Implications of climate change on the aestivating Salamanderfish, *Lepidogalaxias salamandroides* Mees and the Black-stripe Minnow, *Galaxiella nigrostriata* Shipway

Thesis submitted for completion of Honours Degree (Bachelor of Science), School of Veterinary and Life Sciences, Murdoch University

by

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Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

Garry Ogston

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Abstract

Aestivating aquatic fauna are able to survive periods of drying using various unique behavioural, morphological and physiological adaptations. The Salamanderfish, *Lepidogalaxias salamandroides*, and Black-stripe Minnow, *Galaxiella nigrostriata*, are aestivating fishes restricted to the south-western most corner of Australia. The region has experienced a drying climate since the 1970's and this trend is projected to continue due to climate change. The associated reductions in surface flows and groundwater is placing pressure on many of the highly endemic freshwater fishes of the region that are known to have been severely impacted by other anthropogenic stressors. Several of the region's freshwater fishes are listed as threatened, however, the current conservation status of the two aestivating species is in need of updating and threats to both are largely unknown. This study aimed to assess the current conservation status of these species and determine the key physicochemical and hydrological variables associated with their distributions to help project future population viabilities under hydrological change scenarios.

A total of 53 sites were sampled throughout the historical range of *L. salamandroides* and *G. nigrostriata* and a suite of physicochemical and habitat variables measured. Of the sites surveyed, 39 and 28 historically contained *L. salamandroides* and *G. nigrostriata*, respectively. Concerningly, the study revealed that only 26 currently contained *L. salamandroides* (66.67% of their historical distribution) and 20 contained *G. nigrostriata* (71.43% of their historical distribution). Both *L. salamandroides* and *G. nigrostriata* were absent from the four eastern-most sites sampled representing approximate reductions in extent of occurrence (EOO) of 79% and 12% for the two species, respectively. Area of occupancy (AOO) of *L. salamandroides* and *G. nigrostriata* has declined by approximately 71% and 56% for each species, respectively. Both species qualify as Endangered (IUCN

Red List Threatened Category) under A2c), A4 c); population size reductions (past and projected based on AOO and EOO).

Historical sites were predominantly ephemeral wetlands that were acidic (mean pH = 4.77 (±0.12 SE)), had low salinity (mean 0.23 mg/L (±0.01 SE)) and a shallow (<1m) depth to groundwater during the dry period. Species distribution modelling was undertaken to determine those environmental variables that best explained the presence and absence of the species and revealed that sites with greater depth in winter and length of the annual non-wetted period were the variables of highest importance for *L. salamandroides*, with higher connectivity to surrounding wetlands a key variable of importance for *G. nigrostriata*.

Both species exhibited depleted lipid content after aestivation. The change in lipid content across seasons was more pronounced in *L. salamandroides*; however there was also a significant difference between *G. nigrostriata* and the non-aestivating *Galaxiella munda* suggesting that *G. nigrostriata* may also utilise lipid storage as part of its aestivation strategy. Increases in the annual dry period of wetlands may push the limits of such physiological strategies.

Analysis of seasonal stable isotope composition (δ^2H and δ^{18}O) of surface, ground and rain water identified that the wetlands are reliant on winter rainfall for recharge, with high rates of evaporation occurring during summer.

The results of the study suggest that climate change is likely to have been a key factor in the recent decline of these species. It is likely that the viability of populations will continue to be challenged given groundwater models project that the water table within much of this region will decrease by up to 4 m by 2030 and therefore further population losses may be expected. This research has considerable implications for future management of these

aquatic ecosystems, including the management of groundwater levels in the region, reducing anthropogenic modifications and maintaining existing habitats. To help ensure survival of these enigmatic species in light of projected climatic and hydrological change, novel approaches such as creation of artificial wetlands and breeding programs may be necessary to help ensure their survival.

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1 Introduction

1.1 What is aestivation?

The word aestivation is derived from the Latin *aestas* meaning summer or *aestus* meaning heat and is defined as a period of dormancy through either summer or a dry period (Storey & Storey, 2012). Aestivation occurs in a wide range of animal species and a wide range of ecological habitats, both terrestrial and aquatic (Janssens, 1964; Delaney *et al.*, 1974; Eldon, 1979; Pusey, 1989; Withers & Guppy, 1996; Anderson & Smith, 2004; Cranston *et al.*, 2007). Aestivation has evolved in a range of invertebrate taxa including: Cnidaria, e.g. the sea cucumber *Apostichopus japonicus* (Yang *et al.*, 2006; Fangyu *et al.*, 2011); Insecta, e.g. the winter-emerging chironomid *Hydrobaenus saetheri* (Cranston *et al.*, 2007); Annelida, e.g. the tubificid worm *Rhyacodrilus hiemalis* (Narita, 2006) and the earthworm *Hormogaster elisae* (Cosin *et al.*, 2006); and Mollusca, including snails such as the Australian land snail *Rhagada tescorum* (Withers *et al.*, 1997), the Giant African land snail *Achatina fulica* (Rahman & Raut, 2012), and the pond snail *Galba truncatula* (Goumghar *et al.*, 2001). A variety of vertebrates have also evolved aestivation strategies, including amphibians such as the green-striped burrowing frog *Cyclorana alboguttata* (Cramp *et al.*, 2005) and the two-toed salamander *Amphiuma means* (Waller, 2009), and reptilians such as the western-swamp tortoise *Pseudemydura umbrina* (Mitchell *et al.*, 2013).

Several genera of the Teleostei (ray finned fishes) representing a wide variety of families also aestivate (Delaney *et al.*, 1983; Allen & Berra, 1989; Gleeson *et al.*, 1999; Koster, 2003; Chew *et al.*, 2005). These include species from the following genera; *Neochanna* (Galaxiidae) (Eldon, 1979; Gleeson *et al.*, 1999; McDowall, 2003), *Clarias* (Clariidae) (Bruton, 1979), *Pseudapocryptes* (Gobiidae) (Swennen *et al.*, 1995), *Monopterus* (Synbranchidae) (Chew *et al.*, 2005), *Synbranchus* (Synbranchidae) (Bicudo & Johansen, 1979), *Ammodytes* (Ammodytidae) (Tomiyama & Yanagibashi, 2004) and some Australian species in the genera *Galaxiella* (Galaxiidae) (Smith *et al.*, 2002a; Koster, 2003), and *Lepidogalaxias* (Lepidogalaxiidae) (Pusey, 1989). Five species of the Sarcopterygii (fleshy finned fishes) are also known to aestivate, including lungfishes of the genera *Protopterus* (Protopteridae) and *Lepidosiren* (Lepidosirenidae) (African and South American lungfishes respectively) (Delaney *et al.*, 1983). Despite aestivation occurring in a considerable range of fauna, it is relatively rare in fishes, and those that do aestivate have unique adaptations for their demanding life cycles including increased flexibility, cutaneous respiration, and the use of lungs instead of gills (Delaney *et al.*, 1983; Allen & Berra, 1989; Gleeson *et al.*, 1999; Koster, 2003; Chew *et al.*, 2005).

1.2 South-western Australia and its freshwater fishes

The south-west of Western Australia (Figure 1.1) is one of the world's five Mediterranean climatic regions (Cowling *et al.*, 1996) and is recognised as a global biodiversity hotspot (Myers *et al.*, 2000). Rainfall and temperature are highly seasonal, being characterised by hot dry summers and mild wet winters (Bunn *et al.*, 1986). Rivers and streams in the region generally flow intermittently, resulting from seasonal rainfall during winter and spring (Kennard *et al.*, 2010). The remnant fresh lentic systems of the region are found in the western and south-western coastal areas and the extreme south-western part of the region (between Augusta and Albany, Figure 1.1); the latter region being relatively low-lying and containing numerous ephemeral palusplain wetlands (i.e., a flat area that is waterlogged on a seasonal basis) (Semeniuk & Semeniuk, 1995).

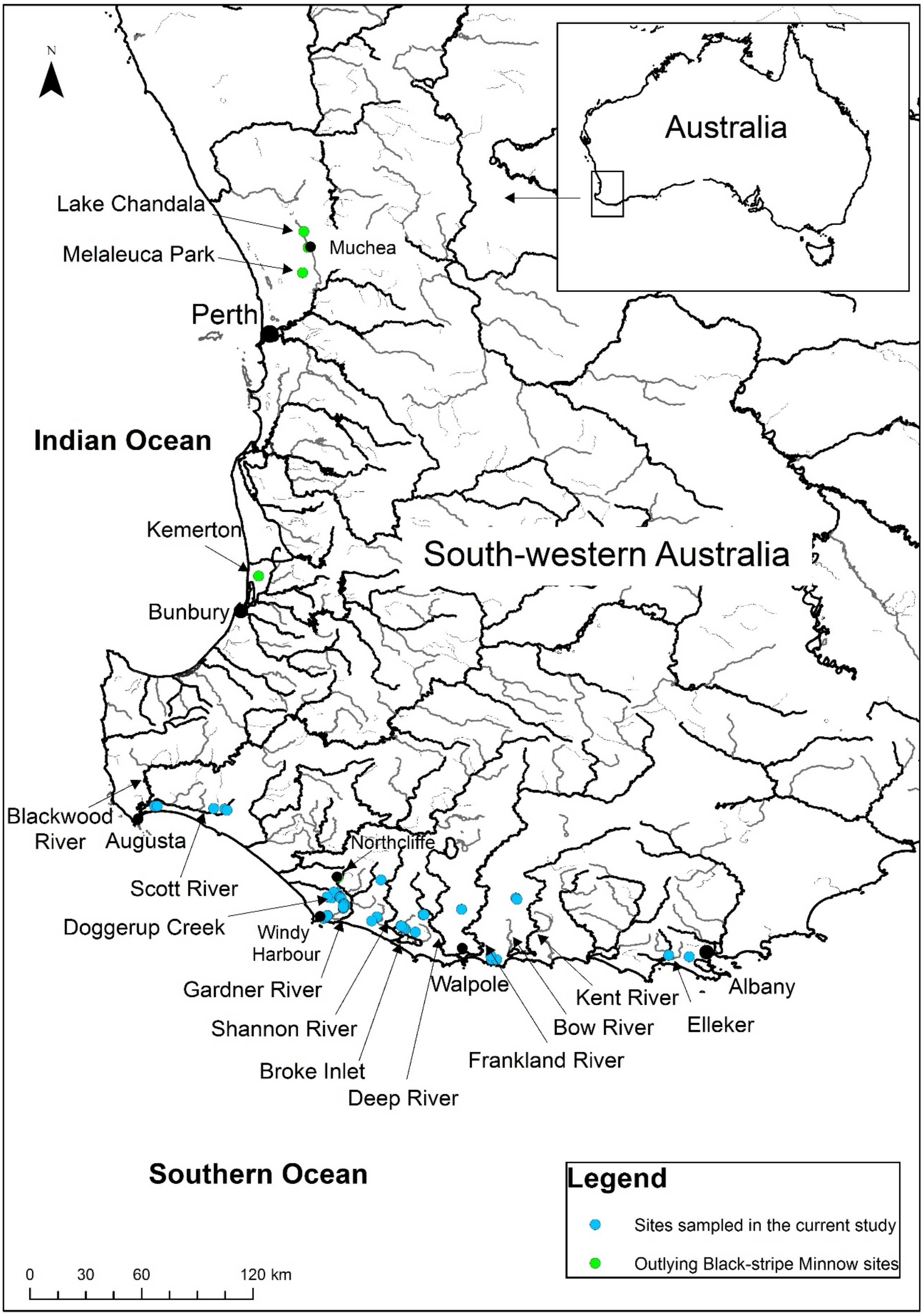

Figure 1.1. A map of south-western Australia identifying key river systems and the sites sampled for Salamanderfish *Lepidogalaxias salamandroides* and Black-stripe Minnow *Galaxiella nigrostriata* in the current study (see Methods). N.B. Also shown are northern outlying populations of the Black-stripe Minnow.

Although the south-west of Western Australia, including the Southwestern Province (Unmack *et al.*, 2012), supports a relatively low number of native freshwater fish species (Morgan *et al.*, 2011), it contains the highest proportion of endemism of any province on the continent (Morgan *et al.*, 2011). Nine of the 11 native freshwater fishes found in the south-west are endemic, including; *Tandanus bostocki*, *Nannatherina balstoni, Nannoperca pygmaea, Nannoperca vittata, Bostockia porosa, Galaxias occidentalis, Galaxiella munda, Galaxiella nigrostriata* and *Lepidogalaxias salamandroides* (Morgan *et al.*, 1998; Morgan *et al.*, 2011; Morgan *et al.*, 2013).

The distinct seasonal hydrological variation of the region has resulted in a number of adaptations of its fishes. These include; having highly seasonal spawning periods coinciding with periods of inundation of lentic systems and seasonal flows in lotic systems, and the aestivation of two species during the dry period (Morgan *et al.*, 2000). The aestivating fishes are the salamanderfish, *Lepidogalaxias salamandroides* (McDowall & Pusey, 1983) and the black-stripe minnow, *Galaxiella nigrostriata* (Smith *et al.*, 2002a; Smith *et al.*, 2002b).

1.2.1 *Lepidogalaxias salamandroides*

Lepidogalaxias salamandroides is a small (maximum 90 mm total length (TL)), cryptically coloured, scaled, freshwater fish restricted to the extreme south-west corner of Australia (Mees, 1961). Within this region it is found in a small area of mostly ephemeral, acidic wetlands between the Blackwood & Kent Rivers (McDowall & Pusey, 1983; Morgan *et al.*, 2000) (Figure 1.1). The species displays sexual dimorphism with the males possessing a modified anal fin for copulation (Figure 1.2) (McDowall & Pusey, 1983).

The species has previously been phylogenetically placed within the Galaxiidae, Esocidae, or within the monotypic Lepidogalaxiidae (Nelson, 2006). Reasons for this confusion can be attributed to its unique morphology; *L. salamandroides* exhibits many differences from the families within which it was initially placed such as possessing scales (unlike members of the Galaxiidae) (Li *et al.*, 2010). Nelson (1994) regarded the family Lepidogalaxiidae as valid but Nelson (2006) regarded it to be a subfamily within Galaxiidae. Based on molecular data, Li *et al.* (2010) unequivocally demonstrated that Lepidogalaxiidae was a valid family basally placed as the sister taxon of all Euteleosteomorpha.

Figure 1.2. A male *Lepidogalaxias salamandroides*, caught in one of the study sites during winter, in a holding bucket prior to being measured.

1.2.2 *Galaxiella nigrostriata*

The Black-stripe Minnow, *Galaxiella nigrostriata*, is a small (maximum 48 mm TL), scaleless freshwater fish that is endemic to the extreme south-western corner of Australia (Berra & Allen, 1989b; Morgan *et al.*, 1998). It is characterised by two black longitudinal bands separated with a yellow/orange to red stripe (McDowall & Frankberg, 1981; Berra & Allen, 1989b; Morgan *et al.*, 1998). *Galaxiella nigrostriata* was initially classified by Shipway (1953) in the genus *Galaxias* as a subspecies to *Galaxias pusilla* (Smith *et al.*, 2002b); a small scaleless galaxiid found in south-eastern Australia, also known to survive periods of drought possibly through aestivation (Koster, 2003). However, it was subsequently classified as its own species in the genus *Galaxiella* (McDowall, 1978).

The species has a restricted distribution between Augusta and Albany and three outlying populations; one located at Kemerton near Bunbury, one north of Perth at Melaleuca Park (Smith *et al.*, 2002a), and one population near Muchea at Lake Chandala (McLure & Horwitz, 2009) (Figure 1.1). *Galaxiella nigrostriata* inhabits ephemeral acidic freshwater wetlands and can survive periods of drought and desiccation, similarly to *L. salamandroides*, through its ability to aestivate in moist substrates (Pusey & Edward, 1990; Smith *et al.*, 2002a). Less is known however about the mechanisms that *G. nigrostriata* utilises to aestivate, due to a lack of obvious morphological adaptations such as those seen in *L. salamandroides* (Thompson & Withers, 1999).

1.3 Challenges faced by aestivating fauna

Aestivating fauna face many challenges as part of their lifecycles, principally water retention and water loss, metabolic waste production and excretion, and energy storage and utilisation (Pusey, 1989). Species used a variety of behavioural (Smith, 1930), morphological and physiological traits (Bruton, 1979; Eldon, 1979; Martin *et al.*, 1983; Pusey, 1986) to cope with the challenges of aestivation.

1.3.1 *Behavioural and morphological traits*

Burrowing is a common behavioural trait associated with aestivation (Smith, 1930; Bruton, 1979; Berra & Allen, 1989a). Physical habitat conditions, particularly soil structure, can impact on burrowing behaviour in aestivating species (Booth, 2006). For example, a study demonstrated the Green-striped Burrowing Frog, *C. alboguttata*, to be reluctant to burrow in clay substrates, and it required significantly longer time to begin burrowing in clay compared to sandy substrates (Booth, 2006). Other behavioural adaptations common in aestivating species include delayed emergence of young from the soil (Doody *et al.*, 2001; Cranston *et al.*, 2007) and utilising vertical migration in the soil profile in order to aestivate (Narita, 2006). Those species that have limited burrowing capability, yet still aestivate, may utilise burrows produced by other fauna (Koster, 2003), or other suitable micro-habitat types such as damp vegetation (McPhail, 1999).

Lepidogalaxias salamandroides has several morphological traits that make it suited to a life of burrowing and re-emergence (McDowall & Pusey, 1983; Berra & Allen, 1989a; Morgan *et al.*, 2000). The head of *L. salamandroides* has secondary eyelids covering the eyes (McDowall & Pusey, 1983), and a wedge-shaped skull (Berra & Allen, 1989a). The skull itself is very robust and may also be an adaptation to support the weight of damp sand during aestivation (Berra, 1997). *Lepidogalaxias salamandroides* is able to bend its head at right angles to its body, made possible due to a large gap between its skull and first vertebra, that may aid in the burrowing process (Berra & Allen, 1989a). Large gaps between the other vertebrae in the spine and reduced ribs are also suited for burrowing by increasing its flexibility (Berra & Allen, 1989a).

Galaxiella nigrostriata does not possess the same morphological adaptations as *L. salamandroides* and is therefore not as efficient at burrowing to survive desiccation (Thompson & Withers, 1999). It has therefore been proposed that *G. nigrostriata* has adapted a different behavioural strategy to survive droughts, including the possibility of using burrows created by freshwater crayfish such as *Cherax preissii* that are often sympatric with the species (Thompson & Withers, 1999).

1.3.2 *Water loss and retention*

Water loss and retention represent a major challenge for aestivating species, with the water potential of the soil surrounding the animal during aestivation impacting on its ability to retain water (Pusey, 1989; Booth, 2006). For example, soil water content of >6% resulted in positive water flux into *L. salamandroides* such that weight was gained during an aestivation period lasting 43 days and upon re-immersion, the gained weight was subsequently lost (Pusey, 1989). Similar findings have been reported for other species, such as the burrowing frog *C. alboguttata* which experienced mass increases when water potential of the soil was -400kPa, however, a loss of body mass occurred when the soil potential was -1000kPa (Booth, 2006). The positive fluxes and water retention in aestivating species that can occur during the initial drying of the soil may offset later water loss as the water potential of the soil decreases (Pusey, 1989).

Secretion of mucous is a trait that plays a key role in reducing water loss during aestivation (Pusey, 1986). *Lepidogalaxias salamandroides* that were induced to aestivate positioned their body into a u-shape and became coated in mucous (Pusey, 1986). Thompson and Withers (1999) supported this observation when exploring the impacts of hypoxia. Mucous bubbles were formed by *L. salamandroides* when placed inside a metabolic air chamber, however, such bubbles did not form in two other non-aestivating freshwater fishes when exposed to the same environment (Thompson & Withers, 1999). Other aestivating fishes, such as the mudskipper, *Pseudapocryptes lanceolatus* (Swennen *et al.*, 1995), the Black Mudfish, *Neochanna diversus* (McPhail, 1999), and the lungfishes, *Protopterus annectens* and *Protopterus dolloi*, (Loong *et al.*, 2008), similarly use mucous during aestivation.

Accumulation of urea in the body fluids in order to increase osmolarity is another common trait of aestivating fauna (McClanahan, 1972). For example, the Spadefoot Toad, *Scaphiopus couchii* accumulates urea in its body fluids following burrowing to increase its internal osmolarity (McClanahan, 1972). The rate and amount of accumulation varies depending on soil water potential. *Scaphiopus couchii* in soils of low water potential (750 mOsm/l) produced significantly more urea over a shorter period than those in soils with a higher water potential (450 mOsm/l) (McClanahan, 1972).

1.3.3 *Waste elimination or storage*

Managing metabolic waste products during aestivation is crucial to avoid potentially toxic internal concentrations. Reducing the amount of ammonia is of particular importance for fish due to its toxicity (Loong *et al.*, 2008). The African lungfishes *P. dolloi* and *P. annectens* significantly decreased the ammonia content in their livers after 40 and 46 days of aestivation in air respectively, whilst significantly increasing their urea concentrations in the tissues (Loong *et al.*, 2008). This has been attributed to a shift to increased urea synthesis and decreased ammonia production in order to reduce the threat of ammonia toxicity (Loong *et al.*, 2008). Decreasing the rate of amino acid catabolism is necessary to decrease the build-up of toxic ammonia and promote urea synthesis (Chew *et al.*, 2004). The amino acid glutamate in *P. dolloi* was observed to be significantly lower in the livers of aestivated versus non-aestivating fish (Chew *et al.*, 2004). Glutamate was likely transformed into glutamine to be utilised as a substrate for urea synthesis (Chew *et al.*, 2004).

Storage of toxic metabolic waste is also important in the African lungfish, *Protopterus aethiopicus,* which only excretes urea during the initial few days of aestivation (Smith, 1930). The cocoon of the aestivating lungfish is stained near the cloaca that is attributed to final expulsion of urine, with no free urine or solid excrement observed in the cocoon (Smith, 1930). *Protopterus aethipocus* relies on protein for energy during aestivation

(Janssens, 1964) and 94 per cent of the nitrogen from degraded protein is metabolised into urea (Smith, 1930). Upon re-immersion in water, a large amount of urea is excreted by *P. aethiopicus*, suggesting nitrogen excretion is ceased and instead storage occurs during aestivation (Smith, 1930).

1.3.4 *Gas exchange*

Aestivating fishes have adapted to respire efficiently when their habitat dries (Urbina *et al.*, 2014). A high surface area to volume ratio allows several species to perform cutaneous respiration (Urbina *et al.*, 2014). This morphological trait is present in several species of aestivating galaxiids, including the Canterbury Mudfish, *Neochanna burrowsius,* which possesses a scaleless integument that maximises the efficiency of cutaneous respiration (Urbina *et al.*, 2014).

Lepidogalaxias salamandroides also has the capacity for cutaneous respiration and this is the predominant pathway for the uptake of O_2 and elimination of CO_2 in air, with the swim bladder being relatively unimportant for gas exchange (Martin *et al.*, 1983; Berra *et al.*, 1989). Berra *et al.* (1989) compared *L. salamandroides* with Australian galaxiids and an American umbrid known to possess well-developed swim-bladders suited for aerial respiration. It was found that the swim bladder of *L. salamandroides* was under-developed compared to the other species, and not suitable for aerial respiration (Berra *et al.*, 1989).

Galaxiella nigrostriata appears to cope with hypoxia less efficiently than *L. salamandroides* but more so than the non-aestivating *B. porosa* (Thompson & Withers, 1999). Thompson *et al*. (1999) found that although oxygen consumption of *G. nigrostriata* decreased in air in comparison to water (unlike *L. salamandroides*) it did not decline to the same degree as *B. porosa* (Thompson & Withers, 1999). These findings suggest that *G.*

nigrostriata has a limited capacity for cutaneous gas exchange, potentially enabling it to survive temporary periods of hypoxia and drought (Thompson & Withers, 1999).

An unusual adaptation for gas exchange is seen in the African lungfish *P. aethiopicus*, which has reduced gills that are inadequate for respiration and instead utilises a lung opening in the oesophagus (Smith, 1930). When in water, *P. aethiopicus* breathes via aerial respiration, exchanging approximately 90% of oxygen and expelling approximately 30% of its metabolic CO_2 via the lungs (Johansen, 1970; Delaney & Fishman, 1977). During aestivation, it encases itself in a cocoon with an opening at the mouth, and becomes completely reliant on the lungs for gas exchange (Smith, 1930; Delaney *et al.*, 1974; Delaney & Fishman, 1977).

1.3.5 *Metabolic suppression*

Metabolic suppression commonly occurs during aestivation (Smith, 1930; Guppy & Withers, 1991). In the lungfish *P. aethiopicus*, rates of oxygen consumption in fish that had been aestivating for 15 to 18 months were much lower (8 cc/kilo/hour) than those in fish that had not undergone aestivation (20 cc/kilo/hour) (Smith, 1930). This difference was attributed to a combination of reductions in muscular activity, respiration, circulation, and accumulation of metabolites (Smith, 1930). Similar findings have been demonstrated in other aestivating fishes such as *Synbranchus marmoratus* (Bicudo & Johansen, 1979), with the depressed metabolic rate in aestivating fishes commonly between 0.2 and 0.3 times the resting rate (Guppy & Withers, 1991).

1.3.6 *Storage and use of carbohydrates and lipids*

Utilisation of energy has also been demonstrated to differ markedly among aestivating, starving, and non-aestivating fish (Janssens, 1964). Starved lungfish were observed to have lost a greater percentage of weight than those that had aestivated (Janssens, 1964). Levels of glycogen in the liver were also much lower in both starved and aestivating fish compared to non-aestivating fish (Janssens, 1964). However, glycogen levels in muscle tissue decreased by 75% in starved fish, but increased by 50% in aestivating fish, with the storage of carbohydrates likely to satisfy the need for energy at the end of the aestivation period (Janssens, 1964).

The lungfish, *P. annectens,* was found to store large quantities of fat (an average of 368 mg dL^{-1} of total lipids) prior to aestivation and once it commenced, a gradual depletion of body weight and lipids was observed over a 12 month period (Okafor *et al.*, 2011). At the end of that period, fish contained an average of only 60 mg dL^{-1} in total lipids; indicating lipids were a predominant source of energy during aestivation (Okafor *et al.*, 2011).

Field observations of *L. salamandroides* also found a significant decrease in total lipids in females and juveniles following aestivation (Pusey, 1990). Female *L. salamandroides* had higher lipid stores than males suggesting that lipids are not only a predominant energy source during aestivation, but are also important in determining both the timing of reproduction and female fecundity (Pusey, 1990).

1.4 Threats facing aestivating fauna

Freshwater systems across the globe are become increasingly threatened; they comprise less than one per cent of global water and these ecosystems are experiencing declines in biodiversity greater than those seen in terrestrial ecosystems (Dudgeon *et al.*, 2006). Several anthropogenic stressors have been identified as the causal factors of these declines, including water pollution, flow modification, degradation of habitat (Dudgeon *et al.*, 2006), habitat loss (Gleeson *et al.*, 1999), damage to riparian vegetation (Pusey & Arthington, 2003; Epaphras *et al.*, 2007; King & Warburton, 2007; Mantyka-Pringle *et al.*, 2014), climate change (Morrongiello *et al.*, 2011; Comte *et al.*, 2013) and the introduction of invasive species (Gleeson *et al.*, 1999; Ling & Willis, 2005).

Given their relatively unusual life-cycles, aestivating fishes have been identified as particularly vulnerable to several of these major stressors. Habitat loss was identified as the greatest threat to the survival for the aestivating mudfishes *Neochanna* spp. (Gleeson *et al.*, 1999). Swamps inhabited by these species were drained and developed for farming and forestry industries resulting in over 90% of wetland habitat within their distribution being lost or severely modified (Gleeson *et al.*, 1999). The remaining habitat is now highly fragmented and the water table has been altered by surrounding farmland, impacting on the drying and wetting regimes likely impacting on the duration of aestivation (Gleeson *et al.*, 1999). Similar habitat destruction has been observed in regions known to contain other aestivating species, such as *G. pusilla*, where drainage of wetlands and water abstraction are two major threats facing the species (Koster, 2003).

Invasive species are considered a threat to aestivating fishes through several means including; predation, competition for resources such as food and shelter, the potential to introduce exotic parasites and diseases (Becker *et al.*, 2005), and the homogenisation (in terms of species composition) of water bodies (Olden *et al.*, 2008). There has been a large increase in introduced freshwater fishes in south-western Australia over the past decade driven mostly by the release of ornamental fishes (Beatty & Morgan, 2013). Such introductions have resulted in the south-west of Western Australia exhibiting the highest level of homogenisation due to invasive species within Australia (Olden *et al.*, 2008).

