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Metapopulation theory explains Black-stripe Minnow (Pisces: Galaxiidae, *Galaxiella nigrostriata*) distribution in seasonal wetlands in south-west Western Australia

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Abstract

The objective of this project was to determine if *Galaxiella nigrostriata* populations could belong to a metapopulation. Metapopulation theory describes how multiple populations with occasional connectivity are a 'population of populations'. Some populations' habitats have optimal conditions (source habitats), others experience regular extinctions (sink habitats). Connectivity allows repopulation of extinct or uninhabited habitats. *Galaxiella nigrostriata* occurred randomly in 11 seasonal wetlands in the Kemerton wetland complex in south-west Western Australia over a 16 year period. The wetlands did not appear to be connected.

Around 70% of wetlands on the Swan Coastal Plain in south-west WA have been filled or degraded since European settlement around 180 years ago. Of those, seasonal wetlands are at most risk from degradation. *Galaxiella nigrostriata* mainly live in seasonal wetlands between Augusta and Albany and in three remnant populations on the Swan Coastal Plain. They are small freshwater fish (<45mm total length), aestivate in moist wetland sediments when wetlands dry and live for about one year. Seasonal wetlands and *G. nigrostriata* are threatened by nutrient enrichment, salinity, introduced fish, landscape modification and changes to hydroperiod by groundwater abstraction and declining rainfall.

Inundated wetlands that previously contained *G. nigrostriata*, and wetlands where they had not been recorded, were sampled throughout south-west WA. Fish and crayfish abundance was surveyed and water samples analysed on site and in a laboratory. Physical characteristics of each wetland and surrounding landscape were also recorded. Information about wetlands was analysed to determine if physico-chemical characteristics accounted for *G. nigrostriata* abundance or distribution between wetlands. Lentocorrals were then established in two Kemerton wetlands prior to inundation. They were sampled following inundation to determine how and where within a wetland *G. nigrostriata* entered the sediment to aestivate. Aestivation was examined to determine whether any physical features may be lacking which could inhibit population persistence. *Galaxiella nigrostriata* specimens from each population had morphological measurements and counts taken prior to tissue being removed for genetic analyses. Two mitochondrial DNA markers were used to investigate divergence and connectivity within and between populations and catchments.

Most wetlands were small (mean 0.6 ha), had tannin-stained water and 41% vegetation cover. All wetlands exceeded guideline values for Fe and Zn and those near agricultural land exceeded guideline values for TN and TP. However, no physico-chemical water properties or habitat features impeded *G. nigrostriata* abundance or distribution between wetlands. Additionally, it was thought there may be a commensal relationship between *G. nigrostriata* and burrowing crayfish, with *G. nigrostriata* using burrows to enter the sediment. No relationship was found between *G. nigrostriata*, crayfish or their burrows, indicating an alternative way for them to enter the sediment. Genetic research and examination of wetland positions in the landscape confirmed *G. nigrostriata* populations (particularly Kemerton) are part of metapopulation. This research showed populations between catchments had not connected for thousands of years but populations in wetland complexes had recent connectivity.

Management of wetlands requires investigation and monitoring of nearby wetlands which may be part of a metapopulation, and may affect population longevity of all wetlands.

Declaration

I certify that this thesis does not, to the best of my knowledge and belief:
(i) Incorporate without acknowledgment any material previously submitted for a degree or diploma in any institution of higher education;
(ii) Contain any material previously published or written by another person except where due reference is made in the text of this thesis; or
(iii) Contain any defamatory material.
(iv) Contain any data that has not been collected in a manner consistent with ethics approval.

David M. Galeotti

November 2013

Acknowledgements

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Thanks also go to Mark Gell, former mine manager at KSS. Mark was also instrumental in getting this project (and funding) off the ground. His concern for positively steering KSS's environment awareness was commendable. Throughout my time dealing with Mark, whether it was through email, phone calls, or onsite asking to borrow the 4WD again, Mark was always friendly, easy going and did whatever he could to help. The rest of the staff at KSS (Gary Meek, Fred Savickis, Paul Williams and Fiona Stockley in particular) were just as friendly and helpful, like a big happy family!

As well as the research funding from KSS, ECU's School of Natural Sciences (SoNS) and Centre for Ecosystem Management (CEM) provided postgraduate and conference (Alice Springs and South Africa!) travel assistance, and additional genetic sampling funding for which I am indebted. I also thank the Australian Society for Limnology for providing funding to attend their conference in Alice Springs.

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Still on genetics (it was supposed to be an easy, small part of a chapter!!), a big thanks to Dr Mark Castalanelli (Dept. of Agriculture/Curtin University and now Western Australian Museum) and Prof. David Groth (Curtin) for the ongoing help in the Biomedical lab at Curtin, coffees, meetings and the paper that is on the way (Don't worry, I haven't forgotten the beer Mark!). Thanks also to the other students using the Biomedical lab for welcoming me into 'their' lab, their help, advice, chilli chocolate ice-cream and yum cha lunches.

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I thank Roger Hearn (Dept. of Environment and Conservation) for suggesting the Poorginup Swamp sites and providing background information along the way.

If I've forgotten anyone I'm sorry, and thank you too!

The two main reasons I undertook this research were: to try establish a career for myself which would 1. Not be such a burden on my body (unlike plastering!), and 2. To try make a difference so that future generations can enjoy what we still have and take for granted today.

To this end, I dedicate this thesis to my beautiful children Isabella, Jamie and Kade.

Publications arising from this research

Refereed Papers

Galeotti D.M., Castalanelli M.A., McCullough C.M., Lund M.A. and Groth D.M. (submitted) Geneotypic and morphological variation between *Galaxiella nigrostriata* (Galaxiidae) populations: Implications for conservation.

Galeotti, D.M., McCullough, C.D. & Lund, M.A. (2010) Black-stripe minnow *Galaxiella nigrostriata* (Shipway 1953) (Pisces: Galaxiidae), a review and discussion. *Journal of the Royal Society of Western Australia*, 93:1, 13-20

Technical Reports

Galeotti, D. M.; McCullough, C. D. & Lund, M. A. (2008). Current state of knowledge of the Black-stripe Minnow *Galaxiella nigrostriata* (Pisces: Galaxiidae) in Western Australia. Centre for Ecosystem Management Report 2008-12. Edith Cowan University, Perth, Australia. Unpublished report to Kemerton Silica Sands. 36 pp.

Other publications

Galeotti, D.M. (2011). A fish out of water. *Bushland News*. Department of Environment and Conservation. Spring edition (79) p. 4.

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1 Introduction

1.1 Metapopulation: a population of populations

Metapopulation theory explains survival or extinction patterns in a group of individual populations that interact through occasional connectivity and where the average rate of extinction is less than that of migration (Levins 1970; Hanski & Gaggiotti 2004; Batzer, Cooper *et al.* 2006). Some of those populations may go extinct from time to time, only to be recolonised later from neighbouring populations. Populations that persist over time are considered to be source populations, these populations are found in habitats that have optimal conditions for their survival (Dias 1996). Populations that regularly become extinct are known as sink populations, which exist in habitats that are unable to sustain a population in the long-term. Metapopulation theory was introduced by Levins (1970), following on from MacArthur and Wilson's work on island biogeography theory (MacArthur & Wilson 1963; 1967).

Hanski and Gilpin (1997) list four requirements for the use of metapopulation theory to be valid for a group of populations. A region thought to contain a metapopulation must be made up of: (1) a mosaic of distinct areas of suitable and unsuitable habitat, (2) each population must at some time be threatened by extinction, (3) there must be some interaction between the populations, and (4) the dynamics of one population should be independent of other population dynamics. Jordán and Báldi *et al.* (2003) examined populations of the endangered *Pholidoptera transsylvanica* Bush Cricket in Hungary, which followed the above criteria. Their results showed the most important dynamic for the cricket populations was connectivity between patches of habitat and habitat size.

Dispersal from source populations may occur prior to or once the carrying capacity has been reached and then flow into potential sink populations (Hoopes & Harrison 1998). Hoopes *et al.* (1998) also suggest that sink populations may ultimately provide the necessary stock to repopulate source populations, should a catastrophic event happen to the source population. Lafferty *et al.* (1999) also emphasise the importance of sink populations. While investigating the affects that small wetlands have on metapopulation dynamics of the endangered *Eucyclogobius newberryi* Tidewater Goby, they concluded that small wetlands need to be incorporated into management plans. They suggested that small wetlands can harbour (sink) populations during low rainfall years and during higher rainfall years provide important migration pathways between larger wetlands containing source populations. This implies that metapopulations are dynamic and require spatial and temporal monitoring in multiple wetlands to understand the seasonal fluctuations that may occur.

Population connectivity may be difficult to establish without genetic analyses (Lowe & Allendorf 2010). Historic or recent dispersal between populations can be inferred by gene flow, as shown by Barson *et al*. (2009) and their research into *Poecilia reticulate* Guppy. Genetic diversity was higher in Guppy sink populations than the source, a common phenomenon due to high death rates (turnover) and migration into the sink population (Gaggiotti 1996). While genetic analysis alone is not effective in detecting metapopulations, it can be a useful tool when used to complement other methods, such as habitat assessments and population surveys (Davis & Roberts 2005; Lowe & Allendorf 2010).

Stochastic environmental conditions (for example: wildfire, climate change or disease), may cause the extinction of a source or sink population or interrupt the connectivity between these populations. Therefore, to understand a species' habitat requirements or manage a species for conservation, information about more than one population may need to be considered. Indeed, if there is more than one population within close range, then all nearby populations should be investigated or managed together. Furthermore, researchers and land managers need to know how much impact there will be on surrounding populations should an area of (populated) habitat be reduced or removed for development (Hanski 1994).

Populations of *Galaxiella nigrostriata* Black-stripe Minnow at the Kemerton wetlands are ideal candidates for testing metapopulation theory, given their intermittent presence in separated wetlands within a wetland complex. The Kemerton wetland populations follow the four criteria for a region to qualify as a metapopulation:

1) A mosaic of suitable and unsuitable habitat - the Kemerton wetlands are relatively heterogeneous in their size, depth, mix of vegetation, soil types and water quality (BEC 2004; Rockwater 2008).

2) Each population threatened by extinction - the hydroperiods of the Kemerton wetlands vary over time, caused by long-term climate change, nearby groundwater extraction regimes and changes to the local topography (through mining) (Rockwater 2008).

3) Interaction between populations - through environmental monitoring records, some Kemerton wetlands appear to maintain *G. nigrostriata* populations while in others their presence is sporadic.

4) All populations are independently dynamic - there are no permanent surface water connections between wetlands. Connectivity may be limited to abnormally high rainfall seasons.

Therefore, metapopulation theory could explain why some *G. nigrostriata* populations are temporary while others are permanent within a wetland complex.

1.2 Seasonal wetlands in south-west Western Australia

Seasonal wetlands are wetlands that have a predictable annual filling and drying regime and are inundated for months in between (Boulton & Brock 1999). Seasonal wetlands in south-west Western Australia (WA) generally begin to fill with the onset of winter rains (April-July) and start to dry as temperatures and evaporation increase from November – January (Balla 1994). Most seasonal wetlands in south-west WA are groundwater dependant, receiving water from unconfined aquifers as groundwater levels rise (recharged by rainfall) and also directly from rainfall and run off (Balla 1994; Gibson, Keighery *et al.* 2005). The majority of seasonal wetlands in south-west WA are found on the Swan Coastal Plain and the Warren bioregion, which includes the Scott Coastal Plain (Gibson, Keighery *et al.* 2005).

Around 70% of wetlands on the Swan Coastal Plain have been drained, filled or otherwise degraded since European settlement over 180 years ago, mostly from urbanisation and agriculture (Seddon 1972; Balla 1994; Davis & Froend 1999). Additionally, on the Swan Coastal Plain between Harvey and Dunsborough, an estimated 95% of wetlands have been filled or drained for agriculture (Riggert 1966). Unfortunately, many of the remaining wetlands are experiencing degradation from clearing or modifying riparian vegetation (replacement by exotic species), poor water quality from increased nutrients and pollution (fertilizers, stormwater runoff) and hydrological changes (e.g. climate change or groundwater abstraction) (Hill, Semeniuk *et al.* 1996; Davis & Froend 1999).

Seasonal wetlands generally have higher genetic diversity and have higher diversity of flora and fauna compared to permanent wetlands (Hill & Del Marco 1996). However, south-west WA has a low diversity of native freshwater fish species, with only 10 species present (Morgan, Gill *et al.* 1998; Allen, Midgley *et al.* 2002). Of those, eight species are endemic, the high degree of endemism is probably due to the long term geographic isolation of this group (Morgan, Gill *et al.* 1998; Unmack 2001). Of the endemic species, there are three representatives of the Galaxiidae: *Galaxias occidentalis*

Western Minnow, *Galaxiella munda* Mud Minnow and *G. nigrostriata* (Morgan, Gill *et al.* 1998).

1.3 Galaxiella nigrostriata

Galaxiella nigrostriata is a small (maximum total length (TL) <50 mm), scaleless freshwater fish with an elongate body that is mostly grey-tan with a white underside (Plate 1) (Morgan, Gill *et al.* 1998; Allen, Midgley *et al.* 2002). From early larval stages *G. nigrostriata* has two black longitudinal stripes that run from the eye to the base of the tail, divided by a yellow to red stripe (Gill & Neira 1994; McDowall & Waters 2004). Each female *G. nigrostriata* lays about 60 eggs per season, possibly over a period of a couple of weeks, between June and September (Pen, Gill *et al.* 1993; Gill & Neira 1994). When larvae hatch they are about 3.5 mm TL and develop their stripes soon after (Gill & Neira 1994; Morgan, Gill *et al.* 1998). *Galaxiella nigrostriata* become sexually mature when nearly one year old and die soon after spawning (Pen, Gill *et al.* 1993).

Plate 1. Adult *Galaxiella nigrostriata* approximately 30 mm total length (courtesy of Gerry Allen)

Migration between water bodies is limited to direct connections such as annual or perennial creeks and rivers and seasonally inundated damplands or floodplains. It is also possible they migrate between waterbodies during temporary sheet flow caused by heavy rainfall, as they have been observed swimming in water 2 mm deep (Beck 1985). *Galaxiella nigrostriata* are typically found in seasonal wetlands with irregular connectivity to nearby water bodies. To survive regular drying of their habitat, *G. nigrostriata* aestivate in the damp sediments when wetlands dry around December and appear again when wetlands inundate around June (Berra & Allen 1989a; Pen, Gill *et al.* 1993; Morgan, Gill *et al.* 1998).

Galaxiella nigrostriata typically inhabit vegetated water that is about 300 mm deep, highly tannin stained and has a wide pH and temperature range of 3.0-8.0 and 11-30°C respectively (Jaensch 1992; Gill & Morgan 1996; Morgan, Gill *et al.* 1998; Allen,

Midgley *et al.* 2002). They are usually found in lentic pools up to 100 km from the coast, with limited connectivity to surrounding wetlands (Pusey & Edward 1990; Morgan & Gill 2000). The main populations of *G. nigrostriata* are in catchments between Augusta and Albany, with the highest prevalence found within the Gardner River catchment near Windy Harbour in D'Entrecasteaux National Park (Morgan, Gill *et al.* 1998; Morgan & Gill 2000) (Figure 1). Until recently, there were only two known remnant populations on the Swan Coastal Plain, one about 30 km NNE of Perth at a Melaleuca Park wetland designated EPP173 and the other 130 km S of Perth in wetlands within the Kemerton wetlands near Bunbury (Morgan, Gill *et al.* 1998; Bamford & Bamford 2002; Knott, Jasinska *et al.* 2002). However, an adult *G. nigrostriata* was captured by Neisha McLure (ECU student) while sampling for aquatic macroinvertebrates at Lake Chandala about 50 km NNE of Perth, which is the first recorded capture north of Melaleuca Park (McLure & Horwitz 2009; Galeotti, McCullough *et al.* 2010).

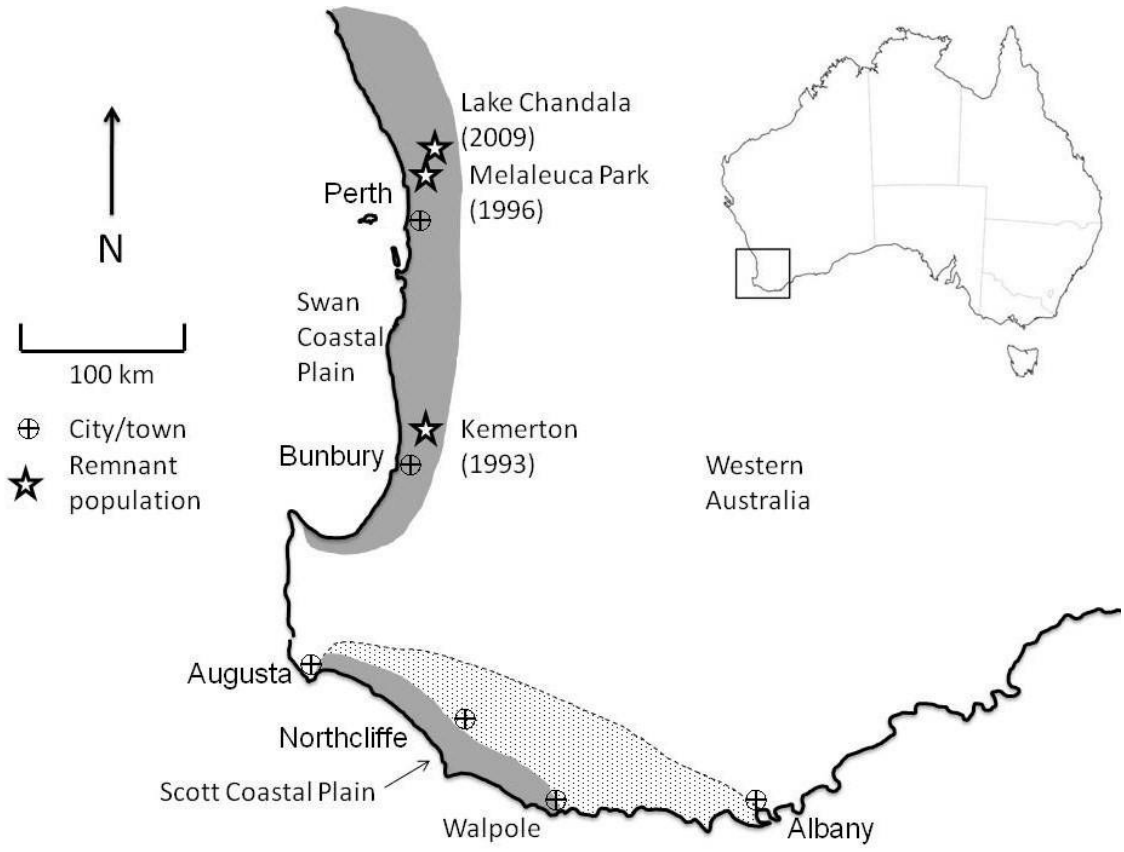

Figure 1. Current approximate distribution of *G. nigrostriata* populations in dotted area, which includes the Scott Coastal Plain (shaded area), and remnant populations shown by stars (date remnant population discovered in brackets) within the Swan Coastal Plain (shaded).

1.4 Threatening processes

The main threats likely to affect *G. nigrostriata* population survival are climate change, habitat modification and destruction. Rainfall in south-west WA is expected to decline by over 40% by 2070 and evapotranspiration is expected to increase (Watterson, Whetton *et al.* 2007). A drying climate will likely affect wetland hydroperiod through decreased run-off and reduced groundwater recharge, with some wetlands becoming permanently dry (Pratchett, Bay *et al.* 2011). Wetlands will also be affected by a predicted rise in mean temperatures which will lead to an increase in evapotranspiration in south-west WA (Watterson, Whetton *et al.* 2007).

A number of land use practices have directly or indirectly caused the loss of wetlands in south-west WA, such as filling or draining for agriculture, urbanisation and roads, forestry, dams and other such infrastructure, mineral and quartzite sand mining under wetlands (Seddon 1972; Balla 1994; Davis & Froend 1999; Smith, Knott *et al.* 2002). Unfortunately, seasonal wetlands are disappearing faster than other wetland types as they are less obvious as a wetland (when dry), making them an easy target for development (Hill & Del Marco 1996). Indeed, the reduced distribution of many native freshwater fish species to relatively pristine areas in the south-west corner of WA is considered due to widespread loss and degradation of wetlands in the region (Morgan, Gill *et al.* 1998; Allen, Midgley *et al.* 2002).

Additionally, groundwater levels at Melaleuca Park's EPP173 have been decreasing since the early 1970's due to declining rainfall, groundwater abstraction and uptake from the nearby pine plantation (WRC 1997). Excessive anthropogenic groundwater extraction can cause unseasonal or extended dry periods in wetlands and decreasing groundwater levels could induce acidification through acid sulphate soils (Smith, Knott *et al.* 2002; Horwitz, Bradshaw *et al.* 2008). Furthermore, altered wildfire seasons and prescribed burning practices can cause organic-rich sediments in seasonal wetlands to burn for long periods, possibly killing fish that may be aestivating within the substrate (Trayler, Davis *et al.* 1996; Semeniuk & Semeniuk 2005; Horwitz, Bradshaw *et al.* 2008). In some areas, an increase in wetland salinity has been caused by massive historical land clearing degrading water quality in rivers and streams (Halse, Ruprecht *et al.* 2003).

Introduced exotic fish species may also impact upon native species through competition for food, aggressive/predatory behaviour that causes displacement, injury or death and by introducing disease (Becker, Laurenson *et al.* 2005; Rowe 2007; Marina, Beatty *et*

al. 2008). For example, introduced *Gambusia holbrooki* Eastern Mosquitofish prefer the shallow still water of wetlands and may show aggressive behaviour (fin-nipping) toward co-habiting species, particularly when water temperature is over 20 °C (Morgan, Gill *et al.* 2004; Rowe 2007). One advantage *G. nigrostriata* have over introduced species is the ability to aestivate during dry periods. Exotic (and most native) fish species known in south-west WA wetlands cannot aestivate, therefore they die or leave an area that dries. However, exotic species could still have a deleterious effect by attacking *G. nigrostriata* populations as water subsides, niche habitats in the water column disappear and competition for food and space increase.

Galaxiella nigrostriata are listed as a species of conservation concern with local, national and international organisations:

Priority 3 (Taxa with several, poorly known populations, some on conservation lands) with the West Australian Department of Environment and Conservation (DEC, unpublished data, 2010)

Restricted with Australian Society of Fish Biology (ASFB 2007)

Lower Risk - Near Threatened with the International Union for Conservation of Nature (Wager 1996).

1.5 Project structure

The objective of this project was to establish if *G. nigrostriata* populations belong to a metapopulation. *Galaxiella nigrostriata* populations were found to occur seemingly randomly in many seasonal wetlands in the Kemerton wetland complex over a 16 year period. The wetlands did not appear to be connected. Research was conducted to systematically demonstrate how metapopulation theory could explain their appearance in the wetlands.

This chapter (Chapter 1) outlines metapopulation theory and how it can be applied to populations of various taxa. It explains how conservation of a species can be managed through monitoring multiple populations or habitats and the importance of sink populations. Background information about wetlands in south-west WA is next, particularly the apparent lack of value placed on them by past (and current) generations. Pertinent ecological and biological facts about *G. nigrostriata* are then given, which include their habitat, distribution and life cycle. Factors that may threaten populations follow, highlighting impacts to habitats from climate change and anthropogenic.

Finally, general features of the wetlands sampled and surrounding areas and climate of south-west WA is discussed to give an understanding of their environment.

Chapter 2 reports on the methods used that are common to more than one chapter. It includes a detailed account of the ad hoc assessment of sampling techniques. This followed the inefficiency of the box traps initially used by the author, based on their use by other researchers for similar sampling. The statistics used in the following chapters are also fully described in this chapter.

The first criterion for metapopulation theory requires areas of suitable and unsuitable habitat. Inundated seasonal wetlands throughout south-west WA were sampled and analysed to determine which factors may limit habitat suitability for *G. nigrostriata* populations (Chapter 3). All factors deemed significant to *G. nigrostriata* population survival were examined and included population presence and abundance, water quality and habitat characteristics. Besides the threatening processes already mentioned in the introduction, any *G. nigrostriata* habitats found to be unsuitable may also satisfy the second metapopulation theory criterion: that each population must at some stage be threatened with extinction.

Dry wetlands were examined next (Chapter 4), given that nearly half of *G. nigrostriata's* life cycle is spent in aestivation. Two experiments investigated how *G. nigrostriata* access the groundwater table, their assumed use of crayfish burrows, and what part of the wetlands is used for aestivation. Knowing if burrowing crayfish are required for *G. nigrostriata* to enter the sediments or if ideal habitats for aestivation are not available in certain wetlands may limit aestivation and therefore reduce long-term survival of *G. nigrostriata* populations.

Chapter 5 examined gene flow between populations to infer connectivity, which would satisfy the third and fourth criterion, that there must be connectivity and that populations should be independently dynamic. Connectivity was investigated between nearby wetlands within a wetland complex, and between catchments to consider how long populations in neighbouring catchments may have been separated. Furthermore, morphology of *G. nigrostriata* was examined to see what effect had come from separation.

The final chapter (Chapter 6) summarises the main findings of this project and discusses those outcomes. How the criteria for metapopulation are met from this research is also discussed. Limitations are considered and recommendations for further research are suggested.

1.5.1 Project aims

The following aims, and the chapter in which they are addressed, are used in this project to provide evidence for *G. nigrostriata* populations belonging to a metapopulation. Each chapter includes hypotheses to lend support to the aims and the overall project objective.

Chapter 3

> Do any specific physical or chemical characteristics of wetlands or their water quality impact on *G. nigrostriata* abundance or distribution? What conditions may cause a habitat to be a source or a sink?

Chapter 4

> Are there specific habitat features/requirements (presence of burrowing crayfish?) for aestivation to be successful? Not all wetlands are exactly the same, which may preclude aestivation survival, creating a sink habitat.

Chapter 5

> Is there connectivity between populations within a wetland complex? Populations require the ability to migrate to be classed as a metapopulation.

2 General methods

2.1 Sampling sites

Sampling sites on the Swan Coastal Plain and the south-west corner of WA (herein the 'south-west region') were mostly groundwater dependant seasonal wetlands that have relatively predictable hydroperiods. The wetlands begin to inundate with the start of the winter rains Only two wetlands contained permanent pools: Melaleuca Park near Perth (EPP173) and Poorginup Swamp north of Walpole (MR1 and MR2) (Knott, Jasinska *et al.* 2002; R Hearn, DEC Manjimup, 2008, pers. comm.). Wetlands sampled ranged from having virtually none to almost impenetrable stands of riparian and wetland vegetation. Several wetlands were also on floodplains and experience occasional flooding. Some of the south-west region wetlands sampled were artificially created in the 1950's as water points for fighting bushfires or used for soil to form roads. Sites sampled were near Perth, Bunbury, Augusta, Northcliffe, Walpole and Albany (Figure 2). More detailed site information including GPS coordinates are in Appendix 8.1.

Wetland names referred to in this thesis are designated by the author, except for three Kemerton Silica Sand (KSS) wetlands (see 2.1.2 Kemerton wetlands) and EPP wetlands (Environmental Protection [Swan Coastal Plain Lakes] Policy 1992) (Balla 1994). Wetlands chosen for sampling sites were from general locations known to contain *G. nigrostriata* from previous fish surveys (Morgan, Gill *et al.* 1998; Knott, Jasinska *et al.* 2002), KSS environmental monitoring reports (Bamford & Bamford 2002; Bamford & Bamford 2006) and Western Australian Museum records (WAM; unpublished data). Similar wetland types where *G. nigrostriata* were not present were also sought within the same regions that the fish had been recorded, to make comparisons to what factors may be limiting their presence.

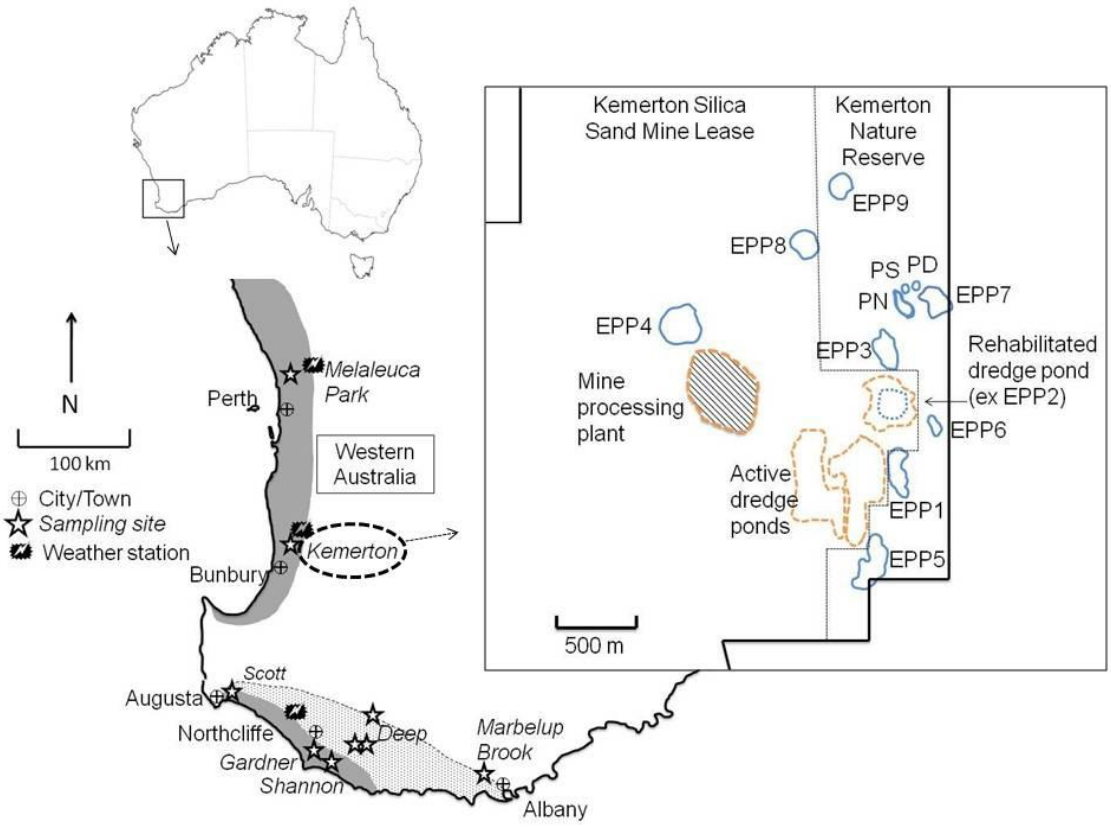

Figure 2. Sampling locations in south-west WA. Kemerton wetlands inset. Only natural wetlands were sampled at Kemerton wetlands. Weather stations shown are where Bureau of Meteorology climate information was obtained.

2.1.1 Melaleuca Park

Wetlands EPP173 and Mb are groundwater fed, seasonally inundated swamps in Melaleuca Park, 30 km NNE of Perth on the Swan Coastal Plain (Knott, Jasinska *et al.* 2002). The wetlands probably used to drain into the nearby Ellen Brook, which is a tributary of the Swan River, prior to landscape modification for agriculture (such as drainage and roads) and when groundwater levels were higher. Wetland EPP173 covers about 1.5 ha and Mb covers about 0.3 ha joining the northern side of EPP173 and both wetlands are thought to be part of a previous single large wetland (Smith, Knott *et al.* 2002). The wetlands are surrounded by *Banksia menziesii* open woodland containing *Eucalyptus marginate* Jarrah, *Corymbia calophylla* and *Xanthorrhoea preissii* Balga or Grasstree on slightly undulating Bassendean sands, are fringed by *Melaleuca preissiana* and EPP173 is dominated by the rush *Baumea articulata* (WRC 1997; Knott, Jasinska *et al.* 2002).

