25th, 75th (lower and upper box lines), 10th and 90th (bars), and 5th and 95th (dots) percentiles for the dissolved oxygen at sites of occupancy for six native and one introduced freshwater fish at sites in south-western Australia. N.B. number of presence sites also shown above species names. Species codes: *Gocc=Galaxias occidentalis, Gmun=Galaxiella munda, Nvit=Nannoperca vittata, Nbal=Nannatherina balstoni, Bpor=Bostockia porosa, Tbos=Tandanus bostocki, Ghol=Gambusia holbrooki.*

3.4   pH

The pH varied significantly among species (p=0.000) with pairwise tests revealing *G. munda* was found at significantly more acidic sites than all other species (mean = 6.11 ±0.09) and *G. holbrooki* occupied sites that were significantly more basic than all other species (mean = 7.59 ±0.05) (Tables 1 and 5, Fig. 6). *Nannatherina balstoni* was found to occupy habitats with a slightly acidic pH (mean = 6.58 ±0.07) (Table 5, Fig. 6). Although variable, *T. bostocki*, *G. occidentalis*, *B. porosa* and *N. vittata* were found in habitats with near neutral pH (Tables 1 and 5, Fig. 6).

Table 5: The mean, range and percentiles of pH at sites of occupancy of six endemic and one introduced freshwater fish in south-western Australia. Species codes: *Go=Galaxias occidentalis*, *Gm=Galaxiella munda*, *Nv=Nannoperca vittata*, *Nb=Nannatherina balstoni*, *Bp=Bostockia porosa*, *Tb=Tandanus bostocki*, *Gh=Gambusia holbrooki*.

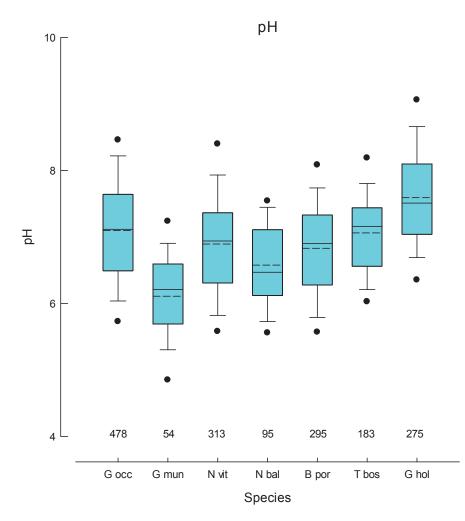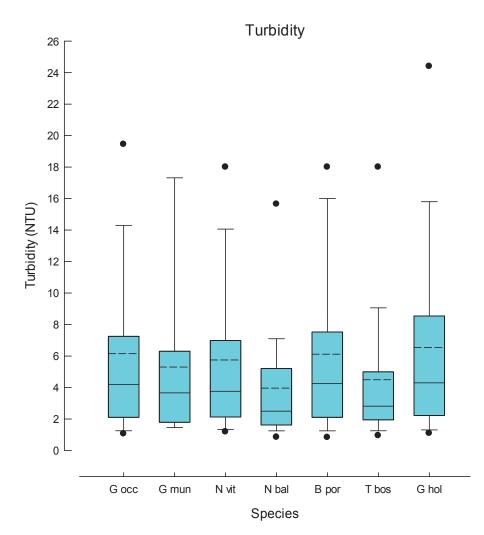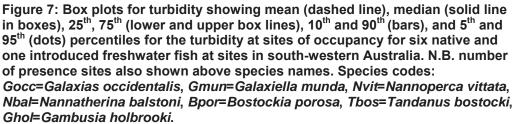|		*Go*	*Gm*	*Nv*	*Nb*	*Bp*	*Tb*	*Gh*
Number of sites		478	54	313	95	295	183	275
Mean		7.10	6.11	6.89	6.58	6.83	7.06	7.59
Std. Error of Mean		0.04	0.09	0.05	0.07	0.05	0.05	0.05
Median		7.12	6.21	6.94	6.47	6.90	7.16	7.51
Range		6.60	3.64	5.49	2.93	5.48	3.02	6.81
Minimum		3.57	3.86	3.86	5.29	3.57	5.44	3.57
Maximum		10.17	7.51	9.35	8.22	9.05	8.46	10.38
Percentile	5	5.73	4.85	5.58	5.56	5.57	6.03	6.36
	25	6.49	5.69	6.31	6.12	6.28	6.56	7.04
	50	7.12	6.21	6.94	6.47	6.90	7.16	7.51
	75	7.64	6.60	7.37	7.11	7.33	7.44	8.10
	95	8.46	7.24	8.40	7.54	8.08	8.19	9.06
Extreme known value of variable if it exceeds range in current analysis		NA	3.0-7.6	NA	3.9	NA	NA	NA