Rising water temperatures are also likely to impact on aestivating fishes, particularly when the temperature exceeds the tolerable limit of the species (Tomiyama & Yanagibashi, 2004). If the water temperature exceeds the threshold of the species, the fish will have to either migrate to cooler waters or decrease their metabolic rate (Tomiyama & Yanagibashi, 2004). In the aestivating Japanese Sandeel, *Ammodytes personatus,* water temperature was one of the cues that led to the commencement of aestivation, and differences in aestivation periods were observed between the same species in different regions due to changes in water temperature (Tomiyama & Yanagibashi, 2004). For many aestivating species, the option of migrating to cooler waters is not possible (Mees, 1961; Eldon, 1979) and it is likely they too will display differences in aestivating behaviour with rising temperatures.

Increased temperatures in many temperate freshwater systems may also favour invasive species that have evolved in warmer climates (Rahel & Olden, 2008). Cold water temperatures can act as a filter, preventing such species adapted to warmer temperatures from successfully colonising cold water systems (Rahel & Olden, 2008). As climate change increases water temperatures, the filter effect will diminish and warm water adapted species will then be more likely to successfully invade and colonise these systems (Rahel & Olden, 2008). For example, more than 50% of introduced fishes in wild systems in south-western Australia are from tropical or sub-tropical regions (Beatty & Morgan, 2013). Increases in water temperature will also impact on other physiological aspects of fishes such as feeding rates (Rahel & Olden, 2008). As fish are ectothermic, consumption of food increases with water temperature within a species' temperature thresholds (Rahel & Olden, 2008). Therefore increasing water temperatures from climate change have the potential to magnify impacts of invasive predatory fish (Rahel & Olden, 2008).

1.4.1 *Threats to Lepidogalaxias salamandroides and Galaxiella nigrostriata*

Lepidogalaxias salamandroides and *G. nigrostriata* are both currently listed as near threatened (Taxa which do not currently qualify for Conservation Dependent, but which are close to qualifying for Vulnerable) under the IUCN Red List of Threatened Species

(Wager, 1996a, 1996b) and both became listed as a fully protected species for recreational purposes during the course of this study (Department of Fisheries, 2015). *Galaxiella nigrostriata* is also listed as “Priority 3” (poorly known species with some on conservation lands) under the Wildlife Conservation Act (1950) (Department of Parks and Wildlife, 2014). While there has been little research specifically quantifying the threats to these species, they are likely to be impacted by loss of riparian vegetation (Keller & Brown, 2008), logging of *Eucalyptus* forests impacting on the watershed (Berra & Pusey, 1997), controlled burning regimes in surrounding forests (Berra & Pusey, 1997), the presence of invasive *Gambusia holbrooki* (Berra & Pusey, 1997; Morgan *et al.*, 2004; Becker *et al.*, 2005), secondary salinisation (Beatty *et al.*, 2011), water extraction and climate change (Morrongiello *et al.*, 2011; Comte *et al.*, 2013).

1.4.1.1 Salinisation

Secondary (i.e. human induced) salinisation has had a major impact on south-western Australia’s freshwater fishes (Morgan *et al.*, 2003; Beatty *et al.*, 2011). This process has caused range reductions of a number of endemic fish species (Beatty *et al.*, 2011) along with other taxa such as invertebrates (Bunn & Davies, 1992; Halse *et al.*, 2003). However, in order to more fully understand the past impacts and future viabilities of populations under various salinisation scenarios, additional research is required to determine the salinity tolerances (acute and chronic) of all of the endemic fishes of the south-west, including both *L. salamandroides* and *G. nigrostriata*, as well as any interactions of salinity with other variables such as temperature (Beatty *et al.*, 2011). Moreover, future projected trends in salinity levels vary between catchments and more research is required to model future salinities in systems in the region (Mayer *et al.*, 2005).

1.4.1.2 Riparian habitat degradation

Riparian vegetation is vitally important to the function of aquatic ecosystems and to native freshwater fishes (Mantyka-Pringle *et al.*, 2014) principally through regulation of light and heat (Pusey & Arthington, 2003; Epaphras *et al.*, 2007), provision of allochthonous food sources and governing the transfer of energy between terrestrial and aquatic ecosystems (Pusey & Arthington, 2003), and maintaining the physical characteristics of the banks of rivers and wetlands (Larsen & Alp, 2015). For example, the population of *G. nigrostriata* at Melaleuca Park (Figure 1.1) would not tolerate temperatures above 26°C (Smith *et al.*, 2002a) and survival of this population is thought to rely on thermal stratification in the wetland and the maintenance of riparian vegetation (Smith *et al.*, 2002).

The degradation of the habitats themselves is also a key threat to both species. Several other species have been impacted by habitat alterations and land clearing (Giam *et al.*, 2012). In particular agricultural land use and construction of roads has led to the alteration and degradation of many wetlands globally (Zedler, 2004) and is a current threat to wetlands in the south-west Western Australia.

1.4.1.3 Introduced species

Alteration of riparian vegetation also favours invasive species over native freshwater fishes, reinforcing the need for maintaining and protecting those habitats (Gill *et al.*, 1999; King & Warburton, 2007; Keller & Brown, 2008). *Gambusia holbrooki* was introduced into Australian waterways as a biological control for mosquitos and has since become one of the most widespread invasive fish species in Australia (Gill *et al.*, 1999; Morgan *et al.*, 2004; Becker *et al.*, 2005). It has been documented to aggressively interact with many native freshwater fishes in south-western Australia, including fin-nipping and causing mortality in *G. nigrostriata* (Morgan *et al.*, 2004) and *N. vittata* (Gill *et al.*, 1999), and thereby represents a major threat to many small-bodied freshwater fishes in this region. However, research exploring interactions between *G. holbrooki* and the aestivating fish, *N. diversus*, found that *G. holbrooki* was only present in permanent water bodies and was absent in the ephemeral waterways housing *N. diversus* (Ling & Willis, 2005), highlighting its inability to aestivate. It is likely then, that *G. holbrooki* will have limited impact on aestivating populations of *L. salamandroides* and *G. nigrostriata* should they be isolated from permanent waterbodies (Gill *et al.*, 1999).

The Yabby, *Cherax destructor*, is an eastern Australian species of crayfish introduced into waterways in south-western Australia (Beatty *et al.*, 2005) that may also impact populations of *L. salamandroides* and *G. nigrostriata*. *Cherax destructor* has invasive life-history traits such as rapid growth rate, long periods of spawning and early maturity (Beatty *et al.*, 2005). It can also consume a wide range of food sources including predation of fishes when numbers are high (Beatty, 2006). These traits make *C. destructor* likely to impact on the seasonal ephemeral wetland systems of south-western Australia through outcompeting native crayfishes for food resources, predating on native fishes and impacting on the benthic environment inhabited by *L. salamandroides*.

1.4.1.4 Climate change

Climate change, resulting in increased temperatures and decreased rainfall, is also a recent and future threat to the population viability and distribution of both *L. salamandroides* and *G. nigrostriata* (Comte *et al.*, 2013). The south-west of Western Australia has experienced climatic drying since the 1970’s (Australian Bureau of Meteorology, 2014a). The total rainfall of the region has declined by between 10 – 50 mm per decade with the greatest decline occurring in the area between Busselton and Augusta (Australian Bureau of Meteorology, 2014a) (Figure 1). In combination with the decreasing rainfall, the region has also experienced an increase in mean annual temperature of between 0.10°C to 0.30°C per decade (Australian Bureau of Meteorology, 2014b).

Projections of climate change for the south-west of Australia indicate a further drying and warming of the region (Suppiah *et al.*, 2007). Depending on future levels of CO_2 emissions, global warming is projected to increase the average temperature within the region by between 0.5 - 1.3°C by 2030 (Suppiah *et al.*, 2007). The number of extreme temperature days is also expected to increase in the south-west with Perth, the capital city of Western Australia, projected to increase from an average of 27 days per year above 35°C to between 29 and 38 days above 35°C by 2030 (Suppiah *et al.*, 2007). Days above 40°C are also projected to increase from a current average of four days per year to between five and eight days by 2030 (Suppiah *et al.*, 2007).

Various models also predict decreases in rainfall ranging from a reduction of as little as 1% to a decrease of 20% by 2030, with a median decrease of 14% (Suppiah *et al.*, 2007; Barron *et al.*, 2012). This is projected to equate to a median reduction in annual stream flow across the region of 25% (range 10-42%) by 2030 (Silberstein *et al.*, 2012). Rainfall declines of up to 40% are predicted to continue to 2070 for the upper range of carbon dioxide levels modelled (Suppiah *et al.*, 2007).

Increased temperatures and decreased rainfall are also likely to impact on groundwater dependent ecosystems in the south-west, with 19% of the current area of groundwater dependent ecosystems under high to severe risk (Barron *et al.*, 2012). One particularly sensitive area is the Scott Coastal Plain (Barron *et al.*, 2012) (Figure 1), which is within the ranges of both *L. salamandroides* and *G. nigrostriata* (Morgan *et al.*, 2000; Smith *et al.*, 2002a). Increasing dry periods due to climate change (Xenopoulos *et al.*, 2005; Mantyka-Pringle *et al.*, 2014) are likely to impact on these species and the time required to survive drought periods through aestivation (Pusey, 1990). The Black Mudfish, *N. diversus*, was found to survive extended periods of aestivation with juveniles surviving up to 11 weeks and adults for at least 20 weeks (McPhail, 1999). These rates were believed to vary under different temperatures and humidity; however weight loss of 45% of their initial body weight during aestivation proved fatal (McPhail, 1999). Such information, however, is not available for *L. salamandroides* and *G. nigrostriata*.

1.5 Knowledge gaps

The conservation status of both *L. salamandroides* and *G. nigrostriata* was last assessed in 1996 under IUCN criteria (Wager, 1996a, 1996b). That time represents the half-way point of the major period of climate change that has occurred in this region. Due to threats to their habitats from climate change (Suppiah *et al.*, 2007; Barron *et al.*, 2012) along with other threatening processes, there is an urgent need to reassess the conservation status of these species and explore the implications of habitat and climate change on their current and future viabilities. Past research has predominantly focused on the ecology (Allen & Berra, 1989; Berra & Allen, 1989a) and biology of both species (Martin *et al.*, 1983; Berra & Allen, 1989a; Morgan *et al.*, 2000; Smith *et al.*, 2002a; Gill & Morgan, 2003). Limited information is available on which environmental factors (e.g. period of inundation, groundwater depth, sediment type) most influence the viability of current populations, inhibiting our understanding of how past and projected changes may impact population viabilities. Projected changes in groundwater levels and reduced rainfall (Suppiah *et al.*, 2007; Barron *et al.*, 2012) may alter the duration of aestivation, with potentially serious implications for their physiological tolerances, timing of reproduction, recruitment and larval development. For example, in order to survive longer periods in aestivation it is likely the species may need to increase their lipid stores; however, limited information is available on lipid storage and use in both of these species (Pusey, 1989, 1990). It is paramount that these key knowledge gaps be filled in order to evaluate the current conservation status of the species, prioritise current and future threats that need to be addressed and help guide management decisions to ensure these remarkable fishes are conserved in their natural habitats.

1.6 Research aims

The overall aim of this thesis was to assess the current conservation status of the two species and investigate how resilient they may be to climate change. The specific aims of the study were:

a) Determine the current distribution and population structure of *L. salamandroides* and *G. nigrostriata* and quantify any change in the number of populations or their geographical range.
b) Elucidate which habitat, hydrological (including relative contribution to surface water and groundwater based on stable isotopes) and physiochemical variables best explain the distribution and viability of remnant populations of *L. salamandroides* and *G. nigrostriata*.
c) Determine the lipid storage strategies of both species and whether they vary between populations.
d) Determine the habitat, hydrological and physiochemical variables required to maintain viable populations.
e) Using the above data, identify key threats and predict how projected climate change may impact the viability of wild populations of *L. salamandroides* and *G. nigrostriata*.

It was hypothesised that the change in climate and anthropogenic disturbances to habitat has led to a decline in the distribution of these aestivating fishes. The variables responsible for the presence and absence of both species are hypothesised to be climatically driven. It was also hypothesised that both aestivating fishes will demonstrate obvious depletions of lipids after aestivation, with a gradual increase prior to the following season. It is hoped that the findings of this thesis will identify any range retractions with the potential for updating the species classification and providing more protection for the species

2 Materials and Methods

2.1 Study area and site selection

The study area lies within the Southwestern Province of Western Australia (Unmack *et al.*, 2012) and included sampling sites between Augusta and Albany (Figure 1.1). The sites included those within the Scott River, Doggerup Creek, Gardner River, Shannon River, Broke Inlet, Deep River, Frankland River, and Bow River watersheds, with a large cluster of sites centred on the area around the small town of Northcliffe.

2.1.1 *Climate of the study area*

Northcliffe is the wettest town in Western Australia, with average rainfall of 1,324 mm per year based on data from 1930-1990 (De Silva, 2004). The entire region is characterised by predictable seasonal changes including high winter rainfall and high evaporation rates throughout the summer period (Figure 2.1).

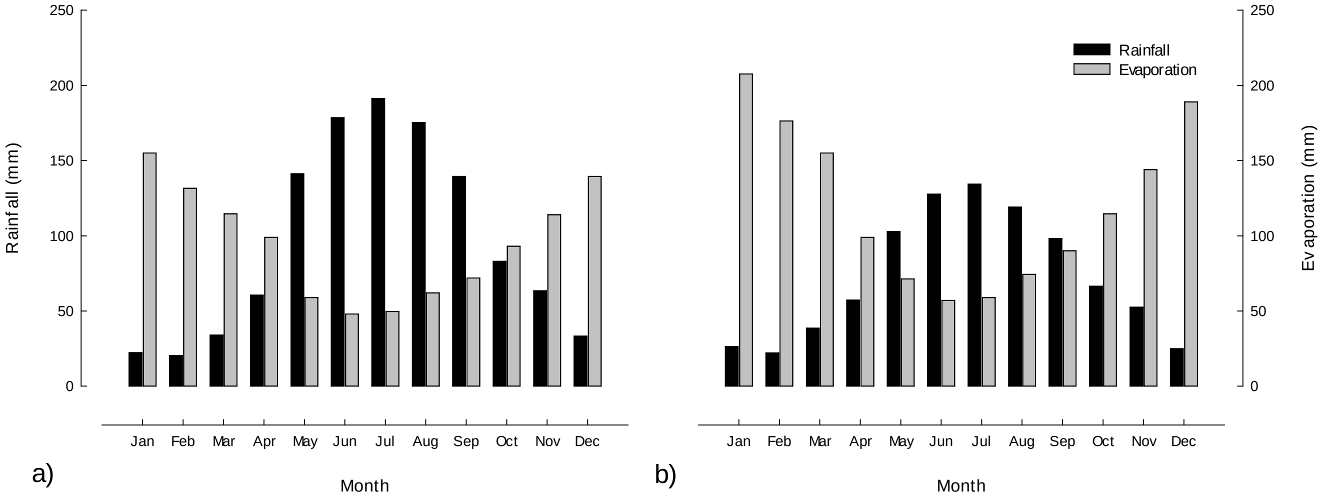

Figure 2.1. Rainfall and evaporation (30 year monthly average 1981 to 2010) across the study area a) Pemberton - near Northcliffe and b) Albany. Data from (Australian Bureau of Meteorology, 2013)

In order to highlight recent climate change within the south-west Western Australia and the study area, data on historical rainfall and temperature anomalies was sourced from the Australian Bureau of Meteorology (Australian Bureau of Meteorology, 2015). Rainfall data for Northcliffe was also sourced for the duration of the study period and compared to the short-term average (Australian Bureau of Meteorology, 2015).

Cumulative deviation from the mean (CDFM) graphs were produced as per Golder Associates (2008). These charts aimed to highlight the cumulative effects of deviation from mean rainfall on groundwater levels across the study area in recent decades, including Augusta, Northcliffe, Walpole, and Elleker (Australian Bureau of Meteorology, 2013).

2.1.2 *Site selection*

A total of 53 sites were selected for sampling for the presence of *L. salamandroides* and *G. nigrostriata* (Appendix A1, Figure 1.1). Sites were mostly ephemeral (Figure 2.2) and the majority were selected based on historical collections (39 of 53 sites) from the Western Australian Museum (WAM), and studies conducted by Christensen (1982), Pusey and Edward (1990) and Morgan *et al.* (1998). When historical sites were inaccessible, surrogates were selected that were as close to the original sites as possible, in both proximity and habitat characteristics. Additional sites were selected where fish were not known historically, to identify new populations and increase the sample size for species distribution modelling. The sites were sampled predominantly across two seasons, with all 53 sites being sampled in June and August 2014 (i.e. winter) and 34 re-sampled in either December 2014 or January 2015 (i.e. summer), just prior to the habitats drying. A sub-sample of six sites was sampled in October (i.e. spring) 2014, and a single site sampled during April and May (i.e. autumn) 2015 to obtain a finer temporal scale of population growth and structure, as well as lipid storage in both species.

Figure 2.2. Examples of sites sampled demonstrating the ephemeral nature of each site; shown during winter sampling (2014) and again in autumn (2015) a) Pool along Chesapeake Rd (site 48) b) Large pool along Chesapeake Rd (site 9) c) Pool along Fouracres Rd (site 26)

2.2 Distribution and population structure of *Lepidogalaxias salamandroides* and *Galaxiella nigrostriata*

Seine nets were used to sample all fishes present at each site (Figure 2.3) as they enabled sampling of all habitat types commonly encountered in peat flat wetlands including both pelagic and benthic zones. This was important as *L. salamandroides* is primarily a benthic dwelling fish (Gill & Morgan, 1999) and *G. nigrostriata* is mostly a pelagic species (Smith *et al.*, 2002a). Seine nets of 3, 5 and 10 m lengths were used depending on habitat conditions (e.g., overall size, presence of snags and vegetation) and all were 1 m in depth, had 3 mm mesh and were weighted with lead line. Nets of 3 and 5 m lengths were the most commonly used. The area sampled varied from 12 m^2 to 300 m^2 depending on the size of the pool with the aim of sampling a proportion (minimum of approximately 5%) of each habitat rather than the entire pool so as to avoid disturbing all fishes that were present.

All fishes captured were initially placed in a large plastic container with a small sub-sample ($\leq$ 10) of each aestivating species then placed into a second bucket for further analysis. The remaining fish were identified and up to 30 individuals of each aestivating species measured to the nearest 1 mm total length (TL) and released at the conclusion of sampling at each site. The sub-sampled fish were then measured to the nearest TL, euthanised in an ice slurry, sealed in plastic vials, and stored in liquid nitrogen. All other native fishes were identified, recorded and returned to the site with all introduced fishes immediately euthanised in an ice slurry.

Sexes of *L. salamandroides* were determined through observation of external characteristics, with males possessing a modified anal fin. Approximate growth rates were determined for *L. salamandroides* and *G. nigrostriata* by calculating the difference between the mid-points of modal lengths in each season. This difference was then divided by the number of months between each sampling season (with the mid way point of each sampling season being used as the start and end point).

Figure 2.3. Seine net being pulled through a wetland along Chesapeake Rd south of Northcliffe (Photo Credit: Stephanie Mugliston)

Extent of occurrence (EOO) was determined by mapping current and historical presence data in ArcGIS Pro 1.0.2 and constructing minimum convex polygons around the perimeter of the sites as per IUCN guidelines (IUCN, 2014). Two polygons were constructed for each species, one for historical sites, and one for current sites found in this study. The area of these polygons were determined for their EOO, with the difference between the two providing an estimated temporal change in EOO for both species (the rule on angles not >180^o were relaxed around coastlines). Area of occupancy (AOO) was also determined as per IUCN guidelines (IUCN, 2014). Two AOO's were determined for each species, one historical and one current. A 1 km^2 grid was inserted as an overlay in ArcGIS with the number of squares overlaying sites summed and multiplied by the area of each square to determine the AOO, with the difference between the two providing temporal change in AOO for both species.

2.3 Physicochemical and habitat variables

In order to characterise the habitats at each site for subsequent species distribution modelling, several physicochemical and habitat variables were recorded at each site in each of the two main seasons sampled (winter and summer). The wetland type at each site was classified using Semeniuk and Semeniuk (1995) and Department of Parks and Wildlife (2003). Physicochemical variables included water temperature, dissolved oxygen (ppm and percentage), conductivity (µS.cm^{-1}), total dissolved solids (TDS), salinity (ppt), pH, and oxidation-reduction potential (mV). All variables were taken at a depth of approximately 30 cm using a water quality meter (YSI), with three replicates taken at various habitats within each site and a mean (±1S.E.) determined. During the summer sampling period, some sites were not of sufficient depth to measure water quality *in-situ* and therefore water was carefully collected in buckets with measurements then immediately taken as described above.

HOBO Pendant Temperature/Light data loggers were used to help identify date of drying in 30 sites. Each logger was attached to a star picket with cable ties and installed at the deepest accessible point between the dates of 23rd June to August 4th 2014. The loggers were programmed to record temperature (C°) and light (lum/ft^2) at an interval of every two hours and were subsequently collected in March and April 2015, with data being downloaded and temperature and light plotted over time for each site. Logged sites were deemed to have dried once a drastic diurnal fluctuation in light level was recorded.

The habitat condition at each site was assessed by scoring for a number of variables including, ‘naturalness’ (ranging from a score of 1 for no anthropogenic modifications to 5 for completely artificial), bank condition (ranging from a score of 1 for pristine to 5 for highly eroded), riparian vegetation naturalness (ranging from a score of 1 for all native to 5 for all introduced species), and connectivity (ranging from a score of 1 for highly connected such as a stream to a score of 5 for a completely isolated pool).

The other habitat variables measured included pool dimensions (using a laser range finder), maximum pool depth (using a metre stick), average bank angle (based on visual observation), percentage of shade cover (based on visual observation), the presence/absence of flow, and the presence/absence of crayfish burrows.

2.4 Groundwater depth and soil field texture

To determine groundwater depth during the dry period (i.e., April, 2015), 47 sites were selected, including sites where fishes where present and where they were absent. At each site one to two cores were drilled using an auger to allow for any minor spatial variation in topography within each site. The cores were drilled until groundwater was reached, or up to a maximum depth of 2.5 m. Upon reaching water the core was left for five minutes for water to settle before groundwater depth was measured. The groundwater depth at each site was measured from the surface to the point of saturation/free-standing water in each core. Depth to consolidation/clay in the core was also recorded.

Benthic substrate was also recorded at each site when dry via texture sampling and visual observation. Grab samples of sediment were taken at several sites, ranging from one to three samples, dependent on depth of core and visual change in soil profiles. Each grab sample was stored in a zip-lock bag, before being sieved and field texture determined. A small handful of the benthic substrate was kneaded into a bolus and rolled out into a ribbon between thumb and forefinger, and the behaviour of the substrate identified for the soil texture grade as per Northcote (1979).

2.5 Species distribution modelling

Species distribution modelling was undertaken to determine environmental parameters influencing presence/absence of each species. Sites were coded for both *L. salamandroides* and *G. nigrostriata* as 1 (species currently present) or 0 (species currently absent). A total of 23 environmental variables were measured across the seasons for each site and current occurrence data were then related to environmental predictor variables using generalised linear models (GLMs) with a binomial distribution and logit link function. Given the importance of variable selection and the need to avoid over-fitting the data during model construction (Burnham & Anderson, 2010), a number of approaches were used to reduce the variable set. Initially, water quality and condition indices were reduced to a smaller set of uncorrelated factors using principal components analysis. A General Linear model using all remaining variables was then fitted and variables with $P > 0.30$ removed before fitting a final global model. The global model was used to generate a set of all possible models using the *R* package MuMIn (Barton, 2013). Models were ranked by the Akaike Information Criterion, corrected for small sample size (AIC_c) (Burnham & Anderson, 2010), and model averaging was performed using MuMIn across all models within four AIC_c values of the best model. The importance of each variable was determined by summing Aikaike likelihood weights across all models within the top-ranked set in which the variable occurred, providing the selection probability that a given variable will appear in the AIC best model (Burnham & Anderson, 2010).

A number of variables, such as depth to groundwater, depth to clay/consolidation, presence of crayfish and drying period, were measured only at a subset of sites. These variables were not included in the GLMs. Instead, their influence on species presence/absence was assessed by univariate logistic regression analysis.

To identify potential environmental predictors that may be responsible for changes in historical distribution for *L. salamandroides* and *G. nigrostriata*, current and historical occurrence were compared across 40 and 27 sites, respectively. Changes in occurrence were coded as 0 (historically present, currently absent) or 1 (historically present, currently present) and related to environmental variables as described above. Environmental variables were not available for historical sites, so any changes in occurrence could only be related to current values taken during this study.

2.6 Isotopic analysis

In order to characterise the relative contribution of rainfall and groundwater to the surface water of sites during winter, oxygen and hydrogen analyses were conducted (Salameh, 2004; Liu *et al.*, 2010; Skrzypek & Ford, 2014). Surface water samples for oxygen isotope and deuterium testing were collected across the two sampling seasons in 2014. Samples were taken from 20 sites in winter and 16 of the 20 sites in summer, with the sites selected providing a wide range from west to east (Appendix 1). Groundwater samples for isotope analysis were also collected (from the 20 sites previously sampled in winter) in April 2015. Rainwater isotope data were used for comparative purposes and sourced from Bowen (2015) with coordinates and elevation used from Site 21 on Chesapeake Road.

Surface water samples were collected as grab samples within 30 cm of the water surface. To sample groundwater, a hand auger (AMS Sand Auger) was used to reach the water table, with a sample then extracted approximately 5 cm below the surface of the free-standing groundwater. The samples were collected using a pre-rinsed (in site water) 30 ml clear glass jar with a HDPE lid. Sample jars were filled and re-capped below the water surface, reducing any breathing space in the sample to a minimum. The jars were immediately wrapped in tin foil and stored at ~4°C prior to isotope analysis.

Analysis occurred using an Isotopic Liquid Water and Continuous Water Vapor Analyser Picarro L1102-i at the West Australian Biogeochemistry Centre, The University of Western Australia (Skrzypek & Ford, 2014). The values of δ^2H and $\delta^{18}O$ were normalised to the Vienna Standard Mean Ocean Water (VSMOW) scale on the basis of three laboratory standards (Coplen, 1996) each replicated twice and reported in parts per thousand (‰) (Skrzypek, 2013). The laboratory standards were calibrated against international standards from the International Atomic Energy Agency (IAEA): VSMOW2, SLAP2, and GISP. The uncertainty (one standard deviation) of the instrument used was 1.0‰ for δ^2H and 0.1‰ for $\delta^{18}O$.

Pearson's correlations were used to interpret the relationship between the values of δ^2H and $\delta^{18}O$. Multiple paired t-tests were performed to compare surface water samples between seasons (winter and summer) and rainwater samples between seasons to account for any seasonal variation. Paired t-tests were also performed for comparisons between surface water and groundwater, surface water and rainwater, and groundwater and rainwater. Seasonal dynamics of the wetlands were identified through analysing relative differences in stable isotope composition, and interpretations of recharge mechanisms within the study area were made from the results of the t-tests. Evaporative loss of surface waters was determined as per Skrzypek *et al.* (2015).

2.7 Lipid extraction

Lipid extraction was performed on retained *L. salamandroides* and *G. nigrostriata,* as well as *G. munda,* as a non-aestivating control. The method of extraction was performed with modifications to Folch *et al.* (1957). One modification based on safety and security reasons, was the substitution of chloroform with dichloromethane as per Cequier-Sanchez *et al.* (2008). The ratios of dichloromethane/methanol (2:1) solution to tissue were kept the same as Folch *et al.* (1957) at 20:1. In summary, the extraction method determined dry weight of the specimens before tissues were macerated (instead of homogenising as in Folch *et al.* (1957)) separately and placed into stoppered test tubes with the dichloromethane/methanol solution at the above ratio. The solution with tissue was then agitated for 15 minutes before being filtered through Grade 1 filter paper and rinsed with 30 ml of solution. Once filtered and rinsed the remaining tissue was again dried to constant weight with the final weight being subtracted from initial dry weight. The final values were then expressed as grams of lipid per gram of dry weight. When dry weight of specimens was deemed too low to determine any change in dry weight after lipid extractions, the fish were pooled together with fish of the same species and age class/gender from the same pool prior to lipid extraction being performed.

