Human impacts on the long-necked turtle
*Chelodina longicollis* (Shaw 1794) in peri-urban Sydney

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

2014

University of Western Sydney
This thesis is dedicated to my beloved Josh and my amazing parents for their endless support, love and encouragement.
Acknowledgements

Firstly I would like to sincerely thank my supervisor Professor Shelley Burgin. I cannot tell you how much I appreciate your enthusiasm, inspiration, advice, friendship, time, and guidance. Your help has been invaluable and has kept me going when everything was falling down. Thank you for all you have given to me and I am so glad that we have such a strong friendship that will continue long after this.

Thank you to my co-supervisor Dr. Ian Wright for your continued friendship, statistical advice and editing. I have really appreciated your support and have loved our ‘chats’, which always made me feel better. I would also like to thank Dr. Paul Somerfield from Primer-e who gave me invaluable support with my data and its analysis using Primer-e. I could not have completed my analysis without his help and guidance. Michael Franklin helped me to navigate Arcview, the GIS program used to organize my roadkill data and I really appreciate all his help. Thank you to Dr Michelle Dalli for all your help with patching up the injured turtles and euthanising the badly injured ones. Thank you also for all your time and advice that you have given me throughout the years. Thank you to David Hall of Hallprint fish tags for donating the shellfish tags used in this research and to Dennis Hitchen, Andrew Norris and my Aunty Cathy Ryan for help in the field.

There are three people whom have provided me all the support, love and encouragement I have needed to get through. I love and appreciate these three people more than words can express. Firstly my mum, Judy Ryan, thank you so much Mum for getting out of bed before dawn and going into the field with me, and especially for keeping me safe from snakes (despite a few close calls). Thank you also for all the hours you have put into reading and editing my draft thesis. Secondly my Dad, Geoff Ryan, thank you firstly for buying me a ute for my field work (the Turtlelux) and for the heavy work of helping me set up my sample sites. I cannot thank you enough for working five days a week then coming into the field on the weekends with me. Finally, thank you to my partner, Josh for supporting me in everything thing that I do and for all your love and help throughout this crazy adventure.
Statement of Authentication

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

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Abstract

It is well documented that humans may have a major impact on native ecosystems including the animals that reside within them. In Australia, over the past 200 years there have been major changes to the landscape, including land clearing, habitat fragmentation, urbanisation, the creation of roadways, and the introduction of exotic species. Freshwater turtles are one taxon that has been affected by both terrestrial and aquatic changes. The long-necked turtle *Chelodina longicollis* (Shaw 1794) is the most widespread, and abundant freshwater turtle in Australia. Because of their broad geographic range, their ability to survive within a diversity of aquatic habitats, urban and rural, and because they have a penchant for terrestrial movement, they are an appropriate species to use as a case study on the effect of urbanisation on the long-term viability of freshwater turtles. In addition, because *C. longicollis* represent the largest vertebrate biomass in many impoundments, their demise is likely to have a major impact on the functioning of these systems.

This study was undertaken in Sydney, the largest city in Australia, in areas that have had significant habitat change due to urbanisation, and continue to have rapid change due to increasing density and extent of urbanisation. It was observed that *C. longicollis* remain the ubiquitous freshwater turtle, although recruitment to the population varied both at different human population density, and among wetlands, and there was little to no recruitment in the urban and suburban sites. Another native freshwater turtle, *Emydura macquarii* was found at all three sites (urban, suburban and peri urban). In the peri urban site there were very few (< 5) of this species netted, whilst at both the urban and suburban sites they were in greater numbers, with *E. macquarii* in greater density than *C. longicollis* in the urban area. Successful recruitment of *E. macquarii* was only observed to have occurred in the suburban site and not in the other two sites (suburban, peri urban). A potential impact on *C. longicollis* was competition from other species such as *Trachemys scripta elegans* the feral red-eared slider that has demonstrated to outcompete native species in many areas of the world, including Australia.
In the peri-urban area it was found that wetland characteristics were influencing the population structure in wetlands, and juveniles were more prevalent in wetlands with low dissolved oxygen and higher percentages of emergent vegetation. It was also observed that over the decade of the study, there was a shift in the population structure to a female bias in the population. Females were also shown to have greater site fidelity than males. Approximately 15% of females were recaptured in the wetland of first capture 13 years after they were first netted in the wetland, although no males were recaptured after this length of time. These females were also in better condition than those that had been netted only in the more recent study period.

Vehicle collision impacted both sexes of *C. longicollis* in all stages of their lifecycle. Females were killed in higher numbers in the nesting season (spring) than at other times, potentially exacerbating the impact on local populations.