Both wetlands are continuous at the height of winter water levels and have a small outflow stream which drains into neighbouring agricultural land during high water level

years (Smith, Knott *et al.* 2002; Cullinane, Wilson *et al.* 2009). An area of several square metres within EPP173 has shallow standing water all year due to groundwater spring seepage, otherwise the remaining wetland is only inundated from about June to January (Knott, Jasinska *et al.* 2002). Prior to this project there were only two records of *G. nigrostriata* from EPP173 (D. Morgan, Murdoch University - Centre for Fish and Fisheries Research (MU-CFFR), unpublished data; Knott, Jasinska *et al.* 2002).

2.1.2 Kemerton wetlands

The Kemerton wetlands or Kemerton wetland complex referred to in this project incorporate wetlands within the Kemerton Nature Reserve and KSS mine lease project area 30 km NNE of Bunbury on the Swan Coastal Plain. The wetlands are 1 km from the Wellesley River, a tributary of the Collie River. Eleven wetlands were sampled, nine within the nature reserve, one on the mine lease and one spanning the boundary between the two. There are 9 EPP wetlands (EPP1-9) and three unofficially named wetlands: Paperbark New (PN), Paperbark Shallow (PS) and Paperbark Deep (PD) following McCullough *et al* (2008)[1]. All EPP wetlands had intermittent yearly monitoring records from 1993. Typically the wetlands are on gently undulating Bassendean sands surrounded by *E. marginata - C. calophylla - Agonis flexuosa* woodlands, and *E. rudis - Melaleuca* woodlands fringe inundated areas (McCullough, Lund *et al.* 2007).

The wetlands range from moderately disturbed from previous agricultural use, to relatively pristine. Most wetlands are managed by Department of Environment and Conservation (DEC) (Bamford & Bamford 2006; McCullough, Lund *et al.* 2007). The environmental importance of the region is enhanced by the presence of Threatened Ecological Communities, Threatened Fauna and Declared Rare Flora, some of which have national and international conservation significance (Bamford & Bamford 2002; DEC 2008). Most of the Kemerton wetlands are groundwater dependant and although some appear perched, all rely on rainfall for seasonal inundation (van Etten, McCullough *et al.* 2008). The wetlands generally remain inundated from July to November (McCullough & Lund 2008).

2.1.3 South-west region

The majority of wetlands sampled in the Augusta to Walpole region were artificially constructed roadside pools dug to supply soil for road construction and maintenance.

[1] Adjacent to four EPP wetlands were three large artificial dredge ponds, about 9-21ha, used for the KSS mining

However, they were seasonally contiguous with surrounding natural floodplain wetland systems with poorly draining sandy soils (Degens & Wallace-Bell 2009). Most wetlands sampled were thought to receive water from rising groundwater and/or rainfall, either directly or through surface flows. About half of wetlands sampled were near Augusta and Northcliffe on the Scott Coastal Plain, which stretches from Augusta to Walpole (Pen 1997).

The Augusta sites were on Scott River Road (site names - SR), 15 km NE of Augusta, in the Scott River National Park and the Northcliffe sites were on Chesapeake Road West (site names - CW) 15 km SSE of Northcliffe and Chesapeake Road and Moores Hut Track (site names - CP) 25 km SE of Northcliffe, all within the D'Entrecasteaux National Park. The remainder of the south-west sites were further inland, 25 km NW and 22 km N of Walpole on Beardmore and Thompson roads (site names - BR and TR), and 45 km N on Myalgelup Rd in the Poorginup Swamp (site names - MR) (part of the Lake Muir-Byenup system) on the southern extremes of the Darling Plateau (Pen 1997). Augusta wetland sites are collectively referred to as 'Scott' (within the Scott River catchment), Chesapeake Road West wetlands as 'Gardner', Chesapeake Road and Moores Hut Track as 'Shannon', and Beardmore, Thompson and Myalgelup Roads as 'Deep'.

Vegetation and topography ranged from heath and sedgelands on the low-lying coastal peat flats, to *E. diversicolor* and *C. calophylla* forests further inland on rolling hills (Christensen 1982; Pusey & Edward 1990). All of the wetlands sampled in this region were within DEC controlled national parks or nature reserves and are recognised as having important conservation values (Pen 1997). The wetlands on the coastal flats are generally flooded from June to December; and the only permanent wetland sampled was Poorginup Swamp (Pusey & Edward 1990; Storey 1998).

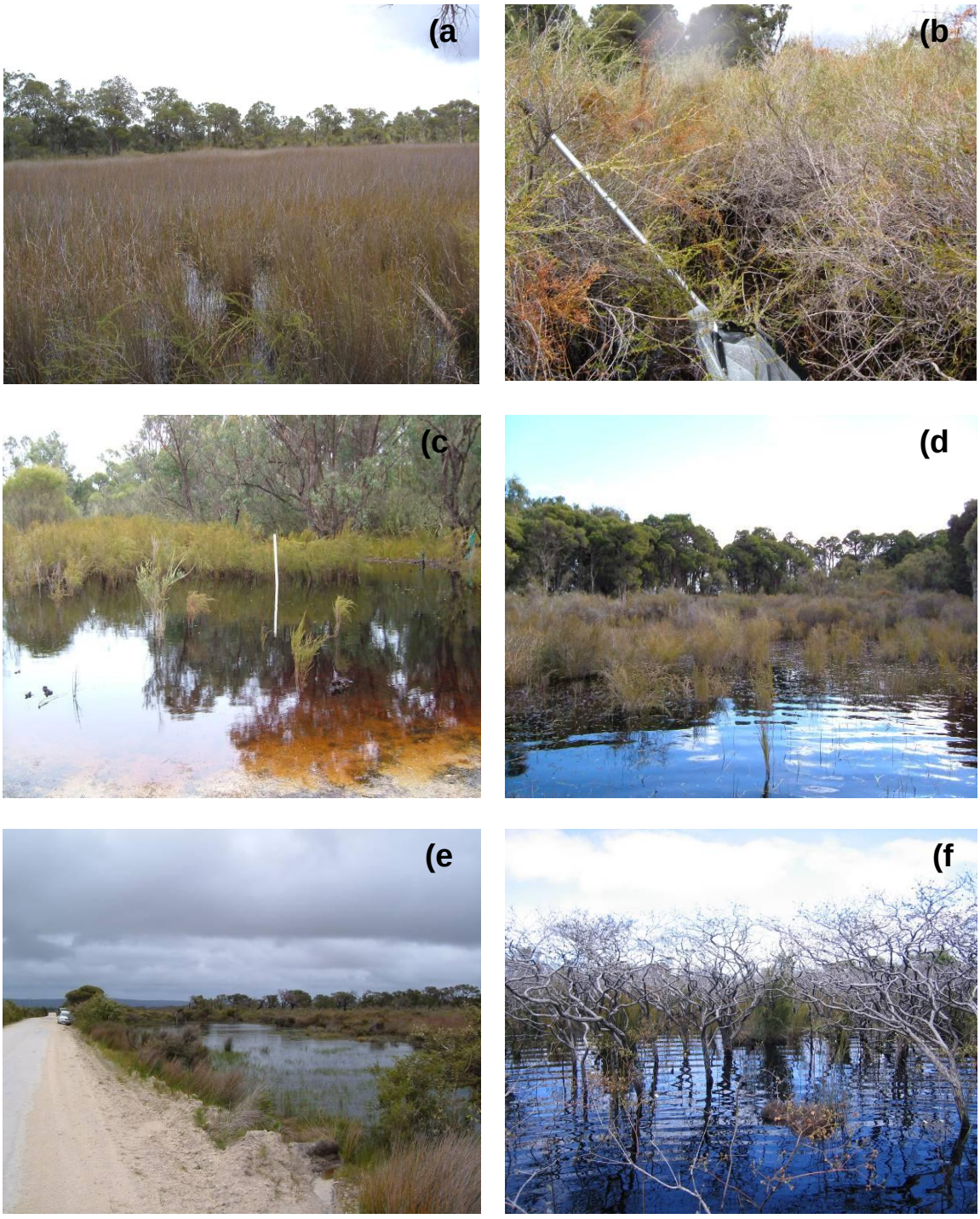

Plate 2. Examples of sampling sites - Melaleuca Park (a) virtually covered in *Baumea articulata* (b) neighbouring wetland with almost impenetrable woody shrubs, Kemerton wetlands (c, d) showing tannin stained water, (e) roadside pool on Scott River Rd, and (f) Chesapeake Rd West.

2.1.4 Climate

Climate and hydrological history of the Kemerton wetlands were compiled from KSS site records, KSS environmental reports and Bureau of Meteorology (BOM) data. All sampling sites listed above are located within a temperate (Mediterranean) climate region (BOM 2008). The region experiences mild wet winters, generally from June to August, and hot dry summers from December to March (Figure 4). The majority of rain falls in June/July and annual rainfall ranges from 690 mm at Melaleuca Park, 950 mm at Kemerton and 1200 mm in the south-west. Combined average annual rainfall data for Kemerton and nearby Wokulup (7 km east of KSS) weather stations show a >5mm decrease in rainfall per year over a 20 year period (Figure 3). Data based on the last 100 years shows a decrease in rainfall of >10 mm every decade and an increase in mean temperature of 0.1°C per decade (Figure 5A, Figure 5B). Climate change is predicted to continue affecting seasonal rainfall which may reduce inflows to catchment areas (Watterson, Whetton *et al.* 2007). For seasonal wetlands in south-west WA, the trends mean shorter hydroperiods through decreased rainfall and therefore lower groundwater recharge, and higher evaporation rates.

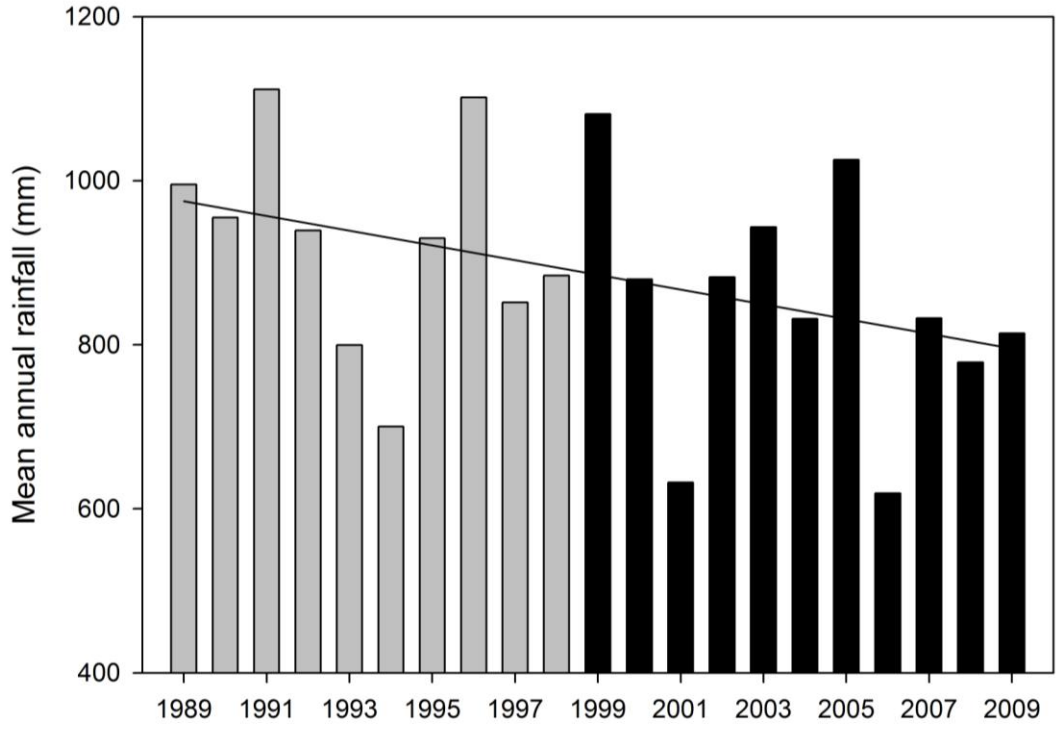

Figure 3. Mean annual rainfall at Wokulup (grey bars) and Kemerton (black bars). Trendline shows declining rainfall over 20 years. (Data for Wokulup from BOM, http://www.bom.gov.au/climate/data/, accessed June 2011; Kemerton from KSS site records)

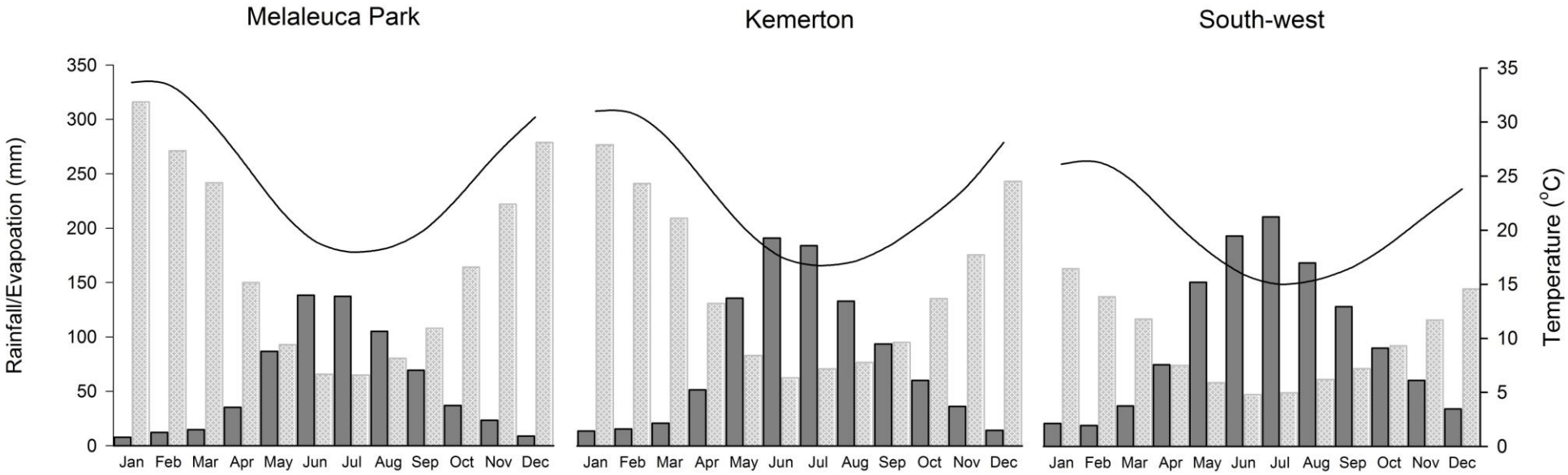

Figure 4. Annual mean climate graphs from weather stations representing Melaleuca Park (RAAF Pearce weather station), Kemerton (Wokalup) and south-west region (Pemberton). Solid bars are mean monthly rainfall, dotted bars are mean monthly evaporation and solid lines are mean maximum daily temperature. Evaporation data for Melaleuca Park taken from Perth Airport weather station. Pearce rainfall data from 1937-2010 and temperature from 1940-2010, Perth Airport evaporation from 1981-2010; Wokalup rainfall from 1951-2010, evaporation from 1969-2000 and temperature from 1951-2000; Pemberton rainfall and temperature from 1941-2010 and evaporation from 1967-2010. (Data from BOM, http://www.bom.gov.au/climate/data/, accessed May 2012)

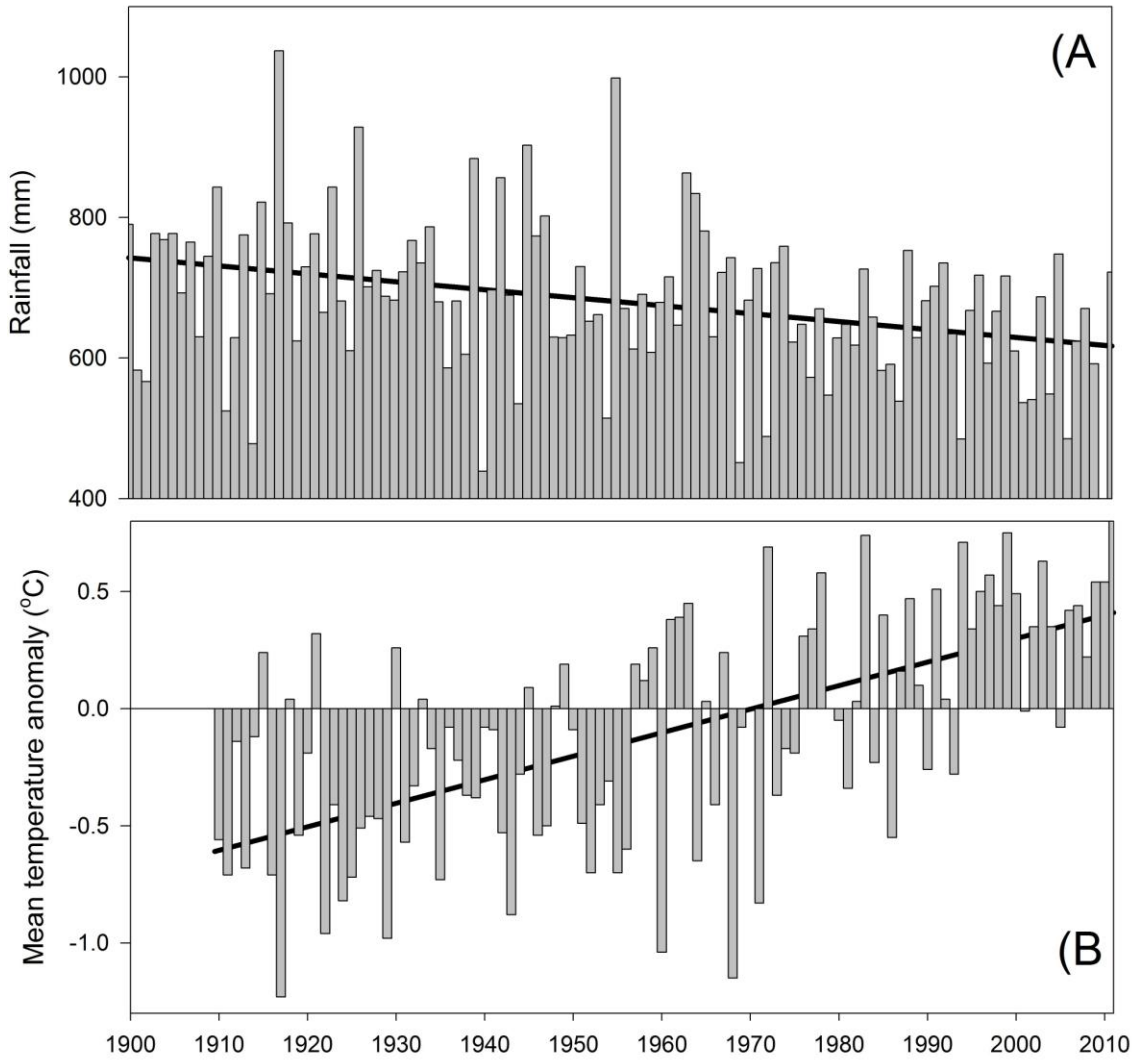

Figure 5. A) Annual rainfall for south-western Australia (1900-2010) with linear regression in bold. B) Annual mean temperature anomaly from average for south-western Australia (1910-2010 with linear regression in bold. (Data from BOM, http://www.bom.gov.au/cgi-bin/climate/change/timeseries.cgi, accessed May 2012)

2.2 Field sampling

Starting in late October 2008 (mid-spring) three sampling trips were conducted over four weeks, which included an initial ad hoc assessment to determine the most efficient capture method for *G. nigrostriata*. Field trips sampled wetlands at Kemerton (11 wetlands, 8 days), Melaleuca Park (2 wetlands, 2 days) and Augusta to Walpole (19 wetlands, 5 days) and took place between 0800 and 1800 h. Each wetland was sampled for fish and crayfish, with water quality and wetland features also recorded. Historical distribution of *G. nigrostriata* was determined from various unpublished and published mining company, DEC, Department of Fisheries WA, WA Museum and National Museum of Victoria (NMV) records, and peer reviewed journal papers (Shipway 1953; McDowall & Frankenberg 1981; Christensen 1982; Jaensch 1992; Morgan, Gill *et al.* 1998; Storey 1998; Smith, Knott *et al.* 2002; Bamford & Bamford 2006; NMV and WAM unpuplished data, 2010). Fish were sampled first, prior to water sampling and setting crayfish traps, to reduce disturbance effects to catch efficacy. To further improve catch efficacy, fish and crayfish were sampled in the three most common habitat types.

2.2.1 Assessment of trapping method

Previous freshwater fish researchers in south-west WA have used a variety of methods for collecting fish, including *G. nigrostriata*, such as seine and sweep nets, electro-fishing and piscicides (Jaensch 1992; Pen, Gill *et al.* 1993; Morgan, Gill *et al.* 1998; Gill & Morgan 2003). Many of the lentic wetlands in this research contained submerged and emergent vegetation, which restricted the use of seine nets (Cooke, Bunt *et al.* 1998). Piscicides, as used by Jaensch (1992) in south-west WA, had been ruled out to reduce the impact on populations of a conservation species (*G. Nigrostriata*) immediately following spawning season and the possible effects on non-target species.

Electrofishing and net traps can complement each other for community structure surveying (McInerny & Cross 2004). However, electrofishing may not prove time and cost efficient when compared to using net traps (McMichael, Fritts *et al.* 1998; McCullough & Hicks 2002). Fine mesh box traps have been successfully used in habitat preference studies of other galaxiid species of similar size and behaviour, for example: *Galaxiella pusilla* Dwarf Galaxias (Koster 2000) and *Neochanna* spp. Mudfish (Hicks & Barrier 1996; Ling & Gleeson 2001).

Box traps were initially used for sampling in October 2008 (mid-spring) in wetlands at Kemerton previously known to contain *G. nigrostriata*. Traps were

250 mm wide x 250 mm high x 500 mm deep, covered in 3 mm wide nylon with a 25 mm diameter funnelled entrance at each end. A minimum water depth of 0.2 m was required for the entrance to be submerged. Normally box traps would be set semi-submerged to prevent air breathing by-catch from drowning, such as turtles or water rats. The possibility of air breathing by-catch was dismissed for the box traps, as the entrance size was too small for these species. Traps were set in pairs (baited and unbaited) 5 m apart, encompassing the three main habitat types throughout each wetland: **open** - bare ground with minimal shading and at least 3 m from the nearest aquatic macrophytes (rushes, small herbaceous bushes/shrubs up to 1.5 m tall) or woody (bushes, trees over 1.5 m tall) vegetation, **vegetated** - immediately adjacent to or within aquatic macrophyte or herbaceous vegetation and **covered** - areas under or within 3 m of the canopy of woody vegetation (Plate 3).

Over two days in four Kemerton wetlands 119 traps were set (Table 1). On the first day 28 traps were set for 24 h in two wetlands, half baited with dry cat food and the other half unbaited (see Hicks & Barrier 1996). *Galaxiella nigrostriata* were only caught in unbaited traps in EPP6 (1 fish) and PN (2 fish). Unfortunately, 39 crayfish (*Cherax quinquecarinatus* Gilgie and *C. preissii* Koonac) were caught in all baited traps and four unbaited traps, which included two in the unbaited traps that also captured *G. nigrostriata.* Crayfish entering the baited traps could affect the catching efficacy by eating or deterring *G. nigrostriata*. To minimise attracting crayfish to the traps, on day two 91 traps were set unbaited. Only one *G. nigrostriata* and four crayfish were caught in the unbaited traps on day two.

The box traps caught very low numbers of *G. nigrostriata* (Table 1). Coincidently, while carrying out the initial sampling, colleagues were conducting macroinvertebrate transects using a fine mesh dip net through neighbouring wetlands. They caught more *G. nigrostriata* than the box trap method, which led to trialling a larger sweep net (500 mm x 500 mm opening x 450 mm deep with 3 mm wide mesh). The number of *G. nigrostriata* caught was exponentially larger using the scoop net than the box traps, while the number of crayfish caught was minimal (Table 1, Table 2). By-catch was mitigated by the sweep net method; any by-catch caught was released immediately at the end of the transect. The success of the sweep net meant it was used in all subsequent sampling.

Plate 3. Different habitat types for fish transects at the Kemerton wetlands - a) open, b) vegetated, c) covered, and d) Crayfish ‘opera house trap’ in open habitat.

Table 1. Efficiency of box traps used for catching *G. nigrostriata* in the initial sampling over two trap nights at the Kemerton wetlands.

Wetland	EPP5	EPP6	PN	EPP7	Total
Number of traps	8	12	39	60	119
Number of *G. nigrostriata* captured	0	1	3	0	4
Number of crayfish captured	5	21	15	2	43

Table 2. Efficiency of a scoop net used for catching *G. nigrostriata* in the initial sampling at the Kemerton wetlands.

Wetland	EPP5	EPP6	PN	EPP7	Total
Number of transects	8	12	12	8	40
Number of *G. nigrostriata* captured	12	41	28	0	81
Number of crayfish captured	0	2	2	0	4

2.3 *Galaxiella nigrostriata* and crayfish sampling

In October/November 2008, 31 wetlands were sampled using a sweep net (500 mm x 500 mm opening x 450 mm deep with 3 mm wide mesh) in six catchments, from Melaleuca Park (Swan River catchment) to near Walpole (Deep River catchment). Eleven of the wetlands sampled were in the Kemerton wetlands, which had extensive wetland monitoring data (including fish surveys) for 1993-2005. Most wetlands sampled between Augusta and Walpole had *G. nigrostriata* recorded in the past 30 years. However, nearby wetlands that appeared to have similar or suitable habitat were also chosen to try select wetlands that did not contain *G. nigrostriata* for comparison.

Wetlands were sampled with 10 m transects using a sweep net in depths of 0.05-1 m. Within each wetland up to four prescribed transects were carried out in a stratified-random sampling design, in random locations representative of the three habitat types, open, vegetated and covered (see 2.2.1 Assessment of trapping method). Additional sweeps were conducted (but not counted as transects) in wetlands where *G. nigrostriata* were not captured, to ensure accuracy of a 'not captured' result. Details about each transect were recorded and included fish species and quantity, average water depth, GPS coordinates and any other species captured such as crayfish or tadpoles. *Galaxiella nigrostriata* caught in each transect, to a maximum of twenty per wetland, were placed into labelled containers and euthanaised immediately in an ice slurry. The ice slurry

method was used to ensure specimens were not contaminated with carbon or nitrogen, which may have interfered with other researchers later planned stable isotope analysis. Ice-slurry has also been shown to be a less stressful method of euthanasia for freshwater fish than the common method of using benzocaine general anaesthetic (Blessing, Marshall *et al.* 2010). Once euthanaised, specimens were kept in an ice cooler prior to storage at -20°C at Edith Cowan University (ECU) Joondalup.

Each wetland was sampled over one night for crayfish in the same habitat types as fish sampling. One to five traps were used per habitat type; sampling effort was on a *pro rata* basis depending upon the size of the wetland. Crayfish were sampled using opera house traps (also known as yabby traps) baited with dry cat biscuits. Each opera house trap was set between 1500 and 1800 h and retrieved between 0800 and 1000 h the following morning giving a maximum of 19 h in the water, although the earliest set trap was generally the first retrieved. Information was recorded upon retrieving each trap including species type, sex, size (Orbital Carapace Length, OCL), abundance and GPS coordinates and water depth at trap, before the crayfish were returned to where they were caught. To be able to compare fish and crayfish capture methods uniformly, catch per unit effort (CPUE) was calculated as the number caught divided by the number of transects/traps.

2.4 Data analyses

2.4.1 Multivariate analyses

Transformations of data and multivariate analyses were performed using PRIMER 6 statistical software with default settings, unless otherwise noted (PRIMER-E Ltd 2006). To smooth the sometimes extreme variability found in this type of data, most data (except descriptive and morphological) was transformed by log(x+1). The logged data was then normalised (variable-mean/SD) to weight all variables evenly prior to statistical analyses (Clarke & Gorley 2006). Samples with missing data were removed from further multivariate analyses.

Principle Component Analysis (PCA) ordinations were produced to give an early indication of whether data separated into groups and if so, which variables were causing the most separation. To examine further which variables contributed to the most dissimilarity between populations or species, a one way Similarity of Percentages (SIMPER) routine was conducted. The Euclidean distance option was used on normalised data for resemblance matrices in SIMPER calculations, which is better

suited to environmental data than Bray-Curtis, which is more suited to biological data (Clarke & Warwick 2001). PRIMER uses standard deviation as part of its calculations and is shown in SIMPER results tables. However, in the main text any numbers following '±' represent standard error unless otherwise noted.

Analysis of Similarity test (ANOSIM) was used to determine if there was a statistical significant difference between variables and groups of data, such as catchment or species. ANOSIM also used a resemblance matrix of the relevant data with the Euclidean distance option, one way with the maximum 9,999 permutations.

2.4.2 Univariate analyses

SPSS 19 was used for all descriptive and univariate analyses (SPSS 2010). To conduct univariate analyses, all untransformed data was first checked for normality ($p > 0.05$) using the Shapiro-Wilk statistic. Normally distributed data was then tested for homogeneity of variances with Levene's test, which determined whether the variance at each site was based on random sampling (SPSS 2010). Data that was normally distributed and had homogenous variances ($p > 0.05$) was tested for significance using ANOVA with a Type I error of 0.05. All mean data is shown with standard error (±) unless otherwise noted.

Any data that was not normally distributed or did not have homogenous variances was tested with the non-parametric Kruskal-Wallis test. Additionally, if ANOSIM results were significant, non-parametric Spearman rank correlations were conducted to investigate if there was any relationship between the variables being analysed. For example, crayfish and fish abundance correlations were investigated for any potential commensal relationship (3.3.2 Crayfish trapping). Spearman rank correlations were chosen due to many variables not being normally distributed; otherwise Pearson correlation would have been used.

3 Habitat requirements of *Galaxiella nigrostriata* in the wet (inundated) phase of seasonal wetlands

3.1 Introduction

Freshwater fishes may be used as indicators of habitat quality, often in conjunction with macroinvertebrates, by comparing presence, diversity and abundance surveys with habitat assessments (Hued & de los Angeles Bistoni 2005; Kennard, Pusey *et al.* 2006; Seilheimer & Chow-Fraser 2006; Weijters, Janse *et al.* 2009). During development of these assessments, knowledge of the tolerance or sensitivity of species to particular conditions is required. For example, *Lepisosteus osseus* Longnose Gar are known to be intolerant of degraded habitats as they rely on aquatic vegetation (Hued & de los Angeles Bistoni 2005), while *Gambusia affinis* Western Mosquitofish are found in mostly degraded sites, having the ability to withstand high salinity, high water temperatures and toxic chemicals that are fatal to other fish (Pyke 2005; Seilheimer & Chow-Fraser 2006). The fish community or specific species population is then surveyed, usually in multiple locations and, if available, a less degraded or modified reference site for comparison. A variety of parameters are simultaneously measured in respect to water and habitat conditions, such as nutrient concentrations, concentrations of toxic metals and physical characteristics of the habitat (for example: size of the waterbody or depth).