General linear models were conducted to analyse differences of lipid content between season and sex/age for each individual species, with post hoc tests of Tukey to identify which levels were significantly different. The entire dataset was pooled with differences between all species, seasons and sexes analysed through the use of a full factorial model with post hoc Tukey test.

3 Results

3.1 Climate of the study area

South-west Western Australia has experienced both warming (Figure 3.1) and drying in recent decades (Figure 3.2). The deviation away from the mean rainfall varies across the study area, with the region around Augusta only experiencing the accumulative effects of negative deviation from the mean across the recent decade, and the central regions of the study area (i.e., around Northcliffe and Walpole) experiencing negative deviation from the mean since the 1970's (Figure 3.3). The region west of Albany near Elleker also demonstrated this negative deviation, but with some fluctuation including higher than average rainfalls during the 1990's before receding again in the last decade (Figure 3.3).

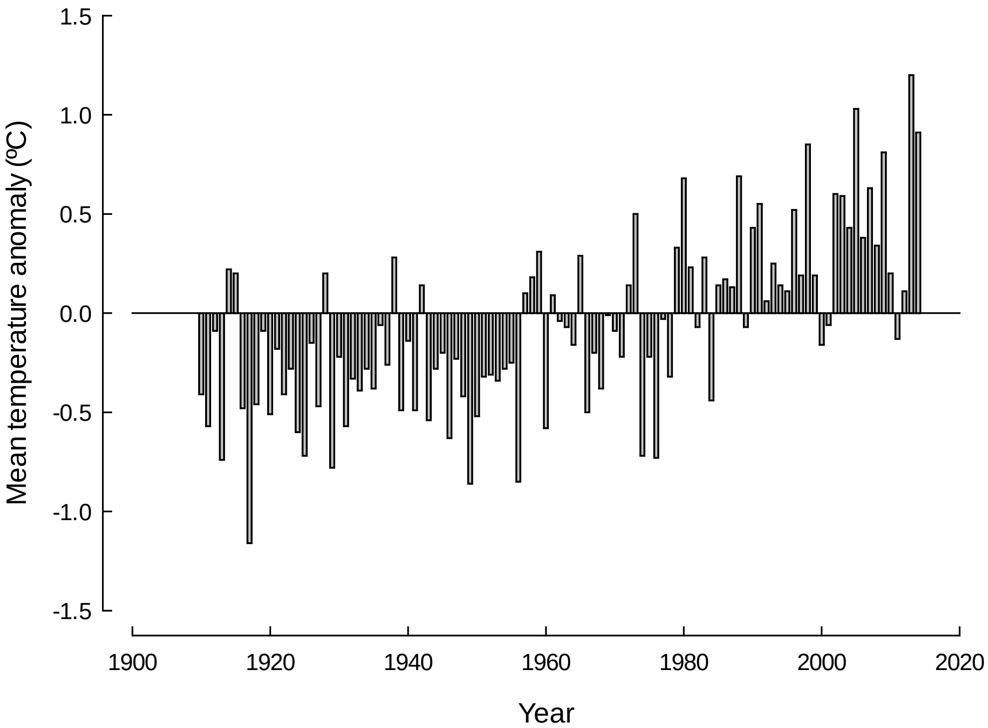

Figure 3.1. Mean temperature anomalies for the south-west of Western Australia based off 30 year average 1961-1990 (Australian Bureau of Meteorology, 2015)

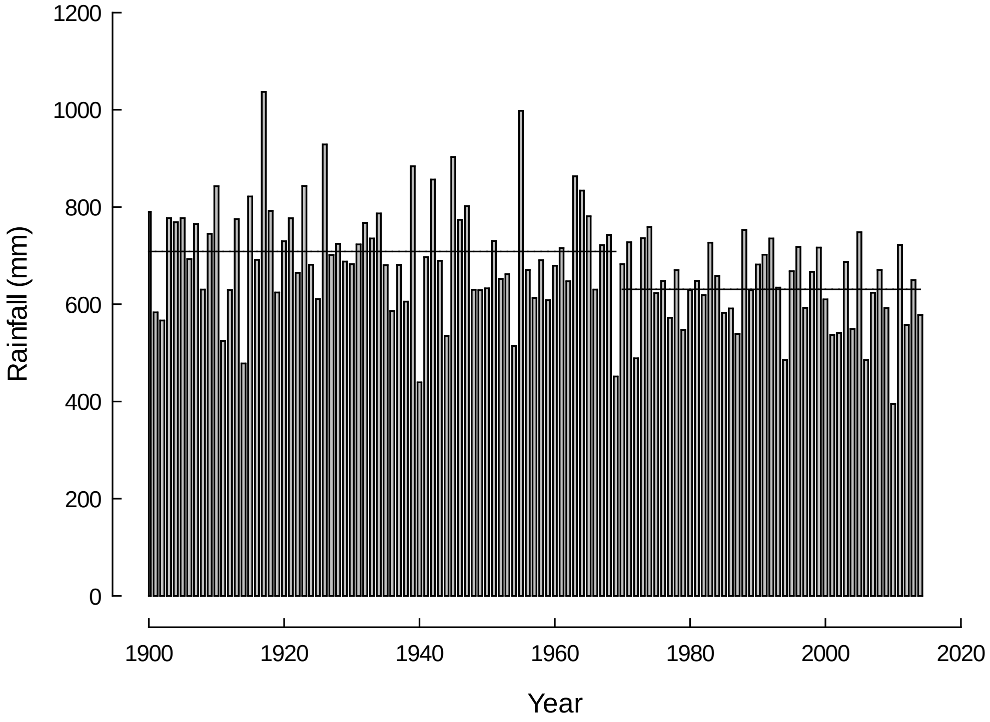

Figure 3.2. Average annual rainfall for the South-western region of Western Australia from 1900 to 2014 with two linear averages showing average rainfall pre 1970 and post 1970. (Data from Australian Bureau of Meteorology (2015)).

Figure 3.3. Cumulative deviation from the mean (CDFM) rainfall (based on average for 1960-2014) a) Augusta (Cape Leeuwin), b) Northcliffe, c) Walpole, d) Elleker (Grassmere) (Australian Bureau of Meteorology, 2013)

Despite the recent drying, the annual rainfall in Northcliffe (closest town to most sampling sites) across the study period (May 2014 to April 2015) was higher (1235 mm) than the short-term (2000 to 2014) average (1232 mm), due largely to two spikes in May and July (Figure 3.4); noting that the actual rainfall however was lower than the average in all months except, March, April, May and July. This early above average rainfall may account for the early re-inundation of several pools in the study area (pers. obs. 25th April 2015; Morgan et al. pers. comm.).

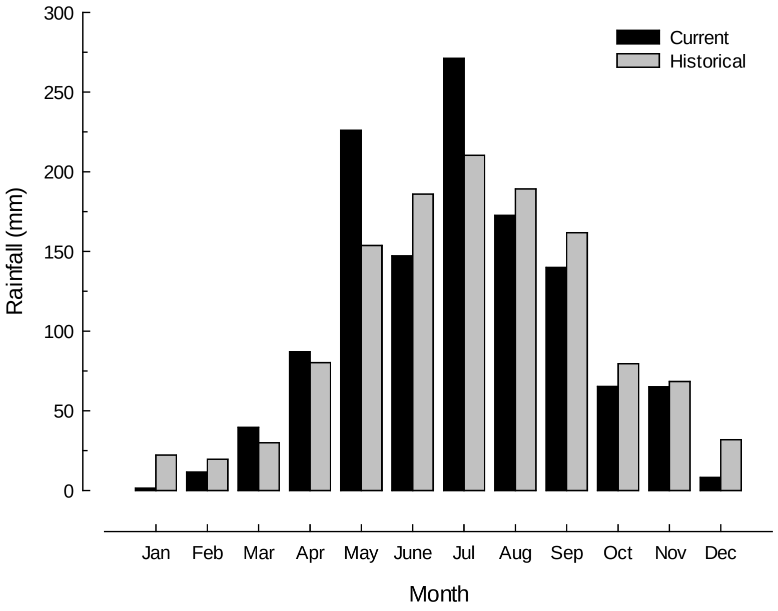

Figure 3.4. Current rainfall versus recent historical average rainfall (2000-2014) for Northcliffe during the study period May 2014 to April 2015

3.2 Presence and population structure of *Lepidogalaxias salamandroides* and *Galaxiella nigrostriata*

Across all seasons, a total of 1,347 *L. salamandroides* were caught at 30 (26 historical) of the 53 sites sampled, and a total of 1,294 *G. nigrostriata* were caught across 24 (20 historical) of the 53 sites sampled (Table 3.1, Appendix A2). By-catch included a total of 283 *Galaxiella munda* (present at 17% of sites), 13 *Nannatherina balstoni* (7.6% of sites), 424 *Nannoperca vittata* (34% of sites), 76 *Bostockia porosa* (13.2% sites) and 44 *Galaxias occidentialis* (11.3% of sites). One introduced species, *Gambusia holbrooki*, was also captured during the study period, with a total of five individuals recorded across two sites in close proximity (site 7 Windy Harbour Rd and site 24 Windy Harbour Rd). The crayfishes *Cherax preissii* and/or *Cherax quinquecarinatus* were also present at most sites (71.7%) sampled.

Table 3.1. Species of fish caught during study each period of sampling including number of sites present in the various seasons and historical sites.

	Total fish caught	**No. sites present (% of sites sampled)**	**No. sites present in winter (% of sites sampled)**	**No. sites present in spring (% of sites sampled)**	**No. sites present in summer (% of sites sampled)**	**Total historical sites present (% of historical sites)**
L. salamandroides	1,347	30 (56.6%)	27 (50.9%)	6 (100%)	21 (61.8%)	26 (66.7%)
G. nigrostriata	1,294	24 (45.3%)	10 (18.9%)	3 (50%)	22 (64.7%)	20 (71.4%)
G. munda	283	9 (17%)	4 (7.6%)	1 (16.7%)	6 (17.7%)	-
N. balstoni	13	4 (7.6%)	2 (3.8%)	-	2 (5.9%)	-
N. vittata	424	18 (34%)	6 (11.3%)	2 (33.3%)	16 (47.1%)	-
B. porosa	76	7 (13.2%)	1 (1.9%)	-	6 (17.7%)	-
G. occidentalis	44	6 (11.3%)	3 (5.7%)	1 (16.7%)	4 (11.8%)	-
G. holbrooki	5	2 (3.8%)	2 (3.8%)	-	1 (2.9%)	-

During the winter sampling period, a total of 500 *L. salamandroides* (37.1% of the total number captured during the study) were recorded in 27 sites (50.9% of total) (Figure 3.5).

During the same period a total of 109 *G. nigrostriata* were caught (8.4% of total) in 10 sites (18.9% of total) (Figure 3.6). Other fish caught during winter included 14 *G. munda* (5% of total), six *N. balstoni* (41.2% of total), 25 *N. vittata* (5.9% of total), one *B. porosa* (1.3% of total), six *G. occidentialis* (13.6% of total) and two introduced *G. holbrooki* (40% of total).

The sub-sample undertaken in October 2014 recorded a total of 82 *L. salamandroides* (6.1% of total) and the species was present in all six sites sampled, and a further 94 *G. nigrostriata* were recorded (7.3% of total) across three sites (50% of total). A further eight *G. munda* were also recorded (2.8% of total), with seven retained for lipid analysis, along with 19 *N. vittata* (4.5% of total), and 17 *G. occidentalis* (38.6% of total).

During the summer period, 696 *L. salamandroides* were caught (51.7% of total) across 21 sites of the 34 sampled (61.8% of total) (Figure 3.7). During this same period 1,042 *G. nigrostriata* were caught (80.5% of total) across 22 sites (64.7% of total) (Figure 3.8). Two hundred and sixty one *G. munda* were also caught (92.2% of total), with a sample of 27 retained for lipid extractions. Other native fishes caught during the summer were seven *N. balstoni* (53.9%) across two sites (5.9% of total), 380 *N. vittata* (89.6% of total) across 16 sites (47.1% of total), 75 *B. porosa* (98.7% of total) across six sites (17.7% of total), 21 *G. occidentalis* (47.7% of total) across four sites (11.8% of total), and three introduced *G. holbrooki* (60% of total) at one site (2.9% of total).

During the April-May (autumn) sub-sample (single site along Chesapeake Rd: Site ID 21), a total of 69 *L. salamandroides* (5.1% of total) and 49 *G. nigrostriata* were recorded (3.8% of total). During the course of the study a record of *G. nigrostriata* in the Mitchell River west of Marbellup Brook was confirmed (Figure 3.6) and subsequently included in calculating EOO and AOO for the species.

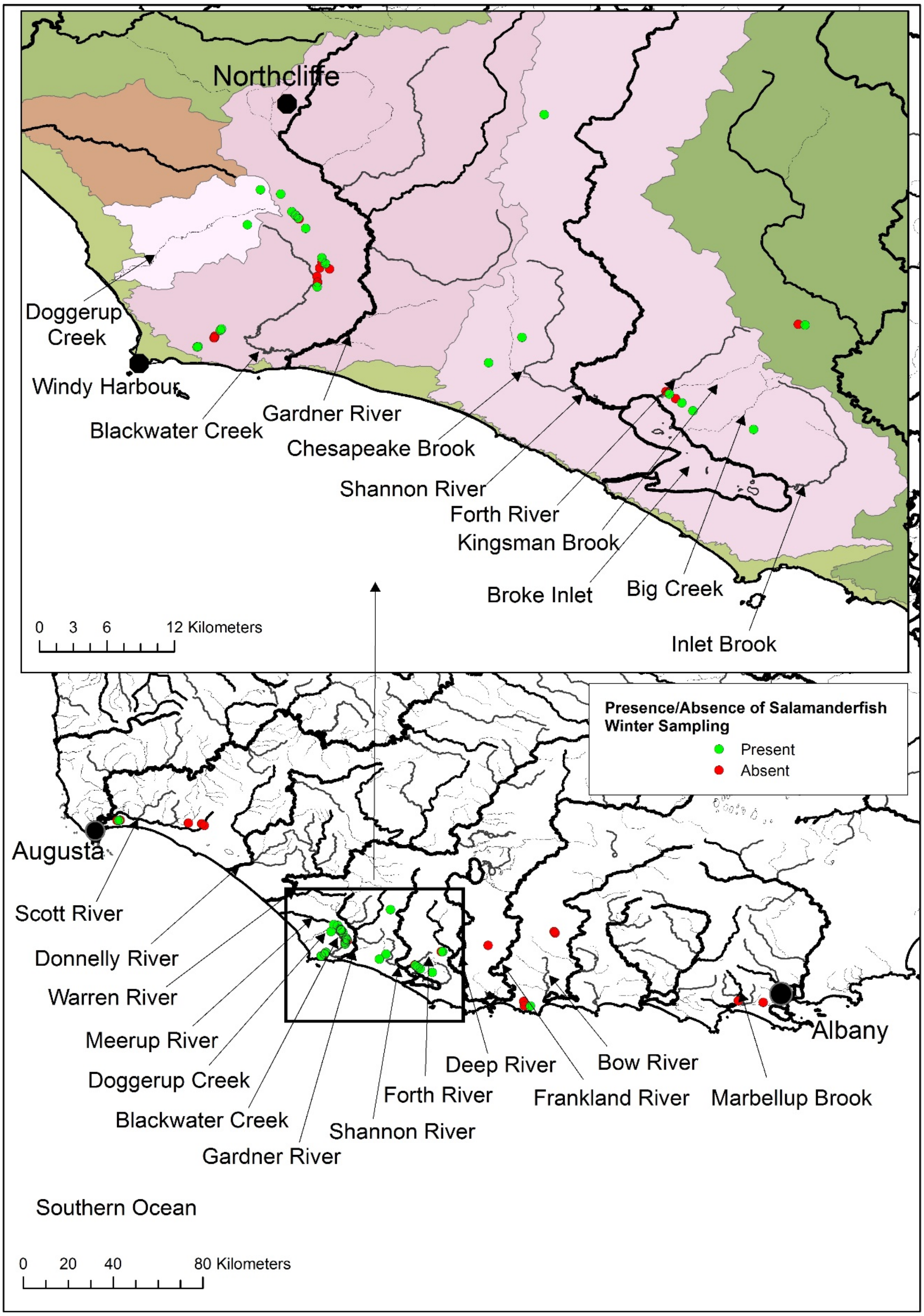

Figure 3.5. Presence and absence sites for Salamanderfish, *Lepidogalaxias salamandroides*, during the winter sampling period

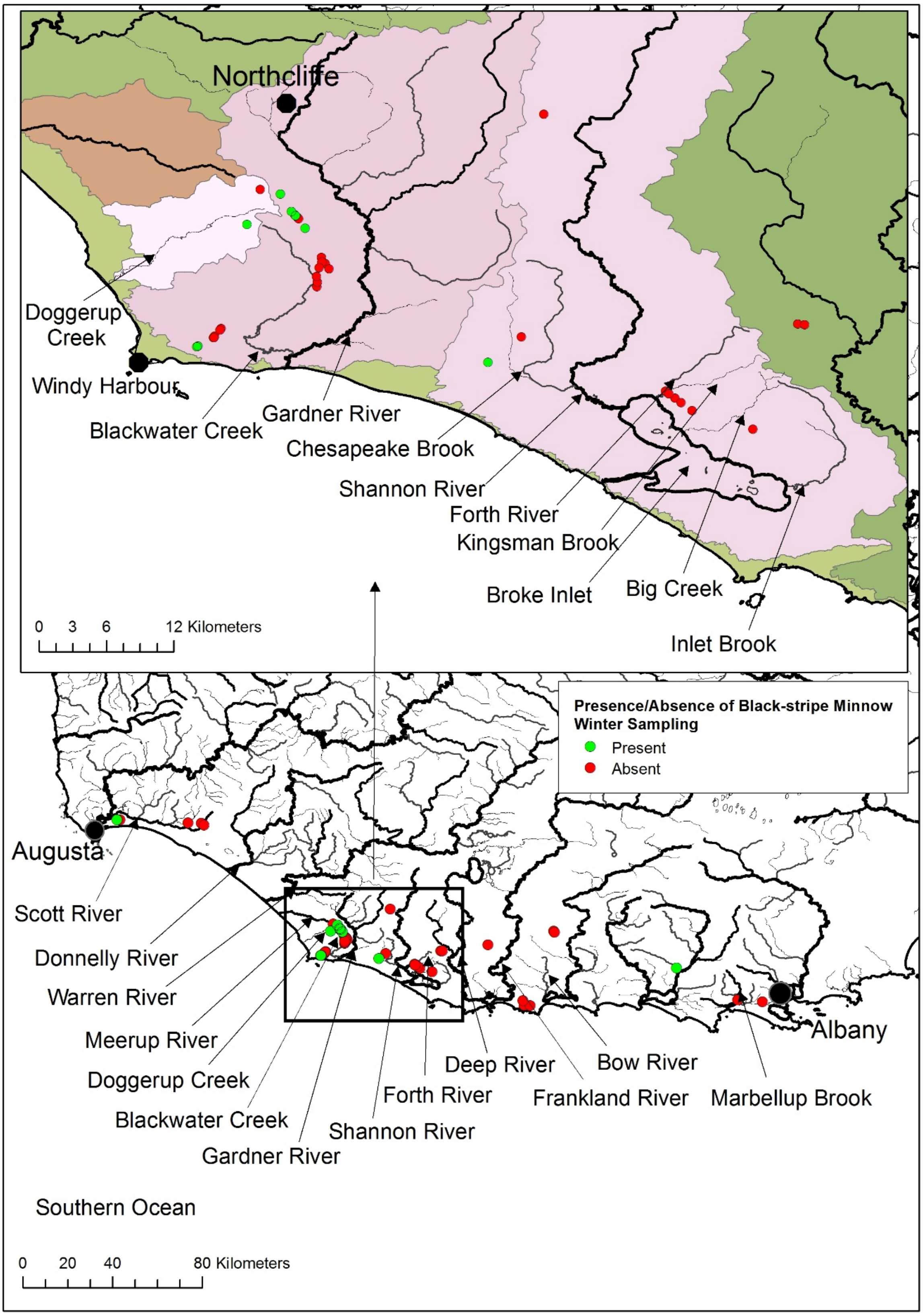

Figure 3.6. Presence and absence sites for Black-stripe Minnow, *Galaxiella nigrostriata*, during the winter sampling period

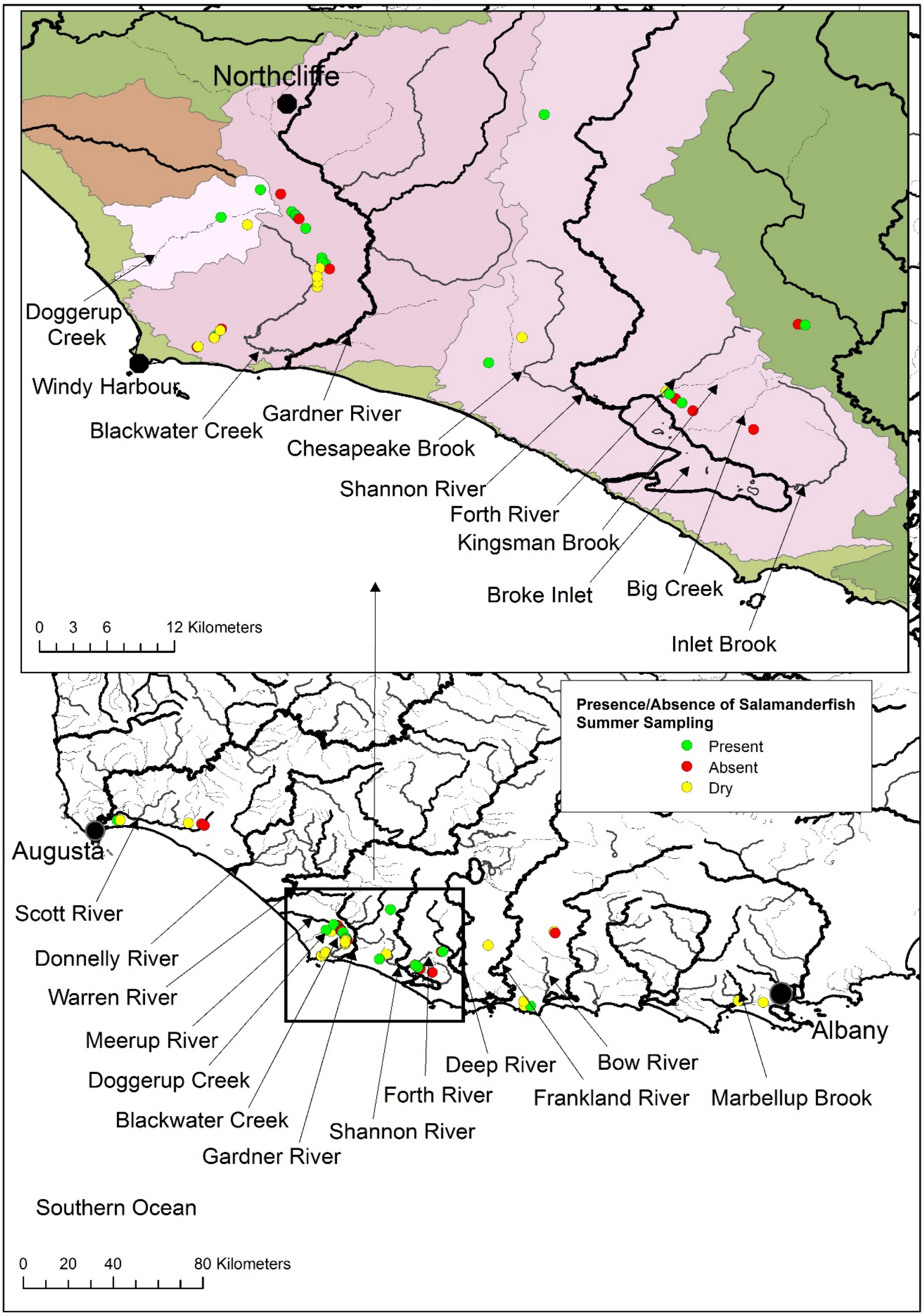

Figure 3.7. Presence, absence and dry sites for Salamanderfish, *Lepidogalaxias salamandroides*, during the summer sampling period

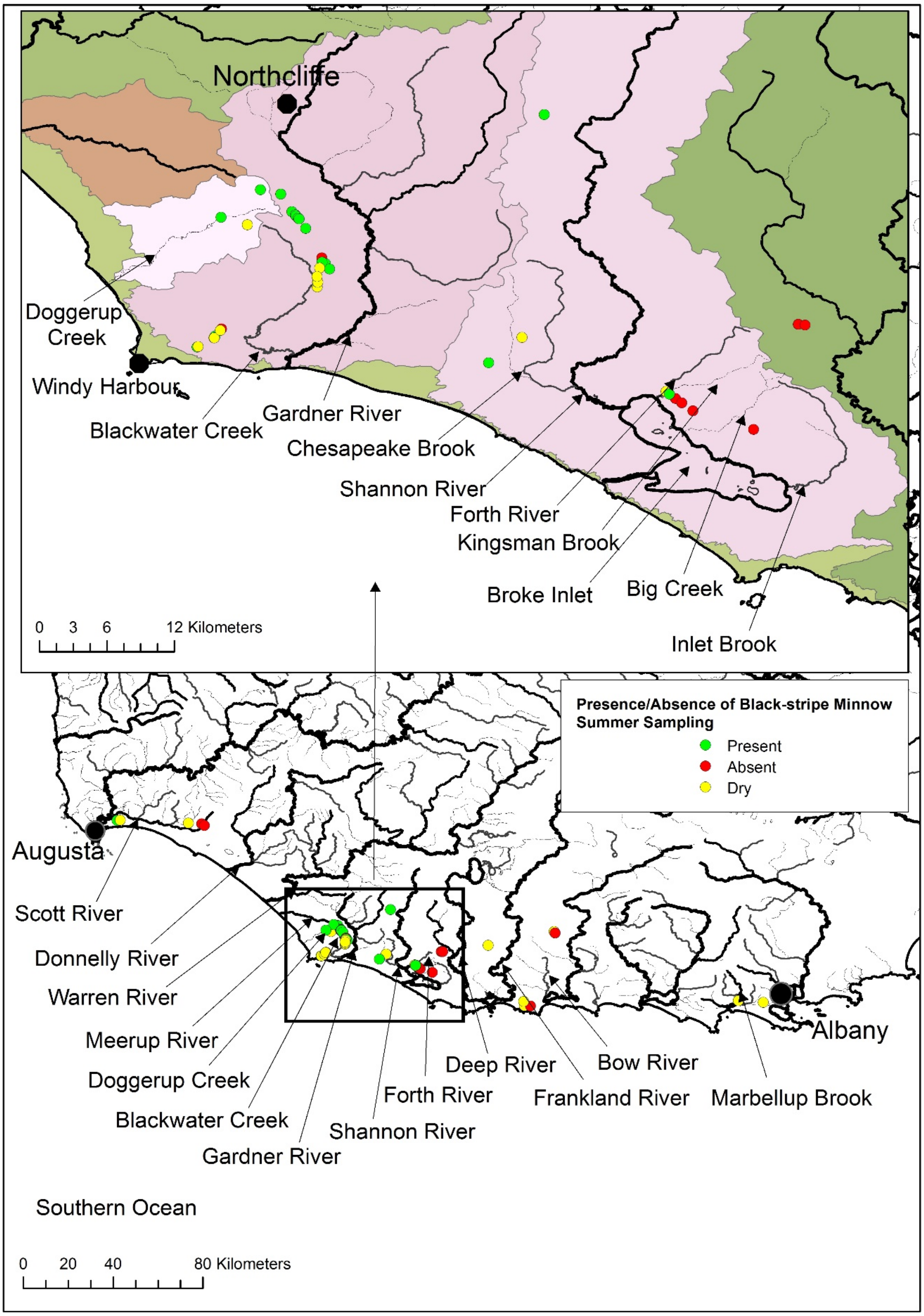

Figure 3.8. Presence, absence, and dry sites for Black-stripe Minnow, *Galaxiella nigrostriata*, during the summer sampling period

Lepidogalaxias salamandroides historically had an EOO of 12,829.53 km^2 (Appendix B1), however, they are now currently only distributed across 2,640.47 km^2 (Appendix B2) (a reduction of 79%). A decrease in EOO was also observed for *G. nigrostriata* that were historically distributed across an area of 47,578.91 km^2 (Appendix B3) and currently have an EOO of 42,017.39 km^2 (Appendix B4) (a reduction of 12%). Area of occupancy (AOO) of *L. salamandroides* declined from a historic 72 km^2 to a present 21 km^2 (a reduction of 71%) The AOO for *G. nigrostriata* also declined, from a historic 55 km^2 to a present 24 km^2 (a reduction of 56%).