Casual observation may indicate that *C. longicollis*, and where they are present, *Emydura macquarii*, have viable populations, even within wetlands within the most densely populated area of Sydney. However, in these long-lived animals, lack of, or limited, recruitment to the population, losses due to vehicle collision during terrestrial movement, supplementary feeding, water quality, and loss of wetlands in the urbanising areas, question whether the populations over the longer term are viable in the urbanised/rapidly urbanising areas of the Sydney Basin. Without knowledge of longevity and age of senescence, it is effectively impossible to predict when the population will become non-sustainable. However, without intervention such as raising the awareness of the plight of freshwater turtles, the provision of safe nesting sites, mitigation measures to reduce vehicle collision, and removal of exotic animals, the population will decline over time.
Chapter 1 – Introduction

The long-necked turtle *Chelodina longicollis* netted in peri-urban western Sydney, (New South Wales, Australia).
1.1 Human impacts on loss of biodiversity

The worldwide reduction of biodiversity is greatly accelerating with time. Definitive rates of extinction cannot be determined globally, as most species have not been formally identified, let alone baseline data gathered. However, it was estimated that the rate of species’ extinction is now between 100 to 1000 times greater than before human dominance (IUCN 2007; Vitousek et al. 1997). The International Union for the Conservation of Nature (IUCN) conducted an assessment of biodiversity based on only 3% of the 1.9 million described species worldwide. It was found that, when the assessment was released in 2007, 15,589 species were considered threatened with extinction (IUCN 2007).

It is widely accepted that a major reason for the accelerated loss of biodiversity is due largely to human impacts. An increase in the human population leads to changes in land use and an increase in urbanisation (Hilty et al. 2006). It is estimated that worldwide the urban population increases by 160,000 people daily (Rosen et al. 2001). This increase results in urban sprawl. The extent of this sprawl has been demonstrated in the United States (US). In 1950, 65% of people in the US lived in urban areas whereas, by 1990, 75% of the population lived in an urban setting. Coincidently, the land covered by urbanisation had almost doubled by the late 1990s (Rosen et al. 2001). Even just this increase in urbanisation could be predicted to have had a major impact on biodiversity due to habitat loss, habitat fragmentation, the expansion of roadways, introduced species, increased use of natural resources, degradation of waterways, and pollution (Eskew et al. 2010; Forrester and Machlis 1996; Hilty et al. 2006; Hitchen et al. 2011b; Vitousek et al. 1997).

1.1.1 Habitat loss and habitat fragmentation

A major issue with human population increase, within and outside of urban areas has been habitat loss which has often altered the landscape such that native ecosystems become unsustainable (Forrester and Machlis 1996). Universally, habitat disruption has become the dominant threat for the majority of species (IUCN 2004). Examples of habitat loss are drained wetlands, removal of native bushland for building, development, cropping or grazing, and the creation (or upgrade) of roadways (Hilty et al. 2006; Jaeger and Fahrig 2004). When this occurs the species that reside in the
Habitat may not be able to sustain viable populations due to lack of food, shelter, changed of water quality, and increased predation risk (Amo et al. 2007).

Habitat loss usually results in habitat fragmentation, which is another contributor to biodiversity loss. Habitat fragmentation is the physical division of one habitat into smaller, remnant habitats (Forrester and Machlis 1996; Hilty et al. 2006). This is usually caused by the removal of vegetation and other habitat for the placement of roadways, grazing and agricultural lands, harvesting of forests and/or urban development (Forrester and Machlis 1996; Hazell 2003; Hilty et al. 2006). Fragments of habitats generally contain lower species richness, smaller population sizes, and an increase in introduced species, and associated lower diversity of native species (Hilty et al. 2006; Kjoss and Litvaitis 2001). For example, Burgin and Wotherspoon (2009) found that in a small area (< 2.5 ha) of highly fragmented natural vegetation within an urban area there was only 21% of the predicted diversity of fauna.

1.1.2 Roadways

One factor that influences the loss of species in fragmented native habitats is roadways. Roadways may affect animal populations in four ways: they decrease habitat quality and quantity, increase mortality due to vehicle collisions, prevent access to resources beyond the road barrier, and isolate populations which, in turn, ultimately reduces genetic diversity (Aresco 2005a; Boarman and Sazaki 2006).

Whilst not comparable with the scale of modern roadways, historical data provides evidence that roadways have always impacted on animal species. For example in an intensive study conducted over three years (1941-1944) on a Nebraskan highway there were more than 100 species and, a total of 6723 animals killed (an average of 0.06 animals killed per kilometre) (McClure 1951). In 1980, there were 200,000 deer killed on US roadways (Danielson and Hubbard 1998). However, annual death tolls on US roads increased significantly by the 1990s (Conover et al. 1995; Romin and Bissonette 1996). In 1997, an estimated 1.5 million deer-vehicle collisions occurred annually (Mastro et al. 2008). In a single month in 2002 across five states in America it was recorded that vehicle collisions were the actual cause of death for 15,000 reptiles, 48,000 mammals and 77,000 birds (Havlick 2004). Although not
quantifiable, some of these data indicates that there has been an upward trend in the death due to vehicle collision, at least in the US. One reason for this change is that mortality is typically greater at higher traffic volumes and speeds (Haxton 2000; Klocher et al. 2006).