Figure 6: Box plots for pH showing mean (dashed line), median (solid line in boxes), 25th, 75th (lower and upper box lines), 10th and 90th (bars), and 5th and 95th (dots) percentiles for the pH at sites of occupancy for six native and one introduced freshwater fish at sites in south-western Australia. N.B. number of presence sites also shown above species names. Species codes: *Gocc=Galaxias occidentalis, Gmun=Galaxiella munda, Nvit=Nannoperca vittata, Nbal=Nannatherina balstoni, Bpor=Bostockia porosa, Tbos=Tandanus bostocki, Ghol=Gambusia holbrooki.*

3.5   Turbidity

The turbidity analyses were based on fewer sites than the above variables and there was no overall significant difference in the turbidity at sites of occupancy between the species (Tables 1 and 6, Fig. 7). Mean and median turbidity ranged from 3.96 ±0.81 and 2.50 NTU (*N. balstoni*) to 6.54 ±0.82 and 4.30 NTU (*G. holbrooki*), respectively (Table 6). Due to the limited number of sites used to generate TIVs for turbidity, along with difficulty in accurately modelling changes in turbidity with groundwater levels, they were excluded from the subsequent BBNs that modelled the likelihood of population declines (see Speldewinde 2013).

Table 6: The mean, range and percentiles of turbidity (NTU) at sites of occupancy of six endemic and one introduced freshwater fish in south-western Australia. Species codes: *Go=Galaxias occidentalis*, *Gm=Galaxiella munda*, *Nv=Nannoperca vittata*, *Nb=Nannatherina*.

		Go	Gm	Nv	Nb	Bp	Tb	Gh
Number of sites		136	13	101	29	102	63	61
Mean		6.15	5.30	5.75	3.96	6.11	4.50	6.54
Std. Error of Mean		0.58	1.66	0.67	0.81	0.58	0.63	0.82
Median		4.18	3.66	3.76	2.50	4.25	2.82	4.30
Range		48.70	22.68	49.70	23.21	29.70	29.70	29.40
Minimum		0.30	1.32	0.30	0.79	0.30	0.30	0.60
Maximum		49.00	24.00	50.00	24.00	30.00	30.00	30.00
Percentile	5	1.07	1.32	1.19	0.85	0.84	0.94	1.10
	25	2.11	1.79	2.13	1.61	2.11	1.95	2.23
	50	4.18	3.66	3.76	2.50	4.25	2.82	4.30
	75	7.26	6.30	6.98	5.20	7.53	5.00	8.55
	95	19.45	.	18.00	15.65	18.00	18.00	24.40
Extreme known value of variable if it exceeds range in current analysis		NA	NA	NA	NA	NA	NA	NA

Figure 7: Box plots for turbidity showing mean (dashed line), median (solid line in boxes), 25th, 75th (lower and upper box lines), 10th and 90th (bars), and 5th and 95th (dots) percentiles for the turbidity at sites of occupancy for six native and one introduced freshwater fish at sites in south-western Australia. N.B. number of presence sites also shown above species names. Species codes: *Gocc=Galaxias occidentalis, Gmun=Galaxiella munda, Nvit=Nannoperca vittata, Nbal=Nannatherina balstoni, Bpor=Bostockia porosa, Tbos=Tandanus bostocki, Ghol=Gambusia holbrooki.*

3.6   Total nitrogen and phosphorous

The analyses of TN and TP were based on a limited number of sites which prevents robust TIVs being generated (Tables 7 and 8) and therefore nutrient TIVs were excluded from the subsequent BBNs that modelled the likelihood of population declines (see Speldewinde 2013). Mean TN ranged from 0.614 ±0.08 mg.l^{-1} (*T. bostocki*) to 1.350 ±0.58 mg.l^{-1} (*G. munda*) (Table 7). Mean TP ranged from 0.044 ±0.014 mg.l^{-1} (*T. bostocki*) to 0.083 ±0.069 mg.l^{-1} (*G. munda*) (Table 8).