A total of 632 *L. salamandroides* were measured (62 in autumn, 246 in winter, 62 in spring, 262 in summer) and ranged from 9 to 75 mm TL (mean = 32.59 (±0.47 S.E.)) with males reaching a maximum of 51 mm TL (Figure 3.9). All fish with lengths greater than 51 mm TL were female. The modal length of juvenile (0+) *L. salamandroides* during autumn (April to May) was 28-32 mm TL and during winter (June to August) was 30-32 mm TL (a growth rate of 0.5 mm/month). In spring (October), the 0+ modal length was 14-16 mm TL, with a secondary modal length of larger individuals of 40-42 mm TL. In the summer sampling period (December to January) the modal lengths of the 0+ cohort of *L. salamandroides* was 24-26 mm TL (a growth from spring to summer of 4 mm/month).

A total of 378 *G. nigrostriata* were measured (15 in autumn, 24 in winter, 24 in spring, 315 in summer) and ranged from 11 to 56 mm TL (mean = 28.45 (±0.34 S.E.)) (Figure 3.10). *Galaxiella nigrostriata* exhibited a modal total length of 28-34 mm in autumn (April to May), followed by a modal total length of 34-36 mm in winter (June to August) (a growth rate of 2mm/month). During spring (October) the modal total length was 18-20 mm TL with a high proportion of individuals also in the length range 40-42 mm TL. During the summer sampling period (December to January) the modal total length was 26-28 mm TL representing a growth rate from spring to summer of 3.2 mm/month.

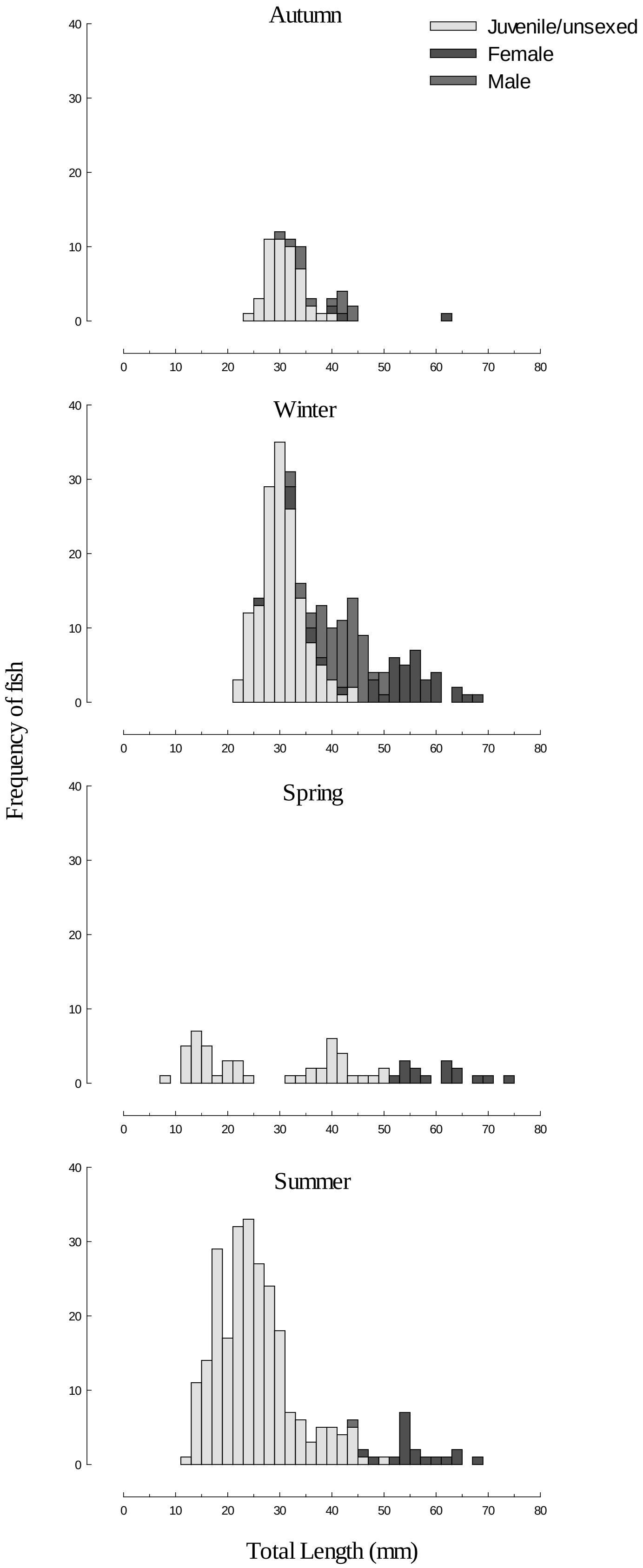

Figure 3.9. Length frequency histograms for *Lepidogalaxias salamandroides* across sampling seasons

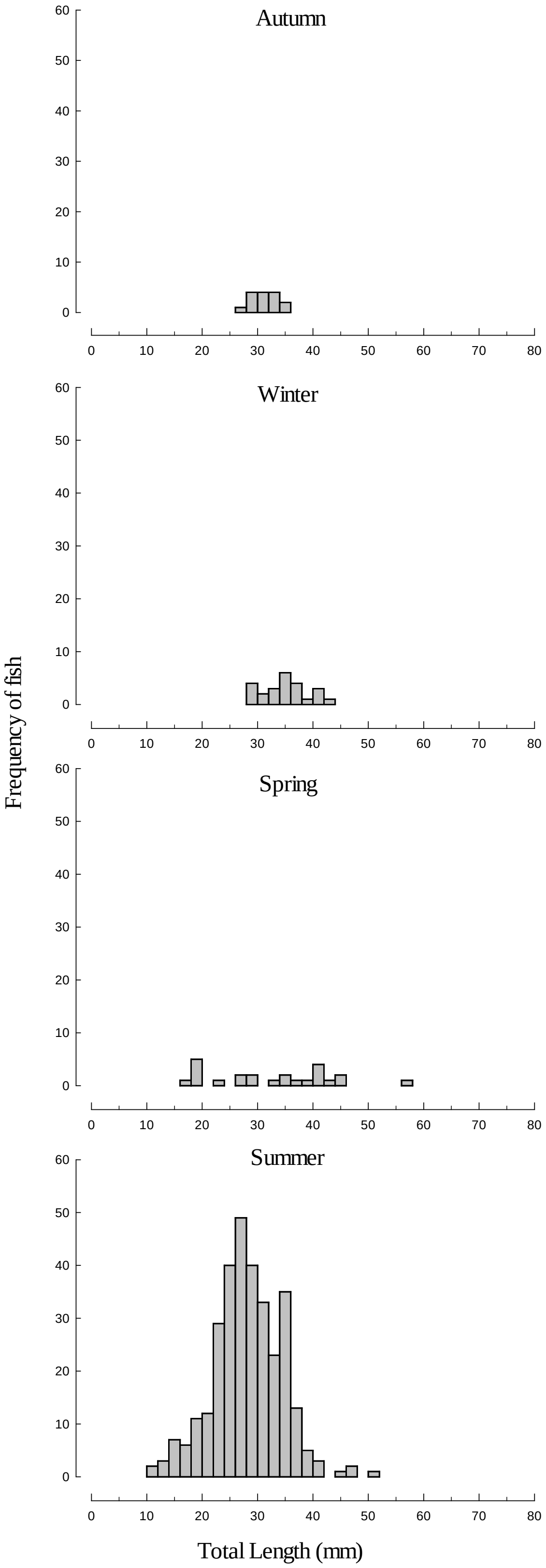

Figure 3.10. Length frequency histograms for *Galaxiella nigrostriata* across sampling seasons

3.3 Physicochemical and habitat variables

Flow was negligible at all sites during all sampling periods. The habitats where the sites were located were predominantly classified as palusplain wetlands (i.e. seasonally waterlogged flats) (Table 3.2). Three sites were classified as floodplains, seasonally inundated flats, which were those on rivers (e.g. Forth River), streams & drain ways/culverts. One site along Broke Inlet Rd was classified as a sumpland, a seasonally inundated basin, and one was site along Windy Harbour Rd south of Northcliffe was identified as a paluslope wetland (i.e., a seasonally waterlogged slope).

All sites were invariably non-saline ≤0.71 mg/L (mean = 0.23 (±0.01 S.E.)). In both winter and summer, sites were predominantly acidic and pH ranged from 3.41-7.69 (mean = 4.77 (±0.12 S.E.)) with both maximum and minimum pH recorded in winter (Table 3.2). Water temperature ranged from a minimum of 9.36ºC in winter to a maximum of 33.47ºC in summer (mean = 17.66 (±0.63 S.E.)). Dissolved oxygen ranged from 1.45 to 9.21 mg/L (mean = 5.50 (±0.18 S.E.)) with oxygen saturation 18.00 - 87.47 % (mean = 56.72 (±1.54 S.E.)).

Temperature/light loggers were retrieved from 25 of the 30 initial sites (five were missing) with one retrieved data logger being no longer functional. Light intensity in most cases remained relatively constant until an obvious diurnal fluctuation was detected, which implied that the logger was now exposed to the air and drying of the site (Figure 3.11, Appendix C). Date of drying varied among sites ranging from the 14th of October 2014 to the 13th of February 2015, with 19 drying between 6th December 2014 and 13th of February 2015 (Table 3.3). Temperature displayed a gradual increase across the seasons until the estimated date of drying when an obvious drastic increase in diurnal variability was observed.

Table 3.2. Physicochemical variables across seasons displaying number of sites (N), minimum and maximum values, mean and standard error (S.E.).

	Season	**N**	**Min.**	**Max.**	**Mean**	**S.E.**
pH	Winter	51	3.41	7.69	4.86	0.15
	Spring	6	3.65	4.95	4.23	0.24
	Summer	33	3.49	7.21	4.71	0.21
Temperature (ºC)	Winter	51	9.63	20.30	13.57	0.32
	Spring	6	15.53	18.10	16.67	0.42
	Summer	33	16.73	33.47	24.16	0.81
DO (mg/L)	Winter	51	2.18	9.21	6.52	0.18
	Spring	6	4.66	6.31	5.50	0.29
	Summer	33	1.45	5.70	3.94	0.18
Oxygen saturation (%)	Winter	51	23.53	87.47	62.69	1.73
	Spring	6	48.30	64.77	56.76	2.93
	Summer	33	18.00	71.70	47.48	2.47
Salinity (mg/L)	Winter	51	0.03	0.47	0.20	0.01
	Spring	6	0.07	0.18	0.12	0.02
	Summer	33	0.12	0.71	0.29	0.03
Conductivity (µS/cm)	Winter	51	42.80	761.00	320.83	21.62
	Spring	6	129.27	315.57	217.16	27.61
	Summer	33	234.00	1668.33	614.60	64.73
TDS (mg/L)	Winter	51	36.18	617.5	268.18	17.43
	Spring	6	96.85	250.03	168.82	22.57
	Summer	33	162.28	936.00	393.41	35.99
ORP (mV)	Winter	51	63.27	301.95	232.89	7.76
	Spring	6	179.33	266.43	213.47	12.70
	Summer	33	68.77	268.07	190.82	10.14

Table 3.3. Earliest estimate for date of drying at each site (sorted west to east) based on increases in temperature and light intensity from data loggers (see Appendix C).

Site	**Site ID**	**Earliest estimated date of drying**	**Data logger increase**	
			Temp (°C)	**Light intensity (lum/ft²)**
Scott River Rd Power Pole 43	2	13/02/2015	29.152	11776
Scott River Rd Power Pole 34	33	16/12/2014	29.652	17408
Scott River Rd (Vegetation site)	1	12/12/2014	28.159	14336
Windy Harbour Rd (pool near lake)	4	6/12/2014	32.911	15872
Small Lake Windy Harbour Rd	6	4/01/2015	24.931	14336
Windy Harbour Rd	23	1/01/2015	33.118	11776
Windy Harbour Rd	8	14/10/2014	33.43	12288
Chesapeake Rd (Sally City)	21	19/01/2015	28.853	10752
Chesapeake Rd	20	13/12/2014	29.853	12800
Chesapeake Rd (1st Large Pool)	9	13/02/2015	23.966	9728
Large Chesapeake pool (opposite other large pool)	31	17/01/2015	34.796	10240
Chesapeake Rd (adjacent to overgrown site)	28	21/12/2014	31.676	19456
Chesapeake Rd (on right hand side outside of Northcliffe)	19	7/02/2015	30.862	3328
Lower Gardner River Rd	41	28/10/2014	31.064	11776
Small pool Lower Gardner River Rd	42	12/01/2015	43.601	17408
Small pool Lower Gardner River Rd	44	28/10/2014	34.268	14848
Chesapeake Rd (log pool)	48	25/12/2014	38.49	16384
Chesapeake Rd	30	19/11/2014	29.953	12288
Pool on Moore’s Hut Track	49	4/01/2015	34.058	14848
Corner of Deeside Coast Rd and Preston Rd	18	10/01/2015	29.652	12288
Pool/stream Broke Inlet Rd	46	30/12/2014	41.692	19456
Beardmore Rd Pools	11	7/02/2015	38.49	15360
Conspicuous Cliff Rd	35	18/12/2014	34.268	10240
Corner Station Rd and Conspicuous Cliffs Rd	37	30/10/2014	30.054	12288

*Dates are the earliest estimated date of drying as several loggers were slightly higher than ground level when installed, meaning they would have been exposed to the air a short time prior to complete drying of the pool occurred.

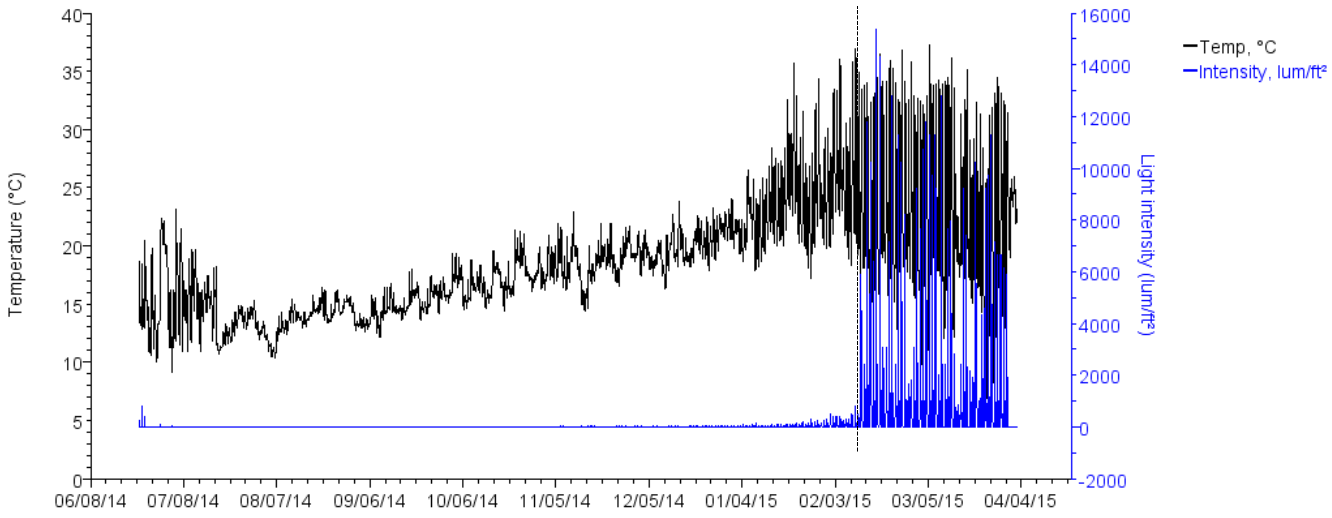

Figure 3.11. Water temperature and light intensity recorded at Scott River Road Power Pole 43 (Site ID 2) displaying a clear increase in temperature and light intensity used to estimate earliest date of drying at the site (dashed line)

In winter sites ranged in area from 24-13,528.5 m² (mean = 997.91 (±290.56 S.E.)) with depths ranging from 12.5–160 cm (mean = 71.22 (±5.02 S.E.)) (Table 3.4). The average bank angle ranged from 5-90° (mean = 22.95 (±2.17 S.E.)) with the bank condition ranging from 1 (pristine condition) to 3 (moderately degraded) with a mean of 1.78 (±0.10 S.E.). Riparian vegetation varied from 1 (all native vegetation) to 3 (moderate presence of invasive species) with a mean of 1.14 (±0.56 S.E.). Shade at all sites was generally quite low ranging from 0 to 80% cover with a mean of 11.67 (±1.89 S.E.) (Table 3.4).

During spring, the sub-sample sites ranged in area from 24–784.56 m² (mean = 299.94 (±117.55 S.E.)) with depths ranging from 82-124cm (mean = 92.33 (±6.66 S.E.)) (Table 3.4). The average bank angle ranged from 10-70° (mean = 32.50 (±10.47 S.E.)) with the bank condition ranging from 2 (near pristine condition) to 3 (moderately degraded) (mean = 2.33 (±0.21 S.E.)). All six sites scored 1 (i.e., all native vegetation) for riparian vegetation. Shade at all sites was again low ranging from 0 to 35% cover (mean = 15.00 (±5.16 S.E.)) (Table 3.4).

In summer, sites ranged in area from 20.91-11,926.20 m² (mean = 870.41 (±372.45 S.E.)) with depths ranging from 5-140cm (mean = 56.83 (±6.96 S.E.)) (Table 3.4). The average bank angle ranged from 5-45º (mean = 17.34 (±1.90 S.E.)) with the bank condition ranging from 1 (pristine condition) to 4 (highly degraded) (mean = 2.03 (±0.10 S.E.)). Riparian vegetation ranged from 1 to 2 (mean = 1.15 (±0.06 S.E.)). Shade at all sites was generally quite low ranging from 0 to 80% cover (mean = 13.45 (±2.95 S.E.)) (Table 3.4).

Table 3.4. Habitat variables across seasons, including the number of sites (N), minimum and maximum values, mean and standard error (S.E.).

	Season	**N**	**Min.**	**Max.**	**Mean**	**S.E.**
Area (m²)	Winter	48	24.00	13,528.50	997.91	290.56
	Spring	6	24.00	784.56	299.94	117.55
	Summer	33	20.91	11,926.20	870.41	372.45
Maximum depth (cm)	Winter	48	12.50	160.00	71.22	5.02
	Spring	6	82.00	124.00	92.33	6.66
	Summer	33	5.00	140.00	56.83	6.96
Bank angle (º)	Winter	51	5.00	90.00	22.95	2.17
	Spring	6	10.00	70.00	32.50	10.47
	Summer	32	5.00	45.00	17.34	1.90
Bank condition (1-5)	Winter	51	1	3	1.78	0.10
	Spring	6	2	3	2.33	0.21
	Summer	33	1	4	2.03	0.10
Riparian vegetation (1-5)	Winter	51	1	3	1.14	0.56
	Spring	6	1	1	1	0.00
	Summer	33	1	2	1.15	0.06
Shade (%)	Winter	51	0.00	80.00	11.67	1.89
	Spring	6	0.00	35.00	15.00	5.16
	Summer	33	0.00	80.00	13.45	2.95

3.4 Groundwater depth and soil field texture

Groundwater depth during baseflow across the sites was relatively shallow (mean = 47.81 (±6.25 S.E.)) and ranged from 0 cm (still expressed at the surface) to >205 cm. Soil type was predominantly sand at most sites, with some exhibiting clayey sand layers, and peat horizons at the surface. Colour of the soil was similar across most sites, ranging from brown to white/grey. Soil was damp or saturated at the surface at all sites during baseflow aside from two that exhibited a dry top layer of sand before the lower horizons became damp (Table 3.5).

Table 3.5. Depth to groundwater (DG), soil characteristics and wetland classification at sites (west to east) across the study area.

Site	**DG 1 (cm)**	**DG 2 (cm)**	**Soil type**	**Soil colour**	**Soil condition**	**Classification**
Scott River Rd Power Pole 43	0	20	Sand	brown/grey	Damp	Palusplain
Scott River Rd	35	40	Sand	brown/grey	Damp	Palusplain
Scott River Rd Power Pole 34	50	55	Sand	brown/grey	Damp	Palusplain
Fouracres Rd*	-	-	-	-	-	-
Fouracres Rd	-	-	-	-	-	Palusplain
Fouracres Rd	>180	-	Sand	brown	Damp	Palusplain
Windy Harbour Rd	90	-	Sand	brown	Damp	Palusplain
Windy Harbour Rd	80	90	Sand	brown	Damp	Palusplain
Pool near small lake on Windy Harbour Rd	34	-	Sand	grey/brown	Damp	Palusplain
Windy Harbour Rd	0	33	Sand	brown	Damp	Palusplain
Small lake Windy Harbour Rd	0	20	Sand	grey	Damp	Palusplain
Windy Harbour Rd	0	-	Sand/gravel	Grey/brown	Saturated	Palusplain
Doggerup Creek	-	-	-	-	-	Palusplain
Windy Harbour Rd	0	-	Sand/gravel	Grey/brown	Saturated	Floodplain
Windy Harbour Rd	99	-	Peat/sand	Light brown	Damp	Palusplain
Chesapeake Rd (1st small pool)	>205	-	Sand/mottled clay	brown/grey	Damp	Paluslope
Windy Harbour Rd	0	10	Peat/sand/clay	grey	Damp	Palusplain
Chesapeake Rd (1st large pool)	50	-	Peat layer/sand	white/brown	Damp	Palusplain
Chesapeake Rd	65	75	Sand	dark brown/grey	Damp	Palusplain^
Chesapeake Rd	15	60	Peat layer/sand	brown/grey	Damp	Palusplain^
Chesapeake Rd	0	20	Sand	brown/grey	Damp	Palusplain^
Chesapeake Rd	0	8.5	Sand	grey/brown	Damp	Palusplain^
Chesapeake Rd	34	40	Sand	grey/dark brown	Damp	Palusplain^
Chesapeake Rd	0	11	Sand	grey/dark brown	Damp	Palusplain
Pool Lower Gardner River Rd	115	-	Organic/sand	brown/grey	Damp	Palusplain

Small pool Lower Gardner River Rd	75	-	Organic/sand	White/grey	Damp	Palusplain
Small pool Lower Gardner River Rd	125	-	Sand	White/brown	Dry/damp	Palusplain
Small pool Lower Gardner River Rd	115	-	Organic/sand	Grey/brown	Damp	Palusplain
Lower Gardner River Rd	128	-	Sand	Brown	Dry/damp	Palusplain
Chesapeake opposite 13.29	0	15	Sand	Grey/dark grey	Damp	Palusplain
Chesapeake	0	-				Palusplain
Lower Gardner River Rd	32	40	Peat/sand	Grey	Damp	Palusplain
Pool 37.6km east end Chesapeake Rd	0	31	Sand	Grey/brown	Damp	Palusplain
Large pool Ches. Rd	0	16	Sand	White/brown	Damp	Palusplain
Pool on Moore Hut Dr	125	-	Clayey sand	Dark brown	Damp	Palusplain
Pool Chesapeake Rd	70	-	Sandy clay/sand	Brown/yellow	Damp	Palusplain^
Crnr Deeside Coast Rd & Preston Rd	0	65	Sand/gravel	Dark grey/white	Damp	Floodplain
Forth River	0	-	Sand/gravel	Grey	Saturated	Floodplain
Small stream 6.6km N of Broke Inlet Rd	0	-	Sand/clayey sand	Grey/brown	Saturated	Palusplain
Chesapeake Road	0	58	Coarse sand/clay	Brown/grey mottling	Waterlogged	Palusplain
Pool/stream Broke Inlet Rd	125	-	Sand/sandy clay	Brown/pale yellow	Damp	Palusplain
Broke Inlet Rd	0	-	Sandy clay	Orange/brown	Waterlogged	Sumpland
Pool Broke Inlet Rd	130	-	Sandy clay	Brown/dark grey	Damp	Palusplain
Beardmore Rd	0	19	Coarse sand	White	Damp	Palusplain
Beardmore Rd pools	0	22	Sand/clayey sand	Dark grey/light grey	Damp	Palusplain
Thomson Rd	-	-	-	-	-	No data
Corner of Station Rd & Cons. Rd	80	-	Sand	White/grey/black	Dry/damp	No data
Conspicuous. Cliffs Rd	70	-	Sand	White/grey/black	Damp	No data
Fiscifolia Rd - Lake off track	70	-	Peat/sand	White/grey/brown	Damp	No data
Nornalup Rd	-	-	-	-	-	No data
Nornalup Rd	-	-	-	-	-	No data
Marbelup Rd north of Elleker	>180	-	Peat/sandy clay	Grey	Damp	No data
Lower Denmark Rd Elleker	50	-	Organic/sand	Grey	Damp	No data

*Site lost during study period through road expansion and subsequently was not sampled for depth to GW

^Sites classified based on inferences from data from Department of Parks and Wildlife (2003) on surrounding pools and definitions in Semeniuk and Semeniuk (1995)

3.5 Species distribution modelling

Initial PCA demonstrated correlations between several water quality variables and these were merged to reduce the number of predictor variables used in model fit: total dissolved solids was correlated with salinity and merged into water quality factor 1, and temperature was correlated with pH and ORP, with these variables merged into water quality 2; dissolved oxygen did not display correlations with other factors and was listed as water quality factor 3 (Table 3.6). Habitat variables also displayed correlations, with bank condition and riparian vegetation correlated as condition factor 1, and naturalness of pool, bank angle, and proportion of shade correlated as condition factor 2 (Table 3.7). All remaining variables were used in model fit as separate factors.

Table 3.6 Water Quality (WQ) Factors 1 to 3 with associated Eigenvalues >1, percentage of variance and cumulative percentage of variability.

Factor	**Eigenvalue**	**Variance (%)**	**Cumulative (%)**
WQ Factor 1	3.0072	50.120	50.120
WQ Factor 2	1.3850	23.084	73.203
WQ Factor 3	1.0477	17.462	90.665

Table 3.7 Condition Factors (CF) 1 to 2 with associated Eigenvalues >1, percentage of variance and cumulative percentage of variability.

Factor	**Eigenvalue**	**Variance (%)**	**Cumulative (%)**
CF 1	1.6626	33.251	33.251
CF 2	1.3537	27.074	60.325

The only variable of significance for presence of *L. salamandroides* on model fit was that of depth in winter (p=0.047; residual deviance 56.136 on 41 degrees of freedom). From the model averaging results the selection probability for depth in winter was 0.85 whereas the remaining variables were <0.29 (Table 3.8). The mean depth observed in presence sites was 78.02 cm versus mean depth in absence sites of 61.70 cm.

Table 3.8 Association between environmental variables and presence of *Lepidogalaxias salamandroides*. Variables are ranked by relative importance (i.e. probability of selection in AIC best fit model), with coefficient estimates and standard errors from model averaging. Asterisk indicates the significant predictor variable (P < 0.05).

Variable	**Importance**	**Estimate**	**SE**
Depth in winter*	0.85	0.02315	0.01166
Condition factor 1	0.29	-0.43619	0.33499
Connectivity	0.14	-0.11798	0.33488

No variables were found to be of significance in determining presence of *G. nigrostriata* on model fit, although soil class, and connectivity appeared of importance (p=0.0778, p=0.1022, respectively; residual deviance 51.170 on 38 degrees of freedom). From model averaging results, the selection probability for soil class and connectivity was 0.42 and 0.77 respectively, indicating connectivity is the most important factor in determining species distribution (Table 3.9). Mean connectivity for presence sites was 3.98 whereas mean connectivity for absence sites was 4.23, with the higher value indicating more isolation.

Table 3.9 Association between environmental variables and presence of *Galaxiella nigrostriata*. Variables are ranked by relative importance (i.e. probability of selection in AIC best fit model), with coefficient estimates and standard errors from model averaging.

Variable	**Importance**	**Estimate**	**SE**
Connectivity	0.77	-0.76936	0.47074
Condition factor 1	0.45	-0.61080	0.38341
Soil class	0.42	2.05306	1.16408
Depth in winter	0.13	0.00788	0.01138
Area in winter	0.13	-0.00013	0.00019

Univariate analyses were also conducted on the relationship between species presence and both depth to clay/consolidation and days of drying. A significant effect on *L. salamandroides* presence was found on length of drying periods ($\chi^2_{1, 24} = 5.09$, $p = 0.0241$), but no significant effect was found for depth to clay/consolidation ($\chi^2_{1, 13} = 2.19$, $p = 0.1392$). No significant effect on *G. nigrostriata* presence was found for either length of drying ($\chi^2_{1, 24} =1.59$, $p = 0.2072$) or depth to clay/consolidation ($\chi^2_{1, 13} = 0.15$, $p = 0.6950$). There was no significant effect of presence of crayfish on the presence of either *L. salamandroides* or *G. nigrostriata* ($\chi^2_{1, 52} = 0.049$, $p = 0.8254$, and $\chi^2_{1, 52} = 0.0000$, $p = 1.0000$, respectively).