However, there may be circumstances where fish presence may not be linked to the abovementioned parameters. Snodgrass *et al*. (1996) and Bouvier *et al.* (2009) found no correlation between species communities and habitat characteristics in temporary wetlands of North America, but connectivity between other wetlands or permanent waterbodies increased species abundance and diversity. Fishes in isolated and temporary wetlands may disperse into adjoining habitats during times of flooding or heavy rainfall causing sheetflow across the landscape. Snodgrass *et al.* (1996) related their findings to source-sink dynamics, as found in metapopulation theory, with the source being the population (habitat) that fish are migrating from. The long-term survival of fish populations in the sinks (wetlands) may then be determined by the conditions of that habitat. Consequently, ongoing management and conservation of fish populations not only requires an understanding of their current habitat features (Lamouroux, Capra *et al.* 1999; Bonnett & Sykes 2002; Hedger, Dodson *et al.* 2005), but also the examination of nearby habitats with which they may interact (Johst, Brandl *et al.* 2002; Rosenfeld & Hatfield 2006).

In a complex of 11 seasonal wetlands at Kemerton WA, *G. nigrostriata* populations were recorded over a 16 year period (1993-2005 – Bamford and Bamford (2006); 2008-

09 – this study). *Galaxiella nigrostriata* have been captured continuously in three of these wetlands and only intermittently in the remaining wetlands (Table 3). *Galaxiella nigrostriata* are known to aestivate in the sediments when the wetlands dry, possibly using crayfish burrows to access the groundwater under the soil surface, which is how they are thought to repopulate these wetlands when they are re-inundated each year. However, aestivation does not explain how *G. nigrostriata* repopulate all Kemerton wetlands, as their appearance in some wetlands has occurred after several years absence and they only have about a one year lifespan (Galeotti, McCullough *et al.* 2010). Aestivation is examined further in Chapter .

All Kemerton wetlands appear comparable by having tannin-stained water and similar aquatic macrophyte and riparian vegetation and do not have any obvious connectivity. Bamford *et al.* (2006) suggested *G. nigrostriata* disperse to other Kemerton wetlands in high rainfall years, but did not undertake habitat assessments or speculate why they did not persist in the other wetlands. A full habitat assessment is required to fully understand what may be the limiting factor for long-term survival of *G. nigrostriata* in these seasonal wetlands.

Table 3. Distribution of *G. nigrostriata* in the Kemerton wetlands. Black filled boxes indicate *G. nigrostriata* presence, shaded boxes are where *G. nigrostriata* were not captured. Wetlands listed in approximate order from north to south (see Figure 2).

Wetland	1993	1997	1998	1999	2000	2001	2002	2005	2008	2009
EPP9										
EPP8										
Paperbark Deep	Not previously sampled									
Paperbark Shallow	Not previously sampled									
Paperbark New										
EPP7										
EPP3										
EPP4										
EPP2		EPP2 mined from 1994								
EPP6										
EPP1										
EPP5										

Bond *et al.* (2003) emphasized that a species habitat preferences should be known prior to conducting habitat use assessment surveys, to ensure all areas are sampled. There is limited information available about the habitat preferences of *G. nigrostriata*, particularly at the within wetland scale (Galeotti, McCullough *et al.* 2008). *Galaxiella nigrostriata* larvae are thought to spend most of their time in shallow water and frequent open deeper water as they mature[2] (Morgan, Gill *et al.* 1998). Adult *G. nigrostriata* are thought to school and spend much of their time in the middle of the water column, which is typically where they feed (Thompson & Withers 1999). However, *G. nigrostriata's* preference for position in the wetland, vegetation structure or type, water depth, nutrient concentration tolerance or possible commensal relationship with burrowing crayfish is unknown.

One of the main theories of how *G. nigrostriata* aestivate is that they use crayfish burrows to access the substrate when wetlands dry in summer (Thompson & Withers 1999; Bamford & Bamford 2003; McDowall 2006). Various taxa are known to use crustacean burrows as a sanctuary when wetlands or riparian zones dry during summer, droughts or between tidal peaks. Even the non-burrowing crayfish *Gramastacus insolitus* Western Swamp Crayfish has a commensal relationship with burrowing crayfish species, by using crevices off the main shaft of occupied burrows (Johnston & Robson 2009). However, the evidence for *G. nigrostriata* using crayfish burrows is currently anecdotal.

[2] In Galeotti *et al.* (2010) it was incorrectly stated that *G. nigrostriata* larvae spend most of their time in open water and move to the vegetated edges as they mature. The opposite is actually true.

3.1.1 Objective and hypotheses

The objective of this chapter is to determine whether habitat characteristics explain the intermittent appearance of *G. nigrostriata* in Kemerton wetlands. Additional wetlands throughout *G. nigrostriata's* range were also sampled to improve sample size and statistical power. The specific hypotheses of this chapter were:

H_0 – Water quality or habitat features have no affect on *G. nigrostriata* abundance or distribution.

H_0 – *Galaxiella nigrostriata* do not have habitat preferences within a wetland.

H_0 –There is no relationship between *G. nigrostriata* and crayfish abundance and distribution.

3.2 Wetland analyses methods

3.2.1 Habitat physical features

Habitat features were recorded at each wetland and included wetland size and average sampling site depth, aquatic macrophyte and woody vegetation cover (after Hicks & Barrier 1996; McCullough 1998; Bonnett & Sykes 2002). Wetland size was also determined with aerial photos and Google Earth (Google Earth 2009). Water depth was estimated by taking depth measurements at the start, middle and end of each transect and calculating the average depth. Average wetland depth relates to the average of all transect depths within a wetland. Therefore wetland depths, where mentioned, pertain to where transects were conducted. The measurements are seen as representative for each whole wetland, as the substrate in most wetlands was quite flat.

3.2.2 Water analyses

Water quality criteria was derived from ANZECC & ARMCANZ (2000) guideline values for protection of 95% of species in slightly/moderately disturbed wetlands in Australia. Guideline values for south-west WA were used if available and any specific guideline conditions were noted. The guidelines indicate at what level physical or chemical stressor values may start to affect fish growth or survival, either directly such as water temperature or salinity, or indirectly by reducing macroinvertebrate prey or vegetation.

Water quality was examined within each wetland by taking measurements *in situ* and collecting samples for laboratory analyses. There was no significant difference in water quality between habitat types within wetlands at Kemerton (McCullough & Lund 2008). Therefore, for each sampling method three replicate sites were chosen to represent the three habitat types and later pooled to form one sample per wetland. Physico-chemical water data was collected *in situ* using a Hydrolab Quanta datasonde (Hydrolab, USA). The datasonde recorded temperature, specific conductivity (EC), dissolved oxygen as mg/L (DO_2) and saturation (DO%), pH and oxidation reduction potential (ORP) from approximately 0.3 m below the surface.

Water samples were pooled from three locations within each wetland (as above) in the one bottle. The collection bottles were detergent (phosphorus free) washed and acid rinsed 1 L polyethylene bottles and then rinsed once with wetland water at the first collection point. Following collection 500 mL of each sample was filtered through 0.5 µm glass fibre filter paper (Pall Metrigard). Samples were filtered on site to

eliminate changes to the chemical structure (ANZECC & ARMCANZ 2000). Unfiltered and filtered sample bottles were then placed on ice in cool-boxes (eskies) for transport to -20°C storage at ECU Joondalup.

All samples were analysed at ECU's School of Natural Sciences Analytical Services. Unfiltered samples were persulfate digested then analysed for total nitrogen (TN-N) and total phosphorous (TP-P). The filtered samples were halved, with one half used for nutrient analysis for ammonia/ammonium (NH_4-N), nitrates/nitrites (NOx-N), filterable reactive phosphorous (FRP-P) and dissolved organic carbon (DOC - measured as non-purgible organic carbon (NPOC)). The other filtrate half was acidified with 1% analytical grade HCl and used for metal analysis. Water samples were analysed for the macronutrient sulfur (measured as total sulfur (TS)) and 18 metals and metalloids: Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, Pb, Se, Zn; using Inductively Coupled Plasma Atomic Emission Spectrophotometry (ICP-AES) (Varian, USA)

3.2.3 Data analyses

Statistics used for wetland analyses are described in General Methods (2.4 Data analyses).

3.3 Fish and crayfish sampling results

3.3.1 Galaxiella nigrostriata transects

Galaxiella. nigrostriata was captured in 16 (52%) of the 31 wetlands sampled (Table 6). The Scott and Kemerton wetlands had the highest CPUE and the lowest was Melaleuca Park with only one *G. nigrostriata* caught in the sweep net transects (Table 4). The mean transect water depth in the wetland habitats sampled - open (n = 89), vegetated (n = 76) or covered (n = 46) - varied between 0.43 ±0.04 m, 0.35 ±0.04 m and 0.29 ±0.4 m, respectively. There was no significant difference (p >0.05) of *G. nigrostriata* CPUE or transect water depth in the different habitat types sampled. However, a negative weak correlation (ρ = -0.32, p <0.05) was found between *G. nigrostriata* CPUE and transect water depth.

Only one wetland, CW2 in the Gardner catchment, contained *G. nigrostriata* that were not caught in transects but in the additional ad hoc sweeps following the prescribed transects. The fish in CW2 were caught by sweep net in vegetated edges surrounding the deep wetland (mean transect depth 0.8 m). In the Deep River catchment 10 fish were caught and initially identified as *G nigrostriata*. However, later genetic analyses

determined them to be *G. munda* (see Chapter 5.3.1 Genetic sequence data). Therefore, no *G. nigrostriata* were caught in wetlands sampled in the Deep River catchment. The presence of *G. munda* in the Deep River catchment is discussed further in Chapter 5.4.3.

Besides *G. munda*, four other endemic fish species were captured while sampling for *G. nigrostriata* (Table 5). Those species were: *Bostockia porosa* Nightfish, *Nannoperca vittata* Western Pygmy Perch, *Nannatherina Balstoni* Balston's Pygmy Perch and *Lepidogalaxias salamandroides* Salamanderfish. The four other species were only caught in the Gardner, Deep and Shannon catchments, with the majority of those caught in Deep river catchment wetlands. *Galaxiella nigrostriata* was only caught with other species in three wetlands, all within Gardner and Shannon catchments.

In addition to fish, 114 small freshwater crayfish (unidentified, 5-25 mm OCL) were also captured during sweep net transects, with an overall mean CPUE of 0.5. The number of crayfish caught in transects varied in the open, vegetated and covered habitats, with a mean CPUE of 0.7 ±0.4, 1.1 ±0.5 and 0.2 ±0.7 respectively. No significant difference (p >0.05) was found between crayfish caught in transects and habitat type. However, similar to *G. nigrostriata*, a negative weak correlation (ρ = -0.34, p <0.01) was found between crayfish CPUE in transects and transect water depth.

Table 4. Catch results from fish sampling in each catchment.

Sampling sites	Number of wetlands sampled (number where *G. nigrostriata* were caught)	Number of transects conducted	Total *G. nigrostriata* caught	CPUE
Melaleuca Park	2 (1)	13	1	0.1
Kemerton	11 (6)	88	110	1.2
Scott	4 (4)	20	25	1.2
Gardner	4 (4)	26	20	0.8
Shannon	4 (1)	23	5	0.2
Deep	6 (0)	43	0	0
Total	31 (16)	211	162	

Table 5. Additional species captured while sampling for *G. nigrostriata.* (-) indicates no fish captured. **G. nigrostriata* captured in prescribed transects.

	Galaxiella nigrostriata	*Galaxiella munda*	*Bostockia porosa*	*Nannoperca vittata*	*Nannatherina balstoni*	*Lepidogalaxias salamandroides*
CW2	6*	-	-	-	2	-
CW3	1	-	1	-	1	4
CP2	7	-	-	-	-	3
BR1	-	3	3	-	-	-
TR1	-	1	-	1	-	4
TR2	-	-	-	-	-	3
TR3	-	6	-	-	-	2
MR1	-	-	3	-	5	-

3.3.2 Crayfish trapping

Burrowing freshwater crayfish *C. quinquecarinatus* and *C. preissii* were found in virtually all wetlands sampled, with either species captured in all but one wetland (MR1, two traps), and only seven out of 163 set traps did not contain crayfish (Table 6). Wetland MR1 had crayfish burrows within or just above the littoral margins and crayfish were caught in nearby Poorginup Swamp. The mean water depth for trap placement in the wetland habitats: open 0.46 ±0.05 m (n = 75), vegetated 0.36 ±0.03 m (n = 51) and covered 0.32 ±0.04 m (n = 37). No statistically significant difference was found between the CPUE of crayfish caught in traps, habitat type or *G. nigrostriata* caught in the same wetland (p >0.05). However, there was a negative weak correlation (ρ = -0.26, p <0.05) between trapped crayfish CPUE and trap water depth.

Table 6. Crayfish trapping results for each catchment.

	Number of wetlands	Number of Traps	Number of crayfish caught	CPUE
Melaleuca Park	2	14	23	1.6
Kemerton	10	73	166	2.3
Scott	4	20	53	2.6
Gardner	4	13	56	4.3
Shannon	4	24	106	4.4
Deep	6	19	78	4.1
Total	30	163	482	

3.4 Wetland analyses results

3.4.1 Wetland bio-physical features

Wetlands sampled ranged in size from 0.03 ha on Chesapeake Road (CP1 - Shannon R. catchment) to approximately 140 ha at Poorginup Swamp (MR2 – Deep R. catchment) (Table 7). All other wetlands were <3 ha and had a mean size of 0.6 ±0.1 ha excluding Poorginup Swamp. Three of the biggest wetlands, EPP173 (1.5 ha), EPP3 (1.9 ha) and Poorginup Swamp contained dense stands of rushes, covering 90-99% of the wetland, which left small areas accessible for sampling. The remaining wetlands were covered by 2-80% of aquatic macrophyte vegetation, and all wetlands contained a mean of 36 ±5.6% coverage.

Woody vegetation provided 5-99% of cover in all wetlands, with a mean of 41 ±5%. The wetlands were typically shallow (range 0.1-1.0 m deep) with a mean depth of 0.4 ±0.04 m. *Galaxiella nigrostriata* were present in one of the two shallowest wetlands (PS - 0.1 m). Additionally, *G. nigrostriata* was observed darting between cover in slowly moving water less than 20 mm deep near CW2 (Gardner catchment). *Galaxiella nigrostriata* was not caught in the sampling transects in the deepest wetland (CW2 - 1.0 m), but were caught in the shallower vegetated edges with additional random sweeps. CW2 was the only wetland where *G. nigrostriata* was not caught in prescribed transects. Water depth, then wetland size, contributed most to habitat differences and were statistically significant different (p <0.005) between sampled regions (Table 7).

Wetland bio-physical features accounted for >70% of total variance between wetlands (Figure 6A, B). *Galaxiella nigrostriata* was found in wetlands with a wide range of bio-physical conditions, particularly aquatic macrophyte and woody cover. There was some separation between wetlands where *G. nigrostriata* was or was not captured, with shallow water and woody cover appearing to increase capture rates (Figure 6A). However, the bio-physical features were not significant factors for explaining *G. nigrostriata* abundance differences between wetlands (ANOSIM Global R = 0.016; p = 0.27).

South-west wetlands had statistically significant different bio-physical features (ANOSIM Global R = 0.139; p = 0.02) than Swan Coastal Plain wetlands (Figure 6B). Swan Coastal Plain wetlands were statistically significantly (p <0.001) shallower and had a smaller surface area than south-west wetlands (Table 7). Wetlands on the Swan Coastal Plain and south-west regions had similar aquatic macrophyte cover (36%), although Swan Coastal Plain wetlands had more woody cover (Figure 6B, Table 7).

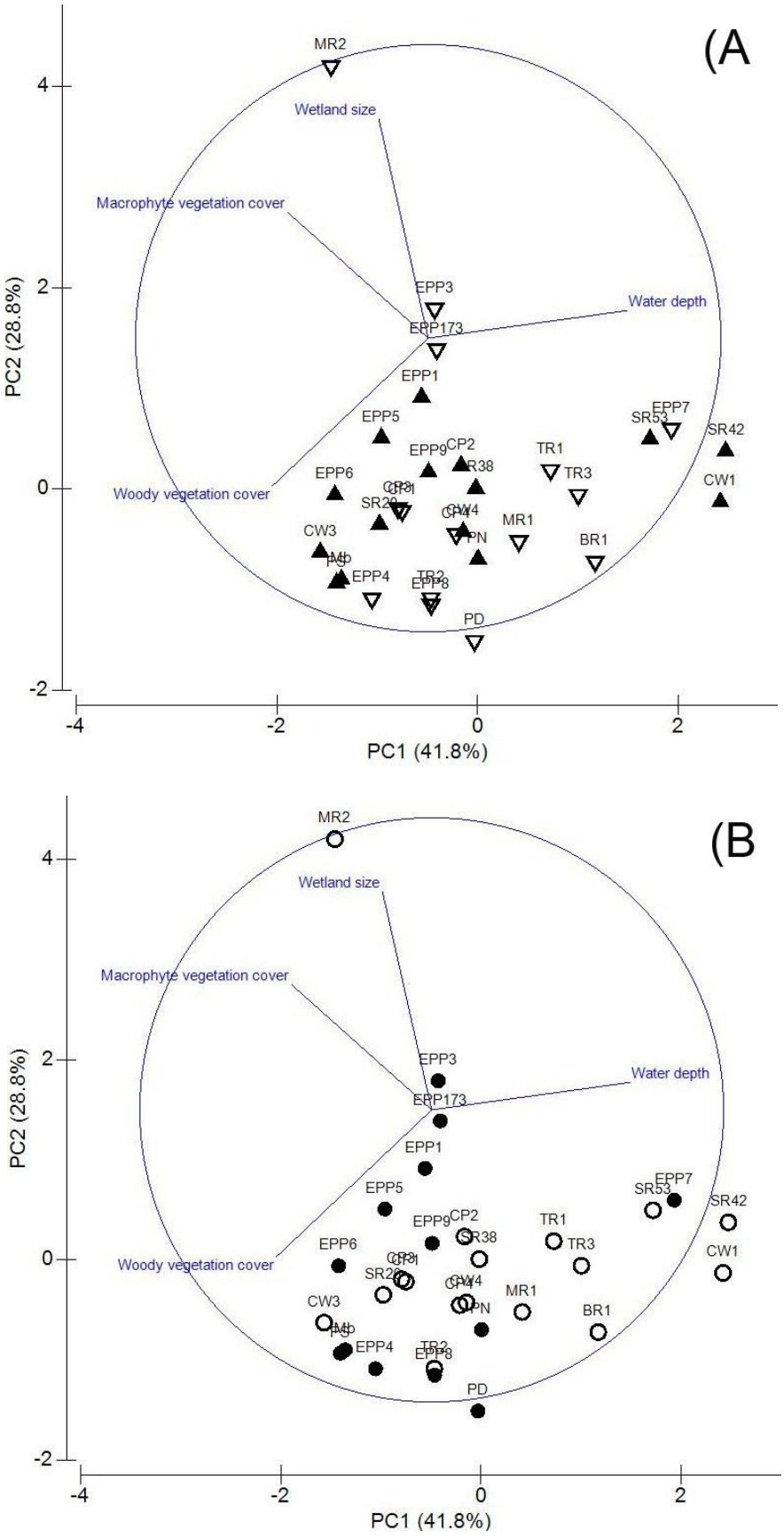

Figure 6. PCA analysis of bio-physical properties of all wetlands. A) Filled triangles show wetlands where *G. nigrostriata* was caught, empty triangles where they were not caught. B) Regions sampled: filled circles show Swan Coastal Plain wetlands, empty circles show south-west region wetlands.

Table 7. Results of SIMPER analyses and statistical testing of bio-physical features in wetlands on the Swan Coastal Plain and the south-west region. Variables are arranged from highest to lowest by mean dissimilarity between regions. Contribution percentage shows how much each variable contributed to overall dissimilarity between regions. Statistically significant differences for bio-physical features between the two regions are shown in bold. Test statistics used were ANOVA (* $F_{1,29}$) and Kruskal-Wallis ($^{\#}\chi^{2}_{1,31}$).

Variable	Mean value Swan Coastal Plain (±SE)	Mean value South-west (±SE)	Mean dissimilarity % (±SD)	Contribution %	Cumulative %	Test statistic	*p* value
Water depth (m)	0.3 ±0.04	0.5 ±0.05	2.45 ±0.78	29.65	29.65	**9.23***	**<0.01**
Woody cover (%)	51.1 ±9.4	34.4 ±5.4	2.04 ±0.83	24.74	54.39	2.67*	0.11
Aquatic macrophyte cover (%)	35.4 ±9.5	36.4 ±7.1	1.99 ±0.87	24.07	78.46	$0.12^{\#}$	0.73
Wetland size (ha)	1.2 ±0.2	7.9 ±7.8	1.78 0±.35	21.54	100.00	**$10.95^{\#}$**	**<0.01**

3.4.2 Nutrients

A wide range of nutrient concentrations was found between all wetlands, with TN and NO_x concentrations the most variable at 481-4,023 µg/L and 29-1,870 µg/L, respectively (mean of 1,587 ±153 µg/L and 297 ±79 µg/L). Concentrations of TN and NOx exceeded ANZECC & ARMCANZ (2000) guideline values in 39 and 65% of wetlands sampled. TP and FRP had similar concentrations ranges (10-679 µg/L and 1-541 µg/L, means 63 ±24 µg/L and 44 ±20 µg/L). TP concentrations had a strong positive correlation (ρ >0.7, p <0.01) with FRP, S, K, Mg, Na and EC. Concentrations of TP and FRP exceeded ANZECC & ARMCANZ (2000) guideline values in Kemerton wetlands only; TP exceeded the guideline values in 73% of Kemerton wetlands and FRP in 55%. TP concentrations also had moderate positive correlations (ρ 0.6-0.69, p >0.01) with Ca, Mn and water temperature. Concentrations of FRP had a strong positive correlation with temperature (ρ = 0.707) and moderate positive correlations with K, Na and Mg (ρ = 0.607-0.674) (all p <0.01).

Concentrations of DOC had a mean of 71 ±9 mg/L (range 10-209 mg/L), a strong positive correlation with TN (ρ = 0.74, p <0.01) and a moderate correlation with FRP (ρ = 0.678, p <0.01). Of all the variables measured in the wetlands, DOC was the only variable that had a statistically significant, although weak positive correlation with *G. nigrostriata* abundance (CPUE) (ρ = 0.395, p = 0.028). Concentrations of the macro-solute S had a mean of 5.7 ±2.4 µg/L (range 0.5-60 µg/L). Chl *a* concentrations were generally low (mean of 1.8 ±0.6 µg/L, range 0.1-13.2 µg/L) compared to the ANZECC & ARMCANZ (2000) guideline value for south-west WA wetlands (>30 µg/L). Only two wetlands contained Chl *a* over 5 µg/L, Mb and PS, which were also two of the most shallow wetlands (0.2 m and 0.1 m). Concentrations of NH_4 ranged between 4-336 µg/L and had a mean of 95 ±14 µg/L. Concentrations of NH_4 were the least variable between wetlands in the two regions sampled and was the only nutrient not significantly different (p = 0.164) (Table 8). Additionally, NH_4 concentrations exceeded ANZECC & ARMCANZ (2000) guideline limits (>40 µg/L) in 77% of all wetlands (Table 8).

Nutrients accounted for >68% of the variability between wetlands (Figure 7A, Figure 7B). *Galaxiella nigrostriata* were found in wetlands with a wide variety of nutrient concentrations. However, wetlands did not differentiate by nutrient content when analysed using a PCA (Figure 7A). Additionally, no significant difference was found between wetlands where *G. nigrostriata* was captured or was not captured (ANOSIM Global R = 0.011; p = 0.28) (Figure 7A).

All Swan Coastal Plain wetlands were surrounded by agricultural land. Nutrient concentrations in these wetlands on average exceeded ANZECC & ARMCANZ (2000) guideline values for all nutrients tested, except chlorophyll *a* (Table 8). Furthermore, concentrations of TN and TP exceeded the guideline values in 77% and 62%, respectively, of Swan Coastal Plain wetlands. There was also great variability in nutrient concentrations between Swan Coastal Plain wetlands compared to south-west region wetlands; particularly TP and DOC, with the largest difference in FRP (Swan Coastal Plain range 4-541, mean 101 ±44 µg/L; south-west region range 1-7 µg/L, mean 3 ±1 µg/L) (Figure 7B). By comparison, nutrient concentrations in the south-west wetlands were at or below guideline values except NH_4, which was still nearly half of Swan Coastal Plain concentrations (Table 8, Figure 7B). The differences between wetlands in the Swan Coastal Plain and south-west regions are clearly shown by two defined groups (Figure 7B, Table 8). The differences are also confirmed by a strong significant difference between all nutrients and Swan Coastal Plain and south-west region wetlands (ANOSIM Global R = 0.697; p <0.001).

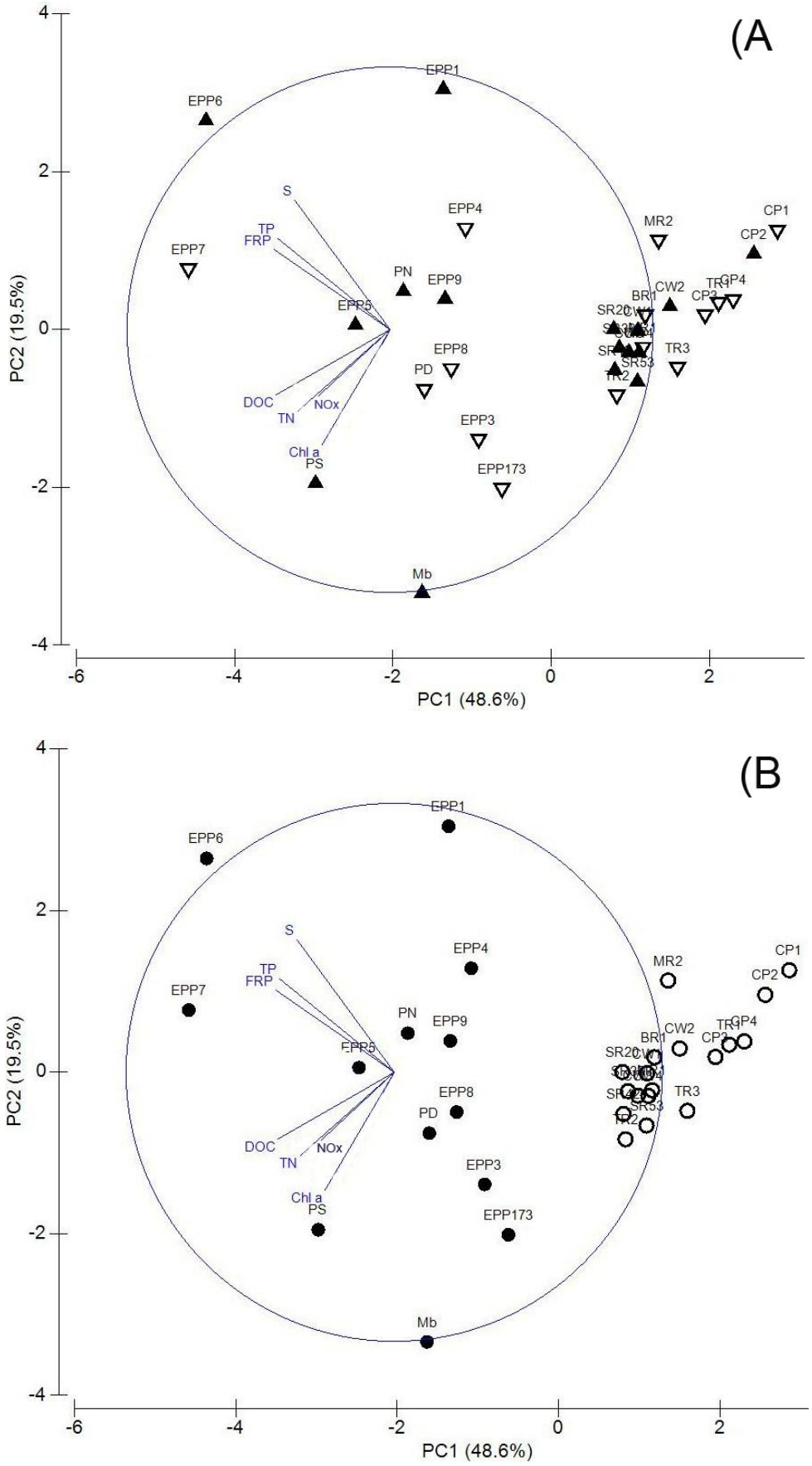

Figure 7. PCA analysis of all nutrients measured in water of sampled wetlands. Only statistically significant variables are shown. A) Filled triangles show wetlands where *G. nigrostriata* was caught, empty triangles where they were not caught. B) Regions sampled: filled circles show Swan Coastal Plain wetlands, empty circles show south-west region wetlands.

Table 8. Results of SIMPER analyses and statistical testing of nutrients in the water of wetlands on the Swan Coastal Plain and the south-west region. Variables are arranged from highest to lowest by mean dissimilarity between regions. Contribution percentage shows how much each variable contributed to overall dissimilarity between regions. Statistically significant differences for nutrients between Swan Coastal Plain and south-west region wetlands are shown in bold. Test statistics used were ANOVA (* $F_{1,29}$) and Kruskal-Wallis ($^{\#}\chi^2_{1,31}$). All variable units are µg/L except DOC (mg/L). Mean values in italics exceed ANZECC & ARMCANZ (2000) guideline values for protection of 95% of species in slightly to moderately disturbed ecosystems.

Variable (µg/L)	Mean value Swan Coastal Plain (±SE)	Mean value South-west (±SE)	ANZECC & ARMCANZ guideline value	Mean dissimilarity % (±SD)	Contribution %	Cumulative %	Test statistic	*p* value
FRP	*101 ±44*	3 ±1	>30	3.14 ±0.78	13.83	13.83	**18.37**$^{\#}$	**<0.01**
DOC (mg/L)	118 ±13	37 ±4	-	3.06 ±1.07	13.49	27.32	**14.78**$^{\#}$	**<0.01**
TP	*133 ±52*	11 ±1	>60	3.01 ±0.70	13.27	40.58	**15.75**$^{\#}$	**<0.01**
S	13 ±5.3	0.5 ±0.3	-	2.97 ±0.79	13.07	53.65	**14.38**$^{\#}$	**<0.01**
NO_x	*571 ±161*	*100 ±14*	>100	2.91 ±0.89	12.80	66.45	**22.39***	**<0.01**
TN	*2245 ±258*	1112 ±77	>1500	2.85 ±0.93	12.53	78.99	**20.70***	**<0.01**
Chl *a*	3.2 ±1.2	0.8 ±0.2	>30	2.54 ±0.64	11.19	90.18	**7.58**$^{\#}$	**<0.01**
NH_4	131 ±27	69 ±12	>40	2.23 ±0.841	9.82	100.00	2.03*	0.16

3.4.3 Metals

Half of the metal concentrations analysed for in wetland waters were below detection: Co, Cr, Hg and Pb (<0.01 mg/L), Ni and Se (<0.02 mg/L) and As, Be and Cu (<0.05 mg/L). Alkali metals Na and K and alkali earth metals Ca and Mg had the highest concentrations and accounted for >58% of variability between all wetlands (Table 9). Concentrations of Na and Ca were most variable, Na had a range of 21-376 mg/L (mean 112 ±16 mg/L) and the range of Ca was 1-117 mg/L (mean 13 ±4 mg/L). These were followed by K and Mg concentrations with ranges of 1-41 and 2.7-69 mg/L and means of 5 ±1 and 14 ±2 mg/L.