Barriers and fencing are often used on roadways as a mitigation measure to reduce vehicle collision with animals (Aresco 2005b; Jaeger and Fahrig 2004; Steen et al. 2006). However, these barriers create a physical barrier that restricts movement between habitats, and between populations. For example, Burgin and Brainwood (2008) found that mortality was higher on roadways with physical barriers on one side of the road than when there were no barriers.

Roadways may also act as a ‘predator haven’, particularly when there is a lack of vegetation and cover on road verges which enhances the predators’ ability to observe, track and capture prey more readily than in adjacent wooded areas (Lalo 1987). This is reflected in the observation that many predatory species including birds (e.g., *Gymnorhina tibicen* magpies, *Podargus strigoides* owls, *Procyon lotor* raccoons, feral and domestic animals (e.g., *Canis familiaris* dogs, *Felis catus* cats, *Vulpes vulpes* foxes (Burgin and Brainwood 2008; Lalo 1987) are road kill statistics. It has also been reported that, in Australia, road kill has a greater impact on native species than to introduced species (Burgin and Brainwood 2008; Doody et al. 2003).

1.1.3 Introduced Species

Worldwide, humans are also increasingly assisting the spread of introduced species. These exotic animals may threaten ecosystem biodiversity via reduction in population due to competition for resources, predation, introduced diseases, and modification of the ecosystem (Edgar et al. 2005; Soule and Orians 2001; Valentine 2006). As a consequence, many habitats are now dominated by competitive non-endemic species because of their ability to out-compete native species (O’Keefe 2005; Perez-Santigosa et al. 2008). Introduced species, particularly those that are translocated, may result in biodiversity loss in native populations, for example, through the hybridisation of the turtle *Emydura macquarii* in the Bellinger River drainage (Georges et al. 2011).
1.1.4 Degradation of Waterways

The degradation of waterways, caused by human impacts, contributes to the loss of terrestrial and aquatic biodiversity. Worldwide, freshwater resources are widely exploited for human activities (e.g., household consumption, irrigation, energy, waste disposal). With the increasing pressure on these aquatic systems humans have greatly altered river systems. For example, by 1997, only 2% of rivers’ flow unimpeded in the US (Vitousek et al. 1997). However, while rivers have been the focus of freshwater research, there has been limited data accumulated on the role of lentic waters in the landscape. For example, there is limited data on farm dams; (cf. ponds) and naturally derived freshwater impoundments, and their role in ecosystem function. This is despite their high density in many areas of the world, occuring in almost every environment including mountains, urban areas, rural areas, agricultural land, and grasslands. Even when their primary purpose for existence is for agricultural or aesthetic reasons (Greenway 2005) they provide an ecological resource for many organisms. Biota that use these lentic systems as an essential component of their lifecycle include macroinvertebrate species, amphibians, aquatic plants, fish, turtles, and many bird species (Roe and Georges 2007; Wood et al. 2003).

Lentic water systems are often considered habitat patches and may provide examples of how fragmentation and habitat loss affect the populations that depend upon them (Boothby 1997). For example, the effect of geographic distance between water bodies affects dispersal and colonisation of species, and essentially indicates how these aquatic organisms cope with fragmentation that is similar to habitat fragmentation in terrestrial organisms. As with fragmented terrestrial habitats, impacts on these discrete bodies of water are caused by surrounding land use. The management practices surrounding wetlands affect the water quality, habitat loss, increased habitat fragmentation and habitat changes (Wood et al. 2003), which all may impact on the loss of biodiversity of fauna.

1.2 Human impacts causing the loss of biodiversity of reptiles

Although internationally mammals and birds have been widely studied (Forrester and Machlhis 1996), in comparison, reptiles have been neglected in scientific literature (Fitzgerald et al. 2005; IUCN 2007; Moore and Seigel 2006). Close to half (45.0%) of the published studies associated with reptiles have been based on North American
fauna (Gardner et al. 2007), despite a significantly greater diversity of reptile and amphibian species throughout the rest of the world (Young et al. 2004). In Australia, the diversity of reptiles is particularly high and relatively unstudied (Kanowski et al. 2006). Despite this relative lack of scientific research, the IUCN 2007 Red List of Threatened Species revealed that reptiles (30%) and amphibians (31%) have the highest number of threatened species listed of all animal groups (IUCN 2007). Scott et al. (2010) suggested that to conserve threatened species it is desirable to determine how human impacts affect reptile populations.

1.2.1 Impact of habitat loss and habitat fragmentation on reptiles

Many researchers have regarded habitat change due to human impacts as the foremost cause of reduction of biodiversity in reptile and amphibian species (Collins and Storfer 2003; Cushman 2006; Gibbons et al. 2000; Hazell 2003; Hitchen et al. 2011b; Sadlier and Pressey 1994).