No predictor variables had a significant effect on the probability of *L. salamandroides* still being found in sites in which it was historically present, although depth in winter again appeared to be the most important factor ($F_{1, 35} = 1.0797$, $p = 0.3059$). Soil class, however had a significant effect on model fit for *G. nigrostriata* ($\chi^2_{2, 26} = 12.728$, $p = 0.0017$). Sites in which *G. nigrostriata* was historically present but is now absent had clayey sand (71.43% of sites), whereas sites in which *G. nigrostriata* was historically present and are currently present had mostly sand (84.21% of sites).

3.6 Isotopic composition

Surface water samples in winter had a mean δ^{18}O value of -3.38‰ (±0.62 S.D.) and a mean δ^2H value of -13.2‰ (±2.9 S.D.), with the values being significantly correlated with a linear fit slope of 3.58 ($r = 0.771$, $p < 0.001$) (Table 3.10, Fig. 3.12). The surface water samples from summer displayed a wider range and more positive isotope values, with a mean δ^{18}O value of 4.08‰ (±4.19 S.D.) and a mean δ^2H value of 20.1‰ (±18.2 S.D.), and again the isotope values were significantly correlated with a slope of 4.30 ($r = 0.991$, $p <$

0.001). Seasonal variation was obvious between the winter and summer samples (Table 3.10) and the means differed significantly for both $\delta^{18}O$ ($t_{15} = -6.9$, $p < 0.001$) and δ^2H ($t_{15} = -7.256$, $p < 0.001$). The wide range of values observed in the summer isotope values are likely due to differing rates of evaporation, and were found to be significantly correlated ($r = -0.851$, $n = 16$, $p < 0.01$) to the depth of pools in winter (Fig. 3.13).

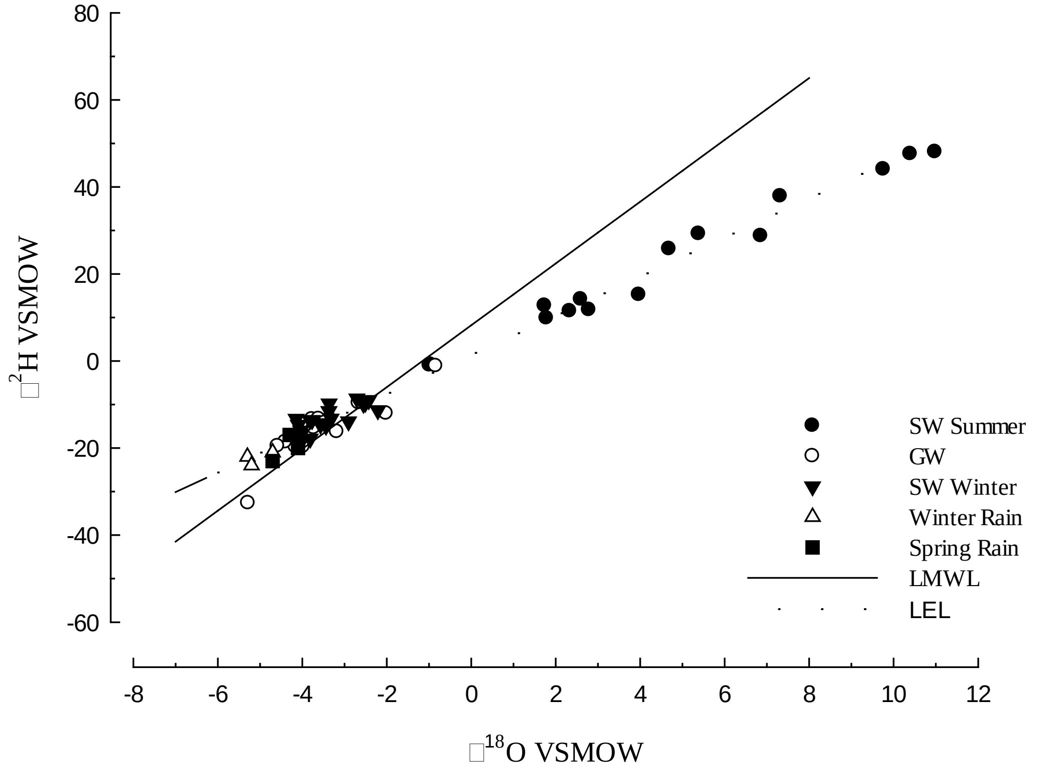

Figure 3.12 Isotope values for groundwater – GW, surface water – SW, and rain water demonstrating relationships between $\delta^{18}O$ and δ^2H. The local meteoric water line (LMWL) was derived from Liu *et al.* (2010).

A local evaporation line, LEL, was defined for all surface water samples as $\delta^2H = 4.5 \times \delta^{18}O + 1.3567$, and the loss due to evaporation between winter and summer was determined to be 0.35 (35%) based on $\delta^{18}O$ and 0.40 (40%) based on δ^2H. The LEL intercepted the LMWL at -2.60‰ $\delta^{18}O$ and at -10.5‰ δ^2H, which would best reflect the mean isotopic value prior to any losses due to evaporation or other fractionation (Figure 3.12).

Table 3.10. Mean and standard deviation (STDEV) isotope values across sample groups with correlations and P-value for isotope values within each group. Asterisks indicate significant correlation at P < 0.05.

Samples	**$\delta^{18}O$**	**STDEV**	**$\delta^{2}H$**	**STDEV**	**Correlation**	**P value**
SW Winter	-3.38	0.62	-13.2	2.9	0.771	<0.001*
SW Summer	4.08	4.19	20.1	18.2	0.991	<0.001*
Groundwater	-3.51	1.16	-15.8	7.2	0.902	<0.001*
Rain Winter^	-5.07	0.32	-22.3	1.5	0.645	0.554
Rain Spring^	-4.37	0.31	-20.0	3.0	0.655	0.546

^Data retrieved from Bowen (2015)

Mean weighted isotope composition in rainwater varied with a $\delta^{18}O$ value of -5.07‰ (±0.32 S.D.) and $\delta^{2}H$ value of -22.3‰ (±1.5 S.D.) in winter and a mean $\delta^{18}O$ value of -4.37‰ (±0.31 S.D.) and $\delta^{2}H$ value of -20.0‰ (±3.0 S.D.) in spring. Seasonal variation is again noticeable with the means in winter and spring differing significantly for $\delta^{18}O$ (t_3 = -4.583, p = 0.044), but not for $\delta^{2}H$ (t_3 = -1.750, p = 0.222). The linear fit slope for rainfall in winter was 3.06 and 6.43 in spring. Importantly rainwater in winter did not significantly differ from surface water in winter for both $\delta^{18}O$ (t_3 = 4.185, p = 0.053) and $\delta^{2}H$ (t_3 = 3.970, p = 0.058).

Groundwater samples had a mean $\delta^{18}O$ value of -3.51‰ (±1.16 S.D.) and a mean $\delta^{2}H$ value of -15.8% (±7.2 S.D.), and the isotope values were again significantly correlated with a slope of 5.59 (r = 0.902, p < 0.001) (Figure 3.12). Groundwater isotope values differed significantly from surface water samples in summer for both $\delta^{18}O$ (t_{12} = -5.408, p < 0.001) and $\delta^{2}H$ (t_{12} = -5.394, p < 0.001). The groundwater values however were not significantly different from surface water samples in winter, $\delta^{18}O$ (t_{12} = -0.686, p = 0.506) and δ^2H (t_{12} = -0.947, p = 0.363), rainwater samples in winter, $\delta^{18}O$ (t_3 = 4.129, p = 0.054) and δ^2H (t_3 = 2.871, p = 0.1.03), or rainfall samples in spring, $\delta^{18}O$ (t_3 =2.141, p = 0.166) and δ^2H (t_3 = -1.282, p = 0.328).

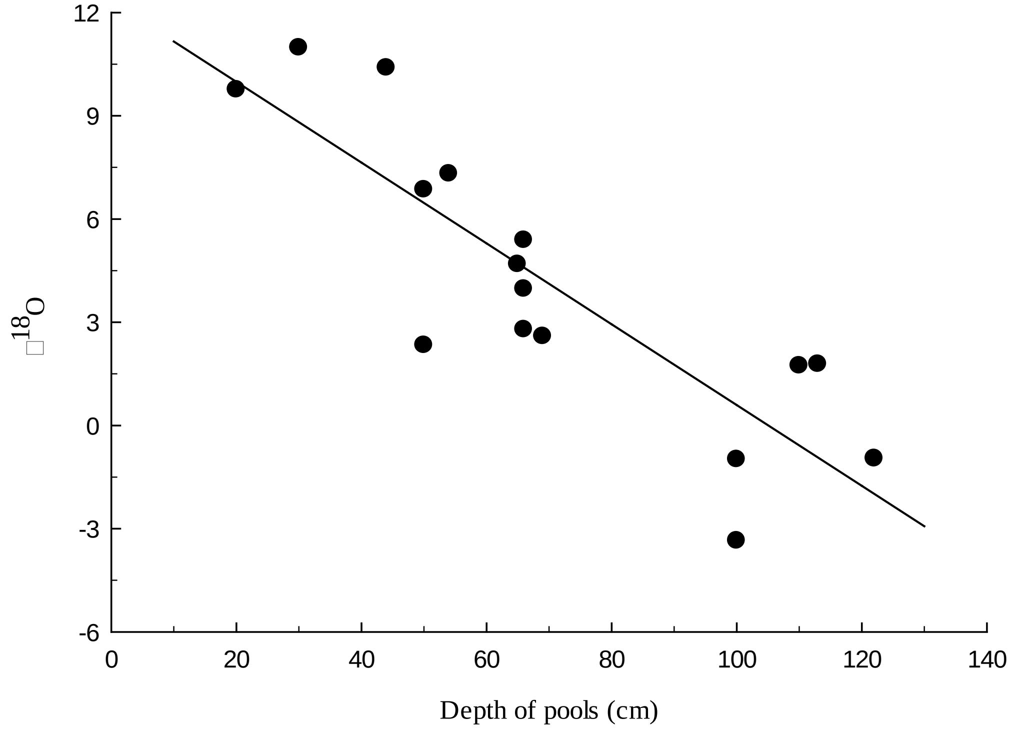

Fig. 3.13 Depth of pools in winter correlated to the oxygen isotope values found in summer with a linear trendline (y = -0.1174x + 12.333).

3.7 Patterns in lipid storage

A total of 279 *L. salamandroides*, 274 *G. nigrostriata* and 48 *G. munda* were retained for lipid extractions. Total lipid content of *L. salamandroides* increased across the seasons with the highest values observed in spring for males (mean = 0.3581 (±0.0546 S.E.)) and females (mean = 0.3215 (±0.0327 S.E.)) and in summer for juveniles (mean = 0.3436 (±0.0274 S.E.)) (Figure 3.14). The lipid content was significantly different among seasons ($F_{3, 68}$ = 4.446, p = 0.007), with a Tukey post hoc test showing a significant difference between winter and summer (p = 0.048). No significance differences were found in lipid content of *L. salamandroides* between juveniles, adult males or adult females ($F_{2, 68}$ = 0.911, p = 0.408), or any interaction between age/sex and season ($F_{5, 68}$ = 0.299, p = 0.911).

Galaxiella nigrostriata appeared to show a small increase in lipid content from its lowest point in autumn for both juveniles (mean = 0.2143 (±0.0000 S.E.)) and adults (mean = 0.2857 (±0.0000 S.E.)) (Figure 3.14), however, no significant difference was found among seasons ($F_{3, 37}$ = 0.949, p = 0.430). No significant difference were seen between juveniles and adults ($F_{1, 37}$ = 0.309, p = 0.583), or any interaction between age and season ($F_{3, 37}$ = 0.411, p = 0.746).

The mean lipid content in juvenile *G. munda* during winter was 0.2563 (±0.0563 S.E.) compared to adults with a mean lipid content of 0.2271 (±0.0438 S.E.). During summer a small increase in lipid content was observed with juvenile *G. munda* having a mean lipid content of 0.2792 (±0.0037 S.E.) compared to adults with a mean lipid content of 0.2833 (±0.0167 S.E.). No significant difference was found in the lipid content of *G. munda* among seasons (Figure 3.14) ($F_{1, 11}$ = 1.115, p = 0.322). Similarly, no significant difference was found between adults and juveniles of *G. munda* ($F_{1, 11}$ = 0.112, p = 0.747), or any interaction between age and season ($F_{1, 11}$ = 0.197, p = 0.669).

In the full factorial general linear model there was not quite significant differences among all species and seasons ($F_{2, 118}$ = 1.928, p = 0.151, $F_{3, 118}$ = 2.572, p = 0.059, respectively). However, a post hoc Tukey test observed a significant difference between *G. nigrostriata* and *G. munda* (p = 0.032).

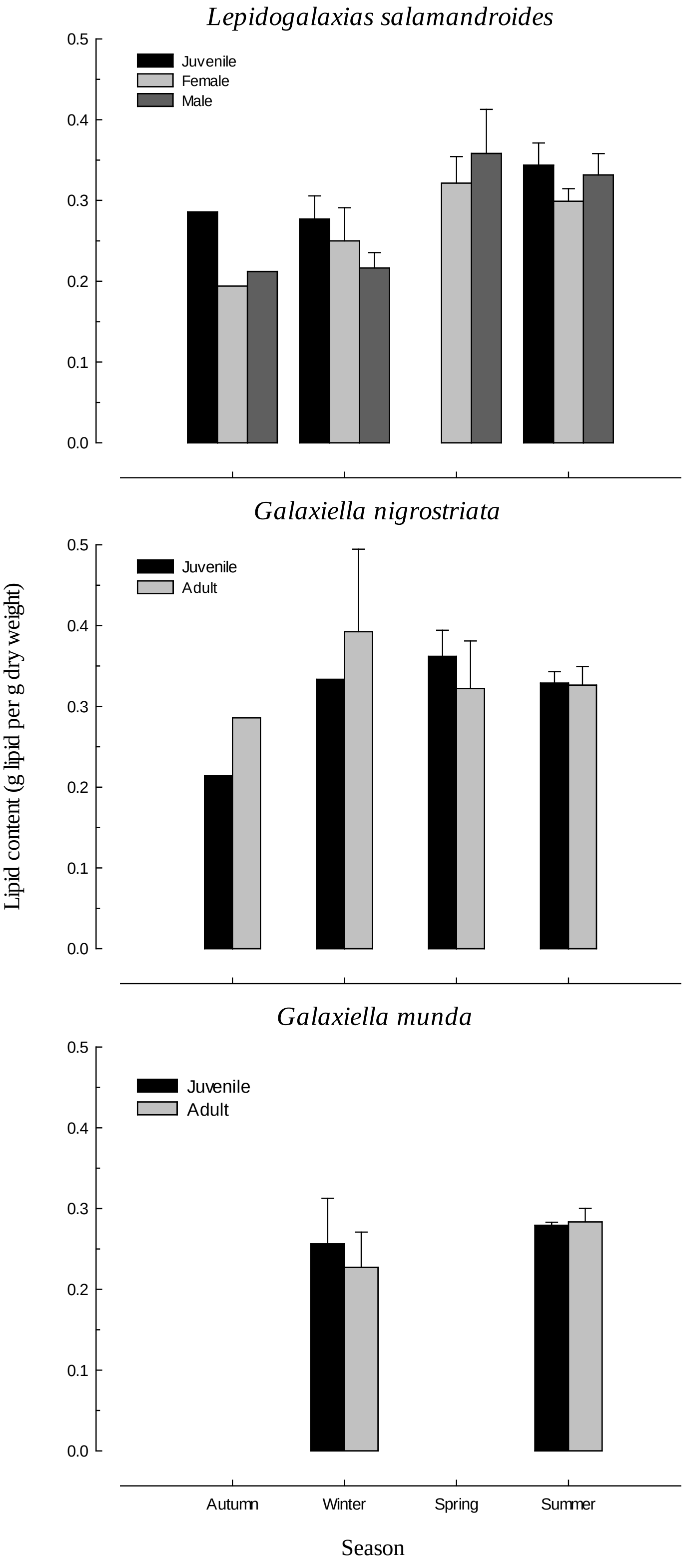

Figure 3.14. Lipid content (g lipid / g dry weight) across the seasons in *Lepidogalaxias salamandroides*, *Galaxiella nigrostriata*, and *Galaxiella munda.*

4 Discussion

4.1 Distribution and population structure of *Lepidogalaxias salamandroides* and *Galaxiella nigrostriata*

4.1.1 *Distribution of Lepidogalaxias salamandroides and Galaxiella nigrostriata*

The current study has demonstrated that over just the past few decades there has been a considerable decline in both the number of extant populations and the geographical distribution of south-western Australia's only aestivating fishes (Christensen, 1982; Pusey & Edward, 1990; Morgan *et al.*, 1998). *Lepidogalaxias salamandroides* and *G. nigrostriata* appear to have been extirpated from 33.33% and 28.57% of sites in which they were historically present. The discovery of an additional four new sites for both species is perhaps not as positive as it would seem as they were located adjacent to sites which historically contained the fishes.

Both species were absent from the four eastern most historic sites near Elleker, and along Nornalup Road (Figure 3.5, 3.6). The loss of these sites represents a large range reduction for both species, with EOO for *L. salamandroides* declining 79% (Appendix B1, B2), and *G. nigrostriata* 12% (Appendix B3, B4). The AOO of each species also declined, with that of *L. salamandroides* reducing by 71% and *G. nigrostriata* 58%. Similar alarming decreases in EOO and AOO have been documented for other freshwater fauna within the south-west of Western Australia (Morgan *et al.*, 2014; Klunzinger *et al.*, 2015).

Changes in community structure were also observed from that which existed historically at several sites. During this study, 21 sites were observed to house both species in sympatry, and of these 18 historically contained both species. Several other sites that historically housed both species, did not have the same sympatry observed during this study. These sites include one pool on Windy Harbour Rd (site 24), one site along Chesapeake Rd (site

39), two sites west of Broke Inlet Rd (site 15 and 46), and one along Beardmore Rd (site 11). Despite previous studies recording both species at the aforementioned sites, only *L. salamandroides* was detected during the current study, aside from site 39 where only *G. nigrostriata* was detected. A second pool on Chesapeake Rd (site 29) was the only historical site that housed *L. salamandroides* but not *G. nigrostriata* and now houses the latter and not the former species.

4.1.2 *Population structure and viability*

The increases in juvenile (0+) modal length over a short period (winter to spring), and also sharp increases in the abundance of juvenile fish in both species during spring support previous studies that have suggested both of these fishes are r-selection strategists (MacArthur & Wilson, 1967), having relatively rapid growth rate and a reproductive strategy that facilitates high recruitment shortly after emerging from aestivation (Pusey, 1990; Morgan *et al.*, 2000). Recruitment through spring can account for the increase in the overall abundance at most sites. Additionally, the large increase in number of sites *G. nigrostriata* was found in during summer added to the overall numbers recorded. One possible explanation for this higher abundance of *G. nigrostriata* during summer is migration from nearby wetlands.

Pen *et al.* (1993) also found high recruitment of juvenile *G. nigrostriata* between the winter and spring period; occurring earlier in comparison to other members of *Galaxiella* (Pen *et al.*, 1993). Juveniles attained up to 88% of total body length prior to the dry period in summer, which was deemed crucial to survival during aestivation (Pen *et al.*, 1993). As in Pen *et al.* (1993), the current study found marked changes in modal lengths between seasons, in particular between autumn and winter (2 mm/month) and again between spring and summer (3.2 mm/month). The greater change between spring and summer is due to the rapid growth of 0+ juveniles in the lead up to summer (Pen *et al.*, 1993).

Galaxiella nigrostriata was only present in 10 sites during winter sampling and eight (80%) of these were from samples with a density of < 0.1 fish/m^2. This changed markedly in summer, with only three of 24 sites (12.5%) having densities < 0.1 fish/m^2. Importantly, Pen *et al.* (1993) also documented a marked differences in presence and abundance of the species between the seasons. One pool that contained high densities and consistent presence of *G. nigrostriata* prior to aestivation, did not yield any in the following winter after heavy rainfalls re-inundated the pool (Pen *et al.*, 1993). Although *G. nigrostriata* has a one year life cycle (Pen *et al.*, 1993), it is unlikely that all of the fish in the latter pool died; instead fish may have migrated to nearby wetlands when the habitats were inundated. Findings similar to this have led to suggestions that some populations of *G. nigrostriata* may in fact be part of a meta-population (Galeotti, 2013) which has considerable management and research implications (see section 4.4).

The patterns in population structure of *L. salamandroides* recorded here are broadly consistent with Pusey (1990), who classified the species as an r-selection strategist. Pusey (1990) found the length of aestivation time appeared to influence survival of juveniles, with higher survival rates recorded in a shorter aestivation period. A lower survival rate of juveniles has also been documented in the aestivating Black Mudfish, *N. diversus* by McPhail (1999), with juveniles predicted to be able to survive only 11 weeks of drought as opposed to an estimated 20 weeks of drought for adults. During the current study however, juvenile *L. salamandroides* (<30 mm TL) that had previously aestivated were found during the autumn and winter sampling. It is likely that rapid development and growth within the first year of life disguises the presence of juveniles that have re-emerged (Morgan *et al.*, 2000), although juveniles may still be at greater risk of suffering mortality than adults.

Further research, similar to that of McPhail (1999) is required into lengths of dry periods that both adults and juveniles can survive.

Importantly, previous research has documented that a large proportion of *L. salamandroides* do not mature until after their second aestivation (only 26% of males and 28% of females matured at the end of the first year of life) (Morgan *et al.*, 2000), whereas *G. nigrostriata* breed and die after only one year (Pen *et al.*, 1993). This is important in terms of potential implications of a drying climate as *L. salamandroides* will be vulnerable to prolonged aestivation periods (most having to aestivate twice prior to breeding for the first time) and may become less capable of maintaining viable populations into the future. However, *G. nigrostriata* would also be vulnerable due to a one year life-cycle; failure of one breeding period would likely result in the loss of a population should re-colonisation not be possible.

4.2 Factors influencing the distribution of *L. salamandroides* and *G. nigrostriata*

The current study demonstrated that depth of pools best explained the distribution of *L. salamandroides*, while connectivity best explained the distribution of *G. nigrostriata*. Soil class was also found to be an important factor influencing historical change of sites for *G. nigrostriata*.

The importance of pool depth may explain why the region around Northcliffe appears to be the remaining hot-spot for *L. salamandroides*, as it is the wettest town in Western Australia (De Silva, 2004), and this depth may also be important in triggering aestivation, as aestivation in *L. salamandroides* commences long before pools completely dry (Morgan *et al.*, 1998). It is likely that climate change may impact on the survivorship of the species within this region through decreased rainfall and subsequently decreasing discharge into groundwater, reducing maximum depth of surface water during winter periods (CSIRO, 2009) (see section 4.4).

Connectivity was the most important variable for presence of *G. nigrostriata*, and this may reflect several of its traits. *Galaxiella nigrostriata* possesses a fusiform body typical of galaxiids (McDowall & Frankberg, 1981), is free-swimming and feeds within the water column and at the surface (Smith *et al.*, 2002a; Gill & Morgan, 2003). It is therefore more likely than *L. salamandroides* (a benthic dwelling species) to disperse when pools connect. This importance has been documented previously through genetic studies suggesting *G. nigrostriata* has a meta-population structure (Galeotti, 2013). Alteration of habitat, through land clearing and pool degradation, and climate change are likely to impact on connectivity (Arthington *et al.*, 2005; Nagrodski *et al.*, 2012), through reducing flow (Silberstein *et al.*, 2012) and surface water recharge in many of the pools (see below).

Soil class was also important when determining the historical change for *G. nigrostriata* (i.e. the disappearance of populations) and could be explained by the morphology of the species. Clay substrates are less favourable than sand for aestivating species that burrow (Booth, 2006) and the species does not have any obvious morphological characteristics that enable it to be an efficient burrower (Thompson & Withers, 1999). It is unlikely that the soil class has changed since the species was documented at the historical sites. Instead, it was likely that the historical sites with clayey sand were never permanent sites for *G. nigrostriata*, and instead were temporary sink (fed by source populations) populations having since been lost due to reductions in connectivity.

4.3 Isotope composition

Each individual sampling group display slopes much flatter than the local meteoric water line, LMWL. Liu *et al.* (2010) demonstrated differences between their data and Dansgaard

(1964) who also obtained a flatter slope (compared to the LMWL) in a study for Perth. The differences were attributed to a small sample size, sampling across just one month and not accounting for seasonal variations.

Seasonal variation however was observed in this study which reflects the ephemeral nature of the wetlands. Evaporation from water bodies initially leave them characterized by high δ^{18}O levels due to the vapour pressure of δ^{16}O being higher than δ^{18}O (Hunt *et al.*, 2005). This saturation of isotopes is obvious from the marked changes between surface water isotope values in winter and summer. The δ^{18}O values are also more impacted by evaporation than deuterium (Hoy & Gross, 1982) which causes the flattening of the slope observed in the summer values of surface water when compared to the LMWL (Figure 3.12).

Stable isotopes have been used globally to help determine various aspects of the water cycle including mechanisms of recharge in wetlands (Salameh, 2004; Hunt *et al.*, 2005; Nyarko *et al.*, 2010; Qian *et al.*, 2014). Importantly in this study the groundwater sampled in the dry period (April 2015) displayed similar values to the rainfall and surface water in winter. The similarities in isotope values and slopes amongst these three sampling groups lend support that the wetlands hydrology relies on winter rain, either through direct input, expression from a superficial aquifer, or both, as opposed to other external inputs such as deep aquifer discharge. The deep Yarragadee aquifer underlying the region has marked differences in isotope values to those observed in this study (Department of Water, 2015). The reliance of rainfall recharging the wetlands sampled underlines the threat posed by climate change to the sites through decreases in rainfall and higher evaporative losses due to increasing temperatures.

4.4 Influence of habitat and hydrological change on the future distribution and population viability of *L. salamandroides* and *G nigrostriata*

As mentioned in section 4.2, depth in winter and length of the period of drying were the key variables that best explained the distribution of *L. salamandroides,* while connectivity best explained the distribution of *G. nigrostriata*. The variables of depth in winter and length of drying days are directly influenced by climatic variables including rainfall, temperature and evaporation, as well as ground water abstraction, whilst connectivity is influenced not only by climate through altered hydrological flow (Silberstein *et al.*, 2012), but also by anthropogenic changes to the landscape, such as creation and widening of roads and habitat clearing/degradation (Nagrodski *et al.*, 2012). Therefore, the future viability of these species will be largely governed by the hydrological characteristics of the remnant habitats quantified in the current study.

The vast majority of sites were classified as palusplain seasonally waterlogged flats, with a shallow depth to groundwater and predominantly sandy soils. Areas with shallow watertable, cleared or shallow rooted vegetation, and sandy soils, favour recharge into groundwater, more so than areas with large rooted perennials, and clayey and gravelly soils (CSIRO, 2009). For areas identified in this study such as the Scott Coastal Plain, with a shallow water table and sandy soils, projected reductions in rainfall from climate change may actually be offset by reductions in groundwater discharge, but also from a reduction in evapotranspiration, potentially buffering changes to groundwater levels (CSIRO, 2009).

According to climate change scenarios, the western portion of the Scott Coastal Plain may actually experience a decrease in depth to groundwater (increase in the water table), apart from the most extreme scenarios (CSIRO, 2009). This area, in particular the location of the three Scott River Rd sites in this study, had high rainfall and sandy soils, and parts are cleared of native vegetation, which facilitates recharge into groundwater. The water table under the western portion of the Scott Coastal Plain will rise by between 0.2 and 1.8 m under all scenarios produced by CSIRO (2009), with the exception of the dry extreme scenario and a scenario combining climate change with groundwater extraction where the water table declines may be up to 1.8 m. This projected increase in the water table may in turn shorten the drying periods in these pools and increase the maximum pool depth in winter making these sites possibly more suitable for *L. salamandroides*.