Table 7: The mean, range and percentiles of total nitrogen (mg.l^{-1}) at sites of occupancy of six endemic and one introduced freshwater fish in south-western Australia. Species codes: *Go=Galaxias occidentalis*, *Gm=Galaxiella munda*, *Nv=Nannoperca vittata*, *Nb=Nannatherina balstoni*, *Bp=Bostockia porosa*, *Tb=Tandanus bostocki*, *Gh=Gambusia holbrooki*.

		Go	Gm	Nv	Nb	Bp	Tb	Gh
Number of sites		73	4	54	6	61	22	34
Mean		0.844	1.350	0.736	1.098	0.807	0.614	0.881
Std. Error of Mean		0.057	0.583	0.063	0.402	0.062	0.084	0.086
Median		0.740	0.780	0.630	0.730	0.700	0.445	0.795
Range		2.136	2.360	2.122	2.570	2.129	1.422	1.854
Minimum		0.064	0.740	0.078	0.530	0.071	0.078	0.046
Maximum		2.200	3.100	2.200	3.100	2.200	1.500	1.900
Percentile	5	0.107	0.740	0.158	0.530	0.171	0.092	0.065
	25	0.505	0.745	0.380	0.658	0.440	0.368	0.438
	50	0.740	0.780	0.630	0.730	0.700	0.445	0.795
	75	1.200	2.525	0.920	1.375	1.100	0.875	1.325
	95	1.760	.	1.775	.	1.890	1.470	1.825
Extreme known value of variable if it exceeds range in current analysis		NA	NA	NA	NA	NA	NA	NA

Table 8: The mean, range and percentiles of total phosphorous (mg.l⁻¹) at sites of occupancy of six endemic and one introduced freshwater fish in south-western Australia. Species codes: *Go=Galaxias occidentalis*, *Gm=Galaxiella munda*, *Nv=Nannoperca vittata*, *Nb=Nannatherina balstoni*, *Bp=Bostockia porosa*, *Tb=Tandanus bostocki*, *Gh=Gambusia holbrooki*.

		Go	Gm	Nv	Nb	Bp	Tb	Gh
Number of sites		73	4	51	6	58	19	34
Mean		0.055	0.083	0.054	0.059	0.061	0.044	0.054
Std. Error of Mean		0.009	0.069	0.012	0.046	0.012	0.014	0.012
Median		0.024	0.016	0.023	0.014	0.024	0.017	0.034
Range		0.474	0.280	0.474	0.281	0.475	0.235	0.365
Minimum		0.006	0.010	0.006	0.009	0.005	0.005	0.005
Maximum		0.480	0.290	0.480	0.290	0.480	0.240	0.370
Percentile	5	0.007	0.010	0.006	0.009	0.006	0.005	0.006
	25	0.013	0.012	0.010	0.011	0.010	0.009	0.017
	50	0.024	0.016	0.023	0.014	0.024	0.017	0.034
	75	0.057	0.221	0.053	0.084	0.065	0.051	0.062
	95	0.240		0.240		0.246		0.272
Extreme known value of variable if it exceeds range in current analysis		NA	NA	NA	NA	NA	NA	NA

3.7   Depth requirements

There is a general paucity of published information on the depth requirements of south-western Australian fishes. However, based on existing published and unpublished data along with expert opinion of the authors, estimates for pool and habitat depth requirements are presented in Table 9. Also presented are the distributions of the various species in the Blackwood River and in the NCCARF groundwater study area.

Table 9: Estimated minimum habitat depth requirements of the six endemic and one introduced freshwater fish in the Blackwood River groundwater study area. Also presented are the distributions of each species in the Blackwood River and their baseflow distributions in the groundwater study area.