Even with recreational activities there are impacts on reptile populations. A longitudinal study of over 20 years in Connecticut US found that two populations of wood turtle (*Clemmys insculpta*) maintained stable populations at 0.94 turtles/10 ha. When recreation (e.g., hiking, fishing) was introduced to the protected area the turtle populations showed a steep decline in density over two years, and no turtles were found in the final year of the study despite maintaining the sampling effort (Garber and Burger 1995). In a recreational area (Pascagoula River US), the yellow-botched map turtle *Graptemys flavimaculata* abandoned nesting sites when boats and humans approached them. For example, of 79 nesting attempts, 15 females successfully completed oviposition without interruption in the presence of humans. It was also shown that females that were interrupted during nesting had a smaller clutch size, and this had a significant impact on the viability of the population due to the smaller numbers of hatchlings (Moore and Seigel 2006).

In addition to indirect human interference (e.g., recreationalist), habitat fragmentation and direct human interference have additional effects. For example, Kjoss and Litvaitis (2001) observed much lower population densities and species abundance in smaller, rather than large, remnant habitat. The abundance was lower and population
structure changed in *Gnypetoscincus queenslandiae* the prickly forest skink (Northern Queensland, Australia) with habitat fragmentation. There was a significantly lower skink abundance, and a smaller proportion of adults, in ‘islands’ of remnant vegetation compared to larger, continuous habitat areas (Sumner et al. 1999). Hitchen et al., (2011b) observed that there was a dearth of large animals in their urban population of *Amphibolurus muricatus* Jacky dragons. They proposed that migration within the adjacent suburban landscape was acting as a sink for emigrants without a commensurate source of immigrants to offset the loss.

1.2.2 *Impact of roadways on reptiles*

In the US and Canada roadways have been shown to have an adverse effects on turtle populations (Boarman and Sazaki 2006; Gibbs and Shriver 2002; Steen et al. 2006; Steen and Gibbs 2004), together with lava lizards (*Microlophus albemarlensis*) (Tanner and Perry 2007), and black ratsnakes (*Elaphe obsoleta*) (Row et al. 2007). The studies indicated that roads increased mortality in the populations of these reptiles with reduced future viability of the populations because of lowered reproductive output.

Studies on American turtle species (e.g., *Chelydra serpentina* snapping turtle, *Pseudemys floridana* Florida cooter, *Sternotherus odoratus* common musk turtle) have shown that females are more susceptible to vehicle collision than males. This occurs because females of most aquatic turtle species nest in the terrestrial environment, and males undertake overland travel relatively less frequently. The outcome in some populations has been that the gender bias in roadkill contributes to a male biased population (Aresco 2005a; Steen et al. 2006; Steen and Gibbs 2004). Roadkill during migration to the nest may, therefore, potentially have a detrimental effect on populations because of lowered reproductive success. As a result there is an increased pressure on the future viability of the species.

1.2.3 *Impact of poor water quality on reptiles*

Unlike gill breathing fish, turtles tend to better withstand poor water quality provided dietary requirements are met, especially; as many turtle species are opportunistic feeders (Georges 1982; Kennett and Georges 1990; Souza and Abe 2000). The ability
of freshwater turtles to withstand pollution in urban waterways has been well demonstrated (Burgin 2006; Burgin and Ryan 2008; Knight and Gibbons 1968; Lindeman 1996; Souza and Abe 2000). However, Cann (1993) noted that despite the widespread degradation of Australia’s water bodies, the study of the effect that reduced water quality has on Australian turtles species has been limited. As an example of the potential impact, he reported over one year 620 dead turtles were collected from the edge of Lake Mokoan (Victoria, Australia), an impoundment with a 80,000 ha surface area. These turtles had died of starvation caused by high turbidity that killed the plant life in the lake, and subsequently reduced the availability of food resources for the resident turtles. Kennett and Georges (1990) also observed food scarcity affected turtles. They observed that the long-necked turtle *Chelodina longicollis* that were resident of lakes with low productivity and, therefore limited food sources, had a lower growth rate compared with animals in more highly productive environments. They also observed that reproduction decreased with lower productivity. It may be assumed that these changes were indirectly due to water quality, which, in turn, affected food availability. This has also been noted for other turtle species worldwide (Browne et al. 2006).

In contrast to the impact of low productivity, when food resources are not limiting, and/or in the presence of optimum water temperature, acceleration of growth rate, and larger body size have been observed in the freshwater turtle *Chrysemys picta* (cf. *Trachemys scripta*). In *C. scripta* this faster development was attributed to increased water temperature that extended the growth period within a year, and associated increased rate and time for digestion (Parmenter 1980). However, this could only have occurred in the presence of an abundance of prey.

At least until the mid-1990s, there have been limited published studies on the effect that recycled water has on turtle populations. Lindeman (1996) conducted a study of two populations of *Chrysemys picta*. One of these populations was resident of a natural lake and the other resided in a wastewater lagoon. A number of differences in reproductive characteristics were noted. For example, females in the wastewater lagoon were observed to double clutch annually, whereas this did not occur in the population from the natural lake. The increased nesting associated with the wastewater lagoon population was reflected in a population that was skewed towards
smaller individuals indicating higher levels of recruitment than in the natural lake. Turtles also experienced faster growth rates in the wastewater lagoon and, therefore, reached sexual maturity quicker than the population in the natural lake (males in wastewater lagoon matured at approximately 2 years, females 6-7; males in the natural lake reached sexual maturity at 3 years, females 8-10).