One issue with the rising water tables however, is the risk of secondary salinisation (Beatty *et al.*, 2011). Several other species within the south-west have already been impacted by secondary salinisation (Beatty *et al.*, 2011), however, little is known of the salinity tolerances for either species in this study, thus urgent research will be required to determine both acute and chronic tolerances. Although the Scott Coastal Plain has been assessed as low salinity risk, regular monitoring of groundwater salinity does not occur in this region (Raper *et al.*, 2014). Due to this area being at the western edge of the distribution for both species, it is an area that may require further monitoring into the future.

Large areas of the Scott Coastal Plain have also been identified as high risk in terms of disturbance of shallow acid sulphate soils causing elevated water acidity (Degens & Wallace-Bell, 2009). It has been concluded that the region has a high risk of widespread environmental impact should those soils be oxidised and that there was evidence that past localised declines in the watertable (through climate change, water abstraction, and land use such as tree cropping and mining) had already caused oxidation of those soils (Degens & Wallace-Bell, 2009). Whilst adapted to acidic waterbodies, the tolerance of both *L. salamandroides* and *G. nigrostriata* should be determined to understand the role that acidification may play in future viabilities.

The three sites along Fouracres Rd, although historically containing both species, had no fishes during winter sampling, and only a small number of *G. nigrostriata* were found in the eastern most pool during summer (that may have immigrated from nearby wetlands). The maximum depth of these pools in winter was low, ranging from 33-98 cm. This may explain the absence of fish, in particular *L. salamandroides*. Depth to groundwater at the middle Fouracres Rd site was greater than 180 cm and consolidated layers were present, likely making it difficult for burrowing. The consolidated layer present was followed by dry sediment, indicating this pool could be a perched wetland system as opposed to an expression of the regional groundwater. No obvious wetland systems were observed in the field or from aerial imagery in close proximity with that site making it unlikely that *L. salamandroides* or *G. nigrostriata* will be able to re-establish viable populations in this pool. The western most historical site on Fouracres Rd was the deepest of the three pools in winter (depth 98 cm), but again neither species was present in the initial winter sampling. During the study period, the site was lost due to road widening ensuring neither species will be able to re-populate (Figure 4.1).

Figure 4.1. Western most Fouracres Rd site surveyed in a) winter, note the dirt road on the top right, b) when visited after summer, clearly showing the site has been lost due to road widening, note the construction vehicle still in the image along with the road in the process of becoming bituminised

Hydrographs produced by CSIRO (2009) indicate groundwater levels around the Albany and Elleker area will decline by 1 to 2 m under mild climate change scenarios, and up to 4 m in the dry and further groundwater extraction scenarios. The sites sampled near Elleker did not contain either species of fish and had low surface water depth in winter (depth 14-39 cm), one with shallow depth to groundwater of 50 cm and the other with a depth to groundwater of >180 cm. Both sites also appeared degraded, in particular the eastern most site which had obvious oil slicks and pollution (e.g. anthropogenic - rubbish present) and was directly adjacent to a railway track.

Upon revisiting the eastern most site in April 2015, it had been further modified, with mulch placed over much of the site, growth of invasive weeds, and it being half-filled in with a drain excavated alongside. Importantly the eastern sites were also found to be absent of both species by Morgan *et al.* (1998) and Galeotti *et al.* (2010). Therefore, it can be concluded that the sites are no longer suitable for the species even if re-introduced. Several other species have been impacted by or are projected to be impacted by similar habitat alterations and land clearing (Giam *et al.*, 2012), including the Australian lungfish, *Neoceratodus forsteri*, another enigmatic air-breathing species that can survive periods of hypoxia (Arthington, 2009). Ongoing losses of habitat and projected future habitat loss has ultimately led to its listing as Vulnerable under the EPBC Act (1999) (Arthington, 2009).

For the other sites studied, potential impacts on both species through climate change and reductions in the water table may actually be buffered by the characteristics of the sites. The vast majority of sites sampled are borrow pits (an area where material and soil has been excavated for use at another location), which are at a greater depth to the surrounding landscape (the palusplain wetlands). Not only do the borrow pits contain deeper free-standing water for longer periods than the surrounding landscape through expression of the water table (Figure 4.2), but also have a shallower depth to groundwater during the dry period. This could provide two benefits to both species; free-standing water remaining for a longer period and at a greater depth shortens the period of drying than would normally be experienced, and also provides them with a shallower substrate to burrow into until they reach suitable moisture levels to survive the dry period.

Figure 4.2. Aerial views of sites along Chesapeake Rd south of Northcliffe. a) Two borrow pits surrounded by inundated wetland in October (Google earth V 7.1.2.2041, 24 October, 2013a) and again in December with the surrounding wetland dry (Google earth V 7.1.2.2041, 27 December, 2011a), b) A single borrow pit also surrounded by inundated wetlands in October (Google earth V 7.1.2.2041, 24 October, 2013b), and again still inundated when the surrounding wetland is dry in December (Google earth V 7.1.2.2041, 27 December, 2011b)

The importance of access to groundwater has also been documented in another species of benthic dwelling burrowing freshwater fish, *Cobitis shikokuensis* (Kawanishi *et al.*, 2013). *Cobitis shikokuensis* survived up to 22 cm below the surface of the streambed in the hyporheic zone of a river system (region of sediment in which mixing between groundwater and surface water occurs) during dry periods (Kawanishi *et al.*, 2013). Morphologically *C. shikokuensis* has many similar traits to *L. salamandroides*, including small total length (40-50 mm) and an elongated frame, believed to aid in mobility in narrow spaces and burrowing to access the hyporheic zone (Kawanishi *et al.*, 2013). The finding of the species within the hyporheic zone and its role in maintaining viable populations provides another example of the critical importance of maintaining groundwater levels for aestivating species.

Despite the potential of the borrow pits to provide refuge under future change, there are still factors that may impact on the survival of even those populations. If the surrounding wetland areas dry too much there may not be enough connectivity to allow either species to immigrate and repopulate other pools upon re-inundation, reducing them to isolated populations in each borrow pit. As connectivity is of high importance for *G. nigrostriata,* this scenario is particularly likely to severely impact this species. Such population fragmentations increases the vulnerability of species to stochastic events such as severe drought, natural disasters, disease, and also can have considerable genetic implications (Knight *et al.*, 2009; Smith, 2009; Galeotti *et al.*, 2015). Those populations may be reliant on unusually large and infrequent rainfall events and flooding for dispersal and population mixing (Kerezsy *et al.*, 2014).

The presence of crayfish and vegetation may also be of some importance should depth to groundwater increase from climate change. It was previously proposed that *G. nigrostriata* may utilise crayfish burrows to help access groundwater (Thompson & Withers, 1999), however, recent research has questioned this (Galeotti *et al.*, 2010). Even so, the burrows themselves may still serve a purpose. One site along Windy Harbour Rd, classified as paluslope, had a depth to groundwater greater than 205 cm and layers of slightly consolidated soils. Despite this depth and consolidation present in the soil, both species were present during sampling, with no obvious signs of nearby wetlands or waterways. This site had large numbers of crayfish burrows (Figure 4.3) creating macropores in the soil. Macropores from roots and gaps between pebbles have been utilised in other species inhabiting temporary waterways, including *N. burrowsius* (Eldon, 1979) and *C. shikokuensis* (Kawanishi *et al.*, 2013); they may serve a similar purpose in these pools.

Figure 4.3. Crayfish burrows at sites on Windy Harbour Road create macropores and may serve as an access way for aestivating fish to the watertable

4.5 Energy storage strategies

Significant changes were found in the total lipid content of *L. salamandroides* across seasons, with a depletion in autumn upon re-emerging after aestivation and a build-up of lipids leading up to aestivation in summer. The findings were broadly in line with Pusey (1990) although spring was not sampled for lipids in that research. Importantly, total lipid content for *L. salamandroides* was at its highest in spring, with a reduction in summer prior to aestivation. One reason for this could be due to energy expenditure during reproduction (Anthony *et al.*, 2000). Other fishes have been documented to have highest lipid content during gonad development with a reduction in lipids occurring after eggs have been spent (Anthony *et al.*, 2000).

Another possible reason for this decline between spring and summer could be due to competition. During spring there are higher prey densities and greater pool depths compared to summer (Pusey & Edward, 1990). This would increase competition for resources in summer and may cause the utilisation of some of the stored lipids during that period. Similar competition in freshwater fishes through partitioning of resources and variation in fish assemblages have been documented in isolated pools globally (Christian & Adams, 2014) and in Australia (Arthington *et al.*, 2005).

No clear differences were found in the lipid content of male and female *L. salamandoides*, although males had slightly higher total lipid content across three of the four seasons. Pusey (1990) documented lower lipid content in males, however males were only analysed in that study during the winter period (July and August), which was also the season in which males had the lower lipid content in the current study. Pusey (1990) proposed that the difference between males and females was due to the females requiring lipid storage for the production of eggs. The current study does not support this, as males not only had a higher overall lipid content, but demonstrated the same seasonal trend as females.

It is therefore likely that males and females may utilise the lipid stores differently. For example, females may have to utilise lipid stores for growth and development throughout their life and balance this with reproduction and surviving dry periods whereas males are likely to utilise lipids for growth only in their first year until they attain maximum length (Morgan *et al.*, 2000); with subsequent years allowing lipid stores to be utilised for reproduction and survival during aestivation. This could explain why males have equally high lipid stores as seen in females during this study.

No significant difference in lipid stores was found between juvenile and adult *L. salamandroides*, which could also be important in explaining the presence of juveniles in autumn and winter. Another aestivating fish, *N. diversus*, was found to have lower survival rates in juveniles than in adults due to losses in percentage of body weight (McPhail, 1999). Results from the current study, however, indicate that juveniles of *L. salamandroides* and *G. nigrostriata* rapidly increase their lipid stores in relation to total body weight to a sufficient level to survive the dry periods. Further research is required to determine percentage of body weight loss that can be tolerated by juveniles versus adults in both species.

Galaxiella nigrostriata had a depletion of lipids in autumn upon re-emergence after aestivation, however there was no significant change across seasons. One reason for this could be rapid growth and development of the species (Pen *et al.*, 1993). Early reproduction and rapid growth allowed juvenile *G. nigrostriata* to attain 78-88% of total body length prior to the dry season (Pen *et al.*, 1993). This same rapid growth could explain the rapid increase in lipid content between autumn and winter, with this level then remaining steady until summer before aestivation.

It appears from the data that the strategy of lipid storage increasing prior to aestivation is more pronounced in *L. salamandroides* than *G. nigrostriata*. The depletion of lipids in autumn in *G. nigrostriata*, however, suggests that lipid utilisation is of crucial importance in the survival of *G. nigrostriata* during the dry season. The reasons for differences between species could be due to life history differences, as mentioned earlier *G. nigrostriata* only undergo one aestivation and survive for a one year period (Pen *et al.*, 1993), whereas *L. salamandroides* usually undergo two aestivations before reaching maturity (Morgan *et al.*, 2000) and therefore utilise and store lipids differently, balancing lipid stores to survive dry periods, growth and development, and reproduction.

Importantly there was a significant difference demonstrated between *G. nigrostriata* and the non-aestivating *G. munda*. This finding supports that *G. nigrostriata* has a different lipid storage strategy to a non-aestivating member of the same genus. Differences in growth and timing of spawning have previously been documented between the two species (Pen *et al.*, 1993) and this likely contributes to the differences seen in lipid storage strategies. The differences between *G. nigrostriata* and *G. munda* requires additional investigation due to the low sample size of *G. munda* in the current study.

The current methods also have some further limitations, including the overall dry weight of samples used for lipid extractions. A similar study pooled specimens until a dry weight greater than 2 g was attained prior to performing lipid extractions (Anthony *et al.*, 2000). This was not possible in this study; a sample with nine pooled adult *G. nigrostriata* only attained a dry weight of 0.81g and 10 juvenile *G. nigrostriata* attained a dry weight of 0.28g prior to lipid extractions.

5 Future research

A wild fire spread through the study area near Northcliffe on the 27th January 2015 (burning for approximately two weeks), encompassing approximately 15 study sites; predominantly those sites along the western portion of Chesapeake Rd, and along Windy Harbour Rd. The most noticeable impact from this fire was the loss of riparian vegetation surrounding the pools (Figure 5.1), which may favour the establishment of invasive flora species, particularly along the roadways. Riparian vegetation has been shown to be important in regulating temperature, and buffering light (Pusey & Arthington, 2003), and is likely to be of importance for both the *L. salamandroides* and *G. nigrostriata* by retaining moisture in the fringes of the wetland systems and providing leaf litter which may act as a refuge for fishes during aestivation; similarly to the Mudfish, *N. burrowsius* (Eldon, 1979).

Figure 5.1. Fire damage seen around the Northcliffe area, a) Entrance sign to the D'Entrecasteaux National Park along Chesapeake Rd, b) One of the sites damaged by fire along Chesapeake Rd, notice the burnt vegetation with some recent regrowth

Another possible impact of fire more generally for both species is from fire-retardant chemicals used. A study by Angeler and Moreno (2006) found impacts on water quality in temporary wetlands after being polluted with fire retardant chemicals with an increase in nutrients and changes in pH and dissolved oxygen recorded (Angeler & Moreno, 2006). Moreover, contaminated pools did not return to pre-contamination conditions after two full hydrological cycles (Angeler & Moreno, 2006). There is public pressure on the

Department of Parks and Wildlife (DPaW) and Department of Fire and Emergency Services (DFES) to commit to regular controlled burns to reduce intensity of future fires (personal observation, 31st March 2015) which could ultimately lead to the pools being contaminated on a more regular basis and not returning to pre-contamination levels. The precarious status of these species revealed here and the possible impacts to them (and other threatened freshwater fishes known to utilise fire-fighting water points) of fire management should be considered during decisions around management of wildfire in this region.

Further research should focus on increasing the resilience of both species given the population losses and associated reductions in EOO and AOO revealed in the current study. The species distribution modelling identified that naturalness of the site and bank condition had no effect on the likelihood of either species being present at a site. As artificial water points already appear to be acting as refuges, the creation of further pools may help protect both species from further population losses and local extinctions. However, more research on the suitability and design of artificial water points to cater for these species is required. Key parameters that need to be investigated include: proximity to nearby sites and wetlands (allowing connectivity for potential source/sink meta-population dynamics which appear to be of importance for *Galaxiella nigrostriata* (Galeotti, 2013)); projected climate change at the finest scale available (including projected change in groundwater levels); depths of the water points; the ability for the water point to recharge in winter (particularly as depth in winter was significant in determining presence of *L. salamandroides*); and potential for the creation of acid-sulphate conditions (Degens & Wallace-Bell, 2009).

Further research will also be required to determine the length of drying period that both species can withstand during aestivation. Research similar to that of McPhail (1999) could be conducted to determine differences in survival rates between mature and juvenile fish across extended drought periods. Determining what percentage of weight loss is deemed fatal during aestivation will allow further understanding to how great the threat of climate change is to both species, and dictate how and when management will be required to save the species from drastic population losses into the future.

Migration and dispersal patters of *G. nigrostriata* need to be determined using mark recapture as has commonly been used on other endangered freshwater fishes (Bird *et al.*, 2014). The use of Visible Internal Elastomer tags (VIE) has recently been used in small native fishes in this region to determine their migration patterns and population abundances (Beatty et al. pers. comm.). This research project will help determine the current level of population dispersal and mixing by the species which would be highly valuable in managing remnant populations or designing translocation programs.

Other areas of future research should include determining which parameters trigger aestivation as climate change could impact water depth, light intensity, temperature, and indirectly impacting several of the physicochemical properties of the pools. Identifying the triggers separately would be important due to *G. nigrostriata* being present when pools are almost dry, while *L. salamandroides* appear to have burrowed before this stage (Morgan *et al.*, 1998). Determining and quantifying the triggers is important in elucidating the ability of both species to survive increasing dry periods. Under future climate change, fishes may commence aestivation earlier than required resulting in them having to survive longer periods than necessary.

The Scott Coastal plain has been shown to be at widespread risk of acidic water being released due to potential acid sulphate soils being disturbed; particularly from localised reductions in groundwater levels due to climate change, water abstractions and land use

(Degens & Wallace-Bell, 2009). Moreover, much of the south-west has already been affected by secondary salinisation that has severely impacted other fishes (Beatty *et al.*, 2011). Therefore, the pH and salinity tolerance (along with other variables) thresholds of both species also requires determination to model future population viabilities.

6 Conclusions

This study determined the current distribution of *L. salamandroides* and *G. nigrostriata* and revealed major loss of populations of both species and associated dramatic decreases in their extent of occurrence and area of occupancy. Therefore, both species require urgent listing for protection under the Wildlife Conservation Act (1950) and EPBC Act (1999); which is currently being prepared for submission based on my findings. Both species qualify as Endangered (IUCN Red List Threatened Category) under A2c), A4 c); population size reductions (past and projected based on AOO and EOO). Climate change is likely to have already negatively impacted the species and will almost undoubtedly continue to do so.

Connectivity was identified as a key variable of importance for the presence of *G. nigrostriata*, and depth in winter and days of drying were identified as the variables of most importance for *L. salamandroides*. These are of high concern, as all variables are directly linked to climate change. *Galaxiella nigrostriata* is also susceptible due to the importance of habitat connectivity. The reliance on connectivity also suggests it may be more capable of adjusting its range to combat impacts from climate change than would *L. salamandroides*. However, the outlying populations of *G. nigrostriata* north of the study area are at great risk, in particular because of low genetic diversity (Galeotti *et al.*, 2015) and projected climate change in that region.

Climate change is likely to impact on both species due to projected decreases in rainfall and warming within the region. The Elleker region should be considered no longer viable due to past degradation and projected decreases in the watertable of up to 4 m. The hotspot in existing distribution is around Northcliffe and those populations require particular management and research attention. Groundwater levels in the western most region of the study area along the Scott Coastal Plain however, may have some resilience to climate change due to potential increases in the water table. However, there is a considerable risk of acid sulphate soils impacting the species and that process may also complicate management actions such as creation of new habitats.

The lipid storage strategies of both species were identified, with both showing depletions in lipid content after aestivation occurs. The strategy appears more pronounced in *L. salamandroides*. *Galaxiella nigrostriata* however demonstrated a significant difference in lipid utilisation between itself and the non-aestivating *G. munda.* Due to increasing dry periods, it is also likely that the lipid storage strategies of both species may be forced to adjust in order to survive longer periods.

It is likely that without action further population losses for both species will occur and novel methods such as establishing new artificial pools, breeding programs and translocations may be required, in combination with protecting existing habitats from anthropogenic modification, to combat potential losses. It is imperative that the key knowledge gaps identified here be addressed in order to develop management actions to halt the dramatic decline of these two fascinating, yet vulnerable, Australian freshwater fishes.

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Appendices

Appendix A. Sites sampled and presence/absence of target species

Table A1. List of sites, west to east, surveyed for presence of *Lepidogalaxias salamandroides* and *Galaxiella nigrostriata* between Augusta and Albany, Western Australia, including installation of data loggers and sites where isotope samples were taken for surface and groundwater. Sites with asterisk indicate those used as a sub-sample in October.

Site name	Coordinates		Historic Site	Isotope sample	Data logger
	South	East			
Scott River Rd Power Pole 43	34°17.063'	115°14.03'	Morgan et al. (1998) 6.15	Y	Y
Scott River Rd	34°17.052'	115°14.410'	Morgan et al. (1998) 6.7	N	Y
Scott River Rd Power Pole 34	34°17.037'	115°14.784'	-	N	Y
Fouracres Rd	34°17.736'	115°31.165'	Christensen (1982) 90	N	N
Fouracres Rd	34°17.859'	115°34.349'	-	Y	Y
Fouracres Rd	34°18.293'	115°34.986'	Christensen (1982) 89	N	Y
Windy harbour Rd	34°49.672'	116°03.021'	Morgan et al. (1998) 13.8	Y	Y
Windy harbour Rd	34°49.649'	116°03.074'	Morgan et al. (1998) 13.9	Y	Y
Pool near small lake on Windy Harbour Rd	34°49.235'	116°03.833'	Morgan et al. (1998) 13.12	Y	Y
Windy Harbour Rd	34°49.238'	116°03.842'	Morgan et al. (1998) 13.13 / Pusey et al. (1990) 9	N	Y
Small lake Windy Harbour Rd	34°49.158'	116°03.878'	Morgan et al. (1998) 13.11	Y	Y
Windy Harbour Rd	34°48.879'	116°04.136'	Morgan et al. (1998) 13.15	N	N
Doggerup Creek	34°43.443'	116°04.172'	Morgan et al. (1998) 12.9	N	N
Windy Harbour Rd	34°48.808'	116°04.198'	Morgan et al. (1998) 13.14	N	N
Windy Harbour Rd	34°43.797'	116°05.444'	Pusey et al. (1990) 8	Y	Y
Chesapeake Rd (1st small pool)	34°36.817'	116°06.05'	-	N	N
Windy Harbour Rd	34°42.112'	116°06.071'	-	N	Y
Chesapeake Rd (1st large pool)	34°42.323'	116°07.041'	Morgan et al. (1998) 13.21	N	Y
Chesapeake Rd*	34°43.174'	116°07.572'	Morgan et al. (1998) 13.23	N	Y
Chesapeake Rd	34°43.328'	116°07.743'	Morgan et al. (1998) 13.24	N	Y
Chesapeake Rd*	34°43.372'	116°07.777'	Morgan et al. (1998) 13.25	Y	Y
Chesapeake Rd	34°43.472'	116°07.877'	Adjacent to Morgan et al. (1998) 13.26	N	Y
Chesapeake Rd	34°43.524'	116°07.925'	Morgan et al. 13.27	N	N
Chesapeake Rd	34°43.972'	116°08.242'	-	N	Y

Pool Lower Gardner River Rd	34°46.290'	116°08.791'	-	N	N
Small pool Lower Gardner River Rd	34°46.789'	116°08.799'	-	Y	Y
Small pool Lower Gardner River Rd	34°46.567'	116°08.811'	-	N	N
Small pool Lower Gardner River Rd	34°46.566'	116°08.816'	-	N	Y
Lower Gardner River Rd	34°45.883'	116°08.913'	Morgan et al. (1998) 13.61	Y	Y
Chesapeake opposite Morgan et al. 13.29	34°45.387'	116°09.012'	Morgan et al. (1998) 13.30	N	Y
Chesapeake	34°45.387'	116°09.017'	Morgan et al. (1998) 13.29	Y	Y
Lower Gardner River Rd	34°45.603'	116°09.032'	-	N	N
Pool 37.6km east end Chesapeake Rd	34°45.670'	116°09.197'	Morgan et al. (1998) 13.33	Y	N
Large pool Ches. Rd	34°45.923'	116°09.391'	Morgan et al. (1998) 13.34	N	N
Pool on Moore Hut Dr*	34°50.430'	116°17.032'	Morgan et al. (1998) 14.1	Y	Y
Pool Chesapeake Rd*	34°49.211'	116°18.642'	-	Y	Y
Deeside Coast Rd & Preston Rd*	34°38.498'	116°19.711'	Morgan et al. (1998) 14.14	Y	Y
Forth River	34°51.819'	116°25.556'	Morgan et al. (1998) 15.1	Y	N
Small stream 6.6km N of Broke Inlet Rd	34°51.937'	116°25.725'	Morgan et al. (1998) 15.2	N	N
Chesapeake Rd	34°52.147'	116°26.017'	Pusey et al. (1990) 3	Y	N
Pool/stream Broke Inlet Rd	34°52.364'	116°26.317'	Morgan et al. (1998) 15.4	N	Y
Broke Inlet Rd	34°52.744'	116°26.847'	Pusey et al. (1990) 2	Y	N
Pool Broke Inlet Rd	34°53.638'	116°29.771'	Morgan et al. (1998) 15.8	N	N
Beardmore Rd	34°48.590'	116°31.917'	Pusey et al. (1990) 1	Y	N
Beardmore Rd pools*	34°48.620'	116°32.247'	Morgan et al. (1998) 16.5	Y	Y
Thomson Rd	34°47.081'	116°43.195'	-		
Station Rd & Cons. Rd	35°00.576'	116°51.691'	-	N	Y
Conspic. Cliffs Rd	35°01.621'	116°51.890'	-	N	Y
Fiscifolia Rd - Lake track	35°01.700'	116°53.355'	Morgan et al. (1998) 18.4	N	N
Nornalup Rd	34°43.792'	116°59.008'	WAM Record	N	N
Nornalup Rd	34°44.121'	116°59.269'	WAM Record	N	N
Marbelup Rd N of Elleker	35°00.417'	117°43.308'	WAM Record	N	N
Road to Elleker by railroad	35°00.803'	117°49.279'	WAM Record	N	N

Morgan et al. refers to site number identified in Morgan, D. L., Gill, H. S., & Potter, I. C. (1998). Distribution, identification and biology of freshwater fishes in south-western Australia.

Christensen refers to site number identified in Christensen, P. (1982). The distribution of *Lepidogalaxias salamandroides* and other small fresh-water fishes in the lower south-west of Western Australia.

Pusey et al. refers to sites identified in Pusey, B. J., & Edward, D. H. D. (1990). Structure of fish assemblages in waters of the southern acid peat flats, South-western Australia.

WAM Record refers to records of specimens at the Western Australian Museum.

Table A2. Presence (+) and absence (-) of *Lepidogalaxias salamandroides* and *Galaxiella nigrostriata* at the sampled sites (sorted west to east) across seasons. Hatched cells indicate site could not be sampled.