	Go	Gm	Nv	Nb	Bp	Tb	Gh
Known distribution of fishes in the Blackwood River and specifically within the NCCARF project's groundwater study area during baseflow.	Wide distribution in Blackwood including all tributaries and throughout main channel. Utilises 1500-2500m of groundwater maintained Milyeannup Brook during baseflow. Also pools in lower sections of Rosa Brook and Poison Gully.	Restricted distribution to fresh tributaries and is absent from the main channel of the Blackwood River. Utilises 1500-2500m of groundwater maintained Milyeannup Brook during baseflow. Also in lower sections of Rosa Brook and Poison Gully.	Present in main channel and tributaries of the lower Blackwood River to at least Nannup. During baseflow utilises 1500-2500m of groundwater maintained Milyeannup Brook. Also known from pools in lower sections of Rosa Brook and Poison Gully.	Restricted distribution in main channel mostly between Milyeannup Brook and Rosa Brook and breeds only within Milyeannup Brook. Utilises main channel and 1500-2500m of groundwater maintained Milyeannup Brook during baseflow.	Present in main channel and tributaries of the lower Blackwood River to at least Nannup. During baseflow utilises 1500-2500m of groundwater maintained Milyeannup Brook. Also known from pools in lower sections of Rosa Brook and Poison Gully.	Found mostly in the main channel from Hutt Pool to at least Southampton Pool. Known to undertake localised migrations through riffle zones during spring and summer including baseflow.	Present throughout the Blackwood River catchment including the salinised upper reaches. Utilises predominantly main channel habitats throughout the study area during baseflow.
Estimated minimum of habitat depths	Riffle: >3 cm Pool: >10 cm	Riffle: >5 cm Pool: >15 cm	Riffle: >5 cm Pool: >15 cm	Riffle: >5 cm Pool: >15 cm	Riffle: >8 cm Pool: >15 cm	Riffle: >12 cm Pool: >40 cm	Riffle: >5 cm Pool: >5 cm
Source of data	Morgan et al. (1998) Beatty et al. (2006, 2007 2009).	Morgan et al. (1998) Beatty et al. (2006, 2009).	Morgan et al. (1998) Morgan and Beatty (2003) Beatty et al. (2006, 2009).	Morgan et al. (1995, 1998) Beatty et al. (2006, 2009).	Morgan et al. (1998) Beatty et al. (2006, 2009).	Beatty et al. (2006, 2009, 2010).	Morgan et al. (1998) Beatty et al. (2006, 2009).

4. DISCUSSION

The current study is the first to characterise the water quality within the habitats of south-western Australian freshwater fishes despite a range of previous studies having documented their distributions (Morgan *et al.* 1998, 2011), aspects of biology (e.g. Pen *et al.* 1991), predation and competition by introduced species (Morgan *et al.* 2004; Beatty and Morgan 2013), impacts of barriers to migrations (Morgan and Beatty 2006; Beatty *et al.* 2007), and the effects of secondary salinisation (Morgan *et al.* 2003; Beatty *et al.* 2011). This study represents an important step in understanding both the drivers of past range declines of freshwater fishes in this region and in predicting future population viabilities under changing environmental conditions such as those brought about by surface flow and groundwater reductions. These data were directly incorporated into Bayesian Belief Networks (BBNs) to model likelihood of population declines of species (see Speldewinde 2103) which were then spatially mapped (see Neville 2013) to examine how populations may respond to projected groundwater level reductions in the Blackwood River.

4.1 Interspecific differences in TIVs

Clear differences existed in several water quality variables at sites occupied by the freshwater fishes in the current study. The difference between the two galaxiids was most notable with *G. occidentalis* occupying sites that were on average 2.7°C warmer and ~1784 µS.cm^{-1} higher in conductivity than *G. munda*. Furthermore, greater differences in the 75th and 95th percentiles for most variables were also revealed. These differences reflect the fact that *G. occidentalis* is the most widespread and abundant native species of freshwater fish in south-western Australia (Morgan *et al.* 1998) and has a range that includes many of the inland, secondarily salinised systems (Morgan *et al.* 2003; Beatty *et al.* 2011) whereas *G. munda* is rarer (protected under the Western Australian Wildlife Conservation Act 1950) and is generally found in smaller, more pristine rivers and wetlands often connected to fresh groundwater.

Beatty *et al.* (2009) found that *G. munda* did not occupy the main channel of the Blackwood River (the region's largest in terms of discharge) being restricted to a small number of mostly forested tributaries in the lower catchment (including those in the current NCCARF groundwater risk project) and proposed that it had been lost from the main channel due to increasing salinity. Furthermore, Phillips *et al.* (2007) found that *G. munda* had significant genetic differences between most tributaries examined in the Blackwood River and proposed that there were historical connections that have been lost. Therefore, whilst the salinity tolerance of *G. munda* has not been determined in the laboratory, given its current distribution, it is likely to be much lower than that of *G. occidentalis*, which was found to have an acute tolerance (effect concentration EC_{50}) of 14.6 ppt (~26500 µS.cm^{-1}) (Table 3).