All alkali metal/earth metal concentrations had moderate to very strong correlations to each other, from Ca-Na ($\rho = 0.674$) to Mg-Na ($\rho = 0.955$; both $p < 0.01$) and strong to very strong correlations with EC (Ca $\rho = 0.806$, K $\rho = 0.857$, Na $\rho = 0.928$, and Mg $\rho = 0.968$; all $p < 0.01$). Concentrations of Ca and K had strong negative correlations to ORP ($\rho = -0.772$ and -0.722, respectively) and Mg and Na concentrations had moderate negative correlations with ORP ($\rho = -0.678$ and -0.606, respectively; all $p < 0.01$). Concentrations of K, Na and Mg, which are the three most common cations that make up total salinity in Australian standing freshwaters (Boulton & Brock 1999; Wetzel 2001), contributed the most to dissimilarity between metals in the water of Swan Coastal Plain and south-west region wetlands (Figure 8B, Table 9). The contribution of the three cations was also reflected in EC measurements which contributed the most to dissimilarity between regions by physico-chemical properties (Table 10).

Of the transition metals, Fe had the highest concentrations with a range of 0.1-8.4 mg/L mean 1.7 ±0.3 mg/L followed by Al with a range of 0.1-2 mg/L and a mean of 0.8 ±0.1 mg/L. Mn concentrations ranged between 0.1-0.8 mg/L, had a mean of 0.6 ±0.1 mg/L and a moderate negative correlation to ORP ($\rho = -0.606$, $p < 0.01$). Concentrations of Zn ranged between 0.1-0.6 mg/L, had a mean of 0.2 ±0.1 mg/L and had a moderately negative correlation with pH ($\rho = -0.670$, $p < 0.01$). The transition metals were the only metals detected that have ANZECC & ARMCANZ (2000) guideline values. Of the transition metals only Fe and Zn concentrations exceeded the ANZECC & ARMCANZ (2000) guideline values and in every wetland. However, Fe and Zn were also the only metal concentrations not statistically significantly different ($p > 0.01$) between wetlands on the Swan Coastal Plain and the south-west region (Table 9). Additionally, *G. nigrostriata* were captured in wetlands throughout the range of Fe

and Zn concentrations, including EPP6 which had the highest Zn concentration (0.63 mg/L).

Galaxiella nigrostriata abundance was not influenced by wetland metal concentrations. Metal concentrations varied greatly between wetlands sampled and there was no distinction between wetlands where *G. nigrostriata* was captured or was not captured (Figure 8A). There were also no statistically significant differences between metal concentrations and wetlands where *G. nigrostriata* was captured or was not captured (ANOSIM Global R = 0.009; $p = 0.31$). Conversely, when metal concentrations were examined there was separation with little overlap between Swan Coastal Plain and south-west region wetlands (Figure 8B). Furthermore, a significant difference was found for metal concentrations between Swan Coastal Plain and south-west region wetlands (Figure 8B, ANOSIM Global R = 0.438; $p < 0.001$). Both regions contained high metal concentration variability, with some variability attributed to a weak negative correlation between Fe and Ca ($\rho = -0.354$, $p = 0.25$) (Figure 8B).

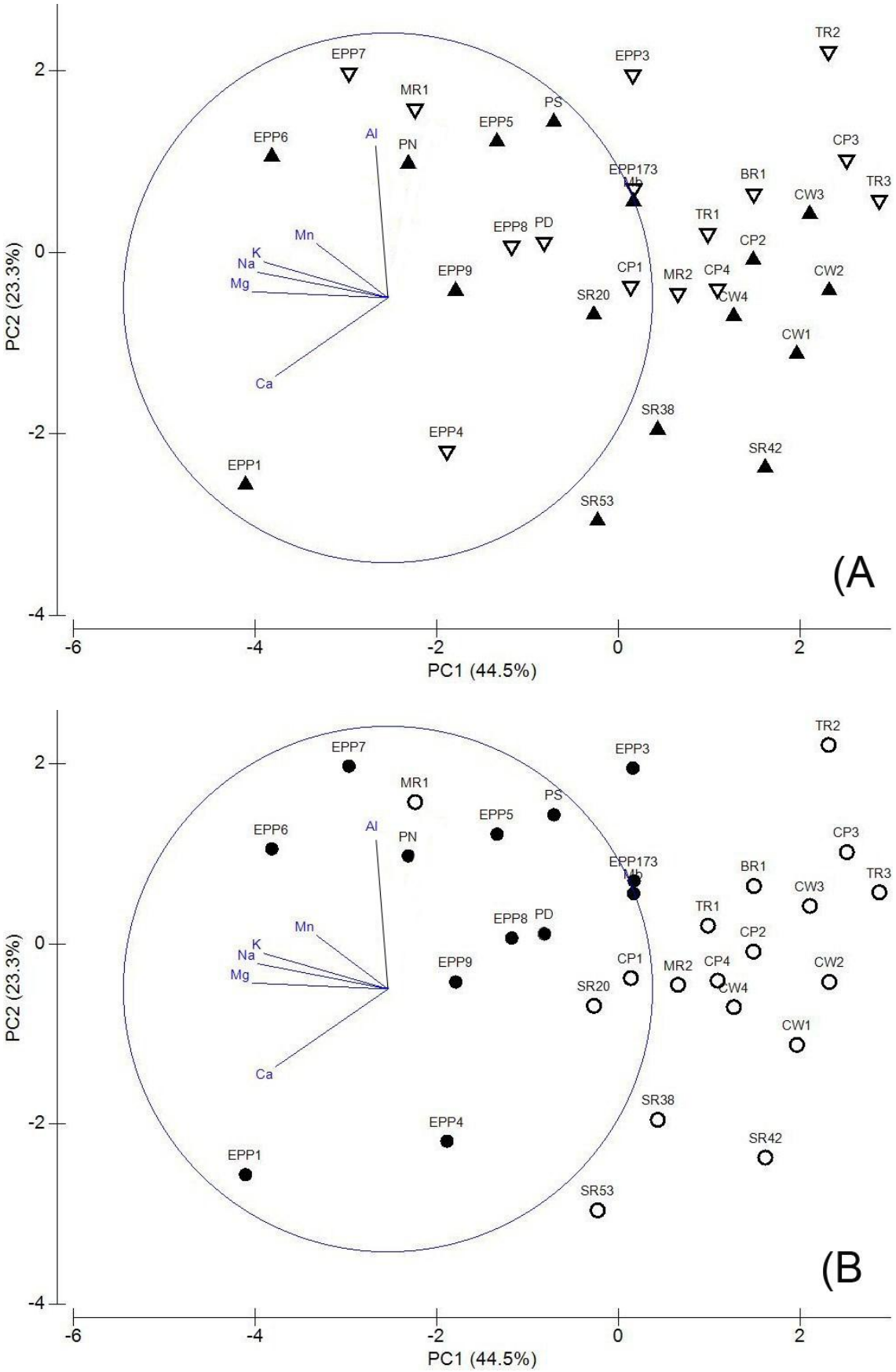

Figure 8. PCA analysis of metal concentrations detected in all wetlands. Only statistically significant variables shown. A) Filled triangles show wetlands where *G. nigrostriata* was caught, empty triangles where there were not caught. B) Regions sampled: filled circles show Swan Coastal Plain and empty circles are the south-west region.

Table 9. Results of SIMPER analyses and statistical testing of metals in the water of wetlands on the Swan Coastal Plain and the south-west region. Variables are arranged from highest to lowest by mean dissimilarity between regions. Contribution percentage shows how much each variable contributed to overall dissimilarity between regions. Statistically significant differences of metal concentrations in water of Swan Coastal Plain and south-west region wetlands are shown in bold. Test statistic used was Kruskal-Wallis ($\chi^2_{1,31}$). All variable and guideline value units are mg/L. Mean values in italics exceed ANZECC & ARMCANZ (2000) guideline values for protection of 95% of species in slightly to moderately disturbed ecosystems.

Variable (mg/L)	Mean value Swan Coastal Plain (±SE)	Mean value South-west (±SE)	ANZECC & ARMCANZ guideline value	Mean dissimilarity % (±SD)	Contribution %	Cumulative %	Statistic	*p* - value
K	9.3 ±2.8	1.8 ±0.2	-	3.22 ±0.82	15.99	15.99	**18.90**	**<0.01**
Na	184 ±26	59 ±7	-	3.14 ±1.07	15.60	31.58	**18.70**	**<0.01**
Mg	23 ±4	7 ±1	-	3.10 ±0.99	15.39	46.98	**18.02**	**<0.01**
Al	1.1 ±0.2	0.5 ±0.1	>4.2	2.46 ±1.00	12.21	59.19	**4.94**	**<0.05**
Zn	*0.2 ±0.05*	*0.1 ±0.01*	>0.008	2.26 ±0.50	11.20	70.39	0.07	0.79
Ca	19 ±8	8.6 ±3	-	2.25 ±0.70	11.16	81.55	**5.77**	**<0.05**
Fe	*1.4 ±0.3*	*2.0 ±0.5*	>0.04	1.92 ±0.67	9.55	91.10	1.26	0.26
Mn	0.07 ±0.03	0.05 ±0.05	>1.7	1.79 ±0.34	8.90	100.00	**10.48**	**<0.01**

3.4.4 Physico-chemical properties

Wetland water was generally acidic with a pH range of 3.5-7.8 (mean 5.3 ±0.2) and only six wetlands recorded pH >6.5. *Galaxiella nigrostriata* were captured throughout the range of measured EC, which was from 140 to 2,290 µS/cm (mean 608 ±86 µS/cm), including the wetland with the highest reading (EPP1). The ORP range was 32-392 mV (mean 221 ±18 mV). pH and EC had a strong negative correlation to ORP (ρ = -0.728, ρ = -0.700, both p <0.01). Wetland water temperature was generally cooler in the south-west region than the Swan Coastal Plain and had an overall temperature range of 12.9-22.7°C (mean 17.6 ±0.5°C) (Table 10).

DO_2 concentrations ranged between 5.1-9.3 mg/L (mean 6.5 ±0.2 mg/L) and the range of DO% saturation was 48-105% (mean 65 ±2%). DO_2 concentrations were on average higher than the ANZECC & ARMCANZ (2000) guideline level (6 mg/L), but were lower than the guideline level in 29% of wetlands sampled. DO saturation was below the ANZECC & ARMCANZ (2000) guideline levels in 94% of wetlands sampled, and half of those contained *G. nigrostriata*.

The physico-chemical properties accounted for 79% of the variability between wetlands (Figure 9A, Figure 9B) and *G. nigrostriata* was found randomly throughout all wetlands (Figure 9A). There was no difference between physico-chemical properties of wetlands for *G. nigrostriata* abundance. Additionally, no statistically significant difference was found between wetlands where *G. nigrostriata* was captured or was not captured (ANOSIM Global R= -0.02; p = 0.65) (Figure 9A). Physico-chemical properties of Swan Coastal Plain wetlands were more variable than those in the south-west (Figure 9B). There was a significant difference in Swan Coastal Plain and south-west region wetlands for physico-chemical properties (Figure 9B, ANOSIM Global R = 0.354; p <0.001). The difference was due to EC and temperature both being higher on the Swan Coastal Plain (Figure 9B, Table 10)

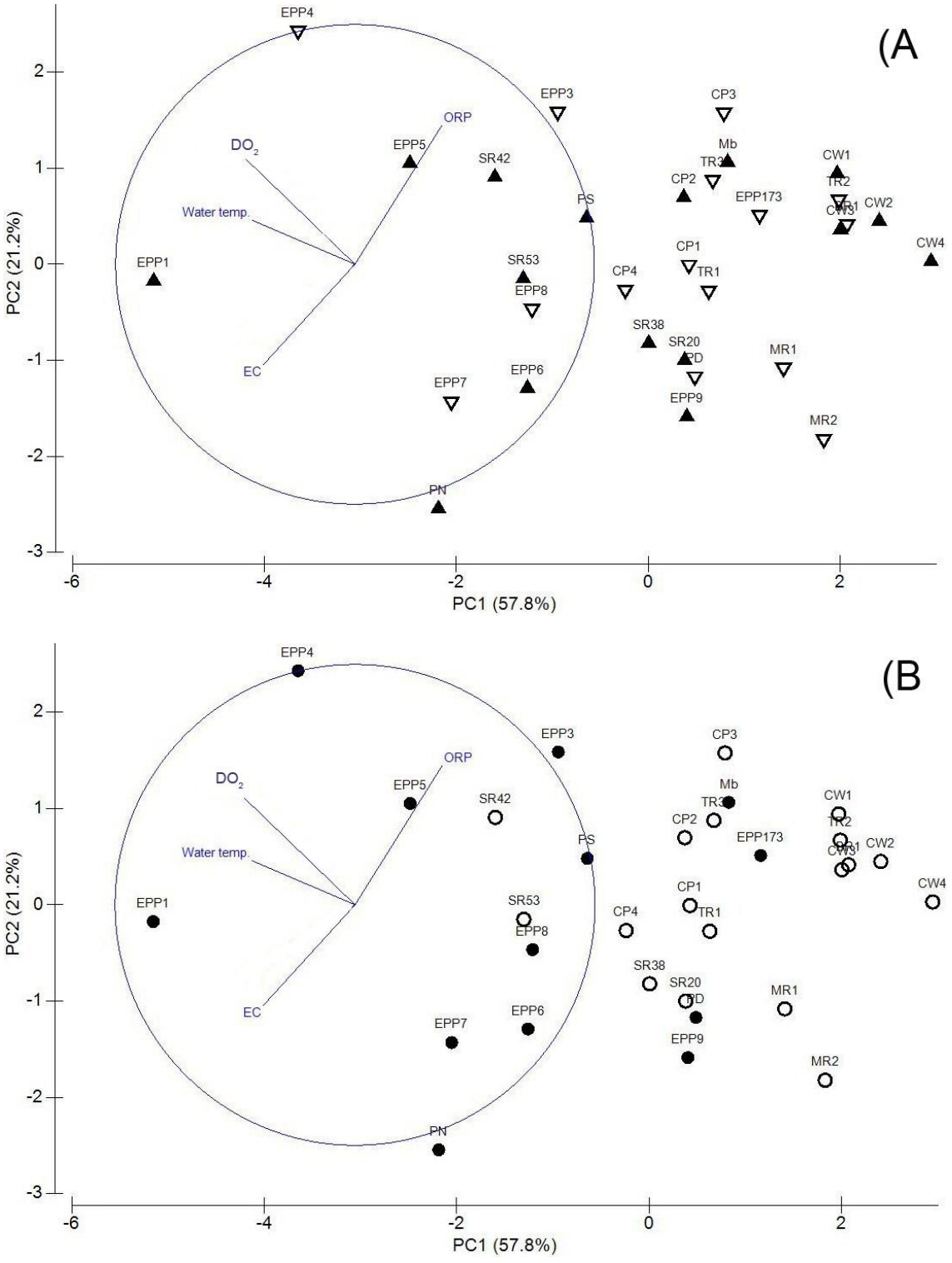

Figure 9. PCA analysis of all wetland water physico-chemical properties. Only statistically significant variables shown. A) Filled triangles show wetlands where fish were caught, empty triangles where they were not. B) Regions sampled: filled circles show Swan Coastal Plain, empty circle show south-west region wetlands.

Table 10. Results of SIMPER analyses and statistical testing of physico-chemical properties of wetlands on the Swan Coastal Plain and the south-west region. Variables are arranged from highest to lowest by mean dissimilarity between regions. Contribution percentage shows how much each variable contributed to overall dissimilarity between regions. Statistically significant differences for physico-chemical properties between Swan Coastal Plain and south-west region wetlands are shown in bold. Test statistics used were ANOVA (* $F_{1,29}$) and Kruskal-Wallis (# $\chi^2_{1,31}$). Mean values in italics exceed ANZECC & ARMCANZ (2000) guideline values. ANZECC & ARMCANZ (2000) guideline values are for protection of 95% of species in slightly to moderately disturbed ecosystems. The guideline value for pH was specifically derived for south-west WA wetlands with highly tannin stained waters.

Variable (unit)	Mean value Swan Coastal Plain (±SE)	Mean value South-west (±SE)	ANZECC & ARMCANZ guideline values	Mean dissimilarity % (±SD)	Contribution %	Cumulative %	Statistic	*p* value
EC (µS/cm)	960 ±150	350 ±0.40	>1500	3.03 ±0.80	20.03	20.03	**16.69#**	**<0.01**
Temperature (°C)	19.6 ±0.6	16.2 ±0.5	-	2.73 ±0.94	18.09	38.12	**18.88***	**<0.01**
ORP (mV)	167 ±27	260 ±21	-	2.57 ±0.68	17.01	55.12	**7.57***	**<0.05**
DO (%)	*71 ±4.2*	*61 ±1.7*	90-120	2.47 ±0.66	16.33	71.46	3.69#	0.05
DO_2 (mg/L)	6.9 ±0.3	6.1 ±0.1	>6	2.38 ±0.64	15.78	87.24	**5.26***	**<0.05**
pH	5.4 ±0.3	5.2 ±0.3	4.5 - 6.5	1.93 ±0.82	12.76	100.00	0.12*	0.73

3.5 Discussion

Wetlands sampled were generally small, shallow, acidic and had a moderate amount of aquatic macrophyte and woody vegetation cover. Physical and chemical features of wetlands and water sampled were typical of south-west WA seasonal wetlands (Gordon, Finlayson *et al.* 1981; Edward & Pusey 1990; McComb & Davis 1993; Balla 1994). The wetlands sampled were also typical of habitat where *G. nigrostriata* are usually found (Pusey & Edward 1990; Morgan & Gill 2000; Knott, Jasinska *et al.* 2002; Galeotti, McCullough *et al.* 2010).

Galaxiella nigrostriata distribution or abundance did not correlate with wetland characteristics, as they were captured throughout the ranges of all variables measured. Concentrations of Fe and Zn exceeded guideline values in every wetland (ANZECC & ARMCANZ 2000). Nutrient concentrations were the most variable of all water chemistry characteristics and all (except NH_4) were statistically significantly different between the two regions (p = <0.006). Additionally, nutrient concentrations in most wetlands sampled on the Swan Coastal Plain exceeded guideline values (for example TP <60 and TN <1500 mg/L) for protection of 95% of species in slightly to moderately disturbed ecosystems (ANZECC & ARMCANZ 2000).

The eutrophic conditions found in the wetlands sampled were characteristic of Swan Coastal Plain wetlands (Wrigley, Chambers *et al.* 1988; Davis & Froend 1999; Sommer & Horwitz 2001). Wetlands in south-west WA, particularly groundwater dependant wetlands on the coastal plains, are susceptible to anthropogenic eutrophication (McComb & Davis 1993). Fertilizers from domestic and agricultural applications can move overland with surface flows or through the sandy coastal plain soils via groundwater, and accumulate in wetlands. Wetlands sampled on the Swan Coastal Plain were close to agricultural lands and are likely to be receiving nutrients from run-off and groundwater (McComb & Davis 1993).

One of the main outcomes of eutrophication is excessive algal productivity, caused by elevated phosphorous and nitrogen concentrations (Wetzel 2001; Smith, Joye *et al.* 2006). However, algae were generally absent from the wetlands, shown by low Chl *a* concentrations and low to moderate dissolved oxygen concentrations (DO_2 >5 mg/L), which was surprising given the high TP and TN concentrations. The low phytoplankton biomass was probably caused by shading from vegetation and dark coloured waters of the wetlands sampled, as light intensity required for algal growth is limited by dark

water. Under these low light circumstances high nutrient concentrations are not necessarily conducive to algal growth (Wrigley, Chambers *et al.* 1988; Davis, Rosich *et al.* 1993).

As algal production was low, dissolved oxygen saturation was lower than the ANZECC & ARMCANZ (2000) guideline levels (>6 mg/L) in nearly all wetlands sampled. The slightly hypoxic conditions recorded in some of the wetlands sampled were probably influenced by respiration and decomposition of plant matter, limited light penetration and high nutrient levels. Fishes are generally adversely affected in slightly hypoxic conditions by a loss of habitat (avoidance), lower growth rates and reproductive rates and exposure to increased availability and toxicity of toxic compounds (ANZECC & ARMCANZ 2000; Diaz & Breitburg 2009). However, behavioural, morphological and physiological adaptations can allow some species to tolerate hypoxic conditions more than others (Farrell & Richards 2009). *Neochanna diversus* Black Mudfish (endemic to New Zealand) survive hypoxic conditions by surface breathing or gulping air when their habitat has almost dried and, as with *L. salamandroides* and possibly *G. nigrostriata*, cutaneous respiration (breathing through their skin) to supplement their oxygen intake (Martin, Berra *et al.* 1993; McPhail 1999; Thompson & Withers 1999).

The physico-chemical water measurements were taken once only at the peak of wetland inundation. Dissolved oxygen concentrations are generally at their peak at the same time, typically falling dramatically as wetlands dry and staying low during initial inundation (Boulton & Brock 1999; Boekman & Bidwell 2007). Thompson *et al.* (1999) demonstrated the ability of *G. nigrostriata* to withstand low oxygen concentrations through laboratory experiments, particularly when removed from water. The ability to withstand low oxygen concentrations presumably helps *G. nigrostriata* survive while emerging from the substrate and as they begin to enter the substrate prior to wetland desiccation. Consequently, low dissolved oxygen concentrations may delay emergence of *G. nigrostriata* from the substrate when wetlands start to inundate (see 4.4.2.1 Paperbark New).

Darker waters may provide protection from predators as visibility is reduced, particularly when *G. nigrostriata* are predominantly found in shallow wetlands. Conversely, the darker water reduces productivity which can affect macroinvertebrate diversity and abundance (Balla & Davis 1995). This may explain why the diet of *G. nigrostriata* can consist of up to 70% terrestrial macroinvertebrates (Gill & Morgan 2003). The large component of terrestrial macroinvertebrates in the diet of

G. nigrostriata also suggests they may favour areas within wetlands where vegetation is prominent and terrestrial macroinvertebrates more abundant (Allan, Wipfli *et al.* 2003). Additionally, aquatic macroinvertebrate abundance is higher in the presence of wetland vegetation (McCullough & Lund 2008), which may influence *G. nigrostriata* to occupy vegetated habitats.

However, no statistically significant difference was found between habitat types sampled and *G. nigrostriata* abundance. This suggests that habitat within the wetlands sampled were homogenous for macroinvertebrate abundance and does not determine the position of *G. nigrostriata*. Terrestrial macroinvertebrate abundance throughout the wetlands may be homogenous because of the relatively small size of the wetlands sampled (mean 0.6 ha) and mean vegetation coverage of 41%. Additionally, *G. nigrostriata* are typically found in the upper water column where terrestrial macroinvertebrates are more available (Gill & Morgan 2003).

The correlations between *G. nigrostriata* and crayfish CPUE, sampling site depth and habitat type were all 'negative weak', although statistically significant. The significance of these correlations may have been due to sampling techniques. In depths over 0.5 m, the size of the sweep net opening, an up and down motion was used along transects to encompass all levels of the water column. Therefore, at shallower depths the sweep net was swept as close as possible to the sediments, in deeper water there was less contact with the sediments. Freshwater crayfish may have been missed in deeper water, as they spend most of their time in the benthic zone. Additionally, as the depth increased, to a maximum working depth of about 1.0 m, momentum through the water was reduced and may have allowed fish to avoid capture.

The crayfish caught, *C. quinquecarinatus* and *C. preissii*, were common to the areas sampled and are known to burrow down to the groundwater (Morgan, Beatty *et al.* 2011). An unexpected result was finding crayfish in virtually all wetlands sampled. It was initially thought *G. nigrostriata* use crayfish burrows to access the moist sediments at or just above groundwater level and crayfish distribution would therefore affect distribution of *G. nigrostriata*. While *G. nigrostriata* may still use crayfish burrows to access groundwater for aestivation, the distribution of *G. nigrostriata* cannot be related to crayfish distribution in this research.

3.6 Conclusion

The distribution of *G. nigrostriata* could not be attributed to any particular physico-chemical variable measured in the wetlands sampled, shown by their capture throughout the wide ranges of the variables measured. Since the water quality and habitat features did not vary enough to impact *G. nigrostriata* populations, metapopulation theory could indeed explain their sporadic presence. Intermittent connectivity facilitates the migration of populations between wetlands and similar physico-chemical properties may maintain long-term populations. However, less than optimal wetland conditions causing population extinction may be due to other factors, as discussed in the following chapters.

Wetlands sampled on the Swan Coastal Plain were impacted by nearby agricultural activities, which increased phosphorus and nitrogen concentrations. By comparison, the south-west region wetlands had relatively low concentrations of nutrients as they were generally located within national parks, further away from anthropogenic influences. The increased nutrients in wetlands sampled on the Swan Coastal Plain did not negatively impact on *G. nigrostriata* abundance or distribution.

Nevertheless, the higher nutrient concentrations may cause a lower diversity and abundance of aquatic macroinvertebrates, influencing the diet of *G. nigrostriata* to contain a large proportion of terrestrial macroinvertebrates. Since physico-chemical properties in the wetlands sampled did not influence the distribution of *G. nigrostriata*, further investigation into their diet may be required, specifically prey availability, as it might affect their survival in some wetlands. Therefore, prey availability may be one of the determining factors to whether a wetland should be classified as a source or a sink for *G. nigrostriata*.

The physico-chemical variables measured in this research cannot be used to infer *G. nigrostriata* survival trigger values, but do indicate some tolerance to degraded conditions. For example: *G. nigrostriata* can tolerate hypoxic conditions (<6 mg/L) and possibly uses cutaneous respiration while aestivating. Given their adaption to aestivation, which requires tolerance to extreme conditions, they are more likely to tolerate relatively extreme physico-chemical conditions than fish that survive in permanent water. More comprehensive sampling and laboratory toxicity experiments would be required to determine their survival threshold for physico-chemical conditions.

There was no statistical significance between the three habitat types sampled and *G. nigrostriata* abundance. The small size, low mean depth and mean vegetation cover of around 40% in wetlands sampled and known typical distribution patterns of aquatic and terrestrial macroinvertebrates within wetlands suggests the three habitat types are relatively homogenous. Freshwater crayfish abundance was also not statistically significant between the three habitat types sample or correlated to *G. nigrostriata* abundance or distribution. There did not appear to be a commensal relationship between *G. nigrostriata* and freshwater crayfish.

4 Habitat requirements for aestivation of *Galaxiella nigrostriata* during the dry phase of seasonal wetlands

With their bent for pathological interpretations, biologists also define aestivation as "the state of torpidity induced in animals by excessive dry heat." **(Shelton 1950)**

4.1 Introduction

Aestivation is a dormancy function used by some animals to survive hot/dry climatic periods, when environmental conditions are not favourable and food resources may be limited (Secor & Lignot 2010). For many of these animals, which include invertebrates, snakes, turtles and salamanders, aestivation involves burrowing or gaining access to the deeper sediments and entering a period of reduced metabolic activity until cooler or wetter conditions prevail (Snodgrass, Ackerman *et al.* 1999; Dietz-Brantley, Taylor *et al.* 2002; Fordham, Georges *et al.* 2008; Nomura, Rossa-Feres *et al.* 2009). Some fish species have also adapted to aestivation as a means of survival, accessing the cool sediments when their habitat dries. Fish such as *Protopterus* spp. African Lungfishes, *Synbranchus marmoratus* South American Marble Swamp Eel and *Lepidogalaxias salamandroides* burrow into the mud and coat themselves with a mucus membrane to retain moisture and can survive for months to years, until they emerge with the next hydroperiod (Pusey 1989; Thompson & Withers 1999; Sturla, Paola *et al.* 2002; Moraes, Altran *et al.* 2005). However, as not all aestivating fish produce a mucus coating they must remain within moist sediments at or below the groundwater level. These include two Galaxiidae genera: New Zealand and Australian *Neochanna* and Australian *Galaxiella* (Thompson & Withers 1999; McDowall 2006).

Of the three *Galaxiella* species, only *G. nigrostriata* and *G. pusilla* are thought to aestivate, *G. munda* does not (McDowall 2006). Anecdotal evidence suggests *G. nigrostriata* use crayfish burrows to access groundwater in the deeper sediment for aestivation when wetlands dry (Thompson & Withers 1999; Bamford & Bamford 2003; McDowall 2006). The congeneric *G. pusilla* was observed retreating to crayfish burrows when disturbed (Beck 1985) and are also thought to use crayfish burrows for aestivation (Chilcott & Humphries 1996). *Galaxiella nigrostriata* are found in similar habitats as *L. salamandroides*, specifically seasonal wetlands, and have been captured while searching for the later species (Berra & Allen 1989a). *Galaxiella nigrostriata* was found while researchers were digging wetland sediments looking for *L. salamandroides* in south-west WA, as wetland pools were starting to dry in December/January (Berra & Allen 1989a). However, *G. nigrostriata* were not the subject of these authors' experiments and were not examined further.

Previous attempts to find aestivating *G. nigrostriata* soon after wetlands dry, in wetlands where they were found while inundated, have been mostly unsuccessful. Smith *et al.* (2002) tried excavating trenches in a wetland north of Perth and sieved the

removed soil to search for *G. nigrostriata*, but did not find any. Pen *et al.* (1993) also tried digging the sediments of a dry wetland (near Northcliffe) but without success. Artificial flooding of dry pools near Northcliffe produced *L. salamandroides* and *Cherax* spp. (freshwater crayfish), but no *G. nigrostriata* emerged (Berra & Allen 1989a). The abovementioned experiments did not examine the requirements for aestivation, such as presence of crayfish or use of their burrows, substrate type, the habitat surrounding where they aestivate or depth to groundwater.

Galaxiella nigrostriata live for just over one year (Pen, Gill *et al.* 1993), of which about six months may be spent aestivating in the sediments (personal observations). Additionally, they only become sexually mature at about one year, after they have emerged from aestivation when winter rain arrives and wetlands refill (Pen, Gill *et al.* 1993). Therefore, *G. nigrostriata* aestivation is a major and critical life stage that requires further investigation. Long-term abstraction by industrial, commercial or residential bores and reduced recharge resulting from a drying climate may cause groundwater levels to decrease in localised areas, resulting in wetland pools remaining dry for longer periods. An extended period of aestivation could be detrimental to *G. nigrostriata* populations, potentially causing them to become extinct. Some populations may be effected more than others, depending on the amount local groundwater levels decrease, water holding capacity of the soil and local climate change.

4.1.1 Objective and hypotheses

The objective of this chapter was to establish what requirements *G. nigrostriata* have for the aestivation stage of their life cycle. Their aestivation requirements may determine whether a wetland can be considered a source or sink population. The following hypotheses were designed to help answer this chapter's objective

H_0 – there is no correlation between the presence of crayfish, their burrows and *G. nigrostriata* abundance.

H_0 – *Galaxiella nigrostriata* aestivate randomly throughout the wetlands.

H_0 – *Galaxiella nigrostriata* enter the sediments immediately prior to the wetland drying.

H_0 – *Galaxiella nigrostriata* emerge from the sediments as soon as the wetlands inundate.

4.2 Methods

4.2.1 Re-wetting experiment

Over five days in March 2009 while the wetlands were dry (late summer), 12 drum enclosures were used for a re-wetting experiment within three KSS wetlands. The experiment was designed to coerce aestivating *G. nigrostriata* to emerge from the substrate by filling drums with water, adapted from the flooding experiment by Berra *et al*. (1989a). The water used for the drum experiment was pre-filtered from the dredge ponds, as all nearby wetlands were dry. The drums were made from 205 L steel drums cut in half (450 mm height x 570 mm width) with a star picket welded across the top opening to be used as a handle to force the drum into the sediment for a watertight seal (see inset Plate 4). Drums were inserted 50 mm into the surface, although where cracks were present some were inserted nearly 200 mm to try maintain a watertight seal. Once in place the drums were manually filled from two larger 'central' drums (200 L each) that were refilled from a truck mounted water tank (500 L) up to 100 m away at the edge of the wetland.