1.3 Lack of studies on Australian reptiles
Australia has a large diversity of reptiles with more than 1,000 described species. They play an important role in almost every ecosystem type from deserts to rainforests (Cogger 2014). However, as observed above, there have been few studies on the impact of humans on the continent’s reptiles.

Freshwater turtles are one group of reptiles considered to be at particular risk of decline due to human impacts. This is because they are affected by changes to both the aquatic and terrestrial ecosystems, they have delayed sexual maturity and low reproductive output compared to many other reptiles (Congdon and Gibbons 1996). In Australia the diversity of freshwater turtles is considered to be high compared with other areas of the world with 7 genera and 21 species (Cogger 2014).

Turtles occur naturally in all jurisdictions of Australia except Tasmania, and they occupy a wide range of aquatic habitats, both lentic and lotic, in rural, peri-urban, suburban and urban areas. They also contribute one of the largest components of the biomass in aquatic ecosystems (Cann 1998). The long-necked turtle *Chelodina longicollis* is the most widespread of all Australian freshwater turtles and inhabits wetlands and waterways on the heavily populated East Coast. For these reasons they are an excellent species to research as a case study into the impact that humans have on a native freshwater turtle taxon.

1.4 Outline of thesis
Whilst there is a common perception that *C. longicollis* are common, there have been no studies that have investigated the impacts on the future viability of the species. Because of their broad range, and their ability to survive within a wide range of aquatic habitats, urban and rural, they are an ideal species to use as a case study for
how human impacts affect biodiversity in both terrestrial and aquatic ecosystems. In rapidly urbanising Western Sydney, the increased use of recycled water and degradation of water bodies, there are potentially increasing pressures on population health, and the long-term viability of these peri-urban populations. *Chelodina longicollis* represents the largest biomass of vertebrates in the impoundments of Western Sydney. As such their demise is likely to have a major impact on the functioning of these systems. This species has, therefore, been used as a model to investigate the impacts of urbanisation on their long-term viability in urban and urbanising areas.

The viability of freshwater turtles in the region where the studies for this thesis are focused have already had significant habitat change, and due to current government policies (e.g., Draft Metropolitan Strategy for Sydney to 2031 (NSW Department of Premier and Cabinet 2012)) will continue to suffer change associated with increasing extent and density of urbanisation.

This thesis has been written as a series of papers, which can be rapidly transferred into journal articles for publication, and as such there is some repetition among some chapters, particularly, in the sections that outline the study species, study site and methodology.

In the Introduction (Chapter 1) the study is placed in the broader perspective of reptile research. Chapter 2 introduces the study species, the long-necked turtle *Chelodina longicollis*. An overview of the study sites is presented in Chapter 3. In Chapter 4, the response of *C. longicollis* to recycled water (stormwater and tertiary treated sewage effluent) is investigated. In Chapter 5, a population of *C. longicollis* in peri-urban Sydney, first sampled in 1994-1995 in a capture-mark-recapture study was resampled to determine the status of this population a decade on. The effects of vehicle collision on the local population of *C. longicollis* surrounding the peri-urban site were investigated in chapter 6, in the context of the long-term viability of the population. In Chapter 7, a comparison of freshwater turtle populations is undertaken across urban, suburban, peri-urban Sydney to compare these populations with different density of human population and the associated pressures placed on these populations due to urbanisation. In the final discussion (Chapter 8) information will be drawn from the
different sub-studies to discuss the human impacts on the freshwater turtles of Sydney. The chapter concludes with a consideration of the future viability of freshwater turtle populations and proposed management priorities for urban populations.
Chapter 2 – The Study Species *Chelodina longicollis*

*Chelodina longicollis* captured in the peri-urban study site in peri-urban Western Sydney (New South Wales, Australia).
2.1 Species description

2.1.1 Distribution

*Chelodina longicollis* (long-necked turtle) is the most widespread of all Australian freshwater turtles. They have an extensive range across Eastern Australia from Adelaide in the south west, throughout Victoria, the Murray-Darling Basin, New South Wales and to above the Tropic of Capricorn (Rockhampton, Queensland; reaching as far north as Charters Towers, and the inland limits approach of Windora (Queensland) and Broken Hill (New South Wales) (Figure 2.1). Their range overlaps much of the most densely human populated areas of Australia and includes the capital cities of Brisbane, Melbourne and Sydney (Cann 1998; Kennett et al. 2009).

![Figure 2.1 – The location of distribution of *C. longicollis* (New South Wales Curriculum and Learning Innovation Centre 2012).](image)

*Chelodina longicollis* occupy a wide range of habitats from shallow ephemeral water bodies and farm dams (Chessman 1988a; Wong and Burgin 1997) to deep flowing
rivers (Chessman 1988a; Kennett and Georges 1990). Their estimated population density has been found to vary between 130 and 400 turtles/ha (Table 2.1).

Table 2.1 – Published estimated population density of *Chelodina longicollis* in natural and modified wetlands (NSW = New South Wales).