There were also differences in the water quality across the sites of occupancy of the three percichthyids. *Nannatherina balstoni* occupied habitats that were on average cooler (≥~1.1 °C) that the other two percichthyids. This species has a relatively restricted range along the south coast and southwest corner of the region (and is listed as Vulnerable under the Federal EPBC Act 1999) and is generally found in more

pristine habitats (Morgan *et al.* 1998; 2011). In the Blackwood groundwater study area, it breeds only within the perennial Milyeannup Brook, which is maintained by Yarragadee Aquifer groundwater during baseflow and has been shown to be more thermally stable than other tributaries (Beatty *et al.* 2009). Although *N. balstoni* was demonstrated to have a far lower acute tolerance to salinity than *N. vittata* in a laboratory tolerance study (Beatty *et al.* 2009), this difference was not generally reflected in the conductivities at their sites of occupancy. Laboratory tolerance studies have not been conducted on *B. porosa*, however, the maximum conductivity they have been recorded in the field (i.e. 29500 μS.cm^{-1}) is far higher than that of *N. vittata* (17500 μS.cm^{-1}) or *N. balstoni* (8700 μS.cm^{-1}). Probably reflecting the fact that *N. balstoni* is found in the highly coloured and acidic peat wetlands along the south coast (Morgan *et al.* 1998), the pH and turbidity at its sites of occupancy was slightly lower than *N. vittata* or *B. porosa*.

The relatively low number of sampling sites where TN or TP was recorded along with species presence prevented a meaningful comparison across all species for these environmental variables. However, sites of occupancy of *G. holbrooki* had the highest mean or median measures of TN and TP compared with other species that had >10 sites of occupancy with those nutrients also measured, i.e. *G. occidentalis*, *N. vittata*, *B.porosa*, and *T. bostocki*. *Gambusia holbrooki* has an extremely wide environmental tolerance and invasive life-history traits (e.g. Pyke 2005) which has facilitated its colonisation of highly degraded habitats throughout south-western Australia (Morgan *et al.* 1998, 2004).

4.2 Implications of environmental change

Setting minimum requirements for aquatic habitat depths for these species was based on a combination of published and unpublished data and more precise quantification of their microhabitat requirements is needed, particularly with regard to pool and riffle depth requirements. However, recent research enables some understanding of how projected rainfall, groundwater and flow reductions (see Suppiah *et al.* 2007; Barron *et al.* 2012; Silberstein *et al.* 2012) may impact the region's highly endemic freshwater fishes. Beatty *et al.* (2010) examined *T. bostocki* passage through riffle zones in relation to discharge and habitat depth during baseflow (when groundwater largely maintained flow) in the Blackwood River and found that total passage impediment would occur at a depth of between 5-18 cm. Therefore, as groundwater intrusion is projected to decline, loss of access to riffle habitat by this species was predicted (Beatty *et al.* 2010). A recent long term study in the Blackwood River demonstrated that most of the other small freshwater endemic species undergo lateral potamodromous migrations into and out of tributaries for breeding and that migration strength during the major flow and breeding periods is explained mostly by the amount of discharge from the tributaries (Beatty *et al.* 2009). Therefore, projected reductions in surface flows due to rainfall decline are likely to reduce recruitment for several species by disrupting the timing and strength of spawning migrations.

Projected increase in temperature in south-western Australia may also have major implications for aquatic fauna, including freshwater fishes (Davies 2010; Morrongiello et al. 2011). The swimming performance at different temperatures has recently been determined for *N. vittata* and *G. holbrooki* (Keleher 2010). That study found that *G.*

holbrooki had a much poorer swimming ability than *N. vittata* and while no major change in swimming performance of *N. vittata* was detected over the modest range of 15-25°C, there are likely to be considerable implications of increased temperature for more sensitive endemic fishes particularly with regard to the increased number of extreme hot days projected for this region (Suppiah *et al.* 2007; Keleher 2010). As temperature increases due to climate change has been shown to impact swimming performance of migratory fishes by reducing their aerobic scope (Farrell *et al.* 2008), reduced groundwater input into refugia (which can thermally buffer surface water) coupled with increased temperatures may reduce the fitness of these populations. Understanding this key physiological trait of freshwater fishes in south-western Australia and indeed elsewhere within Australia is required to predict how future changes in temperature regimes in aquatic ecosystems will affect their migratory ability and population viability.