Plate 4. Half drums waiting to be used in EPP5. Large drum (on right) was filled from a water truck 100 m away and used for buckets to fill the drums (inset).

The drums were used in wetlands that had a high fish CPUE when sampled in spring 2008. Wetlands EPP5, EPP6 and PN were determined as most likely to contain aestivating *G. nigrostriata*. Within the three wetlands drum sites were primarily selected to contain burrows to increase the likelihood of finding aestivating *G. nigrostriata*. Additionally, to examine where *G. nigrostriata* aestivate within the wetland, drum sites

were also selected by three habitat types: open, vegetated and covered (see 2.2.1 Assessment of trapping method). At each drum, site the number and size of burrows and surrounding vegetation type was recorded. A 90 mm x 1 m (optional 1 m extension) hand auger was used to provide a way of measuring groundwater depth at each of the wetlands, central to the main drum sites.

Table 11. Number of drum locations and habitat type where they were placed.

	Open	Vegetated	Covered	Total
EPP5	3	6	11	20
EPP6	10	7	12	29
PN	3	0	2	5
Total	16	13	25	54

Once the drums were installed at each site, 40-80 L of water was added to fill any burrows and leave the drum with about 200-400 mm of water. The water was poured onto a plastic bucket lid on the ground within the drum to minimise disturbance to the sediment. The volume of water used depended on how quickly it was absorbed into the soil or drained down a burrow. After the water was added it was left to absorb/drain into the soil for 10-15 minutes. If the water drained away quickly extra water was added until it stopped draining and maintained a 200-400 mm depth for more than 10 minutes. Once the water settled, each drum was swept with a small scoop net (150 x 180 mm opening x 200 mm deep with 1 mm wide mesh) to collect any emergent animals. The sweeping continued for up to 1½ h at 15 minute intervals.

4.2.2 Lentocorral construction and placement

A lentocorral is a shallow version of a limnocorral, a structure used to isolate a column of water for experiments such as chemical or biological interactions (Chakraborty, Biswas *et al.* 2004; Hinton, Kaplan *et al.* 2006). In this project, the design allowed water to flow through the mesh but stopped fish from escaping. Two wetlands were chosen (EPP6 and PN) that had a high abundance of *G. nigrostriata* when sampled the previous year. Thirty-one lentocorrals were installed in the selected wetlands over five days in mid May 2009 (autumn) while dry.

Plate 5. Vegetated lentocorral in EPP6. Flagging tape was to ward off animals, to prevent injuries to the animals and damage to the enclosure.

The lentocorrals were installed primarily to investigate whether *G. nigrostriata* used crayfish burrows to enter the sediment and if they entered the sediment prior to the water beginning to dry (gradient - anywhere in wetland) or waited until it was almost dry (depression - last remaining pools). Where possible, secondary identifiers relating to habitat type, open, vegetated or covered, were also recorded (Table 12).

A dumpy level (Bear Scientific, USA) was used to determine ground levels, based on nearby established monitoring bores, survey markers and known high water levels from previous records, all using Australian Height Datum (AHD). Finding lentocorral sites in the main section of PN was straightforward, as it consisted of a large deep pool area where gradients and depressions were quite easily observable. However, the northern half of PN and all of EPP6 were quite flat and a level was essential to separate the sites. Unexpected heavy rainfall on the third day of lentocorral construction also made subtle ground contours easier to identify.

Table 12. Habitat properties of lentocorral placement within the wetlands. PN figures in bold.

		Burrows Gradient	No Burrows Gradient	Burrows Depression	No Burrows Depression	Total
Open	EPP6	1	1	1	2	5
	PN	**1**	**2**	**1**	**1**	**5**
Vegetated	EPP6	1	2	1	-	4
	PN	**-**	**1**	**-**	**-**	**1**
Covered	EPP6	2	1	2	2	7
	PN	**2**	**1**	**3**	**3**	**9**
Total EPP6 (**PN**)		4 (**3**)	4 (**4**)	4 (**4**)	4 (**4**)	16 (**15**)

Lentocorrals were constructed with four stakes driven into the sediment 2 m apart and flyscreen wrapped around to a height of either 500 or 800 mm high, depending on expected water depth. The flyscreen ends were joined by overlapping and wrapping around a stake and fastening with cable ties. An extra 100 mm of flyscreen was left at the bottom to help form a seal on the ground. Steel pegs and soil were used to hold the bottom of the flyscreen to the ground.

The number and size of burrows, vegetation cover (rushes, woody, open areas) and position (using a GPS) was recorded for each lentocorral. Two piezometers were installed in each wetland, one on the wetland edge and the other in the approximate wetland centre and about 30 m apart. The piezometers were fitted with a capped 90 mm PVC pipe approximately one metre above ground and two metres below; the first metre of pipe below ground was slotted. A capacitance water level data-logger (Odyssey, New Zealand) was installed into the piezometers in the centre of each wetland to record when groundwater reached ground level, following the onset of winter rain. Following installation and after the winter rains had begun, ground and surface water levels were manually recorded once a week and the data loggers recorded water height hourly until the lentocorral experiment had finished.

4.2.2.1 Lentocorral monitoring

Following the start of winter rains in early June, lentocorrals were sampled twice a week for a ten week period. Clearing of the lentocorrals commenced as soon as they became inundated and continued until the end of the monitoring period, unless they became entirely submerged earlier. As lentocorrals became inundated, they were swept

3-5 times on each occasion using a fish landing net (450 x 450 mm opening x 3 mm^2 mesh), to capture the trapped fish and/or crayfish. All captured *G. nigrostriata* were placed into containers and counted, the first 10 per lentocorral were euthanaised immediately in an ice slurry (for additional specimens for genetic and morphological analyses). If more than 10 fish were caught, the remainder were released outside of the lentocorral. A scoop or flat seine net (3 m x 1 m, 4 mm^2 stretched mesh) were used as required to verify *G. nigrostriata* presence surrounding the lentocorrals in each wetland. Crayfish captured were immediately counted, sexed, measured and released outside of the lentocorral. Other animals. such as frogs or tadpoles, captured during the lentocorral clearing were counted and released outside of the lentocorral.

4.2.2.2 Data analyses

To ensure active burrows with entrances covered or filled in were not counted as 'no burrows' in lentocorral sites, an Independent Samples T-Test was performed. This compared the number of crayfish caught to whether burrows were present in lentocorrals in both wetlands. The interactive effect of different factors, such as habitat type to *G. nigrostriata* aestivation locations within PN, were determined using ANOVA (for full statistical data analyses methods, see 2.4 Data analysis). Fish/crayfish density was calculated by dividing the total number of fish/crayfish caught per lentocorral by four (the surface area of each lentocorral was 4 m^2).

4.3 Results

4.3.1 Re-wetting experiment

Most wetlands were dry by mid-January 2009, except for PN (which still contained *G. nigrostriata*) and EPP7, both of which were about 0.5 m deep. All wetlands were dry by early March 2009 when the drum experiment took place. Groundwater levels ranged from 0.4-1.8 m below surface level, although some bores did not reach groundwater due to the density or composition of the substrate (Table 13). When digging the bore holes the soil was visibly very dry until 200-300 mm above the groundwater level. When filling the drums, occasionally a plume of damp soil would appear up to 0.5 m surrounding the drum. Some drums drained almost immediately, especially those containing large or multiple burrows or a cracked substrate (Plate 4). Other drums took over an hour to drain; one did not drain when left overnight, suggesting an impermeable substrate.

Fifty four drum sites were used in three dry wetlands, however, no fish emerged from any site. When other researchers carried out similar wetland refilling experiments, *G. nigrostriata* (along with the targeted species, *L. salamandroides*) started to emerge within 10 minutes of dry pools being filled to a depth of about 0.2 m (Berra & Allen 1989a; D. Morgan, MU-CFFR, unpublished data). Additionally, *G. nigrostriata* were captured later in the same year in each wetland used for the drum experiment; late winter (August) EPP5 and PN and late spring (November) in EPP6.

Table 13. Groundwater depth from dry wetland surface at the start of autumn (March 2009) from bores drilled into centre of the wetlands. > indicates maximum depth that could be drilled (due to hard substrate) and no water detected.

	EPP1	EPP3	EPP4	EPP5	EPP6	EPP7	EPP8	EPP9	PN	PS	PD
Groundwater depth (m)	1.0	0.9	1.8	>1.5	>1.5	>0.8	0.7	0.8	0.8	0.4	0.8

4.3.2 Lentocorrals

4.3.2.1 Placement of lentocorrals

In EPP6 and PN, a total of 148 juvenile crayfish (5-20 mm OCL, sex/species undetermined) were caught in 15 lentocorrals classified as 'burrows present' and 30 crayfish from 16 lentocorrals classified as 'burrows absent'. A significant difference was found between crayfish caught in lentocorrals with or without burrows ($t_{29,1} = 3.6$, $p < 0.05$) (Figure 10). These results show that lentocorrals classified as 'burrows' did

indeed contain crayfish burrows and lentocorrals classified as ‘no burrows’ in most cases did not contain covered or filled in active burrows.

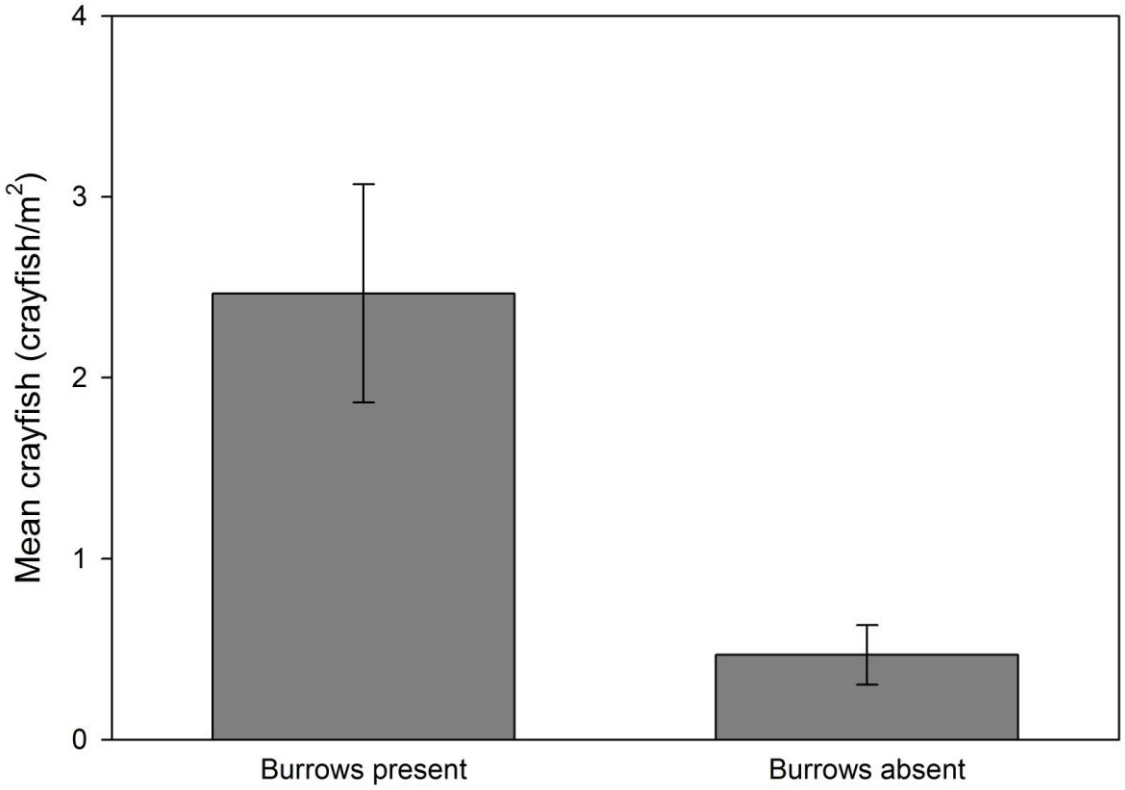

Figure 10. Mean crayfish (±SE) caught in EPP6 and PN while sweeping lentocorrals classified with burrows (*n* = 15) and without (*n* = 16).

4.3.2.2 Lentocorral monitoring results

Due to uneven ground surface within wetlands, not all lentocorrals began to fill at the same time and some became submerged (in the deep pool of PN) before the end of the 10 weeks of monitoring. The first lentocorrals to contain standing water were in PN and by the second week 60% of lentocorrals contained standing water, all in EPP6 by week three, and the last two lentocorrals in PN only contained standing water in week six. The rapidly rising water level in the deep pool of PN caused one lentocorral to submerge after only four weeks, while in the northern flat part of PN one lentocorral was swept for nine weeks. All lentocorrals in EPP6 were swept for eight-nine weeks.

No fish were captured in EPP6, either in the lentocorrals or the surrounding wetland when sampled with a scoop or flat seine net. However, when sampled three months after the aestivation experiment concluded *G. nigrostriata* abundance in EPP6 had the fourth highest CPUE (1.0) out of the five wetlands containing *G. Nigrostriata* (November 2009), despite having the highest CPUE (3.4) in October 2008.

Conversely, 171 *G. nigrostriata* were captured in nine lentocorrals in PN with a mean of 11.3 ±7.8 in each. The highest number of *G. nigrostriata* caught in a single lentocorral was 120 (109 in two weeks), which was situated on a gradient on the edge of the northern section of PN. It also contained two medium burrows (20-50 mm diameter), was shadowed by an overhanging *Melaleuca* sp. and *G. nigrostriata* started to emerge when the lentocorral water depth was about 90 mm. On average *G. nigrostriata* started to emerge 12 ±0.3 days after each lentocorral contained standing water, at an average depth of 337 ±70 mm. Groundwater reached the surface five days after PN began to fill and six days after EPP6 began to fill (Figure 11). By the fourth week all lentocorrals contained standing water and nearly 80% of all fish recorded had emerged. No more fish were recorded emerging after seven weeks (Figure 12). Water level peaked around late September in PN at 1.3 m deep (14.0 m AHD) and EPP6 at 0.3 m deep (12.52 m AHD).

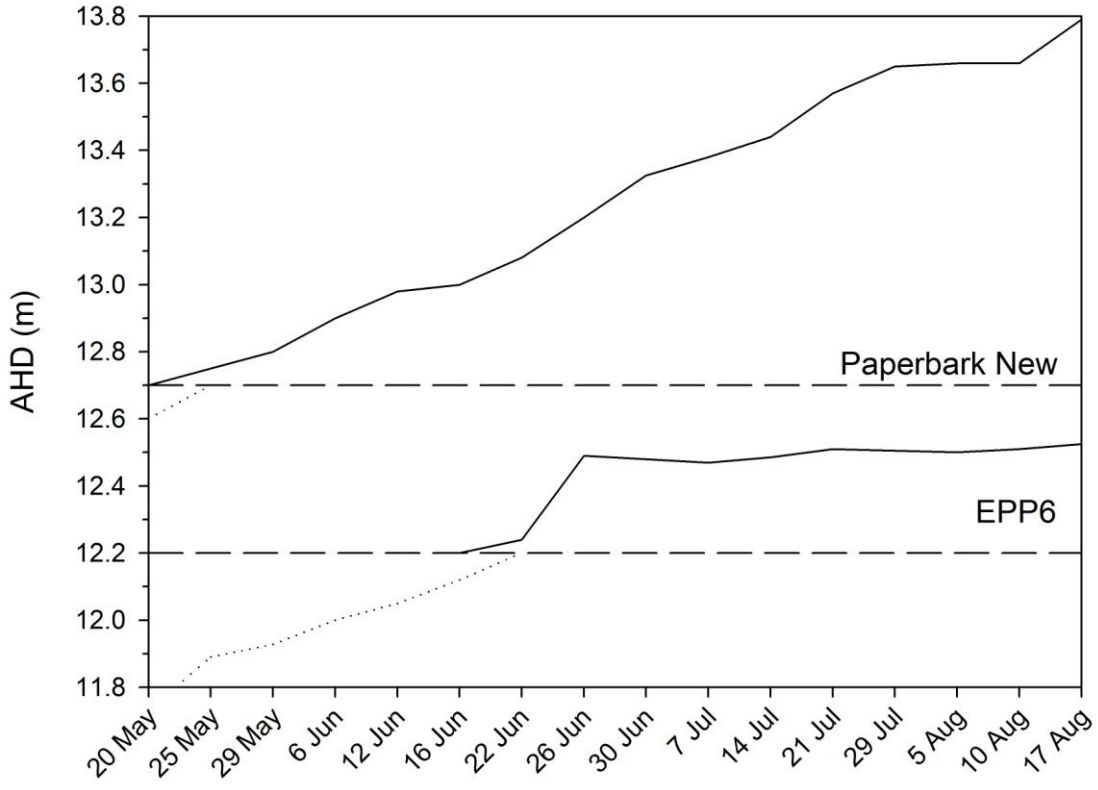

Figure 11. Groundwater/surface interaction in two Kemerton wetlands. Solid lines show water level above ground, dotted lines are groundwater levels below the surface. Horizontal dashed lines show wetland ground surface heights in AHD.

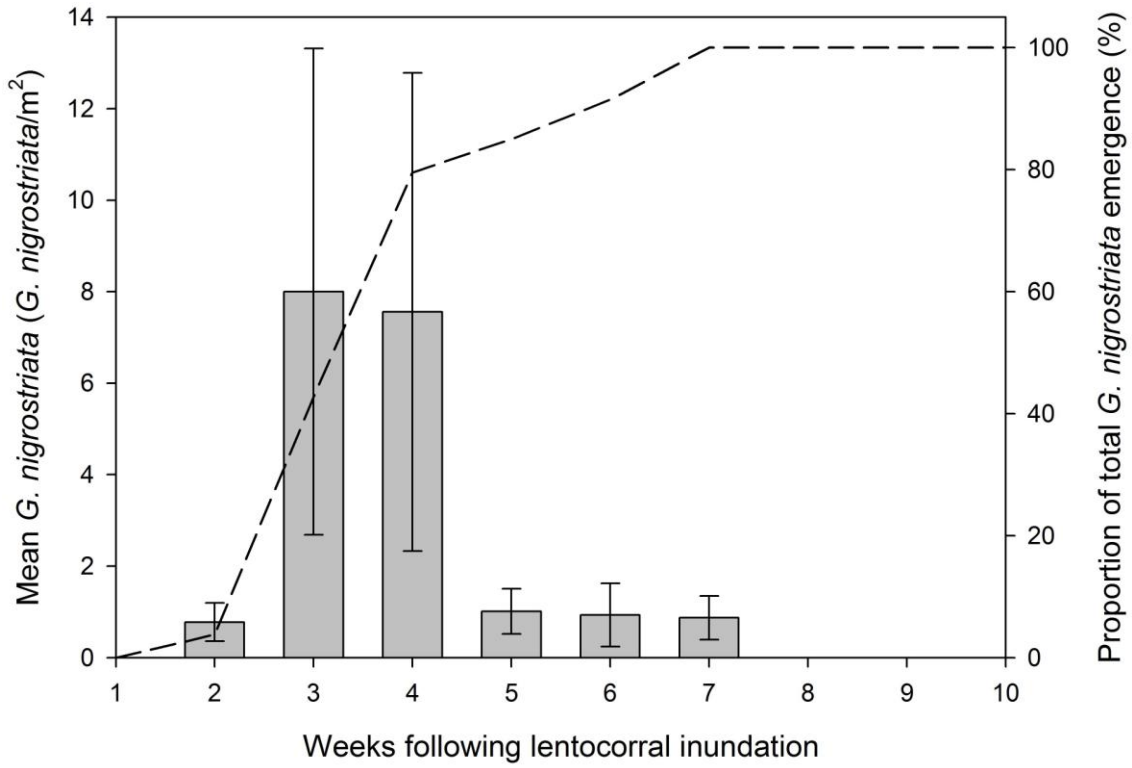

Figure 12. Weeks to emergence for *G. nigrostriata* after lentocorrals become inundated. Bars show mean fish emergence each week (±SE), dashed line shows cumulative emergence over time.

Galaxiella nigrostriata was present in more lentocorrals that had no burrows - 63% (cf. with burrows 57%). Additionally, the correlations between fish and crayfish abundance and burrow presence were not statistically significant (Table 15). There was also no statistically significant (p >0.05) correlation between burrow presence and *G. nigrostriata* abundance within lentocorrals (Table 14). No statistically significant correlations were found between the abundance of fish in relation to the three environmental factors individually or when grouped together (Table 14). *Galaxiella nigrostriata* emerged in 63% of lentocorrals that were in depressions in the ground surface and 67% of lentocorrals directly adjacent to or covered by overhanging vegetation. However, only 50% of lentocorrals that were in depressions and next to or covered by vegetation contained *G. nigrostriata.*

Table 14. ANOVA for various factors and how they influence fish aestivation locations. None of the factors or combinations were significant (p <0.05). Ground topography was where water pools or runs off the surface, Habitat presence is the type of vegetation surrounding each lentocorral (open, vegetated or covered) and burrow presence is presence or absence of crayfish burrows.

Lentocorral factor(s)	$F_{1,15}$	p-value
Ground topography	0.006	0.948
Burrow presence	0.179	0.720
Habitat position	0.293	0.783
Ground topography Burrow presence	16.502	0.154
Ground topography Habitat position	71.632	0.075
Burrow presence Habitat position	8.849	0.206
Ground topography Burrow presence Habitat position	0.029	0.871

Table 15. Correlations between fish, crayfish and burrow presence. There were no statistically significant correlations (p <0.05).

Factors	Burrows	Fish
Fish	r = -0.23 (p = 0.40)	
Crayfish	r = 0.18 (p = 0.53)	r = 0.00 (p = 0.99)

4.4 Discussion

4.4.1 Drum experiment

The absence of *G. nigrostriata* emerging during the drum experiment was surprising, given their appearance in the same wetlands later in the year and the success of similar experiments by other researchers. The water in the drums possibly drained too quickly or was absorbed into the surrounding sediment and failed to create a continuous wet channel down to the groundwater for *G. nigrostriata* to swim to the surface. The drums that held water may have contained blocked or incomplete burrows and only covered a small area (0.25 m^2), particularly when compared to the lentocorrals (4 m^2), so would have been more likely to miss aestivating *G. nigrostriata.* Additionally, *G nigrostriata* did not emerge immediately when wetlands inundated naturally during the lentocorral experiment.

The previous experiments (Berra & Allen 1989a; D. Morgan, MU-CFFR, unpublished data) took place near Northcliffe in an area of higher average rainfall and lower average temperatures and evaporation rates compared to Kemerton (see Figure 4), which could increase soil moisture content and decrease depth to groundwater. In the Northcliffe area *G. nigrostriata* might be closer to the surface while aestivating, either in the groundwater or above it in the damp soil, which allows them to emerge quickly upon filling of the wetlands. By comparison, when the Kemerton wetlands fill it takes longer for the groundwater to rise above the wetland ground and interact with surface water and *G. nigrostriata* appear to take longer to emerge The greater groundwater depth was probably due to lower annual rainfall, which contributes to the wetland's hydroperiod being shorter than the wetlands near Northcliffe.

4.4.2 Lentocorrals

4.4.2.1 Paperbark New

The most unexpected finding of the lentocorral experiment was that there was no relationship between *G. nigrostriata* and crayfish or their burrows, with *G. nigrostriata* found in as many lentocorrals that contained crayfish burrows as those that did not contain burrows. The general theory of *G. nigrostriata* aestivation was that they accessed the damp sediment at or above groundwater level via crayfish burrows. Thus, how do *G. nigrostriata* gain access to the substrate? Other types of animals that may provide burrows and are thought to inhabit the Kemerton area were considered from local species lists (Bamford & Bamford 2000; Bamford & Davis 2003). The only

animal (besides crayfish) that would provide suitable burrows within the wetlands was *Heleioporus* spp. (burrowing frogs) (R. Davis, ECU, 2009, pers. comm.). *Heleioporus* spp. burrow in late Autumn (April - June) before the winter rains start, and up to 1.15 m deep, which in some wetlands may reach the groundwater level (Littlejohn, Roberts *et al.* 1993; Cogger 2000). These burrows may close or collapse during the following hydroperiod and may not be available for *G. nigrostriata* when the water level begins to subside.

The lentocorral with the most *G. nigrostriata* was shaded by overhanging *Melaleuca* trees near the edge of the wetland. Shaded or sheltered habitats provide protection from predators, even if the fish are potentially more visible through the shallower water. Riparian vegetation provides a valuable food source (Baxter, Fausch *et al.* 2005) prior to the wetland drying, with up to 50% of adult *G. nigrostriata's* diet consisting of terrestrial macroinvertebrates (Gill & Morgan 2003). With many of the fish found next to or shaded by overhanging vegetation they may also use underground root cavities, similar to *Kryptolebias marmoratus* American Mangrove Killifish (Taylor, Turner *et al.* 2008). However, it is more probable that *G. nigrostriata* burrow into the sediment themselves[3] like *Lepidogalaxias salamandroides* and *Neochanna* spp. (Berra & Allen 1989a; McDowall 2006). Although, the burrowing capabilities of *G. nigrostriata* are not well understood, but are considered to be poor (Thompson & Withers 1999; D. Morgan, MU-CFFR, pers. comm., 2009) and therefore require further investigation.

The results suggest that *G. nigrostriata* wait until the wetlands have nearly dried before entering the sediment to aestivate and prefer areas shaded by vegetation. Since *G. nigrostriata* do not cocoon themselves with a mucus coating to survive a drying environment, in the same way as *L. salamandroides* or *Protopterus annectens* African Lungfish (Pusey 1990; Thompson & Withers 1999; Morgan, Gill *et al.* 2000; Loong, Ang *et al.* 2008), they need to stay moist to survive. As the wetlands dry, soil temperatures in shaded areas may be cooler and retain more moisture than open areas. However, as *G. nigrostriata* emerged in only half of the lentocorrals in depressions and next to vegetation, more intensive sampling is required to more precisely understand where they can survive aestivation within the wetlands.

[3] In Galeotti *et al*. (2010) it was incorrectly stated that Thompson *et al*. (1999) found *G. nigrostriata* were physically unable to burrow. They merely stated that *G. nigrostriata* did not move as easily as *L. salamandroides* on soil and most likely used crayfish burrows to access underground water when a wetland dried.

As wetlands begin to fill with the onset of winter rains, groundwater levels begin to rise and along with it emerge *G. nigrostriata*. However, the almost two week delay between the groundwater reaching the surface and *G. nigrostriata* emerging may be due to an adjustment period while they regain normal metabolism. Laboratory experiments by Thompson *et al.* (1999) showed that *G. nigrostriata's* metabolism slowed down in hypoxic conditions, the same conditions as would be expected while aestivating underground.

There may also be other triggers for emergence to ensure *G. nigrostriata* emerge at appropriate times, triggers which are not influenced by an unseasonal downpour in the middle of a normally dry period (as demonstrated by the drum experiment). These triggers would act as a safeguard; otherwise *G. nigrostriata* may emerge to a wetland that will not stay wet for long and have minimal shelter and food resources. Triggers that induce emergence have not been studied, but might include changes in groundwater temperature, physico-chemical concentrations, increasing water pressure or zooplankton (food) appearing in the burrows.

4.4.2.2 EPP6

Galaxiella nigrostriata may have gone through a major population contraction/ extinction event while aestivating in EPP6, as no fish were captured in lentocorrals or surrounding water. The extinction was unexpected as EPP6 contained the highest CPUE of *G. nigrostriata* the previous year and they were present for most of the previous monitoring years. Surprisingly, *G. nigrostriata* were present in EPP6 when the wetland was sampled three months after the aestivation experiment. When EPP6 was dry, the groundwater depths were a lot deeper than PN and PS (the northern section of PN is halfway between where groundwater levels were measured at PN and PS). The result of EPP6 drying quickly may be a short hydroperiod and a longer dry period. The amount of time *G. nigrostriata* can survive aestivation is unknown, however, it may be similar to *N. burrowsius* Canterbury Mudfish which is thought to survive for up to five months in the ground (O'Brien 2007).

Wetland EPP6 showed a similar pattern of ground/surface interaction as PN. The sediment of EPP6 is quite flat which makes the wetland uniformly shallow and allows it to dry more rapidly than the deeper pool in PN. However, like EPP6 the northern section of PN is quite flat, but *G. nigrostriata* still emerged in the lentocorrals. The water quality was not analysed during the aestivation experiment, although there was no

obvious reason (wetland modifications or changes to physico-chemical inputs) why the water quality of EPP6 would have changed from the previous year. *Galaxiella nigrostriata* in EPP6 possibly recolonised from EPP1 or seasonal wetlands in the neighbouring private agricultural property during near average rainfall (10 year average rainfall, 1999-2009 - KSS, unpublished data) (Bamford & Bamford 2000). It is also possible they emerged sometime following the aestivation experiment; however this does not seem likely as they would have had to survive an aestivation period of about 8 months.

4.5 Conclusion for aestivation experiments

4.5.1 Drum experiment

The drum experiment did not coerce any *G. nigrostriata* to emerge from aestivation and therefore did not provide any information about their habitat preferences while aestivating. The main reasons were possibly that the groundwater where *G. nigrostriata* were aestivating was too deep, there was not enough water supplied to the drums to create a channel for the fish to swim up, and the water used may not have had the appropriate physico-chemical properties to stimulate the fish into emerging. This method is probably more applicable to regions with higher groundwater levels where fish are not very deep in the wetland sediments, such as the Scott Coastal Plain wetlands in the south-west region. Even though *G. nigrostriata* did not emerge, the drum experiment did provide some additional information to direct future research. Some suggestions are:

- Initially, find sampling sites with high groundwater levels. There would be more chance that aestivating fish will emerge as they don't have as far to travel or for the water to reach them.
- Feed water constantly into drums to maintain standing water, to create a channel for aestivating fish to use to swim to the surface.
- Use water similar to water that fills wetlands, if available.

4.5.2 Lentocorral experiment

The most interesting and unexpected finding was that aestivating *G. nigrostriata* were not correlated with the presence of crayfish burrows. This result questions the long held theory that *G. nigrostriata* use crayfish burrows to enter the sediments. Indeed, the use of crayfish burrows may be important, but further research is required to determine other ways *G. nigrostriata* can enter the sediments. The most probable explanation is *G. nigrostriata* burrow into the sediment themselves, possibly as a last resort after finding no suitable burrows as the wetland dries The composition of the sediments may contribute to the patchiness of their distribution within a wetland or group of wetlands, since clayey soils or areas subject to vehicle access or cattle grazing may be too dense or compacted for a small fish to burrow. The soil density and composition would need to be researched as a possible factor to population survival within a wetland.

Unfortunately, *G. nigrostriata* were not found in every lentocorral in PN and not at all in EPP6, which affected the power of statistical analyses. Additional replicates in more wetlands are required to gain a better understanding of their aestivation habitat preferences. *Galaxiella nigrostriata* may enter the sediments randomly but might not survive aestivation in all habitats. If this is the case, a large proportion of each wetland's population may not survive annual aestivation. The physico-chemical properties of the groundwater and sediments that *G. nigrostriata* aestivate in, including soil moisture content, depth of groundwater and temperature of soil are unknown and may affect their ability to aestivate. Having an understanding of where and how long *G. nigrostriata* can aestivate may help identify priority (source) wetlands and assist land holders in the management of groundwater extraction and wetland rehabilitation design.