<table>
<thead>
<tr>
<th>Region</th>
<th>Population Density (turtles per ha)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>New England Tablelands</td>
<td>400</td>
<td>Parmenter (1976)</td>
</tr>
<tr>
<td>NSW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gippsland Victoria</td>
<td>236</td>
<td>Chessman (1978)</td>
</tr>
<tr>
<td>Murray River (NSW/Victoria)</td>
<td>159</td>
<td>Thompson (1993)</td>
</tr>
<tr>
<td>Richmond NSW</td>
<td>130</td>
<td>Dalem (1998)</td>
</tr>
<tr>
<td>North-west Sydney NSW</td>
<td>360</td>
<td>Burgin et al. (1999)</td>
</tr>
</tbody>
</table>

2.1.2 Growth and longevity

Whilst in their first year hatchlings may grow rapidly, as with all freshwater turtles, *C. longicollis* growth usually slows after the first year. Although they have indeterminate growth, typically the growth rate averages 6.2 mm/year (under optimum conditions in inland NSW) (Kennett and Georges 1990). Registered growth may, however, be as low as 0.89 mm annually during suboptimum conditions (Roe and Georges 2008a). Females grow to a slightly larger size than males and reach a carapace length of 28.0 cm (weight 1.8 kg), while males attain 25 cm and 1.2 kg (Chessman 1978; Thompson 1993).

Lifespan is effectively unknown. There is one recording of an individual in captivity reported to have lived for approximately 36 years (Goode 1967) and in wild *C. longicollis* it has not been determined. However, as they are slow growing and sexual maturity may take a decade to achieve, and their lifespan may be more than 100 years (Parmenter 1976; Thompson 1993).

2.1.3 Diet

*Chelodina longicollis* can only capture its prey within the aquatic environment. This is because they can only move their jaw upwards and, therefore, are unable to lower
their head to capture terrestrial prey (Chessman 1984b). In water they use a strike and gape action in order to catch fast prey (Kennett and Georges 1990). The species is carnivorous and seldom incorporate vegetation, and then it is considered accidental. For example, Georges et al. (1986) found that few individuals (2.4%, n = 209) had dead plant material among their stomach contents and then it only constituted a small proportion of the stomach contents (< 5%). Their diet consists of aquatic invertebrates, such as odonatan, plecopteran and dipteran larvae, worms (such as bloodworms), aquatic beetles (Hemiptera), frog eggs and tadpoles, small fish, and fish carrion (Beck 1991; Chessman 1984b; 1988a).

2.1.4 Nesting and reproduction

The onset of sexual maturity is dependent on size, not age. In general, females reach sexual maturity at a carapace length of 165 mm and males at a carapace length of 145 mm (Kennett and Georges 1990). Mating usually occurs in September, and eggs are laid in late spring and early summer (Kennett and Georges 1990; Kennett et al. 2009). Females usually lay their eggs nocturnally, and have been observed to lay during inclement weather (Parmenter 1976; Vestjens 1969). Nests have been recorded up to 500 m from the water’s edge (Burgin 2006; Cann 1998; Kennett et al. 2009; Thompson 1983). Parmenter (1976) suggested there are two factors *C. longicollis* use to select a nesting site: a steep slope to allow for adequate drainage so the eggs do not become water logged, and an area relatively bare of grass and vegetation.

Female *C. longicollis* usually only have one clutch annually (Parmenter 1976; Thompson 1993; Vestjens 1969), although some individuals have been recorded to double clutch (Kennett et al. 2009). Clutches of between 6 and 23 eggs have been identified. Such variation may be due to differences in female body size. In addition to reptile size, the productivity of the wetlands in which the individual resides may also impact on fertility (Burgin 2006; Kennett et al. 2009). Eggs incubate in the nest for 120 - 150 days (Parmenter 1976), and hatchlings usually emerge between March and May (Vestjens 1969; Parmenter 1976; Cann 1998). Turtles that hatch late in the season may ‘overwinter’ in the nest to emerge in October, when the weather is warmer (Beck 1991; Burgin and Dalem 1996).
2.1.5 Predation

In the lifecycle of *C. longicollis*, juveniles and eggs are the most susceptible to predation. For example, it was estimated that in the Murray River Basin up to 96% of *C. longicollis* eggs are taken by predators (Thompson 1983). While predators differ depending on location within the species’ range, the major predators of Australian freshwater turtles eggs are *Vulpes vulpes* foxes, *Corvus coronoides* ravens, *Hydromys chrysogaster* water rats, *Varanus varius* and *Varanus gouldii* goannas and *Felis catus* domestic cats and *Canis familiaris* dogs (Beck 1991; Burgin 2006; Parmenter 1976; Thompson 1983).

The ability of adult turtles to completely draw their limbs and head under their hard shell provides some protection, and so adult *C. longicollis* have few predators. They are also thought to deter predators with secretions from their well-developed musk glands. When the turtle perceives danger it may secrete from these glands. However, the function of the secretion is unknown (Cogger 2014) although its effect on predators has been investigated and found not to deter predators, such as *Canis lupus* dingos and ravens (Kool 1981).