Site name	Site ID	*L. salamandroides* present		*G. nigrostriata* present	
		Winter	Spring Summer	Winter	Spring Summer
Scott River Rd Power Pole 43	2	-	+	+	+
Scott River Rd	1	+	+	-	+
Scott River Rd Power Pole 34	33	-	▨	-	▨
Fouracres Rd	27	-	▨	-	▨
Fouracres Rd	26	-	-	-	-
Fouracres Rd	25	-	-	-	+
Windy harbour Rd	3	+	-	+	+
Windy harbour Rd	4	+	▨	+	▨
Pool near small lake on Windy Harbour Rd	5	-	+	-	+
Windy Harbour Rd	5a	-	▨	-	▨
Small lake Windy Harbour Rd	6	-	+	-	+
Windy Harbour Rd	24	+	▨	-	▨
Doggerup Creek	54	▨	+	▨	+
Windy Harbour Rd	7	+	-	-	+
Windy Harbour Rd	8	+	▨	+	▨
Chesapeake Rd (1st small pool)	55	-	-	-	-
Windy Harbour Rd	23	+	+	-	+
Chesapeake Rd (1st large pool)	9	+	-	+	+
Chesapeake Rd*	19	+	+	+	+
Chesapeake Rd	20	+	+	-	+
Chesapeake Rd*	21	+	+	+	+
Chesapeake Rd	28	+	+	-	+
Chesapeake Rd	29	-	-	-	+
Chesapeake Rd	30	+	+	+	+
Pool Lower Gardner River Rd	45	-	▨	-	▨
Small pool Lower Gardner River Rd	42	+	▨	-	▨
Small pool Lower Gardner River Rd	43	-	▨	-	▨
Small pool Lower Gardner River Rd	44	-	▨	-	▨
Lower Gardner River Rd	41	-	▨	-	▨
Chesapeake opposite 13.29	32	+	+	-	+
Chesapeake	31	+	+	-	+
Lower Gardner River Rd	40	-	+	-	+
Pool 37.6km east end Chesapeake Rd	38	+	+	-	+
Large pool Ches. Rd	39	-	-	-	+
Pool on Moore Hut Dr*	49	+	+	+	+

Pool Chesapeake Rd*	48	+	+	-	-
Crnr Deeside Coast Rd & Preston Rd*	18	+	+	-	+
Forth River	17	-	▨	-	▨
Small stream 6.6km N of Broke Inlet Rd	47	+	+	-	+
Chesapeake Road	16	-	-	-	-
Pool/stream Broke Inlet Rd	46	+	+	-	-
Broke Inlet Rd	14	+	-	-	-
Pool Broke Inlet Rd	15	+	-	-	-
Beardmore Rd	10	-	-	-	-
Beardmore Rd pools*	11	+	+	-	-
Thomson Rd	34	-	▨	-	▨
Crnr. Station Rd & Cons. Rd	37	-	▨	-	▨
Conspic. Cliffs Rd	35	-	▨	-	▨
Fiscifolia Rd - Lake off track	36	+	+	-	-
Nornalup Rd	53	-	▨	-	▨
Nornalup Rd	52	-	▨	-	▨
Marbelup Rd north of Elleker	51	-	▨	-	▨
On road to Elleker next to railroad	50	-	▨	-	▨

Appendix B. Extent of occurrence for *Lepidogalaxias salamandroides* and *Galaxiella nigrostriata*

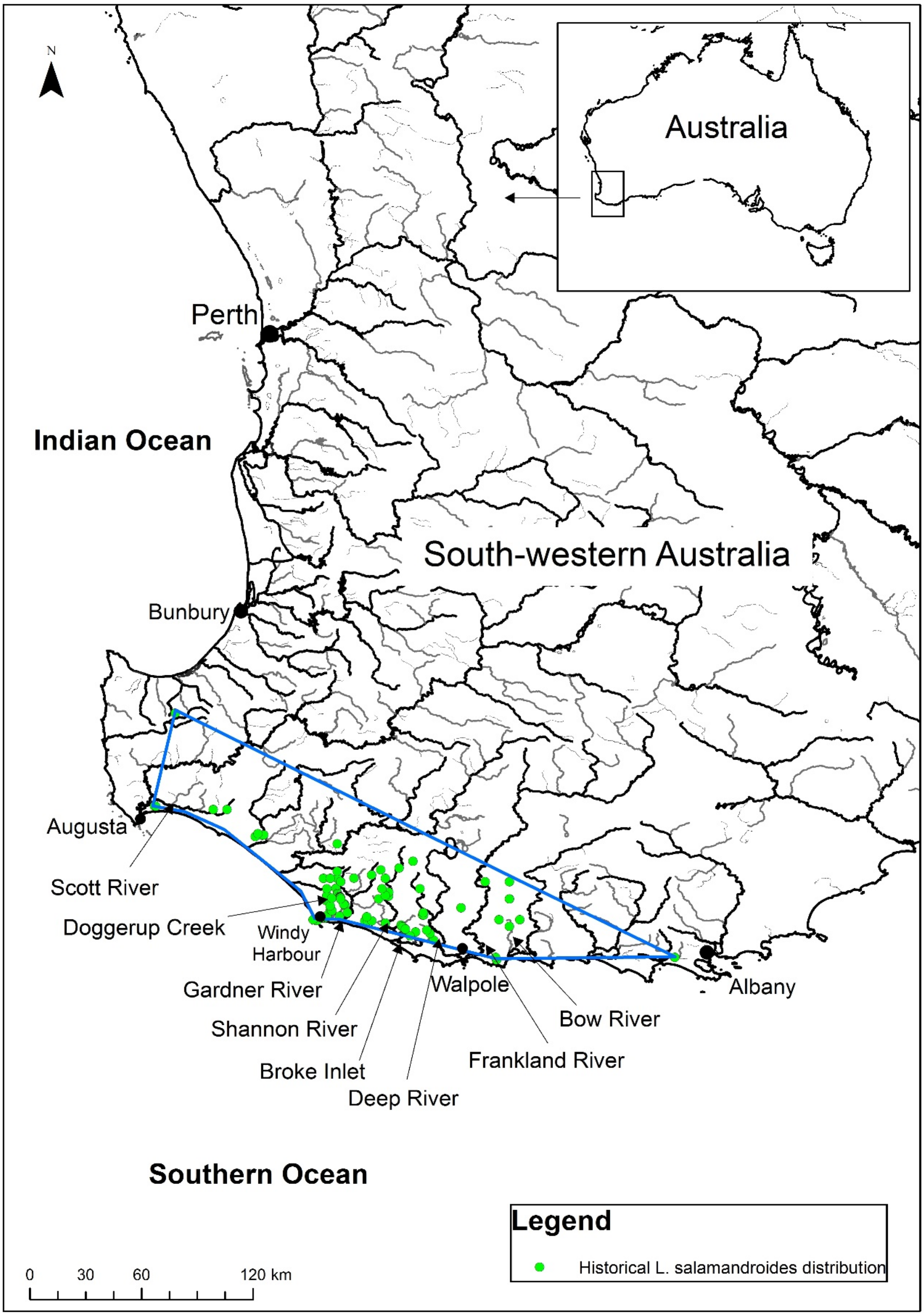

Figure B1. Historical extent of occurrence (EOO) for *Lepidogalaxias salamandroides*

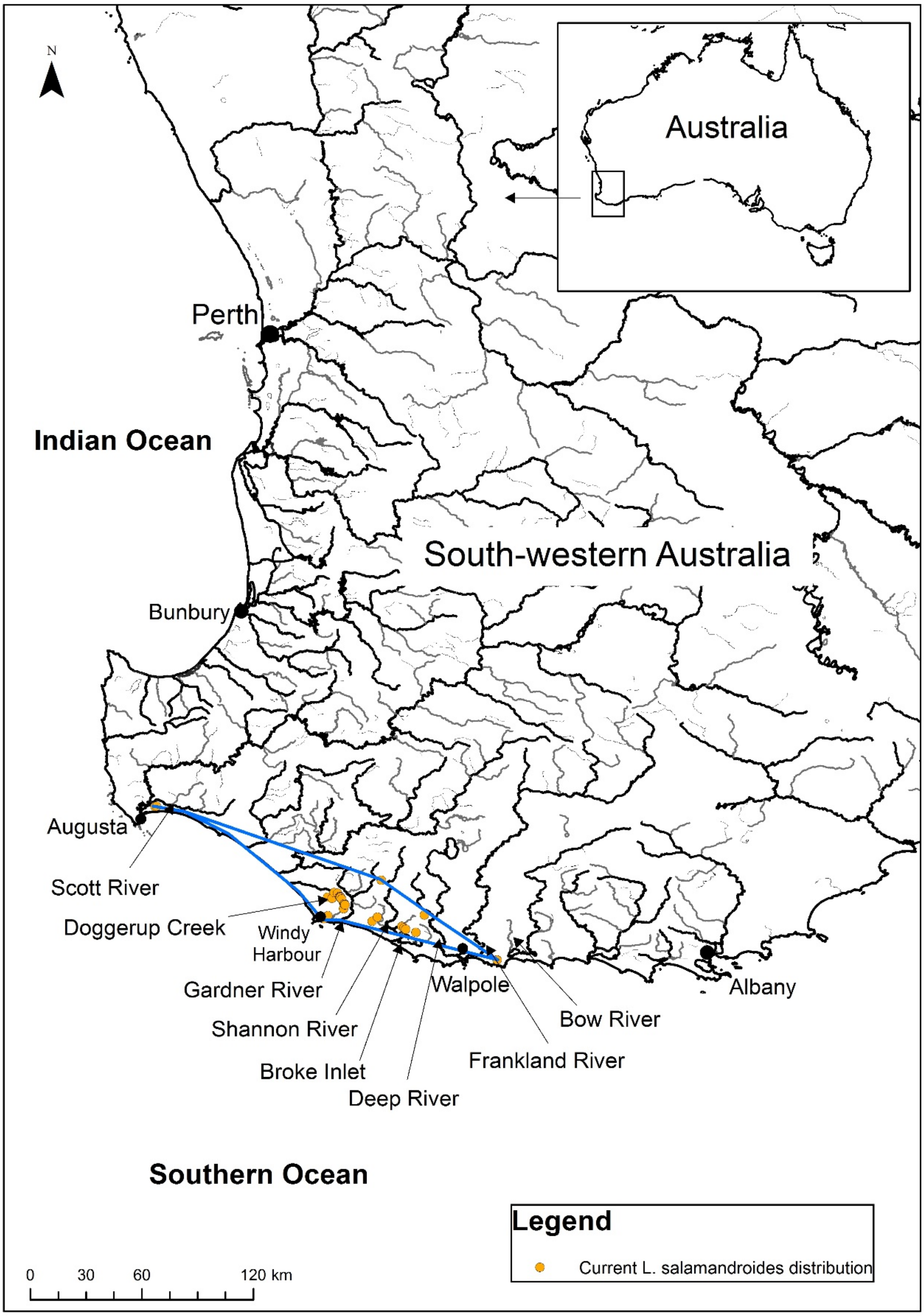

Figure B2. Current extent of occurrence (EOO) for *Lepidogalaxias salamandroides*

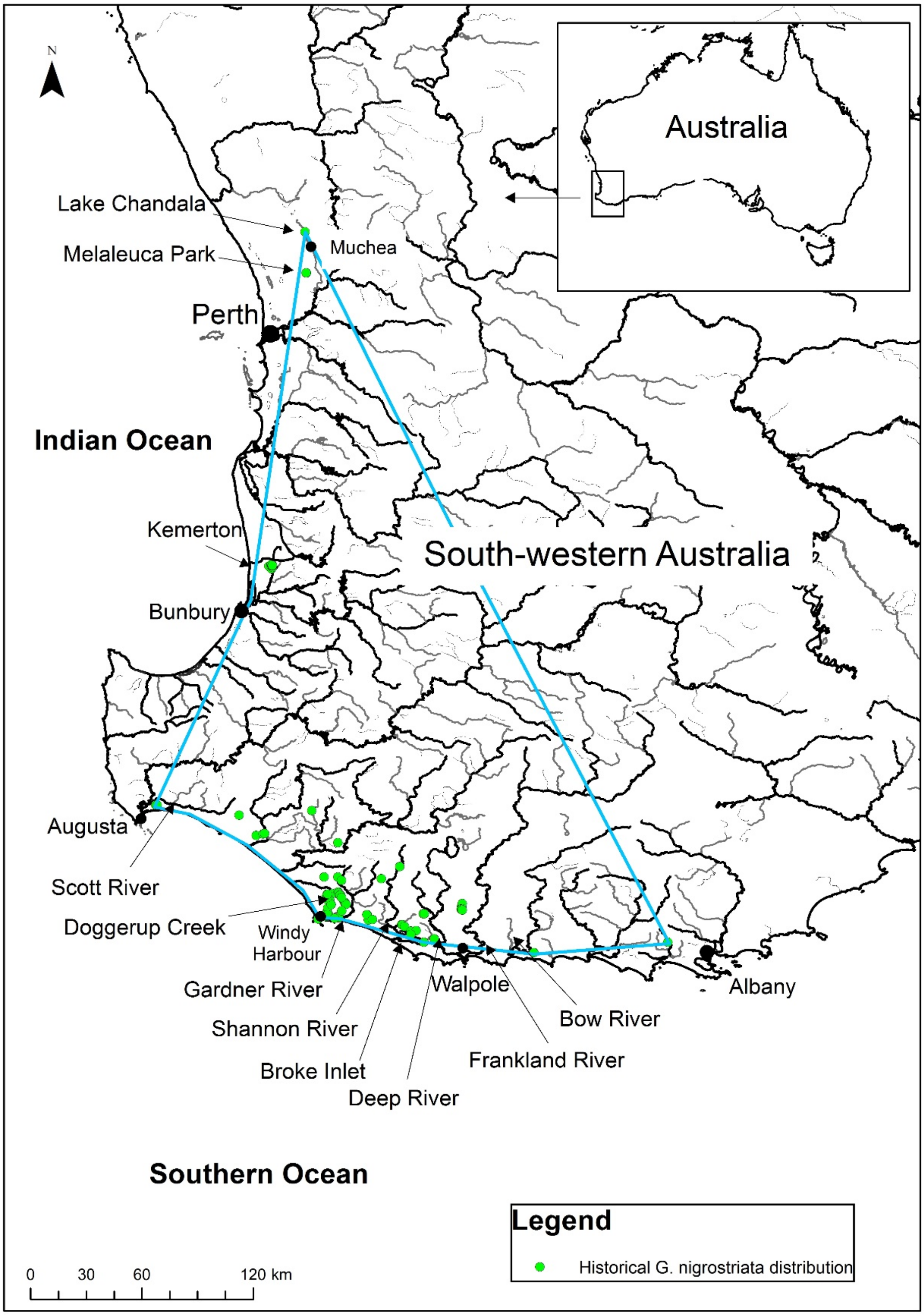

Figure B3. Historical extent of occurrence (EOO) for *Galaxiella nigrostriata*

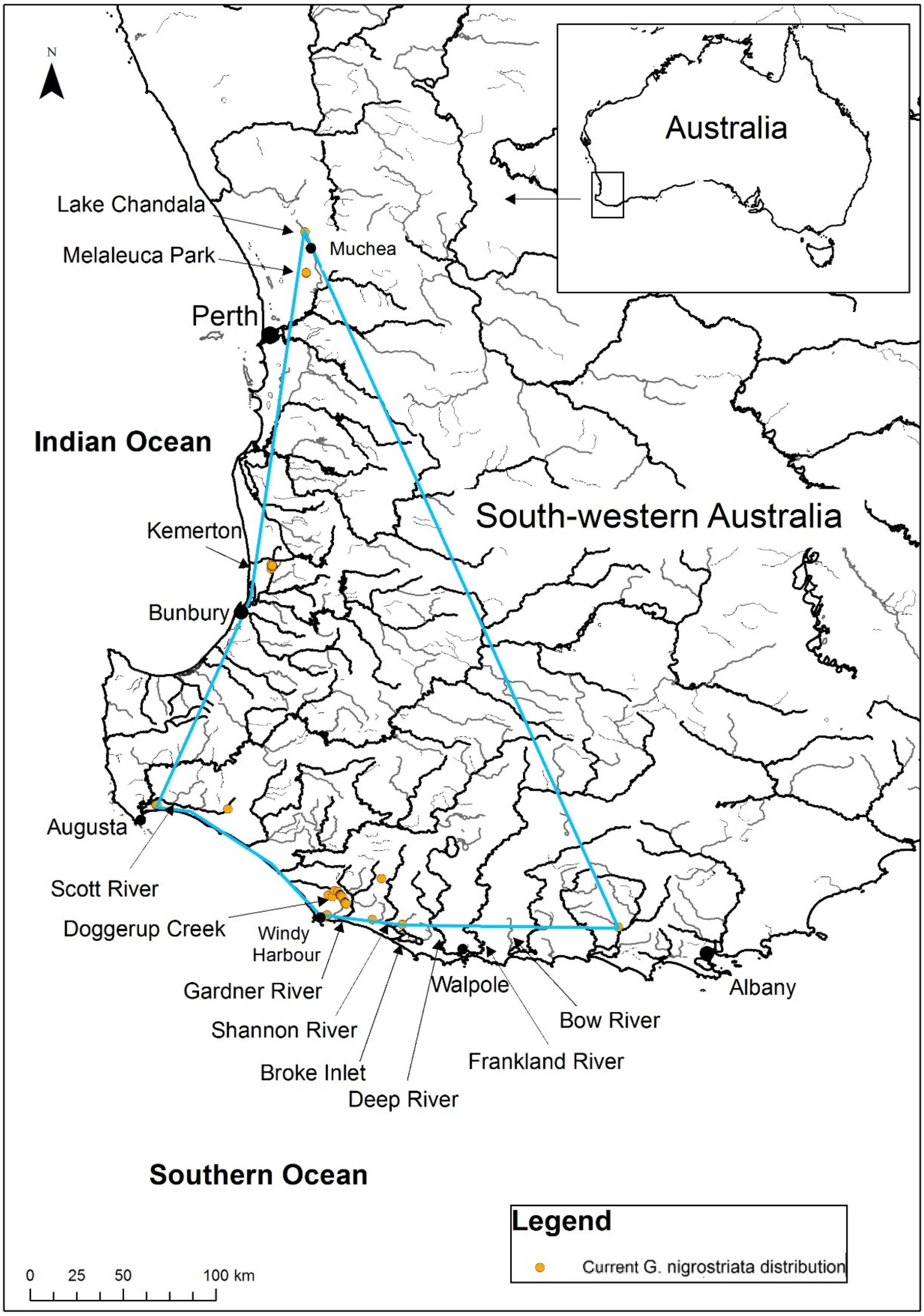

Figure B4. Current extent of occurrence (EOO) for *Galaxiella nigrostriata*

Appendix C. Temperature/light logger charts

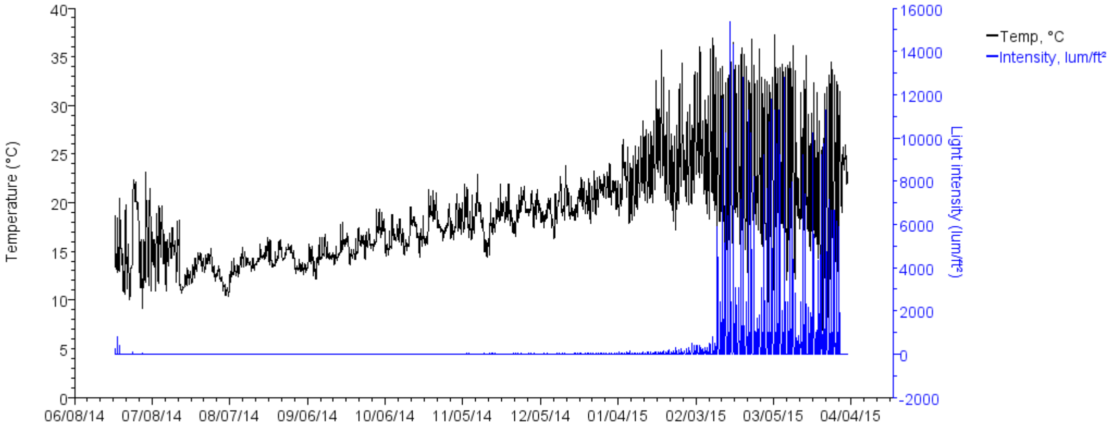

Figure C1. Water temperature and light intensity recorded at Scott River Road Power Pole 43 (Site ID 2) displaying a clear spike in temperature and light intensity used to estimate earliest date of drying at the site

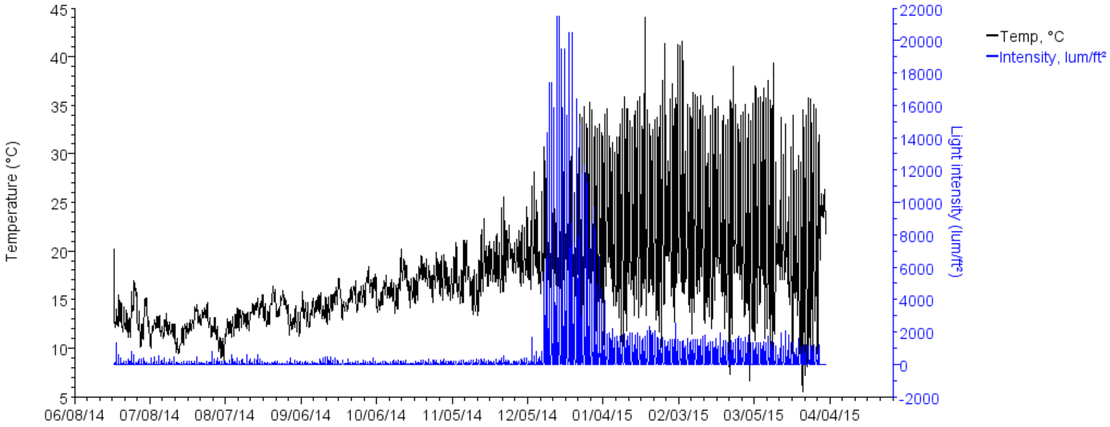

Figure C2. Water temperature and light intensity recorded at Scott River Rd (Site ID 1) displaying a clear spike in temperature and light intensity used to estimate earliest date of drying at the site

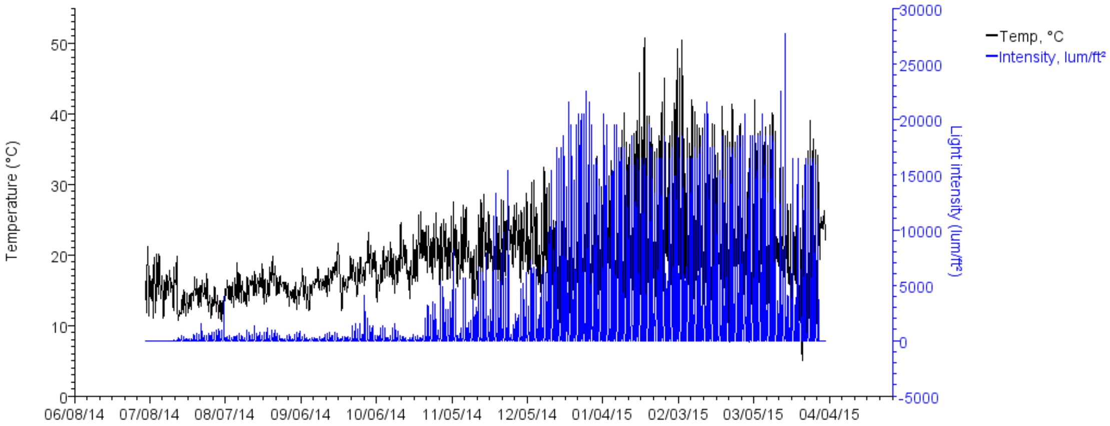

Figure C3. Water temperature and light intensity recorded at Scott River Rd (Site ID 33) displaying a clear spike in temperature and light intensity used to estimate earliest date of drying at the site

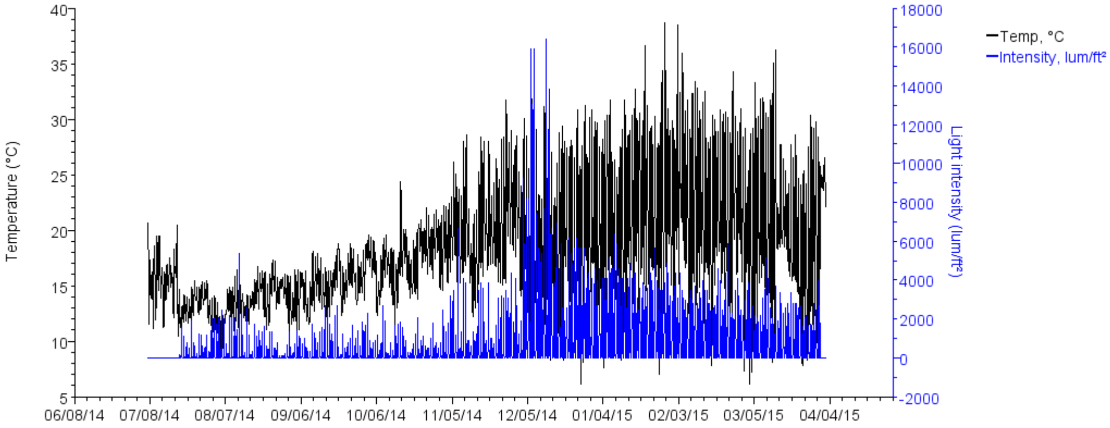

Figure C4. Water temperature and light intensity recorded at Windy Harbour Road (Site ID 4) displaying a clear spike in temperature and light intensity used to estimate earliest date of drying at the site

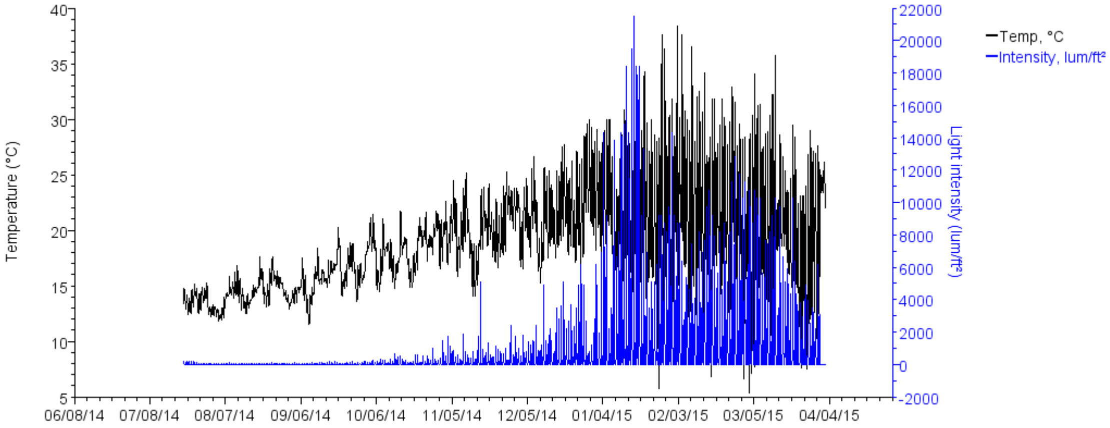

Figure C5. Water temperature and light intensity recorded at Windy Harbour Road (Site ID 6) displaying a clear spike in temperature and light intensity used to estimate earliest date of drying at the site

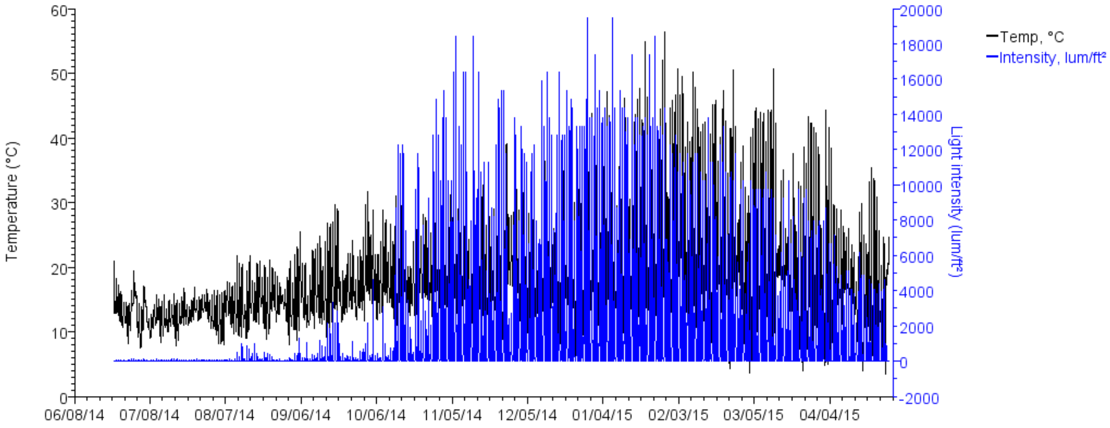

Figure C6. Water temperature and light intensity recorded at Windy Harbour Road (Site ID 8) displaying a clear spike in temperature and light intensity used to estimate earliest date of drying at the site

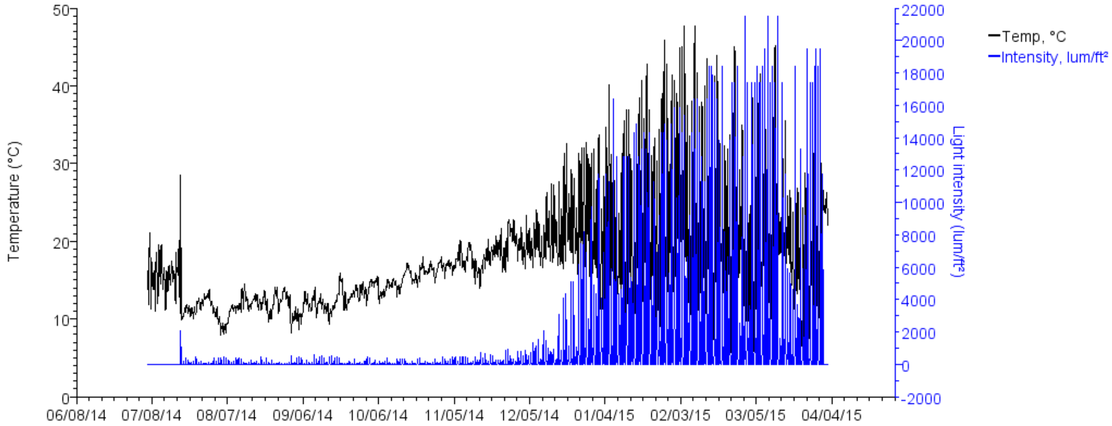

Figure C7. Water temperature and light intensity recorded at Windy Harbour Road (Site ID 23) displaying a clear spike in temperature and light intensity used to estimate earliest date of drying at the site

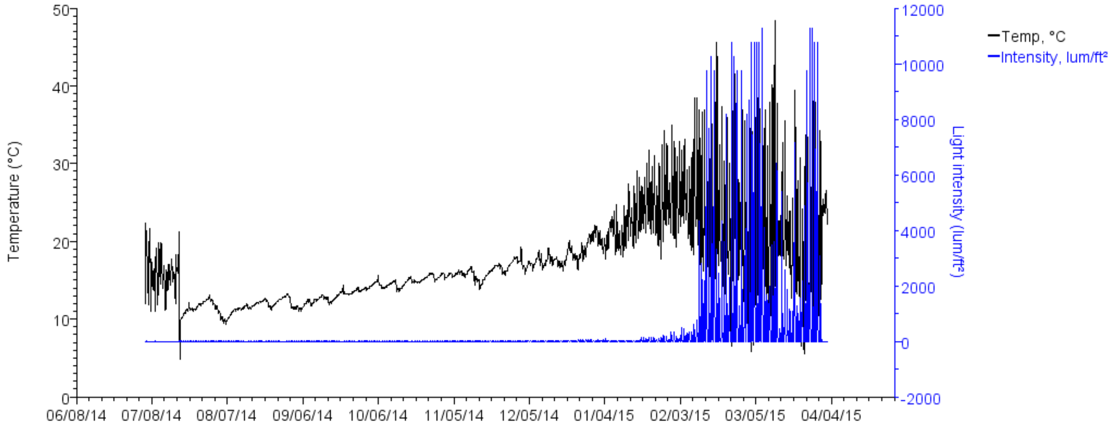

Figure C8. Water temperature and light intensity recorded at the first large pool on Chesapeake Rd (Site ID 9) displaying a clear spike in temperature and light intensity used to estimate earliest date of drying at the site