5 Population genetic structure and morphological differences of *Galaxiella nigrostriata*

5.1 Introduction

South-west WA is internationally regarded as having high biodiversity, with estimates up to 49% of flora and 22% of terrestrial vertebrate fauna being endemic (Hopper & Gioia 2004). Additionally, nine of the 11 native freshwater fish found in the south-west are also endemic (Morgan, Beatty *et al.* 2011). Unfortunately, south-west WA is also listed as one of the most vulnerable 'biodiversity hotspots' in the world, for having very high endemism and experiencing considerably high loss (>70%) of natural habitat (Myers, Mittermeier *et al.* 2000; Malcolm, Liu *et al.* 2006; Horwitz, Bradshaw *et al.* 2008).

To assist with the conservation of a species, it is important to have an understanding of the elements most critical to their survival, which could include their habitat requirements (Bonnett & Sykes 2002), physiology and reproductive behaviour (Hardie, White *et al.* 2007), and genetic diversity (Vrijenhoek 1996; Moritz 2002; Schwartz, Luikart *et al.* 2007). Knowledge of the genetic structure of a population is a vital tool for modern conservation efforts as a finding of low heterozygosity could infer less resilience to disease, reduced fertility, inbreeding depression and an overall lower chance of population survival (Vrijenhoek 1996). Furthermore, genetic analyses can reveal evolutionary, geographic and migratory pathways through gene flow inferred by genetic diversity values. Fragmented habitats and therefore populations, while still under threat, may still be relatively safe from extinction if connectivity pathways exist and are managed as metapopulations. Investigating the circumstances shaping a populations past can help direct future conservation efforts (Chen, Zhang *et al.* 2009).

Fish in fragmented populations can also go through slight physical changes, evolving due to local environmental pressures (Scalici & Gibertini 2009). This can make identification of a species across a broad geographic range difficult, especially when the species are known to coexist in similar habitat types as congeneric species (Beatty, Morgan *et al.* 2009; Galeotti, McCullough *et al.* 2010). For example, the major differences of *G. nigrostriata* and *G. munda* were published to aid identification (Berra & Allen 1989b). However, the small size of the fish (max. TL 50 mm and 60 mm respectively) can still make identification difficult and may require genetic analysis for more certain species determination (Galeotti, McCullough *et al.* 2010).

Plate 6. A) Adult *Galaxiella nigrostriata* (~35 mm TL), and B) adult *G. munda* (~40 mm TL). When viewing the two species side by side, their differences are more noticeable, specifically the positioning of the dorsal fin origin compared to the anal fin. (Photos courtesy of G. Allen)

When *G. nigrostriata* was described in 1953 (Shipway 1953), they were known to inhabit wetlands in the south-west corner of WA, between Augusta and Albany and mostly within the Scott Coastal Plain (Gill & Morgan 1996). Since the 1993 discovery of a remnant population near Bunbury, two additional remnant populations were discovered even further north on the Swan Coastal Plain at Melaleuca Park (1995) and Lake Chandala (2009) (Smith, Knott *et al.* 2002; McLure & Horwitz 2009). The recent discoveries suggest G. nigrostriata was once more widespread in south-west WA and specifically the Swan Coastal Plain and have since faced a regional decline. The mass destruction of wetlands over the past 150 years has left G. nigrostriata habitats patchy and now disconnected (Morgan, Gill *et al.* 1998; Smith, Knott *et al.* 2002). Without putting conservation management strategies in place, their survival may be threatened.

The Swan and Scott Coastal Plains are separated by the Blackwood Plateau, which bisects the two coastal plains between Busselton and Augusta (Beard 1999). Historically, rivers from both coastal plains may have had more connectivity during the last glacial maximum (18,000 - 20,000 years before present) when sea levels were >130 m below current sea level and south-west WA river deltas and estuaries were more widespread (Lambeck & Nakada 1990; Unmack 2001). Recent connectivity for

G. nigrostriata between neighbouring waterbodies within the two coastal plains is limited to heavy rainfall causing sheetflow over floodplains or damplands, or possibly when winter rains flush freshwater into estuaries (Unmack 2001).

Furthermore, *G. nigrostriata* are known to inhabit half of the seasonal wetlands at the Kemerton wetlands, near Bunbury WA. There is limited connectivity between the wetlands, possibly only during well above (current) average winter rainfall. Examining gene flow between the wetlands may show how much migration between wetlands is required to maintain source wetlands using metapopulation concepts (Johst, Brandl *et al.* 2002; Thomas & Hanski 2004). Understanding recent or past connectivity would give the opportunity to maintain current or provide new migration pathways that could be utilised by *G. nigrostriata* between priority wetlands.

Until recently, allozyme electrophoresis and morphometric studies were the primary methods for establishing genetic connectivity between fish populations, including *G. nigrostriata* (Watts, Storey *et al.* 1995; Chaplin, Baudains *et al.* 1998; Smith, Pen *et al.* 2002). However, as mitochondrial DNA (mtDNA) analyses using PCR sequencing has become cheaper and more efficient in recent years, its use has become more widespread for determining the population genetic structure of fish in general (Zhang & Hewitt 2003; Chen, Zhang *et al.* 2009) and in studies of galaxiids (McDowall 2002; Davey, O'Brien *et al.* 2003; Phillips, Chaplin *et al.* 2007). A finding of genetically divergent populations may justify applying a higher conservation status and more intensive management of each deme (Ling, Gleeson *et al.* 2001; Cook, Bunn *et al.* 2007; Phillips, Chaplin *et al.* 2007). Additionally, genetic diversity is enhanced through appropriate management of populations and metapopulations (Lindenmayer & Burgman 2005).

5.1.1 Objective and hypotheses

The objective of this chapter is to explore the historic and current potential connectivity between populations of *G. nigrostriata* to test their status as a metapopulation. To achieve this, topography, genetic diversity between populations (to infer gene flow) and potential morphological changes due to isolation were examined. The following hypotheses were designed to help answer this chapter's objective.

H_0 – There has been recent gene flow between and within all catchments.

H_0 – Populations of *G. nigrostriata* have diverged enough to be regarded as subspecies.

H_0 – *Galaxiella nigrostriata* have not undergone physical changes due to recent population isolation.

H_0 – *Galaxiella nigrostriata* can be easily identified in the field against the congeneric *G. munda*.

5.2 Methods

5.2.1 Genetic analyses

Galaxiella nigrostriata specimens collected during the habitat requirements field trips (see 2.3 *Galaxiella nigrostriata* and crayfish sampling) were used for genetic analysis. Mitochondrial DNA markers were chosen because they have high rates of mutation, are maternally inherited with no detectable recombination and have become a relatively cheap and easy analysis method (Moritz, Dowling *et al.* 1987; White, Wolff *et al.* 2008; Galtier, Nabholz *et al.* 2009). Two mtDNA genes were used, control region and cytochrome *b*, which are proven reliable markers for population genetic structure research in freshwater fish (Thacker, Unmack *et al.* 2007; Huey, Baker *et al.* 2008; Sousa, Penha *et al.* 2008). Tissue was taken from 126 specimens from 6 populations, resulting in 88 concatenated sequence pairs (control region and cytochrome *b*) from 87 *G. nigrostriata* and one *G. munda*. A known *G. munda* sequence was obtained (from N. Philips, Murdoch University) for comparison with *G. nigrostriata* sequences, to eliminate species misidentification. Following the determination (from genetic analyses) that all Deep River catchment specimens were *G. munda*, one *G. munda* sequence was used as the outgroup for analysis of the *G. nigrostriata* sequences.

All wetlands where fish were collected for genetic analyses had topographical features recorded to assess potential connectivity pathways. These features were examined by taking measurements of distance and heights between wetlands from aerial photos, satellite imagery and on-site using GPS, tape measures and a dumpy level working from existing AHD markers. Historic geomorphological changes to wetlands, river systems and topography in south-west WA were also examined using peer-reviewed journal papers to consider past connectivity between catchments.

5.2.1.1 DNA extraction

Galaxiella nigrostriata muscle tissue was removed from each specimen immediately following morphometric measurement (see 5.2.2 Morphological analyses) and placed into a 2 mL microtube for storage at -20°C. Approximately 2 mm^3 of muscle tissue from each fish was used for genomic DNA extraction using EDNA HISPEX™ (Fisher Biotech, Australia) following the manufacturers methods. To check DNA concentration levels following extraction, a 1.5% agarose solution gel block in TAE buffer was loaded with 5 μL template (extracted DNA) and 2 μL dye per lane, and 5 μL of 100 kb ladder (Promega, USA) in a separate lane for comparative sizing. The gel then ran for about 45 minutes at 80 V. The gel block was then removed and placed into a TAE/ethidium

bromide solution for 20 minutes to dye the block, then placed into a UV light box to be photographed for analysis.

5.2.1.2 PCR amplification

Polymerase chain reaction (PCR) was used to amplify a portion of the control region and cytochrome *b* for further analysis. Control region amplification used universal fish primers H16498 5' CCTGAAGTAGGAACCAGATG 3' (Meyer, Kocher *et al.* 1990) and 5' AACTCTCACCCCTAGCTCCCAAAG 3' (N. Philips, MU-CFFR, 2009, pers. com.). Primers used for cytochrome *b* were H15149 5' CCCTCAGAATGATATTTGTCCTCA 3' from Waters *et al.* (2000) who reduced the length from the original primer in Kocher *et al* (1989), and L14724 5' CGAAGCTTGATGAAAAACCATCGTTG 3' (Pääbo 1990). Each PCR reaction was made up of Fast Start buffer 10x, 5 units of Fast Start Taq and 25 μM of $MgCl_2$, (all Roche, Switzerland), 0.2 μM of primer (Geneworks, Australia), 200 μM each of dNTP (Roche, Switzerland) and DNA template (concentration of 50 ng/μL) and made to a total of 25 μL per sample with PCR-grade water. An Eppendorf thermocycler was used to amplify the target section of the two regions using the following protocol: Initial denaturation at 95°C for 10 min then 45 cycles of: 30 s denaturing at 95°C, 30 s annealing at 46°C (control region) or 48.8°C (cytochrome *b*), 1 min extension at 72°C, and a final extension at 72°C for 7 min. The PCR protocol was adapted from Philips *et al.* (2007) from their work on *G. munda*.

To assess PCR amplification, 5 μL of each amplified product were loaded onto a gel block (Figure 13) as per the DNA extraction analysis method above. Any samples that did not amplify on the first attempt were run through another PCR using a different dilution until the suitable dilution was determined. Once the required number of samples amplified successfully, they were run through a final PCR using Hifidelity Taq in preparation for sequencing. The protocol was similar to the previous PCR except for 1 μL of combined Taq buffer and $MgCl_2$, 10x (Roche, Switzerland) and 5 units/μL of Hifidelity Taq (Roche, Switzerland), and made to a total of 25 μL per sample with PCR-grade water per reaction.

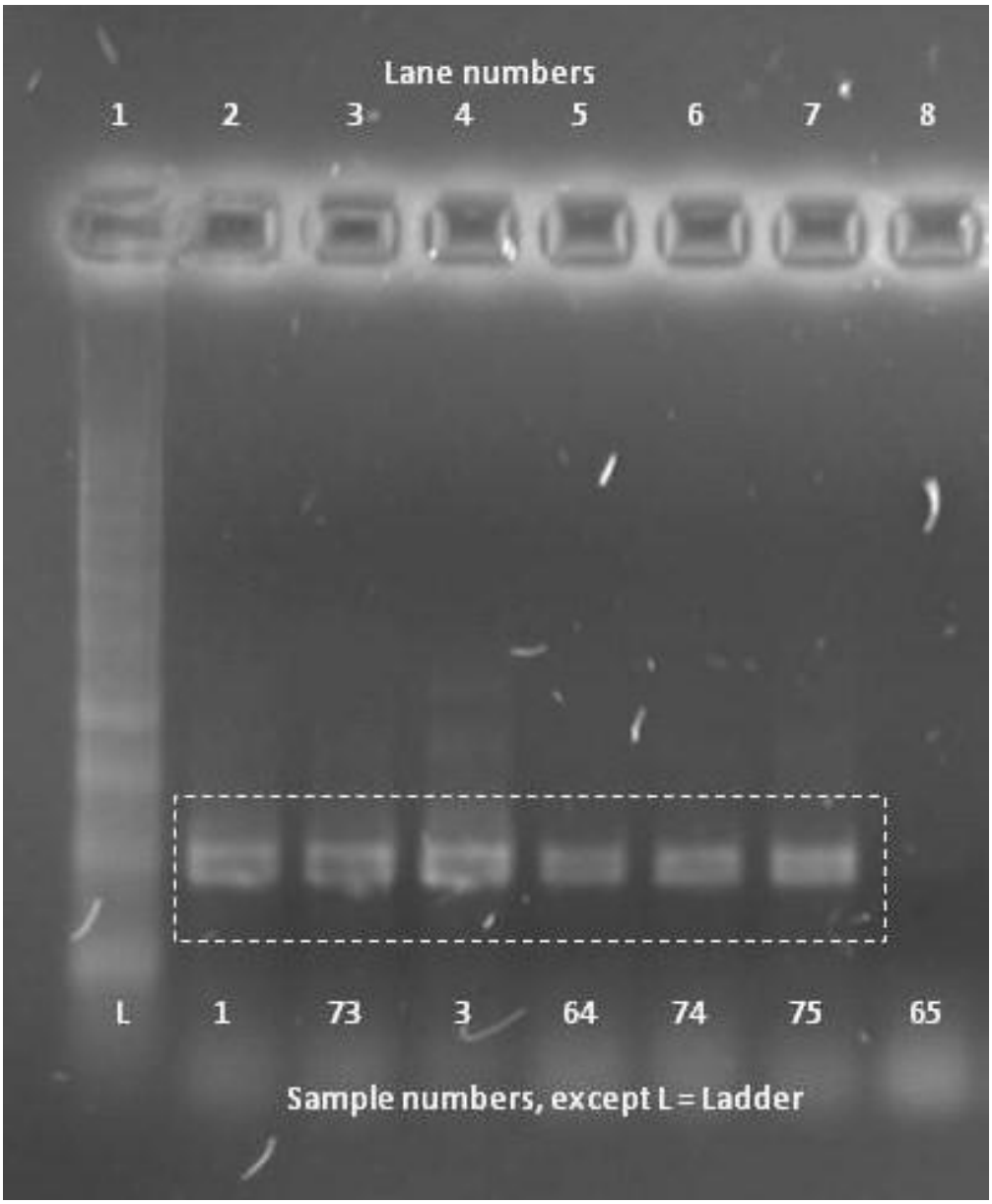

Figure 13. Example of a gel to test the success of a PCR on seven samples using control region primers and hifidelity Taq. Lane 1 is a 100kb ladder and the next six lanes contain samples that are viable for sequencing (inside dashed box). Lane eight did not amplify.

Twenty two samples (of both control region and cytochrome *b*) would not amplify after changing dilution rates or using the Hifidelity Taq, so a PCR was tried using Phusion Taq (Finnzymes, Finland) in the reaction mixture. The Phusion reaction mixture was made using: Taq buffer 10x (Finnzymes, Finland), 25 μM of $MgCl_2$, 0.2 μM each of forward and reverse primers, 0.2 μL dNTP (Roche, Switzerland), 0.1 μL of Phusion Taq (Finnzymes, Finland) and 1 μL of DNA template (concentration of 50 ng/μL) and made to a total of 25 μL per sample with PCR-grade water per reaction. The PCR protocol was initial denaturation at 98°C for 2 min then 45 cycles of 10 s denaturing at 98°C, 20 s annealing at 60°C (both control region and cytochrome *b*), 30 s extension at 72°C

and a final extension at 72°C for 5 min. Any samples that did not amplify after using this method were disregarded for future analysis.

Both the Phusion and Hifidelity Taq were not used for initial trial PCR reaction mixtures as it is considerably more expensive. Phusion Taq was only used as an alternative to Faststart Taq if initial amplification was unsuccessful. Hifidelity Taq is generally only used when reactions have been successfully amplified using standard Taq, and immediately prior to sequencing.

5.2.1.3 PCR Clean-up and sequencing

Successfully amplified reactions were then cleaned up prior to sequencing. Each reaction had 10 units of Exonuclease I and 2.5 units of Antarctic Phosphatase (both NEB, USA) added and was incubated for 30 minutes at 37°C then inactivated by heating the reaction to 80°C for 20 minutes. Approximately 20 μL of each reaction was then sent to Macrogen Inc. (South Korea) in a 96 well plate for sequencing, as they provide a fast, reliable and competitively priced service compared to local sequencing services. Each sample was sequenced in both directions using forward and reverse primers, resulting in two strands of DNA. Sequencing was carried out by Macrogen Inc. (South Korea) using an Applied Biosystems ABI 3730 48-capillary DNA analyser using Big Dye Terminator Technology according to the manufacturers protocols (Applied Biosystems, USA).

5.2.1.4 Analysing sequences

Each forward and reverse sequence was analysed initially with CodonCode Aligner 3.5.2. (CodonCode Corporation) for each *G. nigrostriata* specimen. CodonCode Aligner was used to trim the ends, assemble and align each forward and reverse sequence and finally assemble all of the sequences into one group alignment (for each gene), allowing all sequences to be compared. Sequence analysis for intra- and inter-population pairwise sequence distance (divergence) were calculated using the p-distance model with standard defaults in MEGA5 (Tamura, Peterson *et al.* 2011). Sequences were concatenated using DAMBE (Xia & Xie 2001) and grouped by haplotype to eliminate multiples of common haplotypes.

5.2.2 Morphological analyses

Galaxiella nigrostriata specimens that were collected during the habitat requirements field trips were also used for morphological analysis (see 2.3 General Methods). Specimens were collected from six populations: Melaleuca Park (*n* = 12), Kemerton (*n* = 22), Scott (*n* = 12), Gardner (*n* = 9), Shannon (*n* = 6) and Deep (*n* = 7). Prior to genetic analyses, all specimens collected were originally thought to be *G. nigrostriata*, therefore six *G. munda* specimens were borrowed for morphometric comparison. Three *G. munda* specimens were collected from Buayanyup Creek (S. Beatty, Murdoch University) and three from Marbelup Brook (P. Unmack, Brigham Young University). The seven specimens collected from the Deep population were determined by genetic analyses to be *G. munda*, so were used for morphometric comparison. All specimen collection sites are shown in Figure 14.

Galaxiella nigrostriata specimens used for morphological analyses were > 18 mm TL (juvenile to adult size) to ensure all features examined were fully formed; the pelvic fin is the last feature to form fully by 16.2 mm standard length (SL) (Neira, Miskiewicz *et al.* 1998). The pelvic fin is also last to form in *G. munda*, however, they were still not fully formed by 16.3 mm SL when collected during research by Gill and Neira (1994). The post-flexion size of *G. munda* is thought to be 12.8 - 16.3 mm SL (Gill & Neira 1994), therefore, specimens collected over 18 mm were expected to be fully formed and were used in this project.

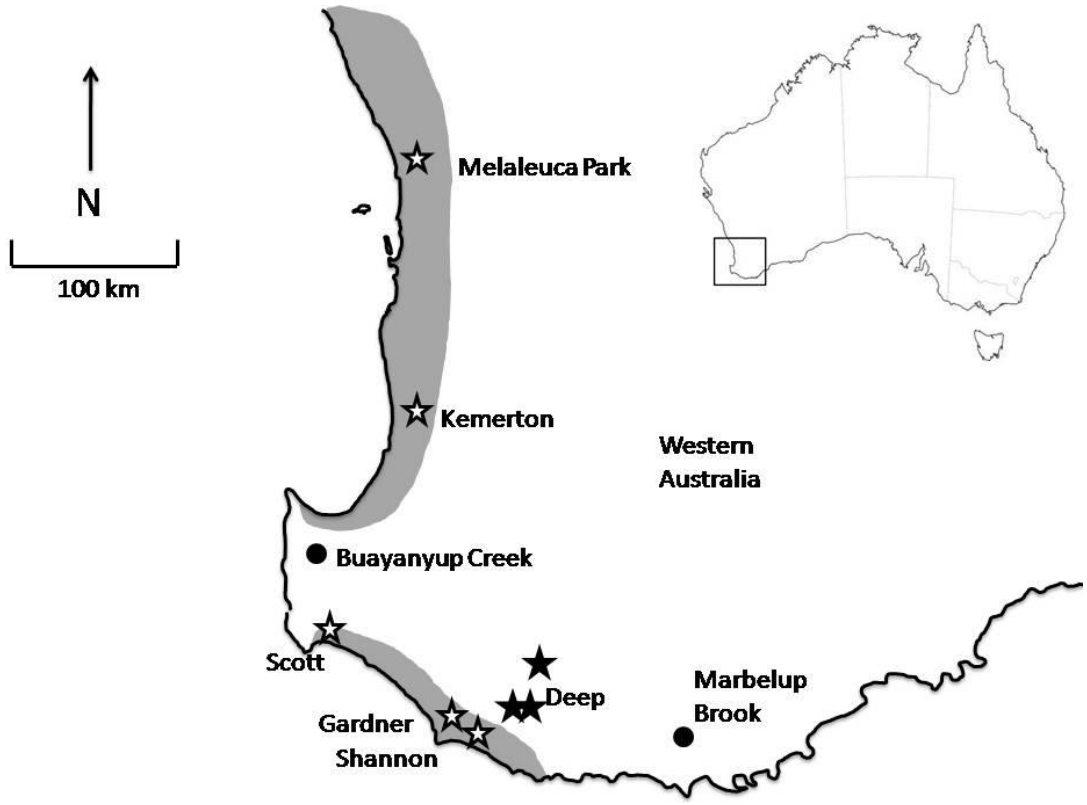

Figure 14. Specimen collection sites in south-west WA, with site (population) names given. Open stars are where *G. nigrostriata* were collected; full stars where *G. munda* were collected; full circles where borrowed *G. munda* were from. Northern grey shaded area is the Swan Coastal Plain; southern grey shaded area is the Scott Coastal Plain.

5.2.2.1 Morphometric measurements and meristic counts

Each specimen was thawed, padded dry and weighed to the nearest milligram. Each specimen also had tissue taken at this time for genetic analysis, so were placed on a tray sitting on ice while measurements were taken. Lowering the temperature reduces enzymatic activity in tissue, which would otherwise degrade DNA (Nagy 2010; Rodriguez-Ezpeleta, Mendibil *et al.* 2013). All morphometric measurements and meristic counts were taken under a dissecting microscope and from the left side of the fish. Meristic counts were taken by counting the number of rays (spines) in each of the following fins: pectoral (P), pelvic (Pv), anal (A), dorsal (D) and caudal (C).

Morphometric measurements were taken using digital callipers (Starrett, limit of detection 0.02 mm) of the following 11 features (see Figure 15): total length (TL), head length (HL), snout length (SnL), eye diameter (ED), length of base of dorsal fin (DL), length of pectoral fin (P_1L), length of pelvic fin (P_2L), length of base of anal fin (AL), length of caudal peduncle (CL), depth of caudal peduncle (CD) and body depth at anus (BD). The protocol for whether total length (tip of snout to posterior of tail fin) or standard length (tip of snout to the origin of the tail fin) is measured varies among

researchers. This project measured total length of all fish due to their small size and their tail fins were all in good condition[4]. Fin-nipping was not expected as *G. nigrostriata* was generally the only species found in each wetland. The sex of each specimen was not determined during examination. The meristic counts and morphometric measurements were adapted from methods by Gill and Neira (1994), Watts *et al.* (1995), Ling and Gleeson (2001), Smith and Knott (2002) and Tseng *et al.* (2009).

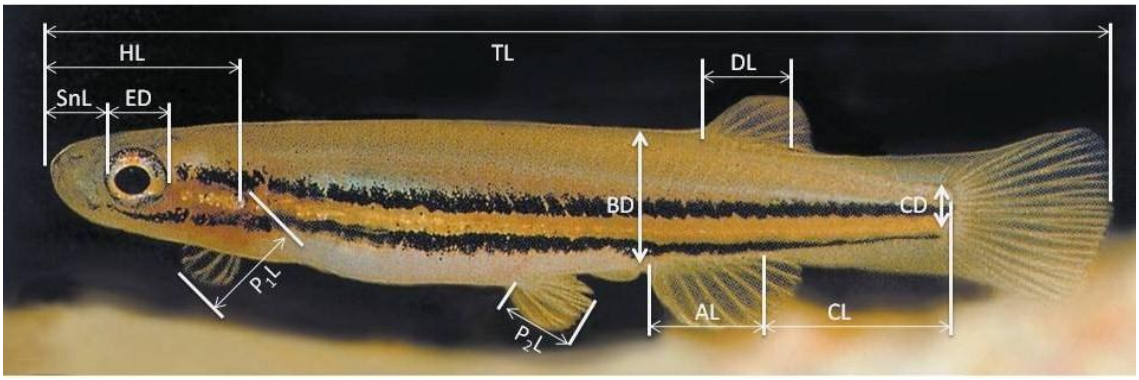

Figure 15. Morphological measurements on *G. nigrostriata*. TL = total length, HL = head length, SnL = snout length, ED = eye diameter, P_1L = length of pectoral fin, P_2L = length of pelvic fin, DL = length of base of dorsal fin, BD = body depth at anus, AL = length of base of anal fin, CL = length of caudal peduncle, and CD = depth of caudal peduncle. (Base photo courtesy of G. Allen)

[4] However, standard length is preferred by most researchers.

5.2.2.2 Data analyses

To be able to compare fish of different sizes, linear regression analysis tested whether each measured feature grew relative to total length, for example, total length was compared to body depth (r^2 = 0.86 ±0.26). After a significant linear regression was confirmed, all measurements were then standardised by dividing each variables' measurement by the total length, except for snout length and eye diameter which were divided by head length. Tables show all length data as standardised values, except total length. Ray counts are shown as mean values but are otherwise untransformed data. Standardised measurement data was normalised and count data underwent square root transformation once imported into PRIMER v6, (Clarke & Gorley 2006; O'Hara & Kotze 2010). Full statistical data analyses methods are in General Methods (2.4 Data analyses).

Data was compared by individual catchment to investigate recent morphological changes brought about by population isolation. To examine historic morphological changes of *G. nigrostriata* between coastal plains, catchment data was grouped within the Swan or Scott Coastal Plains.

5.3 Results

5.3.1 Genetic sequence data

5.3.1.1 All populations

Control region sequences resulted in 13 haplotypes from 366 base pairs (bp), of which 14 bp were variable, from 87 *G. nigrostriata* specimens. Control region inter-population sequence divergence ranged from 0.6 ±0.3% between Melaleuca Park and Kemerton, to 1.7 ±0.6% between Melaleuca Park and Scott and had a mean sequence divergence of 0.9 ±0.3% (Table 16). Haplotype diversity was higher in Kemerton (eight from 54 specimens) and the lowest was at Melaleuca Park (one from five specimens).

Cytochrome *b* sequences had more haplotypes and higher sequence diversity than control region sequences. Cytochrome *b* sequences produced 23 haplotypes from 374 bp, of which 34 bp were polymorphic, from 87 *G. nigrostriata* specimens. Cytochrome *b* inter-population sequence divergence ranged from 0.5 ±0.3% between Melaleuca Park and Kemerton, to 4.3 ±1.0% and had a mean sequence divergence of 2.1 ±0.4% (Table 16). Similar to control region, the highest diversity of Cytochrome *b* sequences was found at Kemerton (seven from 54 specimens) and the least at Melaleuca Park (one from five specimens).

Concatenated sequences resulted in 31 haplotypes, the most in Kemerton with 15 haplotypes followed by Scott and Gardner with six each, Shannon with three and Melaleuca Park had the least with one. Concatenated inter-population sequence divergence ranged from 0.5 ±0.2% between Melaleuca Park and Kemerton populations to 2.7 ±0.6% between Gardner and Shannon and had a mean sequence divergence of 1.4 ±0.3% (Table 17). All specimens collected from the Deep River catchment that were thought to be *G. nigrostriata* were determined by genetic analysis to be *G. munda*.

Table 16. Intra- and inter- population pairwise sequence divergence (%) for *G. nigrostriata* populations and one *G. munda* specimen for comparison. Inter-population figures are for mean divergence. Control region (unshaded) and cytochrome *b* (shaded).

		Cyt *b* Intra				CR Inter					
		n	min	max	mean	Melaleuca Park	Kemerton	Scott	Gardner	Shannon	*G. munda*
CR Intra	n					5	54	12	11	5	1
	min					0.0	0.0	0.0	0.0	0.0	-
	max					0.0	1.1	0.0	0.3	0.0	-
	mean					0.0	0.6	0.0	0.1	0.0	-
Cyt *b* Inter	Melaleuca Park	5	0.0	0.0	0.0		0.6	1.7	1.5	1.4	23
	Kemerton	54	0.3	0.8	0.4	0.5		1.6	1.3	1.0	23
	Scott	12	0.0	0.5	0.4	1.3	1.8		1.3	1.4	23
	Gardner	11	0.0	0.8	0.5	2.8	3.3	1.9		0.7	23
	Shannon	5	0.3	0.5	0.4	3.8	4.3	2.9	1.9		23
	G. munda	1	-	-	-	14	15	14	15	15	

Table 17. Mean pairwise divergence (%) for concatenated sequences for each population shown in the shaded area, unshaded area is SE.

	Melaleuca Park	Kemerton	Scott	Gardner	Shannon
Melaleuca Park		0.2	0.4	0.5	0.6
Kemerton	0.5		0.4	0.5	0.6
Scott	1.5	1.6		0.4	0.5
Gardner	2.1	2.2	1.6		0.4
Shannon	2.6	2.7	2.1	1.3	

5.3.1.2 Kemerton wetlands

The Kemerton wetlands contained haplotypes unique to both individual and multiple wetlands. Concatenated sequences produced 15 haplotypes with an average sequence divergence of 0.1 ±0.1%. One haplotype was common to all wetlands and nearly half of all specimens (Table 18). Four haplotypes were unique to two wetlands north of the dredge ponds, while 10 were unique to the three wetlands to the south.

Table 18. Distribution of concatenated control region and cytochrome *b* haplotypes in the Kemerton wetlands. Shaded haplotypes indicate southern wetland populations.

Haplotype #	EPP9 *n* = 11	PN *n* = 12	EPP6 *n* = 11	EPP1 *n* = 9	EPP5 *n* = 11	Total *n* = 54
1	1					1
2		1				1
3		1				1
4	3	2				5
5				2		2
6			1	3	1	5
7				1	1	2
8					1	1
9			2			2
10					1	1
11					1	1
12			1			1
13			1			1
14			2			2
15	7	8	3	4	6	28

5.3.2 Population connectivity

5.3.2.1 Melaleuca Park

When sampled in late spring 2008, the Melaleuca Park wetlands (EPP173 and Mb) were connected by extremely shallow streams (<20 mm). The nearest waterbody, Ellen Brook which feeds into the Swan River, is ~5 km east of the Melaleuca Park wetlands. The ephemeral creek connecting the wetlands and Ellen Brook has been heavily modified due to agricultural use of the land.

5.3.2.2 Kemerton wetlands

Wetlands north of the dredge ponds were on undulating land, compared to wetlands south of the dredge ponds. Consequently, water level height varied more between the northern wetlands (1.3 m), compared the southern wetlands (0.4 m) (Table 19).