In contrast, to the relative lack of predators of larger individuals, small juveniles have a soft shell and are ready prey for ducks, snakes, and fishes that reside in the water bodies. Typically most of these predators swallow small juveniles whole although some species of birds may break open the turtle shell (Parmenter 1976).

Unique to *C. longicollis*, individuals of the species aquatically bask at the surface of the wetland with legs and head extended. They have seldom been observed to bask out of water on logs, earth banks, and rocks that project above the water. Not being reliant on hauling out of the water to bask, as is the situation with all other freshwater species (Beck 1991), would diminish the threat from terrestrial predators compared to other species of freshwater turtles.

2.1.6 Terrestrial movements
Chelodina longicollis must leave the water to nest but they also frequently undertake overland migration between water bodies (Kennett and Georges 1990; Stott 1987). Migration is usually instigated by environmental stress, for example, drought, pollution, and a reduction in the amount of available prey (Chessman 1984a; Kennett and Georges 1990; Wong and Burgin 1997). It has also been observed that C. longicollis may use multiple wetlands within a year, due to different wetlands offering different resources (Roe and Georges 2007).

Because C. longicollis have a much lower rate of evaporative water loss than any other Australian freshwater turtle species, individuals are not as prone to desiccation as individuals of any other species of equivalent dimensions (Chessman 1984a; 1988a). Chessman (1984a) suggested that these turtles had the ability to reabsorb water from the bursae and thus delay dehydration when out of water, thus reducing the incidence of desiccation in this species compared to other freshwater turtles. This allows them to travel over relatively long distances compared to individuals of other freshwater turtle species and thus they may inhabit water bodies remote from the main river channel where competition from fish and other turtle species may be minimal (Chessman 1988a). They have been recorded to travel up to 556.0 m in a day (Stott 1987). However, there is no correlation between the distance travelled and the number of movements made between males and females (Beck 1991; Chessman 1978; Roe and Georges 2007).

Terrestrial movement in C. longicollis is non-random. This is evidenced by their movement in effectively a straight line between wetlands, and they often return to their place of origin. They have been found to maintain a home range over 1 – 4 years (Parmenter 1976; Roe and Georges 2007; Stott 1987). However in peri-urban Sydney only 11.9% (n = 572) of the animals captured in 1995 and 1996 were recaptured in the same wetland at least a decade on (Ryan and Burgin 2007).

2.1.7.a Aestivation

Aestivation is defined by Beck (1991) as a state of reduced activity that allows the animal to conserve body reserves, food and water. Aestivation usually occurs when the temperature of the water is above preferred temperature, and enables the turtle to
escape from unfavourable conditions, such as a substantial drop in the water level in a wetland (Kennett and Georges 1990). Typically the turtle leaves the water and selects a site in shade, well covered with leaf litter. The individual then buries itself within the leaf litter and/or the surface layer of soil (Beck 1991).

Chessman (1983) monitored terrestrial movements of one *C. longicollis*. He found that the turtle aestivated twice, once for approximately 40 days, and then travelled for a few weeks before it returned to approximately the same spot, and estivated for a month. Roe and Georges (2008a) found that while a large proportion of turtles die during extended terrestrial estivation, at least one turtle in their study survived up to 480 consecutive days out of water. This ability to aestivate allows the turtle to survive in remote, and drying water bodies (Chessman 1988a) when other turtle species are likely to perish.

2.1.7.b Hibernation

*Chelodina longicollis* cease activity for approximately three months of the year, at the beginning of June (Parmenter 1976). Hibernation begins when the water temperature drops to approximately 7.5°C, and hibernation ends when the water temperature begins to rise again, usually in late August (Beck 1991). *Chelodina longicollis* hibernate in the water, and there is no record of hibernation on land (Cann 1998). Green (1994) stated that during the three years of research, no turtles returned to the same site of hibernation as the previous year.

2.2 Health of the population

The health and the long-term viability of any turtle population may be dependent on the population structure. As turtles are long-lived, slow growing animals that are able to reproduce over the majority of their life (Haxton 2000), the structure of the population provides an indication of the future health and viability of the population. Indicators of health of the population include population size, physical deformities, skewed sex ratios, numbers of juveniles, and clutch size (Brown and Hecnar 2007).

The numbers of juveniles in a population are an indication of whether the population is increasing or decreasing. For example, a high proportion of juveniles may indicate
that the population is increasing, whereas if there are a low proportion of juveniles in
the population, it may indicate that the population is decreasing.

Another indicator of future viability and health of a population in freshwater turtles is
sex ratio. It can be expected that most *C. longicollis* populations approximate a 1:1
sex ratio at sexual differentiation of the embryo; however, a number of studies have
observed that this is not the case. There have been incidences of highly skewed sex
ratio (Burgin 2006; Chessman 1978; Parmenter 1976; Ryan and Burgin 2007; Wong
and Burgin 1997). This may indicate that there are environmental factors that affect
the sexes differently. Sampling bias has often been proposed as a reason for such bias
(Gibbons 1970; Kennett and Georges 1990). Other potential issues for skewness in
sex ratio include increased mortality of females due to propensity to undertake
overland migration to nest (Steen et al. 2006), greater mortality among males due to
overland migration to mate (Gibbons and Lovich 1990), or males may be more
effected by severe food limitations than females (Kennett and Georges 1990).