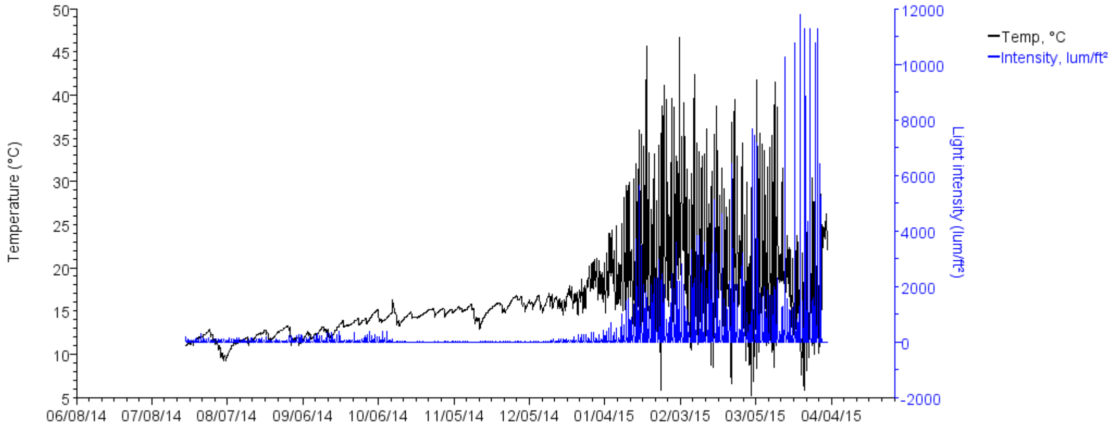

Figure C9. Water temperature and light intensity recorded at Chesapeake Rd (Site ID 19) displaying a clear spike in temperature and light intensity used to estimate earliest date of drying at the site

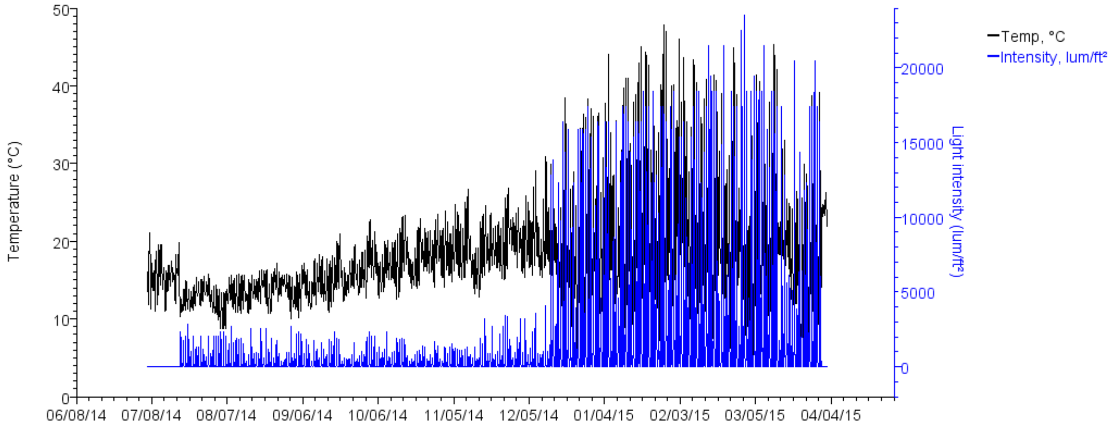

Figure C10. Water temperature and light intensity recorded at Chesapeake Rd (Site ID 20) displaying a clear spike in temperature and light intensity used to estimate earliest date of drying at the site

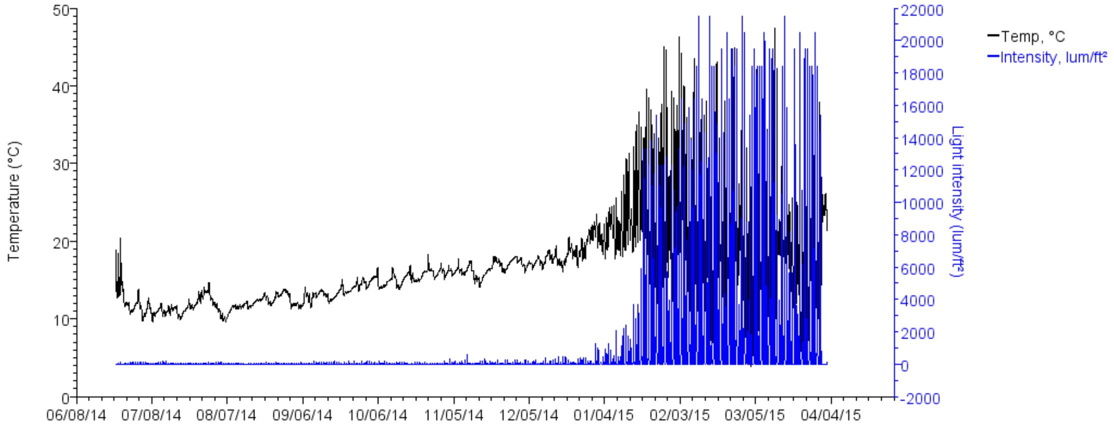

Figure C11. Water temperature and light intensity recorded at Chesapeake Rd (Site ID 21) displaying a clear spike in temperature and light intensity used to estimate earliest date of drying at the site

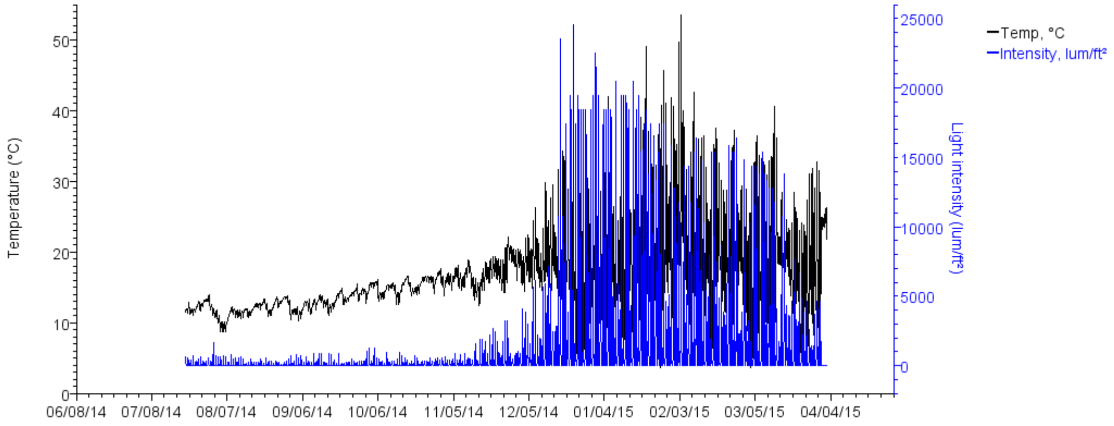

Figure C12. Water temperature and light intensity recorded at Chesapeake Rd (Site ID 28) displaying a clear spike in temperature and light intensity used to estimate earliest date of drying at the site

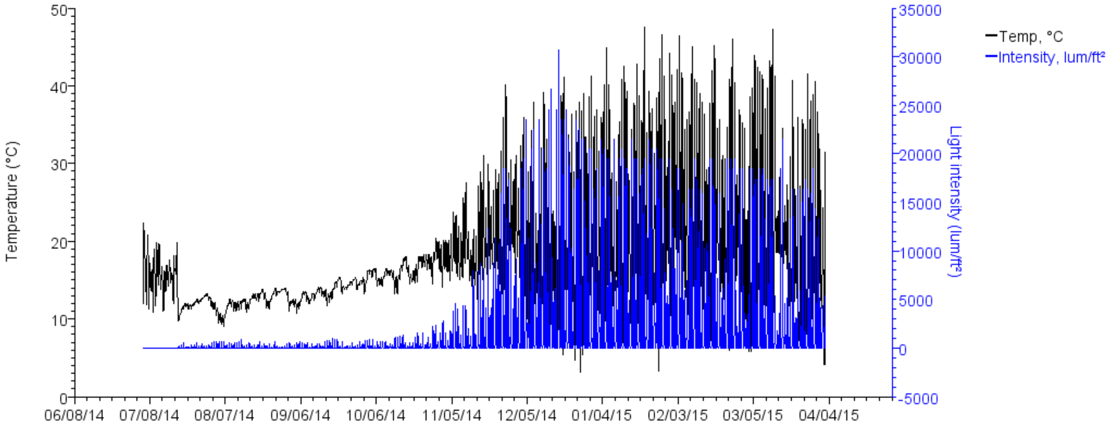

Figure C13. Water temperature and light intensity recorded at Chesapeake Rd (Site ID 30) displaying a clear spike in temperature and light intensity used to estimate earliest date of drying at the site

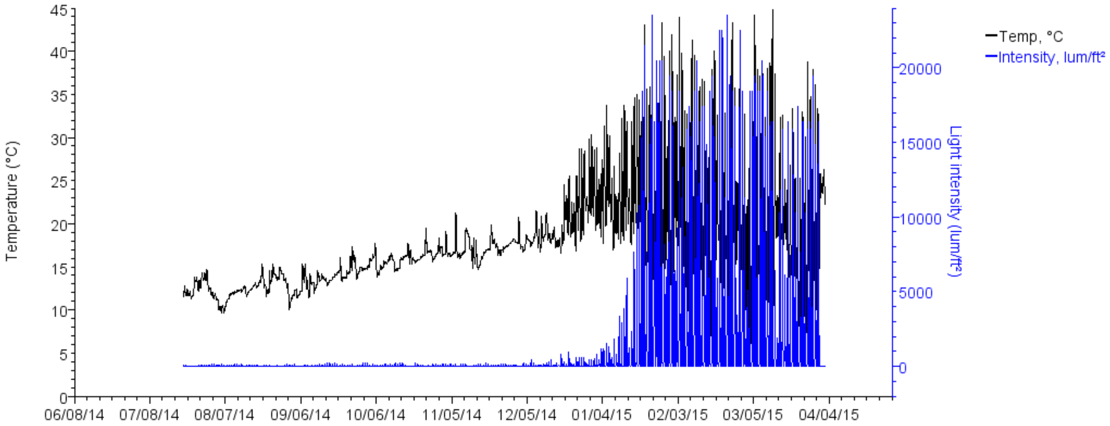

Figure C14. Water temperature and light intensity recorded at Chesapeake Rd (Site ID 31) displaying a clear spike in temperature and light intensity used to estimate earliest date of drying at the site

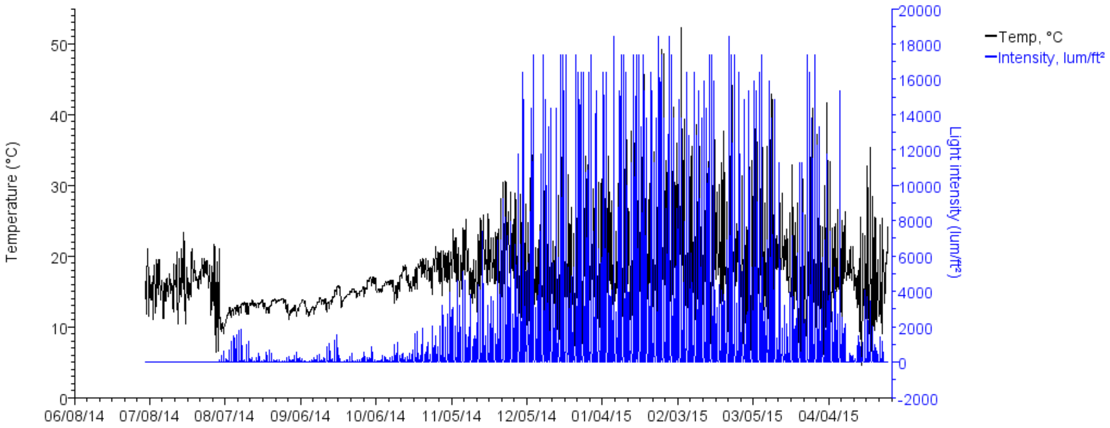

Figure C15. Water temperature and light intensity recorded at Chesapeake Rd (Site ID 48) displaying a clear spike in temperature and light intensity used to estimate earliest date of drying at the site

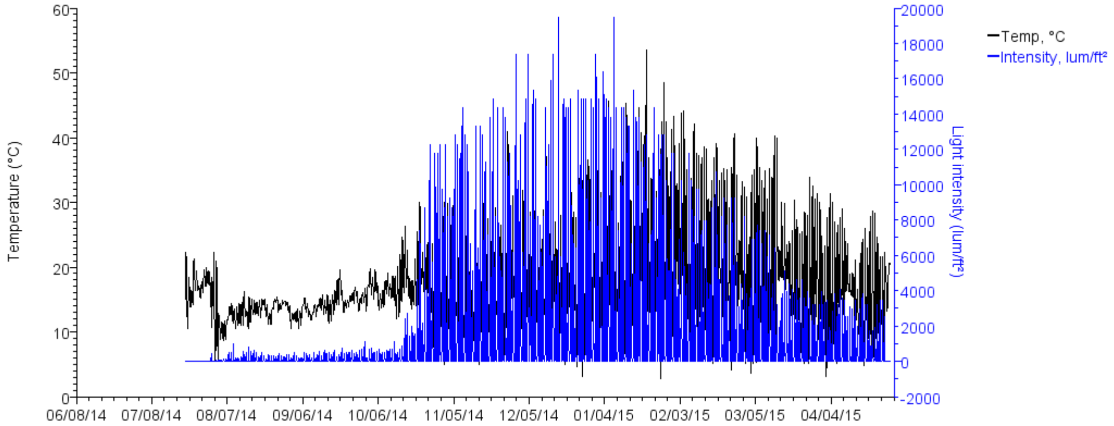

Figure C16. Water temperature and light intensity recorded at Lower Gardner River Road (Site ID 41) displaying a clear spike in temperature and light intensity used to estimate earliest date of drying at the site

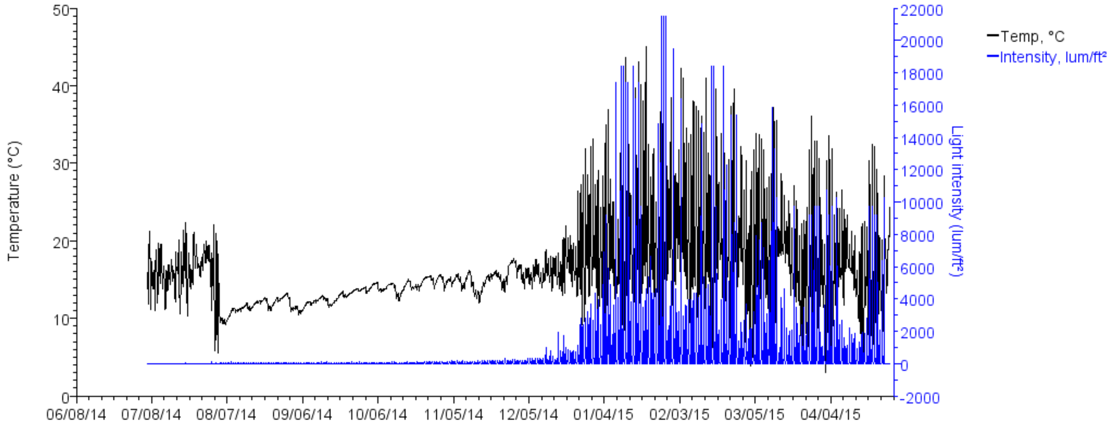

Figure C17. Water temperature and light intensity recorded at Lower Gardner River Road (Site ID 42) displaying a clear spike in temperature and light intensity used to estimate earliest date of drying at the site

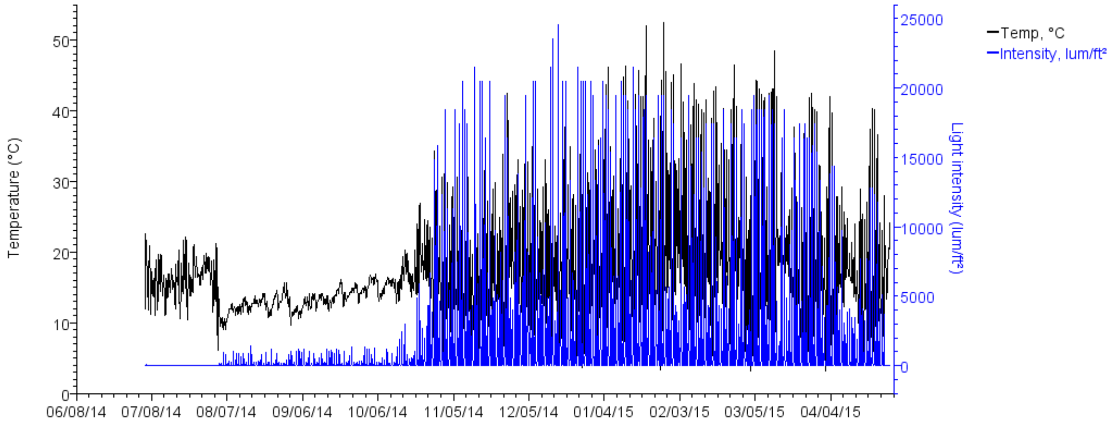

Figure C18. Water temperature and light intensity recorded at Lower Gardner River Road (Site ID 44) displaying a clear spike in temperature and light intensity used to estimate earliest date of drying at the site

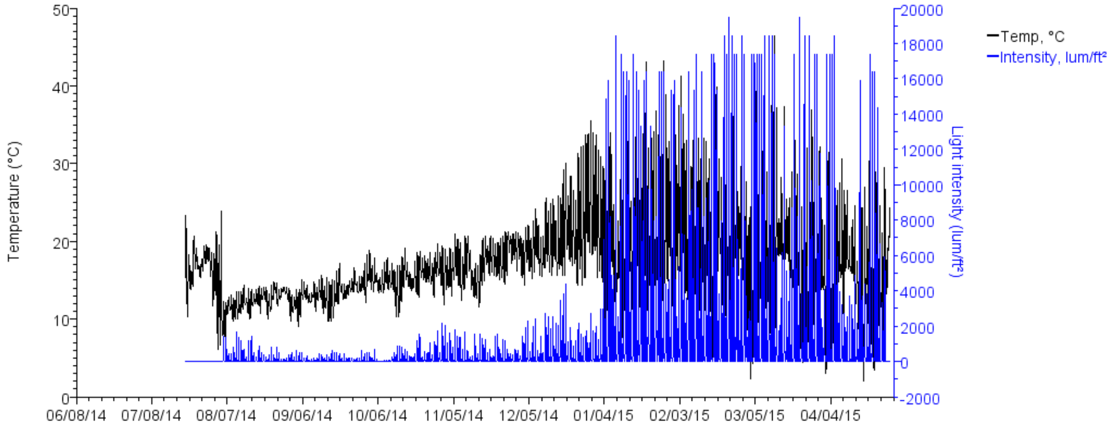

Figure C19. Water temperature and light intensity recorded at Moore's Hut Track (Site ID 49) displaying a clear spike in temperature and light intensity used to estimate earliest date of drying at the site

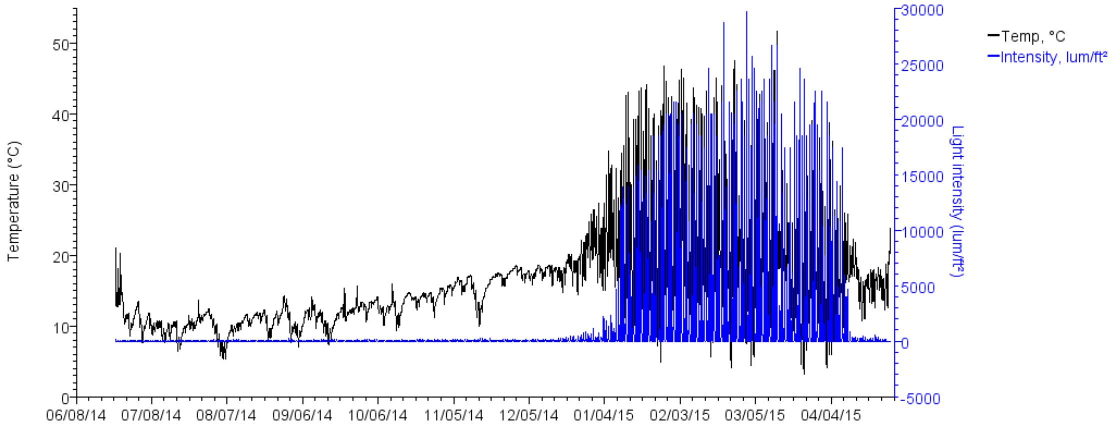

Figure C20. Water temperature and light intensity recorded at Deeside Coast Rd junction with Preston Rd (Site ID 18) displaying a spike in temperature and light intensity used to estimate earliest date of drying

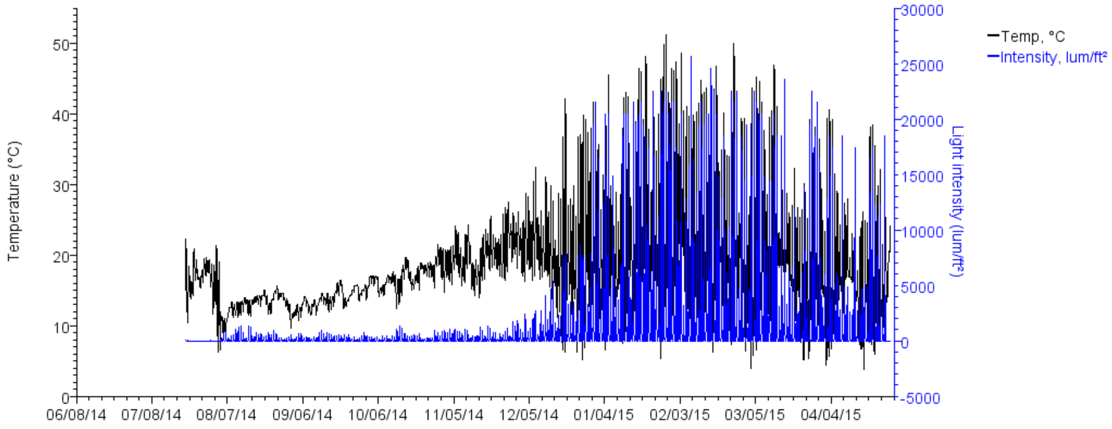

Figure C21. Water temperature and light intensity recorded at pool along Broke Inlet Road (Site ID 15) displaying a clear spike in temperature and light intensity used to estimate earliest date of drying at the site

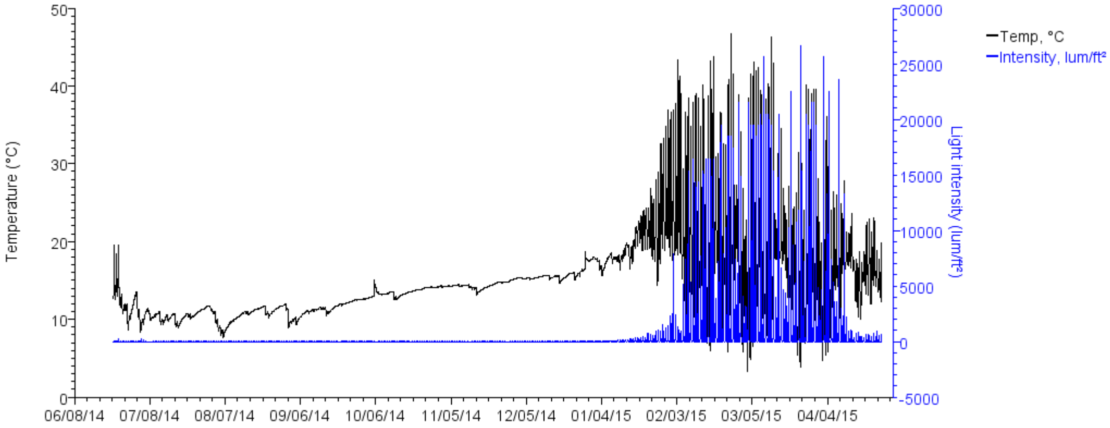

Figure C22. Water temperature and light intensity recorded at Beardmore Pools (Site ID 11) displaying a clear spike in temperature and light intensity used to estimate earliest date of drying at the site

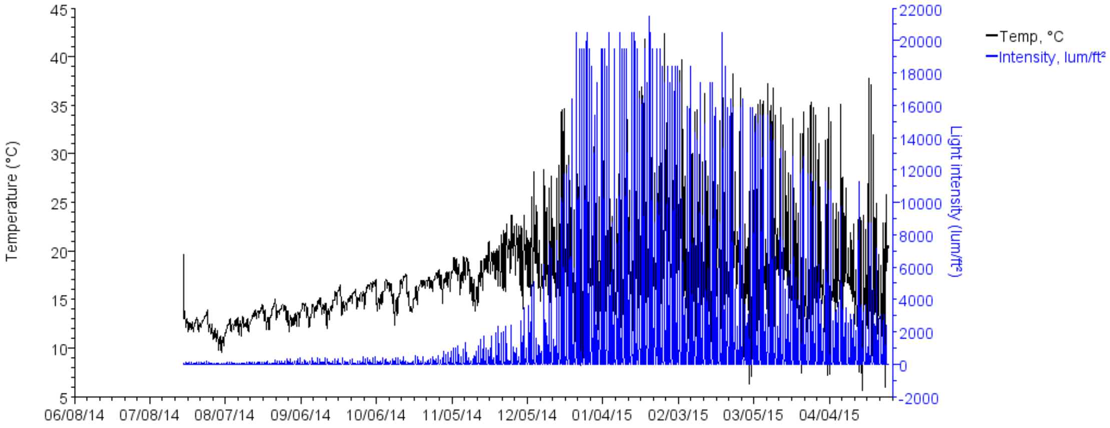

Figure C23. Water temperature and light intensity recorded at Conspicuous Cliffs Rd (Site ID 35) displaying a clear spike in temperature and light intensity used to estimate earliest date of drying at the site

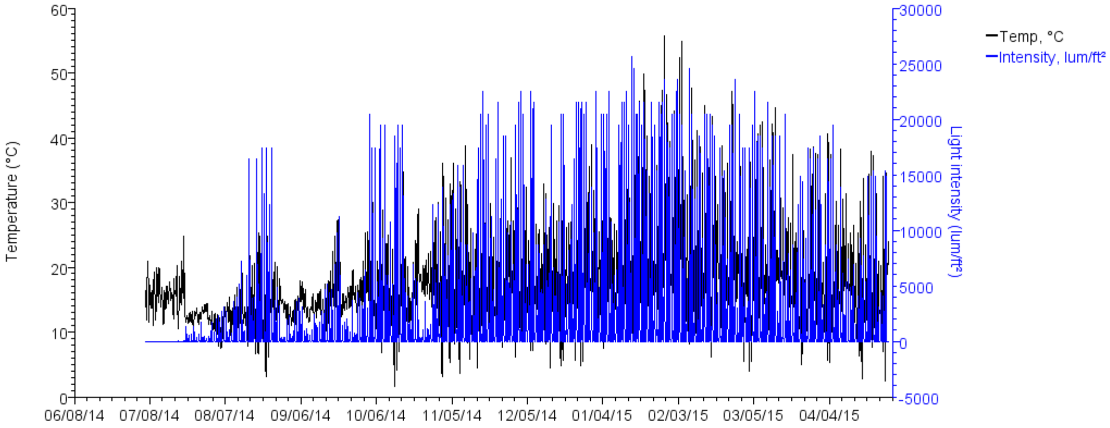

Figure C24. Water temperature and light intensity recorded at corner of Conspicuous Cliffs Rd and Station Rd (Site ID 37) displaying spikes in temperature and light intensity used to estimate earliest date of drying