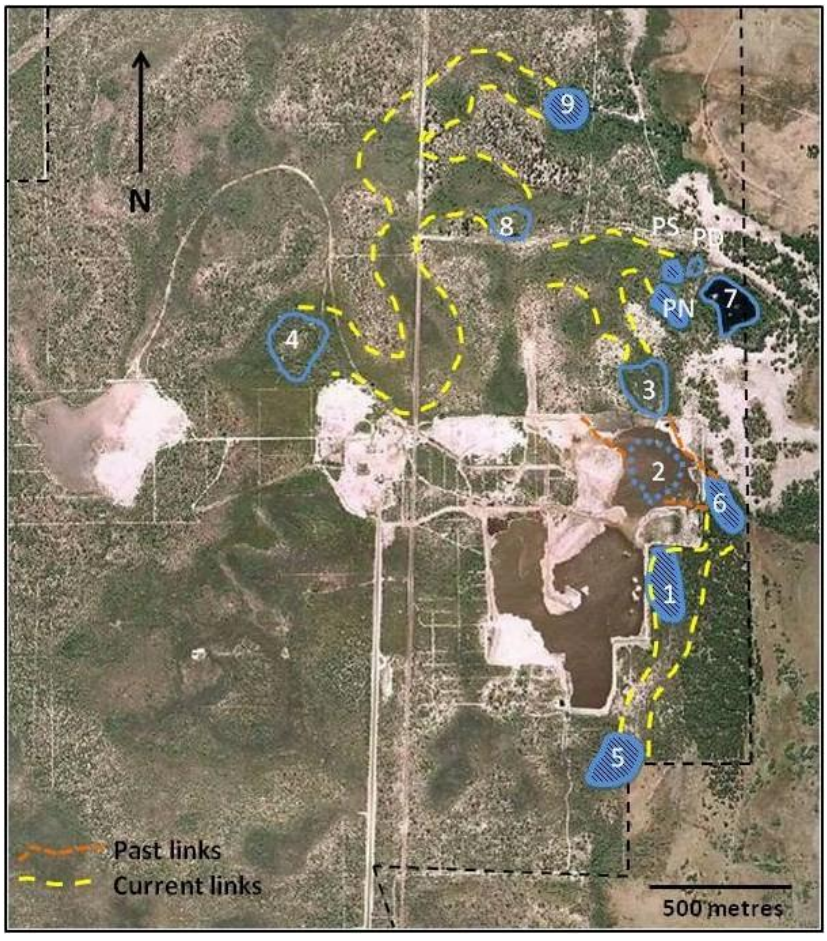

Figure 16. Potential connectivity between Kemerton wetlands. Numbered wetlands are EPP wetlands. Links follow natural low-lying ground or man-made drains. Striped wetlands contained *G. nigrostriata* during 2008/09 sampling. EPP2 is now an ex-dredge pond. Wetlands names from McCullough *et al.* (2008).

Table 19. Topographical features surrounding the Kemerton wetlands, listed north to south.

Wetland	*G. nigrostriata* present	Wetland water level Nov 2009 (m AHD)	Nearest wetland (Distance - Table 20)	Landscape between nearest wetland
EPP9	Yes	14.5	EPP8	Undulating
EPP8	No	14.7	EPP9	Undulating
PD	No	13.9	PS	Undulating
PS	Yes	13.9	PD	Undulating
PN	Yes	13.9	EPP7	Flat
EPP7	No	13.7	PN	Undulating
EPP4	No	15.0	EPP8	Flat
EPP3	No	13.7	PN	Undulating
EPP6	Yes	12.5	EPP1	Flat
EPP1	Yes	12.3	EPP6	Flat
EPP5	Yes	12.1	EPP1	Flat

Table 20. Distance (m) between the Kemerton wetlands. Lineal distances shown, actual exploitable distances through low-lying areas or drains would be greater.

	EPP9	EPP8	PD	PS	PN	EPP7	EPP4	EPP3	EPP6	EPP1
EPP8	340									
PD	700	690								
PS	690	660	30							
PN	810	690	200	150						
EPP7	800	745	85	75	75					
EPP4	1 280	920	1 530	1 490	1 405	1 525				
EPP3	1 060	840	520	475	275	340	1 300			
EPP6	1 510	1 300	890	860	680	625	1 710	390		
EPP1	1 675	1 410	1 150	1 115	910	920	1 605	570	290	
EPP5	2 350	2 025	1 885	1 830	1 625	1 650	1 905	1 290	995	675

5.3.2.3 Scott Coastal Plain

Scott River

The roadside pools sampled were along a 4.5 km stretch on Scott River Road and were within damplands surrounding the Scott River. There are numerous pools dug for construction/maintenance of the unsealed Scott River Road and natural wetlands on the relatively flat plain, which are thought to dry every summer. The wetlands/pools are linked via culverts under the road, drainage ditches and temporary streams or sheet flow with heavy or constant rain, and are about 1.7 km from the Scott River. The only other fish species captured in these wetlands during this project were *L. salamandroides*. *Lepidogalaxias salamandroides* is another aestivating species which are also mostly found in seasonal wetlands (Allen & Berra 1989).

Gardner River

The Gardner population was found in roadside pools and wetlands spread 1.5 km along Chesapeake Road West, 2.4 km west of the Gardner River and in very similar conditions to the Scott River Road population. These wetlands contained *G. nigrostriata* and *L. salamandroides*, however, *N. balstoni* and *B. porosa* were also captured which suggests connectivity to a permanent waterbody (Morgan & Gill 2000).

Shannon River

Galaxiella nigrostriata and *L. salamandroides* were the only fishes found and only in one of the four Shannon wetlands sampled. The wetlands sampled were a mix of roadside pools and wetlands and were 6.5 km west of the Shannon River and 9.5 km east of the Gardner River. The Shannon wetlands had the highest height variation, 22-44 mAHD; the fish were caught in the lowest wetland; and the greatest distance between wetlands was 5.2 km. The fish were found in the lowest wetland, which was in an area of damplands with numerous small (probably intermittent) streams and other wetlands nearby.

Table 21. Properties of wetlands sampled for genetic analysis. Connectivity frequency - Regular: often during average winter rainfall, Occasional: short periods most years during average - above average winter, Rarely: maybe short periods in a season, many years apart, only above average winter rainfall

Catchment	Number of wetlands sampled (*G. nigrostriata* present)	Number of wetlands within each complex	Average lineal distance (m) between wetlands	Height range between wetlands (m AHD)	Possible connectivity modes within wetland complex	Connectivity frequency	Surrounding landscape
Melaleuca Park	2 (1)	2	20	0	Surface	Regular	Slightly undulating, crown land neighbouring agricultural land
Kemerton	11 (7)	11	>100	3	Contours	Rarely	Slightly undulating, mining lease/nature reserve, neighbouring agricultural land
Scott	4 (4)	>6	>100	4	Culverts/ surface	Occasional	Flat, floodplain, national park
Gardner	4 (4)	>6	>30	6	Culverts/ surface	Occasional	Flat, damplands, national park
Shannon	4 (1)	>6	>3 000	21	Culverts	Occasional	Undulating, lower areas contain damplands, national park

Table 22. Distance (km) between catchment populations sampled. Lineal distances shown, actual exploitable distances through watercourses or adjoining wetlands would be greater.

	Melaleuca Park	Kemerton	Scott	Gardner
Kemerton	157			
Scott	293	136		
Gardner	340	182	96	
Shannon	349	194	112	14

5.3.3 Morphometric analysis

The minimum linear distance between *G. nigrostriata* populations in separate catchments was 15 km (Gardner - Shannon) and maximum 350 km (Melaleuca Park - Shannon). A total of 74 specimens had meristic counts and morphological measurements taken prior to tissue removal for the genetic analyses. Some specimens could not be included for all counts or measurements due to damage while in storage or their fragility once thawed (numbers used are listed with each graph). All morphometric measurement and meristic count data are listed in Appendix 8.2 and 8.3 respectively.

Genetic analyses determined seven of the specimens collected during habitat requirement field trips were actually *G. munda*, all from Deep River populations. The wetlands sampled for this project in Deep River catchment had been sampled previously and contained either both species (BR1, MR1-2) or only *G. nigrostriata* (TR1-3) (Morgan, Gill *et al.* 1998; Storey 1998). No wetlands sampled contained *G. nigrostriata* and *G. munda* cohabitating. Therefore, of the 74 specimens used for morphometric analysis mentioned above, 61 were *G. nigrostriata* and 13 were *G. munda* (seven from the habitat requirements field trip and six borrowed).

5.3.3.1 *Galaxiella nigrostriata* morphology in south-west Western Australia

A significant difference was found for morphological differences between all populations when analysed together (ANOSIM Global R = 0.247; p <0.001). The most similar populations were Melaleuca Park and Kemerton, both in the Swan Coastal Plain and 157 km apart, with an average dissimilarity of 30.39. The Melaleuca Park and Gardner *G. nigrostriata* populations, separated by 340 km, were the most morphologically different with an average dissimilarity of 40.44.

The biggest statistically significant morphological difference between Melaleuca Park and Gardner *G. nigrostriata* specimens was the number of dorsal fin rays. Melaleuca Park specimens had a mean of 8.4 ±0.2 (range 7-9), compared to with 7.0 ±0.2 (range 6-8) (Figure 17, Table 23). Pelvic fin length had a statistically significant difference, being longer in Gardner specimens with a mean of 7.0 ±0.1% (range 6.1-7.6%) (standardised - percentage of total length) and Melaleuca Park had a mean of 5.3 ±0.3 (range 3.7-6.5%). Body depth and caudal peduncle depth were both deeper in Gardner with means of 13.5 ±0.5% (range 11.7-16%) and 17.3 ±0.5% (range 14.8-19.5%) respectively, compared with Melaleuca Park's body depth mean of 12.0 ±0.2% (range 11.2-12.7%) and caudal peduncle depth mean of 5.7 ±0.2% (range 5.1-7.0%).

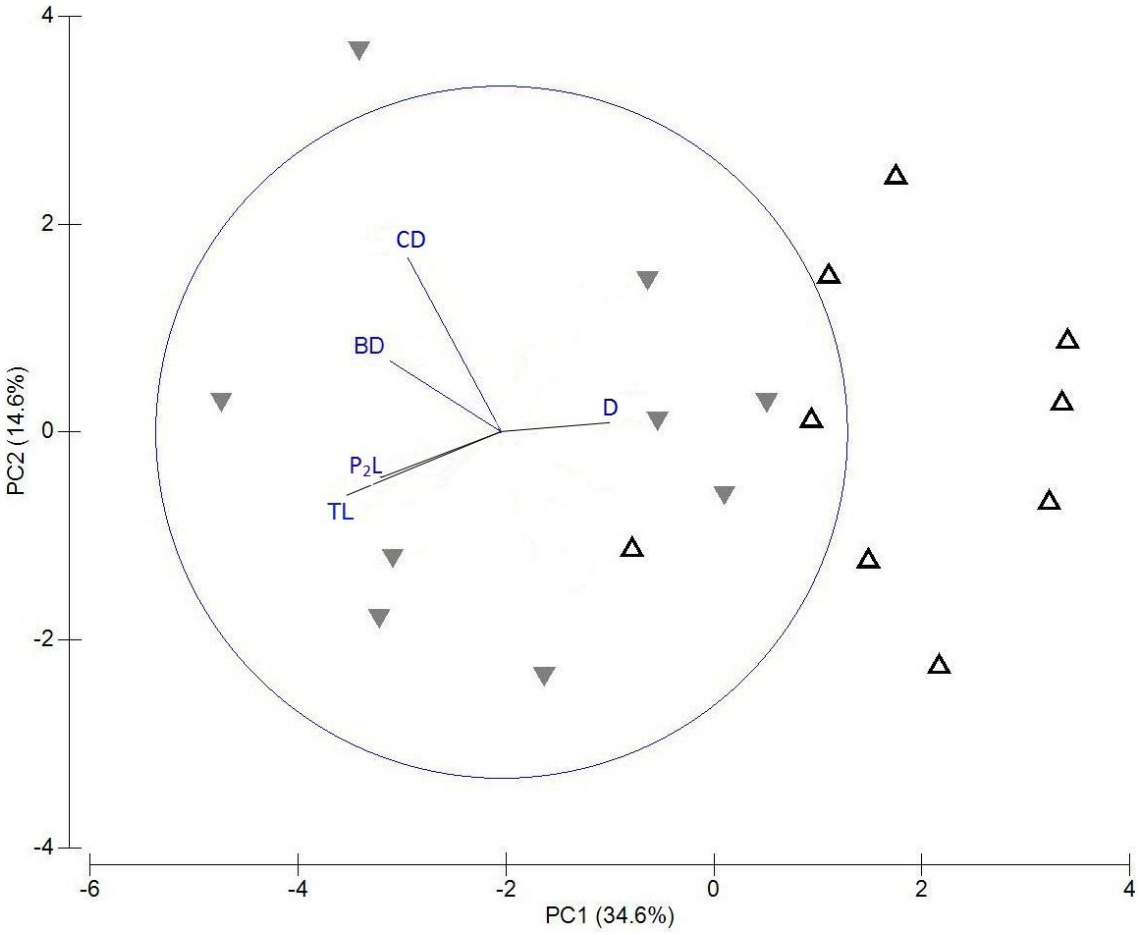

Figure 17. PCA analysis of *G. nigrostriata* morphological variables from Melaleuca Park and Gardner populations. Only statistically significant variables contributing >6% morphological dissimilarity between the two populations are shown. Black open triangles are from Melaleuca Park (*n* = 9), grey filled triangles are from Gardner (*n* = 9).

Table 23. Results of SIMPER analyses and statistical testing for *G. nigrostriata* morphological variables between Melaleuca Park and Gardner populations. Variables are standardised values (variable/TL), except for TL (mm) and D (ray count). Variables are arranged from highest to lowest by mean dissimilarity between regions. Only variables with >6% contribution listed. Contribution percentage shows how much each variable contributed to overall dissimilarity between regions. Statistically significant morphological differences between Melaleuca Park and Gardner wetlands are shown in bold. Test statistics used were ANOVA (* $F_{1,19}$) and Kruskal-Wallis ($^{\#}\chi^2_{1,20}$).

Variable	Mean standardised value Melaleuca Park (±SE)	Mean standardised value Gardner (±SE)	Mean dissimilarity % (±SD)	Contribution %	Cumulative %	Statistic	*p* value
D (*n*)	8.4 ±0.2	7.0 ±0.2	3.84 ±1.02	9.41	9.41	**10.08**$^{\#}$	**<0.01**
P_2L	5.2 ±0.25	7.0 ±0.14	3.46 ±1.04	8.49	17.90	**22.07***	**<0.01**
W	0.27 ±0.04	0.47 ±0.07	3.25 ±0.79	7.97	25.86	2.43$^{\#}$	0.12
BD	12.0 ±0.2	13.5 ±0.5	3.21 ±0.77	7.87	33.74	**13.85***	**<0.01**
TL (mm)	22.0 ±0.9	25.8 ±1.6	3.11 ±0.82	7.63	41.37	**4.78***	**<0.05**
CD	5.7 ±0.2	6.3 ±0.2	2.99 ±0.65	7.33	48.70	**4.69**$^{\#}$	**<0.05**
DL	6.7 ±0.2	6.3 ±0.3	2.77 ±0.96	6.79	55.49	1.67*	0.24

5.3.3.2 *Galaxiella nigrostriata* morphology from Swan and Scott Coastal Plains

No statistically significant difference was found for morphometric data analysed between populations within either the Swan or Scott Coastal Plains. However, a significant difference was found when the morphological data was compared between the grouped populations from the Swan Coastal Plain and Scott Coastal Plain (ANOSIM Global R = 0.189, p <0.001). The most statistically significant difference between the coastal plain populations was the number of dorsal fin rays, with the Swan Coastal Plain mean of 8.1 ±0.1 (range 7-9) and the Scott Coastal Plain mean was 7.2 ±. 0.1 (range 6-9) (Figure 18 and Table 24). Head length and dorsal fin base length had the next most statistically significant differences, with Swan Coastal Plain means of 19.8 ±0.2% (range 17.3-21.8%) and 6.7 ±0.1% (range 5.3-7.6%) respectively, compared to Scott Coastal Plain head length means of 18.9 ±0.3% (range 15.8-23.0%) and dorsal fin base length mean of 6.2 ±0.1% (range 5.2-7.9%).

The pelvic fin was longer in Scott Coastal Plain populations, with a mean of 6.6 ±0.2% (range 5.2-8.4%) compared to the Swan Coastal Plain with a mean of 5.7 ±0.2% (range 3.7-7.6%). Eye diameter was larger in Swan Coastal Plain specimens with a mean of 34.6 ±0.7% (range 28.8-42.3%) compared to a mean of 33.4 0.8% (range 24.5-40.6%) on the Scott Coastal Plain. Even though some *G. nigrostriata* features were longer or larger in the Swan Coastal Plain, on average Scott Coastal Plain specimens were larger overall (Table 25). *Galaxiella nigrostriata* total length had a statistically significant difference between coastal plains (ANOVA $F_{1,68}$ = 6.29, p <0.01), with average total lengths increasing in each southern population (Table 25).

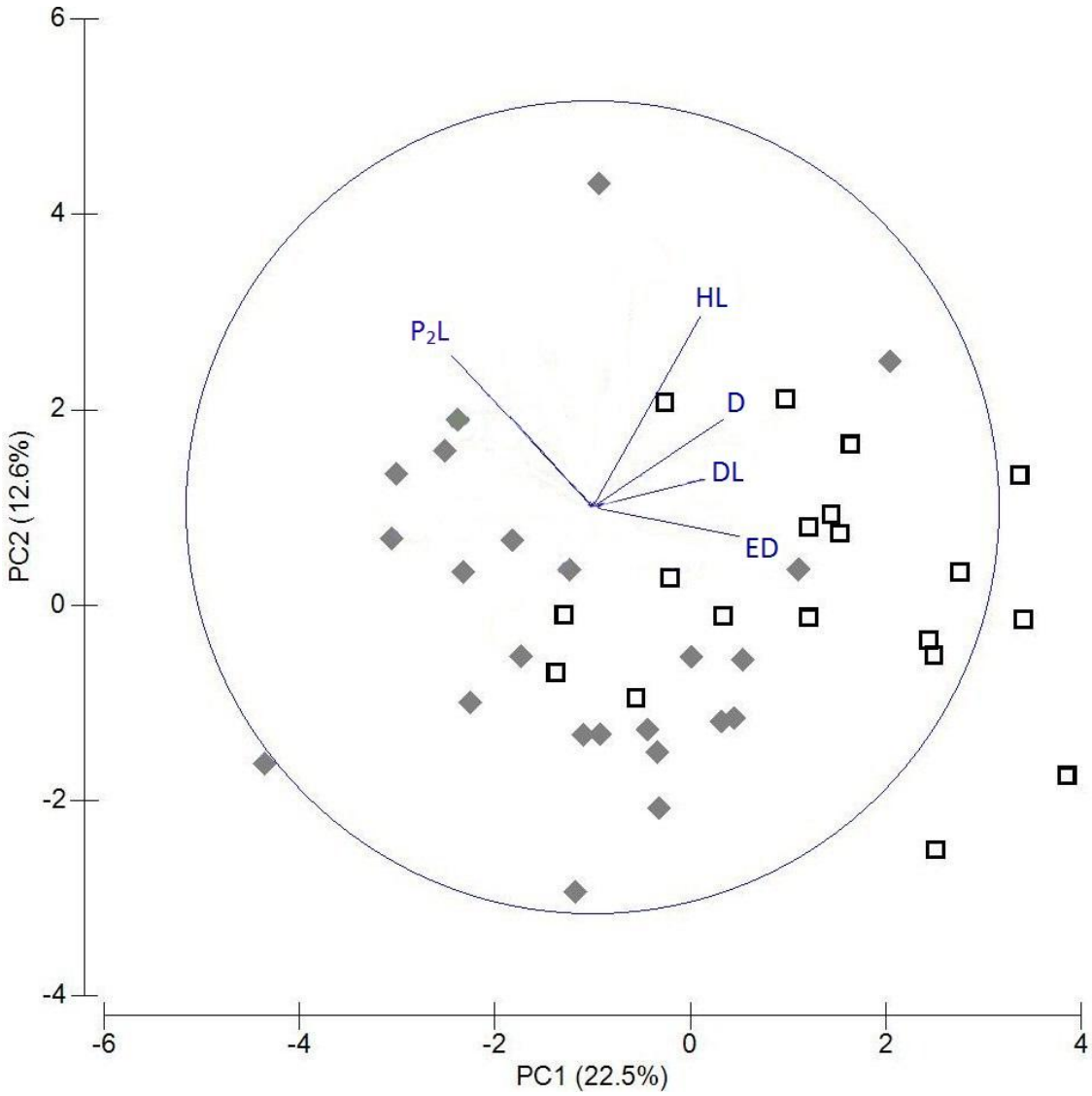

Figure 18. PCA analysis of all *G. nigrostriata* morphological variables showing differences between Swan and Scott Coastal Plain populations. Black open squares are from the Swan Coastal Plain populations (*n* = 26), grey filled diamonds are from the Scott Coastal Plain (*n* = 26). Only statistically significant variables contributing >6% difference are shown.

Table 24. Results of SIMPER analyses and statistical testing of *G. nigrostriata* morphological differences between grouped populations within the Swan and Scott Coastal Plains. Variables are standardised values (variable/TL) except for D (ray counts), and are arranged from highest to lowest by mean dissimilarity between regions. Only variables contributing >6% listed. Contribution values show how much each variable contributed to the overall differences between coastal plains. All variables were statistically significant and are shown in bold. Test statistics used were ANOVA (* $F_{1,51}$; ^ $F_{1,56}$) and Kruskal-Wallis ($^{\#}\chi^2_{1,57}$).

Variable	Mean standardised value Swan (±SE)	Mean standardised value Scott (±SE)	Mean dissimilarity % (±SD)	Contribution %	Cumulative %	Statistic	*p* value
D (*n*)	8.1 ±0.1	7.3 ±0.1	2.79 ±1.00	7.65	7.65	**13.58**$^{\#}$	**<0.01**
P_2L	4.7 ±0.4	6.6 ±0.2	2.49 ±0.81	6.83	14.48	**12.64***	**<0.01**
DL	6.7 ±0.1	6.2 ±0.1	2.35 ±0.97	6.45	20.93	**10.14**$^{\wedge}$	**<0.01**
ED	6.8 ±0.1	6.3 ±0.1	2.28 ±0.74	6.26	27.19	**9.10**$^{\wedge}$	**<0.01**
HL	19.8 ±0.2	18.8 ±0.3	2.25 ±0.75	6.18	33.37	**7.64**$^{\wedge}$	**<0.01**

Table 25. Total length and weight of *G. nigrostriata* within individual populations and coastal plains, listed approximately from north to south.

Coastal Plain	Catchment (sample size)	Total Length (mm)		Weight (g)	
		Range	Average (±SE)	Range	Average (±SE)
	Melaleuca Park (*n* =12)	18.5 - 30.2	22.6 ±1.1	0.03 - 0.19	0.07 ±0.01
	Kemerton (*n* =19)	17.3 - 31.1	23.0 ±0.9	0.01 - 0.19	0.07 ±0.01
Swan (*n* = 31)		17.3 - 31.3	22.8 ±0.7	0.01 - 0.19	0.07 ±0.01
	Scott (*n* =11)	18.8 - 30.8	26.1 ±1.2	0.02 - 0.17	0.10 ±0.01
	Gardner (*n* =9)	20.2 - 33.9	25.8 ±1.6	0.04 - 0.29	0.13 ±0.03
	Shannon (*n* = 6)	22.2 - 29.7	26.0 ±1.0	0.05 - 0.16	0.08 ±0.01
Scott (*n* = 26)		18.8 - 33.9	26.0 ±0.8	0.02 - 0.29	0.11 ±0.01

5.3.3.3 Morphological differences between *Galaxiella nigrostriata* and *Galaxiella munda*

Of all the variables measured, weight and total length accounted for over 16% of the dissimilarity between the species, but neither was statistically significant (Table 26). *Galaxiella munda* was heavier and longer with means of 0.13 ±0.03 g (range 0.02-0.41 g) and 28.0 ±2.3 mm (range 18.3-46.1 mm) respectively, compared to *G. nigrostriata* mean weight of 0.09 ±0.01 g (range 0.01-0.29 g) and mean total length of 24.4 ±0.5 mm (range 17.3-33.9 mm). The longest *G. nigrostriata* recorded was 34 mm from Gardner and *G. munda* was 46 mm from Marbelup Brook (Table 26).

The next most dissimilar variable that did have a statistically significant difference was pectoral fin rays; *G. nigrostriata* had more with a mean of 12.6 ±0.1 (range 11-14) compared to a mean of 10.9 ±0.1 (range 10-12) for *G. munda. Galaxiella munda* had a statistically significant longer anal fin base, mean 11.5 ±0.4% (range 9.2-13.7%) compared to *G. nigrostriata* with a mean of 9.8 ±0.2% (range 6.8-12.8%) (Table 27). Consequently, *G. nigrostriata* also had less anal fin rays with a mean of 10.3 ±0.1 (range 9-12) and *G. munda* had 11.4 ±0.2 (range 10-13) (Kruskal-Wallis $^{\#}\ \chi^2_{1,68} = 15.38$, $p < 0.01$). A statistically significant morphological difference was found when all measurements and counts were compared between *G. nigrostriata* and *G. munda* (ANOSIM Global R = 0.685, $p < 0.001$) (Figure 19, Table 27).

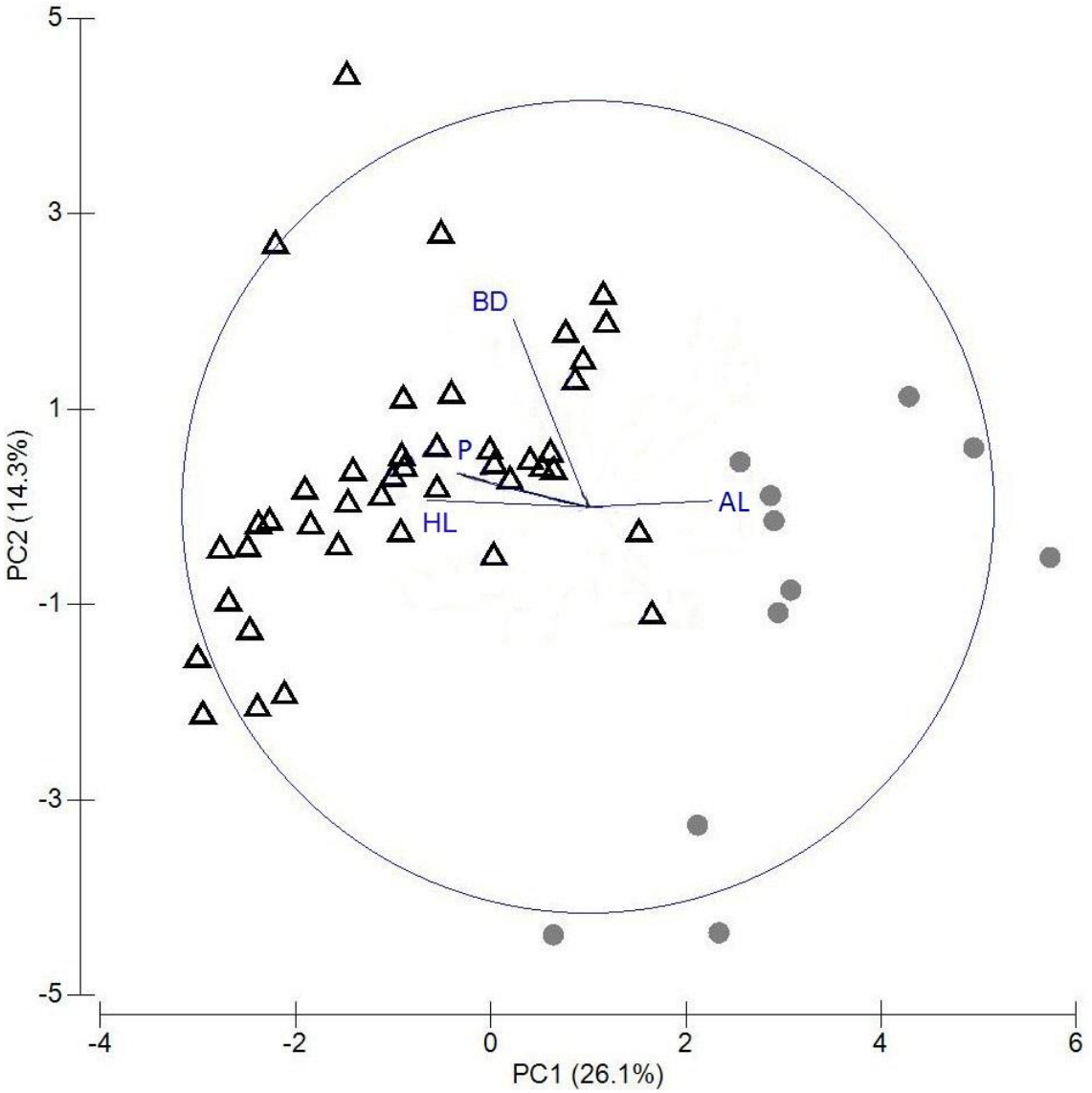

Figure 19. PCA analysis of all morphological differences between *G. nigrostriata* (black open triangles, *n* = 55) and *G. munda* (grey filled circles, *n* = 12). Only statistically significant variables contributing >6% difference shown.

Table 26. Total lengths and weights of all *G. nigrostriata* and *G. munda* used for this study.

	Total length (mm)		Weight (g)	
	Range	Average (±SE)	Range	Average (±SE)
G. nigrostriata (*n* = 61)	17.3 - 33.9	24.4 ±0.5	0.01 - 0.29	0.09 ±0.01
G. munda (*n* = 13)	18.3 - 46.1	28.0 ±2.3	0.02 - 0.41	0.13 ±0.03

Table 27. Results of SIMPER analyses and statistical testing of morphological differences between *G. nigrostriata* and *G. munda*. Variables are standardised values (variable/TL) except for TL (mm) and P (ray counts), and are arranged from highest to lowest by mean dissimilarity between regions. Only variables contributing >6% listed. Contribution values show how much each variable contributed to the overall differences between the two species. Variables that were statistically significant are shown in bold. Test statistics used were ANOVA (* $F_{1,68}$; $^{\phi}$ $F_{1,67}$) and Kruskal-Wallis ($^{\#}$ $\chi^2_{1,69}$, § $\chi^2_{1,67}$).

Variable	Mean standardised value *G. nigrostriata* (±SE)	Mean standardised value *G. munda* (±SE)	Mean dissimilarity % (±SD)	Contribution %	Cumulative %	Statistic	*p* value
W	0.33 ±0.02	0.39 ±0.08	4.40 ±0.58	9.18	9.18	0.31$^{\#}$	0.58
P (*n*)	12.6 ±0.1	10.9 ±0.1	3.74 ±1.17	7.80	16.98	**30.44§**	**<0.01**
HL	19.4 ±0.2	16.5 ±0.3	3.58 ±1.04	7.46	24.44	**46.54***	**<0.01**
TL (mm)	24.4 ±0.5	28.0 ±2.3	3.56 ±0.65	7.43	31.86	1.74$^{\#}$	0.19
BD	12.6 ±0.1	10.8 ±0.2	3.22 ±0.81	6.72	38.59	**25.68$^{\phi}$**	**<0.01**
AL	9.8 ±0.2	11.5 ±0.3	2.95 ±0.85	6.14	44.73	**17.84$^{\phi}$**	**<0.01**

5.4 Discussion

5.4.1 Genetic diversity in all populations

The low genetic divergence rates between *G. nigrostriata* populations between each coastal plain indicate that gene flow has not occurred for possibly thousands of years. The low genetic divergence rates support the results of Smith *et al.* (2002), who compared *G. nigrostriata* populations at Melaleuca Park and near Northcliffe. However, the consequences of low genetic divergence rates are not limited to the remnant populations on the Swan Coastal Plain. *Galaxiella nigrostriata* dispersal is restricted between catchments due to their preference for isolated seasonal wetlands and lentic waters (Pusey & Edward 1990; Morgan & Gill 2000). Without the use of lotic systems to migrate between wetlands, gene flow is significantly affected. Consequently, metapopulation theory cannot be applied to *G. nigrostriata* populations in different catchments, since one of the requirements is for occasional interaction between populations.