4.3 Limitations and areas of future research

Accurately undertaking analyses of environmental niche breadths for TIVs of freshwater fishes clearly depends on the amount and quality of existing datasets on species distributions and matching environmental variables, specifically in the case of the current NCCARF study, water quality variables that would be influenced by groundwater decline. In Australia, similar datasets would exist for most regions, particularly eastern Australia, and may also allow standardisation of species densities in order for weighted averaging of variables to occur, providing more robust estimates of tolerance indicator values (e.g. Meador and Carlisle 2007). Kennard *et al.* (2006) incorporated fishes in predictive modelling to assess ecosystem health and the impacts of human disturbances on water quality, riparian and instream habitat conditions in south-east Queensland. However, as far as we are aware, assigning specific TIVs for individual species on a regional scale has not previously been conducted elsewhere in Australia.

Use of fish communities as predictors of ecosystem health can be limited by the number of species available for use in multivariate analyses. However, Kennard *et al.* (2006) concluded that whilst only 24 species were used in the model, the level of precision was similar to those that utilised macroinvertebrates, diatoms or habitat categories. They excluded rare species to help overcome the effect of temporal variations in fish assemblage composition in predicting species composition through time. In depauperate regions such as south-western Australia (total of 11 freshwater fish species), accounting for low species diversity and rarity in similar predictive models would be more challenging. Reach scale stressors such as embeddedness and turbidity (in turn influenced by slope and forest cover) were the variables that had the strongest influence on fish descriptors in wadeable streams in Georgia, USA (Walters *et al.* 2009). The latter study also incorporated macroinvertebrate communities to assess stressors and suggested that, due to those faunal groups having different sensitivities to various stressors, the use of multiple assemblages provides a complete and accurate assessment of ecological health. Beatty and Morgan (2010) also proposed that the use of both macroinvertebrate and freshwater fish communities in south-western Australia would allow more rigorous assessment and monitoring of ecosystem health.

Field derived TIVs, such as those in the current study, can be used in both predicting future viabilities and classifying species into broad tolerance categories (Karr *et al.* 1981). However, their use needs to be considered in light of several limitations, most notably the presence of sampling bias towards less extreme environments and the number of sites used in the analysis. There are also the problems of a limited sub-set of variables being analysed, and the issue of co-variability and interactions within and between physical and chemical habitat components (Meador and Carlisle 2007). Meador and Carlisle (2007) found that classification of freshwater fish into three tolerance groups based on 773 stream sites in the U.S. was influenced mostly by tolerances to suspended sediments, specific conductance, chloride and TP. Conversely, tolerance to factors such as temperature, dissolved oxygen, and pH were less important. Incorporating a broader set of water quality variables (e.g. nutrients) and others influenced by human pressures particularly hydromorphological variables (e.g. degree of channelisation), and other instream and riparian habitat conditions with those examined in the current study would generate a more complex set of TIVs that would have value in more sensitive modelling of population viabilities under future environmental conditions.

The specialisation-disturbance hypothesis proposes that specialised species are less tolerant to habitat disturbance than generalists (Vázquez and Simberloff 2002). Sergurado *et al.* (2011) were the first to demonstrate this for freshwater fish, as they found niche breadth was positively correlated with fish tolerance estimates. Whilst further work is required in terms of both field and laboratory based studies, the results of the current study suggest that restricted species, particularly *G. munda*, are likely to be less tolerant to water quality decline than more widespread species. However, whilst more restricted species that are naturally found in less disturbed sites may be assumed to be intolerant to pressures, this may not always be the case and undertaking complementary bioassays would enable a better understanding of tolerances.

Another major limitation in the application of TIVs to predict population viabilities under altered future physiochemical conditions is variability in the sensitivity of life-history stages. In the current study, along with the inability to use weighted averages to account for relative abundances between sampling sites, no consideration was given to whether there was evidence of recruitment at the various sample sites. As a consequence, we deliberately selected rather conservative percentiles as TIVs and indicators of population decline (i.e. 25th, 75th) for the subsequent BBNs and GIS modelling conducted in the current NCCARF project to err on the side of caution in terms of recruitment viability.

5. CONCLUSION

The current study has utilised a relatively large dataset of species distributions and environmental variables in south-western Australia to assign field derived TIVs for most species of native freshwater fishes of the region. The species included were those that are known from the Blackwood River groundwater study area, which will therefore enable the trialling of the risk assessment and decision framework to manage groundwater dependent ecosystems. This study has demonstrated considerable interspecific differences in TIVs for several environmental variables, which will have implications for the subsequent BBN modelling of the likelihood for population declines and the spatial mapping of those likelihoods within the groundwater study area. Complementary bioassays to determine effects of concentrations of key water quality variables to various life-history stages of freshwater fishes are recommended to provide more robust TIVs in future population viability modelling.

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