The divergence rate within Melaleuca Park was zero, with only one haplotype found. Even though there was a small sample size at Melaleuca Park (n = 5), the Shannon wetlands produced three haplotypes from the same sample size. Furthermore, all populations except for Melaleuca Park had a similar intrapopulation divergence rates for cytochrome *b* (>0.5%) regardless of sample size. It is possible haplotypes were missed at Melaleuca Park. However, it is also possible a bottleneck has occurred since it is an isolated single wetland and the nearest known population is nearly 200 km away, which was also suggested by Smith *et al* (2002). Additionally, the use of two genes increases statistical power and reduces the possibility of missing haplotypes. Prolonged isolation and lack of genetic diversity generally leads to inbreeding depression and deleterious mutation accumulations within the population, which greatly raises the risk of extinction (Gaggiotti & Hanski 2004).

The haplotype numbers in each catchment suggest that single wetlands have low genetic diversity of *G. nigrostriata*, while wetlands that are part of a complex have higher diversity, most likely due to intermittent migrations between neighbouring populations. The lower genetic diversity shown at Melaleuca Park shows limited gene flow when compared to other populations sampled. For example, even though the wetlands within Kemerton are not close enough to connect on a regular basis, occasional large storms or extended wet periods could facilitate migration enabling transfer of genetic stock.

When examining concatenated sequences in the three Scott Coastal Plain populations, Gardner and Shannon had the least inter-population genetic divergence (1.3%). The two populations are in neighbouring catchments with small hills and numerous wetlands between them. It is interesting though that concatenated sequences in Melaleuca Park and Kemerton had lower genetic divergence (0.5%) than the Scott Coastal Plain populations but were 10 times further apart and have three major river systems separating them. The difference in divergence suggests *G. nigrostriata* populations on the Swan Coastal Plain had extensive connectivity much more recently than Gardner and Shannon. However, given the physical distances involved, further investigation is required to substantiate this possibility.

Similarly, the two Swan Coastal Plain populations have lower genetic divergence with Scott than with Gardner or Shannon populations. Beard (1999) explained how the Beaufort and Arthur Rivers used to drain into the Collie River (on the Swan Coastal Plain) prior to uplift of the Jarrahwood Axis around Late Eocene (40 - 34 Mya). Following this event the Beaufort and Arthur Rivers diverted to the Blackwood River, which drains through the eastern boundary of the Scott Coastal Plain today. Considering Gardner and Shannon are more divergent to the other populations, *G. nigrostriata* may have originated on the Swan Coastal Plain. However, this does not agree with the migration theory of Chilcott *et al.* (1996) that *Galaxiella* moved from east to west across southern Australia. Alternatively, Gardner and Shannon populations may have gone through a bottleneck similar to *G. pusilla* during the last glacial maximum about 20,000 years ago (Unmack, Bagley *et al.* 2012). Further genetic analyses and biogeographic research would be required to support this new theory.

The interpopulation divergence rates for *G. nigrostriata* (0.6-1.7% for control region) reflect rates from closely related Galaxiid species such as *Neochanna diversus* (Gleeson, Howitt *et al.* 1999) and *N. burrowsius* (Davey, O'Brien *et al.* 2003). These species are found in marginal wetlands in lowland areas and also have the ability to aestivate. Conversely, Waters *et al.* (2000) determined interspecific divergence (cytochrome *b*) of 7.8% for the lacustrine *Paragalaxias* and 17.2% for *Neochanna*, which is comparable to the 15% divergence reported here between *G. nigrostriata* and *G. munda*. Therefore, the divergence determined from this research do not suggest speciation is occurring between *G. nigrostriata* populations.

5.4.2 Genetic diversity within the Kemerton wetlands

Two of the Kemerton wetlands contained six haplotypes each, two thirds of all haplotypes found in the five wetlands sampled. The finding of one common haplotype within all wetlands suggests they have all connected in recent time. Similarly, of the remaining haplotypes two thirds were unique to the southern wetlands, the other third only found in the northern wetlands. This division suggests limited or no recent connectivity between the northern and southern wetlands. The genetic division precedes the modification of EPP2 into a dredge pond, which implies there was another barrier to migration. Currently, it is unlikely *G. nigrostriata* would traverse the dredge ponds as they are permanently inundated and contain potentially aggressive or predatory species such as *Bostockia porosa*, *Nannoperca vittata*, *Gambusia holbrooki* and *Galaxias occidentalis*. Given the relative flatness of the area, it is very possible connectivity is still occurring between populations within northern and southern wetlands during times of high winter rainfall. Analyses using mtDNA microsatellite markers would provide more precise data on immigration patterns of *G. nigrostriata* within the Kemerton wetlands (Selkoe & Toonen 2006).

5.4.3 Morphological differences of Galaxiella nigrostriata

Melaleuca Park and Gardner were identified as the two catchments having the biggest morphological differences and were also the greatest geographic distance apart. Based on the level of genetic divergence between the two populations, they have been separated for possibly thousands of years, and may have adapted to local conditions. *Galaxiella nigrostriata* were slightly, although statistically significantly, larger in the Scott Coastal Plain wetlands which may be due to different spawning times (see below). However, dorsal fin ray counts were higher on Swan Coastal Plain specimens. Latitudinal effects on fish morphology can be influenced by environmental factors such as temperature changes, decreased hydroperiod and connectivity between wetlands and prey availability (Baber, Childers *et al.* 2002; Munch & Conover 2002; O'Reilly & Horn 2004).

Sampling took place over four weeks, starting on the Swan Coastal Plain and finishing in the south-west region (including the Scott Coastal Plain). Collecting was carried out in this order to negate the effects of variable spawning times. Spawning starts around June on the Scott Coastal Plain (Gill & Neira 1994; Morgan, Gill *et al.* 1998) and around July - August on the Swan Coastal Plain (Smith, Knott *et al.* 2002; Bamford & Bamford 2003). The spawning delay between the two coastal plains may be due to the

earlier onset of winter rains in the south. Additionally, wetlands on the Scott Coastal Plain have a longer hydroperiod than the Swan Coastal Plain because winter rainfall starts earlier and there is higher annual rainfall (see Figure 4). Therefore, *G. nigrostriata* have an extended growing period on the Scott Coastal Plain. The size difference may need to be considered when planning sampling trips and conducting further research, as *G nigrostriata* are generally larger on the Scott Coastal Plain.

5.4.3.1 Identification of *Galaxiella nigrostriata*

All wetlands sampled were chosen as they contained or were in close proximity to wetlands that contained *G. nigrostriata* during past sampling efforts. It is interesting that some wetlands, even greatly separated wetlands within the same catchment (such as the Deep River catchment) that previously contained *G. nigrostriata*, instead contained *G. munda*. Most previous sampling took place 10 - 30 years ago, so of course it is feasible that the fish populations have simply moved, been displaced or become locally extinct. However, it is also possible that the fish observed/collected were misidentified.

Gerry Allen collected *G. nigrostriata* from Marbelup Brook (near Albany) in 1976, 15 years before he helped publish the major differences between *G. nigrostriata* and *G. munda* (WAM, unpublished data, 2008; Berra & Allen 1989b). In June 2009, only *G. munda* were caught in Marbelup Brook (this research). The wetlands sampled during this research in the Deep River catchment contained all *G. munda*, while past sampling found half of the wetlands to contain *G. nigrostriata* (Morgan, Gill *et al.* 1998; Storey 1998). Specimens collected by S. Beatty from Buayanyup River in 2008 were thought to be *G. nigrostriata*, following records from 1972 (Griffiths 1972; Beatty, Morgan *et al.* 2009), but DNA analysis proved they were *G. munda*. Curiously, of thousands of sites sampled in south-west WA, only four contained *G. nigrostriata* and *G. munda* cohabitating, and both were in the Shannon River catchment (Morgan, Gill *et al.* 1998). While not all of the above are necessarily misidentifications, more attention may need to be made to verify and amend historically incorrect species determination.

The morphological differences between *G. nigrostriata* and *G. munda* shown in this research may not be sufficient to make field identification easier. However, knowing major physical differences between *G. nigrostriata* and *G. munda* could help when comparing specimens in the field, particularly when collected from different wetlands. Of the significant features found during this research, head length and overall shape of the fish (*G. nigrostriata* is more streamlined) may assist in identification in the field, but only if the two species are compared together. Less mature specimens that lack

distinctive stripes can be very difficult to determine. When viewed individually the most prominent difference is the origin of the dorsal fin, which is anterior to the 5th ray in the anal fin for *G. nigrostriata* and posterior for *G. munda* (see Plate 6), as described by Berra *et al* (1989b). When research is for conservation management purposes or collecting distribution data, it is highly recommend that a sample of the specimens collected be subjected to mtDNA analysis to guarantee correct identification.

5.5 Conclusion

5.5.1 Genetics

Galaxiella nigrostriata populations in distinct catchments cannot be part of a metapopulation, due to having no connectivity. The low genetic divergence within populations and absence of common haplotypes across catchments of *Galaxiella nigrostriata* suggest that gene flow between catchments has not occurred for probably thousands of years. When compared to other Galaxiid species the low inter-population divergence rates (<1.8%) show that speciation has not taken place. Restricted dispersal between catchments has caused the lack of gene flow, probably because *G. nigrostriata* prefer lentic seasonal waterbodies.

Gene flow is limited to nearby wetlands within catchments that have intermittent connectivity. For example, unique and common haplotypes were found in northern and southern populations of *G nigrostriata* populations in the Kemerton wetland complex indicating connectivity. Consequently, wetlands that are part of a group or complex of wetlands have higher diversity due to migrations during intermittent connectivity. By comparison, single wetlands with no connectivity have more chance of extinction from reduced gene flow and therefore are more susceptible to genetic bottlenecks, inbreeding and less resistance to environmental changes or disease (Vrijenhoek 1996). Additionally, the removal and degradation of wetlands on the Swan Coastal Plain restricts the migration of G. *nigrostriata* between wetlands. This lack of connectivity will cause further genetic divergence and possible speciation of populations in separated catchments.

This project only sampled relatively close wetlands (<6 km apart) within each catchment. Sampling *G. nigrostriata* populations using the abovementioned genetic analysis throughout each catchment would give a better indication of how recently populations have been connected. Additionally, more intense within catchment sampling may also help determine *G. nigrostriata* dispersal characteristics including their use of lotic waterbodies or other means of connectivity.

5.5.2 Morphology

Migrations have not occurred between catchments sampled for *G. nigrostriata* populations sampled for possibly thousands of years. Local adaptations and a lack of migration appear to have influenced differences in *G. nigrostriata* morphology. Morphological and genetic divergence is known to occur when populations become

segregated (Crow, Waters *et al.* 2009). The greatest morphological differences are between the two most separated populations by distance, Melaleuca Park and Gardner. *Galaxiella nigrostriata* are on average smaller in the northern populations but have more dorsal fin rays. These morphological changes may affect the correct identification in the field. *Galaxiella nigrostriata* may have been misidentified for *G. munda* in the past, leading to incorrect distribution records being published.

6 Metapopulation theory explains *Galaxiella nigrostriata's* distribution across a wetland complex

The aim of this research was to see if metapopulation theory could explain *G. nigrostriata* population distribution in south-west WA wetlands. The Kemerton wetland complex was chosen as a sampling site as it was easily accessible and there were a large amount of environmental reports available that detail *G. nigrostriata* distribution and observations within the wetlands. The literature review examined current knowledge of *G. nigrostriata's* biology, known habitat preferences and distribution.

6.1 Limitations of this project and recommendations for future research

The scope of this research examined nearly all facets of *G. nigrostriata's* ecological requirements, to acquire as much data as possible to determine their status as a metapopulation. There were only two components not covered during this research which may add weight to *G. nigrostriata's* metapopulation status, diet and sediment analyses. Dietary requirements of *G nigrostriata* has been researched (Gill & Morgan 2003), although the availability of preferred prey may impact upon their nutrient intake and even preclude their survival in wetlands. *Galaxiella nigrostriata* may also avoid wetlands with inadequate macroinvertebrate diversity. Sediment composition may impede *G. nigrostriata's* ability to enter the sediment, particularly if they are burrowing themselves. *Galaxiella nigrostriata* are not thought to be fully physiologically adapted to burrowing (Thompson & Withers 1999) and may not succeed in burrowing through less friable clay soils.

Other *G. nigrostriata* populations quite likely form components of their own local metapopulations, although further intensive sampling of surrounding wetlands would be required for this to be confirmed. The information provided during this research could be used to assess other likely populations as candidates for metapopulations. Consequently, when conducting environmental assessments for developments in wetland areas, consideration should be given to all wetlands surrounding those to be affected. Passageways constructed between wetlands could assist fish migration and structures such as sand bunds (mounds) can restrict unwanted native or introduced species from entering wetlands (Hohausová, Lavoy *et al.* 2010)

6.2 Conclusions

Field sampling examined the only known remnant *G. nigrostriata* populations on the Swan Coastal Plain (at time of sampling) and the breadth of their distribution in the south-west region (including the Scott Coastal Plain). Physico-chemical properties of inundated wetlands were investigated to find why some wetlands contained permanent *G. nigrostriata* populations while others were more temporary. Habitat requirements were investigated (Chapter 3) and encompassed *G. nigrostriata* and freshwater crayfish abundance, wetland bio-physical features (for example: macrophyte coverage, wetland size) and physico-chemical properties of the water.

The analyses revealed that *G. nigrostriata* abundance in near-pristine wetlands impacted by nearby agriculture (increased nutrient levels) were not statistically significantly different to populations in mostly artificial (but naturalised) wetlands within nature reserves. *Galaxiella nigrostriata* populations in wetlands that contained potentially toxic iron and zinc levels were also not statistically significantly different to populations in wetlands with low levels. Similarly, no statistically significant correlation was found between crayfish and *G. nigrostriata* abundance. Therefore, habitat requirement analyses did not highlight any variables that affected *G. nigrostriata* abundance or distribution.

Aestivation was determined to be an under-researched but very important period in the life cycle of *G. nigrostriata* (Chapter 4). As with the habitat requirement chapter, understanding aestivation requirements may indicate how optimal the wetland is for *G. nigrostriata* survival, and therefore how aestivation requirements affect their distribution in a metapopulation. The aestivation experiments concluded that *G. nigrostriata* probably enter the sediments close to vegetation (for shade/cool ground temperatures and high macroinvertebrate abundance) and when the wetland is close to total desiccation.

Prior anecdotal evidence suggested *G. nigrostriata* use crayfish burrows to enter the sediments to aestivate while the wetlands dry. Crayfish sampling was an integral part of the aestivation experiments, to either prove or dispel the theories. Surprisingly, no statistically significant correlation was found between crayfish burrows and *G. nigrostriata* abundance in the lentocorrals. *Galaxiella nigrostriata* must find another way into the sediment to aestivate, possibly through smaller unnoticeable burrows made by frogs or macroinvertebrates, or they are capable of burrowing themselves.

The final chapter investigated the movement (migration) of *G. nigrostriata* between wetlands and catchments and whether those migrations are still occurring (Chapter 5). Migrations are a vital criterion for populations to be recognised as a metapopulation. The genetic analyses showed that *G. nigrostriata* have not migrated between catchments for probably thousands of years, but specimens in wetland complexes have migrated more recently. It is highly unlikely that migration will happen again between populations on the Swan Coastal Plain or between the coastal plains, due to declining rainfall and habitat modification/destruction that has already occurred. Consequently, the lack of gene flow will cause an increase in genetic divergence and changes to morphology between catchments, eventually creating sub-species. However, it is still possible migrations could occur between Scott Coastal Plain populations if habitat modification can be minimised.

Not surprisingly, individual wetlands have low genetic diversity due to a lack of gene flow, causing bottlenecks and inbreeding, compared to wetlands in a complex where at least occasional migration can occur. Within the Kemerton wetlands a division seems to be affecting north-south gene flow and further land modification and changes to wetland hydroperiods may limit future migrations. Additionally, the lack of connectivity and migration of *G. nigrostriata* populations between catchments is becoming apparent through changes to body size. Specifically, *G. nigrostriata* have more dorsal fin rays in the northern remnant populations.

The information obtained during this research indicates metapopulation theory can indeed be used to describe *G. nigrostriata* populations, primarily those in the Kemerton wetlands. *Galaxiella nigrostriata* populations in the Kemerton wetlands, when examined in conjunction with the data obtained from all wetlands, satisfy the four requirements (listed in italics) to be considered a metapopulation listed by Hanski *et al* (1997).

1. *A mosaic of distinct areas of suitable and unsuitable habitat*. The Kemerton wetlands sampled were made up of 11 seasonal wetlands of different shapes and sizes and varying degrees of aquatic macrophyte and terrestrial riparian vegetation. The habitat characteristics examined here show *G. nigrostriata* were found in wetlands with a wide range of physico-chemical properties, but did not differentiate between optimal or unsuitable habitat. It is most likely the depth to groundwater (and consequently hydroperiod) that plays an important role to their survival during aestivation, and therefore their long-term survival in any one wetland. The aestivation experiment, while

only conducted in two wetlands, showed *G. nigrostriata* survived aestivation in just one wetland even though both wetlands contained high abundances the previous year.

2. *Each population must at some time be threatened by extinction.* All Kemerton wetland populations are subject to variable groundwater depths which are affected by changing rainfall patterns/dry periods and local groundwater abstraction. With only a one year lifespan, *G. nigrostriata* may not survive aestivation during an extended dry period. Indeed, the absence of *G. nigrostriata* in EPP6 during the aestivation experiment may indicate their sensitivity to longer dry periods during aestivation.

3. *There must be some interaction between the populations*. The genetic analyses indicate that *G. nigrostriata* migrations are occurring between the Kemerton wetlands, with common haplotypes being found in all populations. This is supported by *G. nigrostriata* presence being reported seemingly randomly throughout the wetlands over a 16 year period. The Kemerton wetlands are located on relatively flat land surrounded by damplands and palusplains (BEC 2004). These land features and topography could provide passageways between wetlands during high rainfall seasons, as *G. nigrostriata* have been observed swimming in shallow water (<5mm) (pers. obs.) as does the congeneric *G. pusilla*

4. *The dynamics of one population should be independent of other population dynamics*. The physical characteristics (such as size, shape and depth) vary in each of the Kemerton wetlands which means they are subjected to differing drying regimes, specifically, deeper wetlands have a longer hydroperiod. Similarly, different types and amounts of vegetation within and surrounding each wetland impact on the microclimate (for example: shading and water temperature), macroinvertebrate availability and shelter. Additionally, the lack of regular connectivity limits interactions between populations that may affect the dynamics. One population may experience a decrease in population due to disease from exotic species or high nutrient input from surface flows. However, these influences may not affect neighbouring populations.

In conclusion, this research has demonstrated that *G. nigrostriata* populations in the Kemerton wetland complex satisfy the criteria to be a metapopulation. It is likely other catchments in south-west WA also contain groups of wetlands that harbour *G. nigrostriata* metapopulations.

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8 Appendix

8.1 Location information for all sample sites.

Wetland locality	Sampling site name	Species caught Full names below	GPS waypoints (WGS84)		Wetland height (m AHD)
Melaleuca Park	EPP173	-	S31°42'14.8"	E115°57'48.1"	54
Melaleuca Park	Mb	*Gn*	S31°42'10.5"	E115°57'46.0"	54
Kemerton wetlands Kemerton Nature Reserve	EPP1	*Gn*	S33°07'59.8"	E115°47'45.9"	12
Kemerton wetlands Kemerton Nature Reserve	EPP3	-	S33°07'39.4"	E115°47'47.2"	14
Kemerton wetlands KSS	EPP4	-	S33°07'32.1"	E115°46'53.3"	15
Kemerton wetlands Kemerton Nature Reserve	EPP5	*Gn, Gh*	S33°08'20.4"	E115°47'38.4"	12
Kemerton wetlands Kemerton Nature Reserve	EPP6	*Gn*	S33°07'51.6"	E115°47'56.6"	12
Kemerton wetlands Kemerton Nature Reserve	EPP7	-	S33°07'27.5"	E115°47'56.6"	14
Kemerton wetlands KSS	EPP8	-	S33°07'15.5"	E115°47'26.3"	15
Kemerton wetlands Kemerton Nature Reserve	EPP9	*Gn*	S33°07'05.1"	E115°47'33.3"	15
Kemerton wetlands Kemerton Nature Reserve	PN	*Gn*	S33°07'28.4"	E115°47'49.4"	14
Kemerton wetlands Kemerton Nature Reserve	PS	*Gn*	S33°07'22.4"	E115°47'51.9"	14
Kemerton wetlands Kemerton Nature Reserve	PD	-	S33°07'21.4"	E115°47'53.7"	14
Ironstone Gully Buayanyup River catchment	IG	*Gm* (borrowed)	S33°49'48.03"	E115°14'27.64"	118
Scott River Rd Scott River NP	SR20	*Gn*	S34°17'00.4"	E115°16'04.3"	12
Scott River Rd Scott River NP	SR38	*Gn*	S34°17'02.7"	E115°14'24.4"	14
Scott River Rd Scott River NP	SR42	*Gn*	S34°17'04.2"	E115°14'57.3"	16
Scott River Rd Scott River NP	SR53	*Gn*	S34°17'20.6"	E115°13'06.9"	16
Chesapeake Rd West Gardner R. Catchment D'Entrecasteaux NP	CW1	*Gn*	S34°45'22.1"	E116°09'00.1"	49
Chesapeake Rd West Gardner R. Catchment D'Entrecasteaux NP	CW2	*Gn, Nb*	S34°45'52.7"	E116°09'22.2"	45

Wetland locality	Sampling site name	Species caught Full names below	GPS waypoints (WGS84)		Wetland height (m AHD)
Chesapeake Rd West Gardner R. Catchment D'Entrecasteaux NP	CW3	*Gn, Ls, Nb, Bp*	S34°45'54.3"	E116°09'24.6"	44
Chesapeake Rd West Gardner R. Catchment D'Entrecasteaux NP	CW4	*Gn*	S34°46'02.3"	E116°09'25.6"	43
Chesapeake Rd Shannon R. Catchment D'Entrecasteaux NP	CP1	-	S34°49'12.8"S	E116°16'51.5"	44
Moores Hut track Shannon R. Catchment D'Entrecasteaux NP	CP2	*Gn, Ls*	S34°50'25.2"	E116°17'01.5"	22
Chesapeake Rd Shannon R. Catchment D'Entrecasteaux NP	CP3	-	S34°49'04.5"	E116°18'04.0"	30
Chesapeake Rd Shannon R. Catchment D'Entrecasteaux NP	CP4	-	S34°48'46.0"	E116°14'43.0"	43
Beardmore Rd Deep R. Catchment Mt. Frankland NP	BR1	*Gm, Bp*	S34°48'36.07"	E116°32'04.42"	67
Myalgelup Rd Poorginup swamp Deep R. catchment Mt. Frankland NP	MR1	*Nb, Bp*	S 34°32'49.6"	E 116°43'48.7"	182
Myalgelup Rd Poorginup swamp Deep R. catchment Mt. Frankland NP	MR2	-	S34°32'50.17"	E116°44'32.29"	177
Thomson Rd Mt. Frankland NP	TR1	*Gm, Ls, Nv*	S34°45'37.22"	E116°43'10.38"	109
Thomson Rd Mt. Frankland NP	TR2a	*Ls*	S34°46'56.73"	E116°43'11.20"	131
Thomson Rd Mt. Frankland NP	TR2b	*Ls*	S34°47'09.25"	E116°43'11.52"	129
Thomson Rd Mt. Frankland NP	TR3	*Gm, Ls*	S34°45'52.41"	E116°43'09.66"	114
Marbelup Brook Marbelup North Rd	AL	*Gm* (borrowed)	S34°56'25.0"	E117°42'41.8"	36

Gn- Galaxiella nigrostriata, Gm - G. munda, Ls - Lepidogalaxias salamandroides, Nb - Nannatherina balstoni, Nv - Nannoperca vittata, Bp - Bostockia porosa, Gh - Gambusia holbrooki (Gh is an introduced species)

8.2 Morphometric measurements of all specimens

	Head length % of TL Range, mean ±SE, number of specimens	Snout length %of HL	Eye diameter % of HL	Body depth at anus % of TL	Length of base of dorsal fin
Melaleuca Park	17.9-21.8% 20.1 ±0.3% *n* = 12	14.9-27.8% 22.2 ±1.0% *n* = 12	30.5-39.7% 34.8 ±0.8% *n* = 12	11.2-12.7% 12.0 ±0.2% *n* = 12	5.3-7.6% 6.8 ±0.2% *n* = 12
Kemerton	17.3-21.1% 19.6 ±0.2% *n* = 19	13.6-27.7% 21.7 ±0.9% *n* = 19	28.8-42.3% 34.5 ±1.0% *n* = 19	9.1-14.4% 12.2 ±0.2% *n* = 19	5.5-7.5% 6.8 ±0.1% *n* = 19
Scott	15.8-23.0% 19.4 ±0.6% *n* = 11	16.1-25.7% 21.3 ±1.1% *n* = 11	24.5-39.4% 32.4 ±1.4% *n* = 11	11.7-15.5% 13.0 ±0.3% *n* = 11	5.4-7.1% 6.3 ±0.2% *n* = 11
Gardner	16.7-19.8% 18.7 ±0.3% *n* = 9	19.5-26.2% 22.0 ±0.7% *n* = 9	30.8-39.5% 34.5 ±1.1% *n* = 9	11.7-16.0% 13.5 ±0.5% *n* = 9	5.6-7.9% 6.3 ±0.3% *n* = 9
Shannon	16.1-19.1% 18.1 ±0.4% *n* = 6	17.2-24.7% 21.5 ±1.0% *n* = 6	30.0-40.6% 33.7 ±1.5% *n* = 6	11.5-13.4% 12.6 ±0.3% *n* = 6	5.2-6.3% 5.8 ±0.2% *n* = 6
G. nigrostriata	15.8-23.0% 19.4 ±0.2% *n* = 57	13.6-27.8% 21.8 ±0.4% *n* = 57	24.5-42.3% 34.1 ±0.5% *n* = 57	9.1-16.0% 12.6 ±0.2% *n* = 57	5.2-7.9% 6.5 ±0.1% *n* = 57
G. munda	15.1-19.1% 16.5 ±0.3% *n* = 13	17.1-26.5% 22.4 ±0.8% *n* = 13	26.0-41.5% 34.5 ±1.2% *n* = 13	9.5-11.8% 10.8 ±0.2% *n* = 12	4.9-8.6% 6.5 ±0.3% *n* = 13

	Length of pectoral fin Range, mean ±SE, number of specimens	Length of pelvic fin	Length of base of anal fin	Length of caudal peduncle	Depth of caudal peduncle
Melaleuca Park	9.1-10.9% 9.8 ±0.2% *n* = 12	3.7-6.5% 5.3 ±0.3% *n* = 12	6.8-10.2% 8.9 ±0.3% *n* = 12	16.3-18.7% 17.5 ±0.2% *n* = 12	5.1-7.0% 5.7 ±0.2% *n* = 12
Kemerton	8.5-13.5% 10.0 ±0.2% *n* = 19	3.9-7.6% 6.0 ±0.3% *n* = 14	9.0-12.8% 10.6 ±0.3% *n* = 19	15.0-19.8% 17.7 ±0.3% *n* = 19	4.9-6.4% 5.7 ±0.1% *n* = 19
Scott	8.9-10.5% 9.6 ±0.2% *n* = 11	5.4-8.4% 6.7 ±0.3% *n* = 11	7.7-10.9% 9.3 ±0.3% *n* = 11	15.6-20.7% 17.0 ±0.4% *n* = 11	4.5-6.8% 5.7 ±0.2% *n* = 11
Gardner	8.9-11.2% 10.1 ±0.2% *n* = 9	6.1-7.6% 7.0 ±0.1% *n* = 9	8.7-11.3% 10.3 ±0.3% *n* = 9	14.8-19.5% 17.3 ±0.5% *n* = 9	5.8-7.7% 6.3 ±0.2% *n* = 9
Shannon	7.9-10.4% 9.1 ±0.4% *n* = 6	5.2-6.9% 5.8 ±0.3% *n* = 6	7.8-11.1% 9.7 ±0.5% *n* = 6	15.4-20.0% 18.3 ±0.7% *n* = 6	5.0-6.6% 5.9 ±0.2% *n* = 6
G. nigrostriata	7.9-13.5% 9.8 ±0.1% *n* = 57	3.7-8.4% 6.1 ±0.1% *n* = 52	6.89-12.8% 9.8 ±0.2% *n* = 57	14.8-20.7% 17.5 ±0.2% *n* = 57	4.5-7.7% 5.8 ±0.1% *n* = 57
G. munda	7.6-10.6% 9.1 ±0.2% *n* = 13	4.4-9.1% 7.0 ±0.4% *n* = 13	9.2-13.7% 11.5 ±0.4% *n* = 12	13.8-17.0% 15.7 ±0.3% *n* = 13	4.4-6.1% 5.2 ±0.1% *n* = 13

8.3 Meristic counts of all specimens

	Pectoral			Pelvic			Dorsal			Anal			Caudal		
	Range, mean ±SE, number of specimens														
Melaleuca Park	12-14	13 ±0.1	*n* = 12	5-7	6.1 ±0.2	*n* = 10	7-9	8.4± 0.2	*n* = 12	9-11	9.9 ±0.1	*n* = 12	20-22	20.4 ±0.2	*n* = 12
Kemerton	11-13	12.4 ±0.1	*n* = 17	5-6	5.5 ±0.2	*n* = 11	7-9	7.9 ±0.2	*n* = 19	9-12	10.3 ±0.2	*n* = 19	19-21	19.9 ±0.1	*n* = 15
Scott	12-14	12.7 ±0.6	*n* = 11	5-6	5.4 ±0.5	*n* = 9	7-9	7.5 ±0.7	*n* = 11	9-11	10.2 ±0.9	*n* = 11	19-22	20.2 ±1.0	*n* = 10
Gardner	12-14	12.9 ±0.3	*n* = 9	5-7	5.8 ±0.3	*n* = 9	6-8	7.0 ±0.2	*n* = 9	10-12	10.6 ±0.2	*n* = 9	18-21	20.2 ±0.3	*n* = 9
Shannon	11-12	11.8 ±0.2	*n* = 6	5-6	5.5 ±0.2	*n* = 6	7-7	7.0 ±0	*n* = 7	10-11	10.7 ±0.2	*n* = 6	19-22	19.7 ±0.5	*n* = 6
G. nigrostriata	11-14	12.6 ±0.1	*n* = 54	5-7	5.7 ±0.1	*n* = 45	6-9	7.7 ±0.1	*n* = 58	9-12	10.3 ±0.1	*n* = 57	18-22	20.1 ±0.1	*n* = 52
G. munda	10-12	10.9 ±0.1	*n*= 13	5-7	5.8 ±0.2	*n*= 13	7-9	7.6 ±0.2	*n*= 13	10-13	11.4 ±0.2	*n* = 12	19-21	20.0 ±0.2	*n*= 13