### 2.3 Implications for the future

*Chelodina longicollis* are present in urban, suburban, peri-urban and rural areas within
their range and they may be affected by deteriorating water quality within urban
areas. The use of recycled water, heavy metals, unusual levels of dissolved oxygen,
pH, salinity, nitrates and ammonia may potentially impact on populations of aquatic
species. The propensity for terrestrial movement for both males and females within *C.
longicollis* makes them more susceptible to vehicle collision than other species of
Australian freshwater turtles. Whilst it has been suggested that thousands of *C.
longicollis* are killed annually on the roads (Parmenter 1976) there are no published
data on the affect of road kills on any population of *C. longicollis*. An increase in
human interactions with the turtles, such as supplementary feeding, the release of pet
turtles, and increases in predation rates due to domestic animals can also place
pressure on turtle populations that are associated with human habitation. There have
been studies that have investigated urban (Burgin 2006; Burgin and Ryan 2008;
Robey et al. 2011), suburban (Rees et al. 2009; Roe et al. 2011) and peri-urban areas
(Burgin et al. 1999; Ryan and Burgin 2007). However, comparison of populations of
Australian freshwater turtles among such sites has not been undertaken.
Chapter 3 – Overview of study sites used to sample *Chelodina longicollis* in the Sydney region

A wetland in the peri-urban site sampled during the study, (near Richmond, New South Wales, Australia).
The study was undertaken in the Sydney Basin, New South Wales, Australia (33.8600° S, 151.2111° E) (Figure 3.1).

![Map of Sydney Basin and Sample Sites](image_url)

Figure 3.1 - The Sydney Basin and the location of the three sample sites for freshwater turtles, Centennial Park (Central Business District, ‘urban’ site), Eagle Vale (suburban site) and near Richmond (peri-urban site) and the Sydney Central Business District (Source: maps.google.com).

The climate of the area is temperate but the winter temperature does vary across the Region. For example, in 2013, in the Central Business District, the maximum temperature recorded was 44.2°C and minimum was 3.7°C, while in outer Sydney; for example; in Richmond, the maximum temperature recorded was 44.9°C and the minimum was -4.8°C (Bureau of Meteorology 2013).

The urban site has a dense human population. For example, Randwick, an adjacent suburb to the urban study site, had a human population density of 39.16 persons per hectare in 2013. The suburban area (Eagle Vale) had a population density of approximately 4.95 persons per hectare, while Richmond, adjacent to the in peri-urban study site in 2013 had a population density of approximately 1.17 persons per
hectare (Australian Bureau of Statistics 2013). With this difference in population density, there is also a difference in density of infrastructure such as roadways and traffic, with greater densities in the urban areas compared to the other two sites.

In this thesis, the data for chapters 4, 5 and 6 were collected in the peri-urban area most closely associated with Richmond (Figure 3.2) although the data for chapter 6 were collected over a greater area (Figure 3.3). The data for chapter 7 also included the peri-urban areas adjacent to Richmond, but also included the suburban site (Eagle Vale, Figure 3.4) and the urban site (Centennial Park, Figure 3.5).

Figure 3.2 – A typical wetland and surrounding area in the peri-urban site, (near Richmond, New South Wales, Australia).
Figure 3.3 – A wetland and surrounding area in peri-urban Sydney (near Richmond, New South Wales, Australia).

Figure 3.4 – The suburban wetland sampled (Eagle Vale, New South Wales, Australia).
Figure 3.5 – A wetland at the urban site, Centennial Park, New South Wales, Australia (Centennial Park and Moore Park Trust 2011).
Chapter 4 – Investigation of the effects of recycled water on a population of the native freshwater turtle *Chelodina longicollis* (long-necked turtle).

A juvenile *Chelodina longicollis* netted in peri-urban Sydney (New South Wales, Australia).
4.1 – Introduction

Climate change and a rapid increase in human population growth has increased demand for reliable freshwater worldwide. The use of recycled sewage and harvested stormwater to ensure a reliable source of reusable freshwater has been a high priority for politicians, environmental managers, and regulators (Dimitriadis 2005). In Australia, the driest inhabited continent, there is an increasing demand for alternative sources of water (Willis et al. 2011), and the use of recycled water is gaining in popularity (Toze 2006). Some reuse systems collect untreated stormwater from surrounding urban, suburban, and industrial areas and/or tertiary treated sewage effluent, from sewage treatment plants. Waters from such reuse systems have been used to irrigate, for example, pastures, golf courses, and sporting fields (Booth et al. 2003; Coutts 2006; Greenway 2005) in place of potable water (Derry et al. 2003). Frequently, before use for irrigation, these waters (e.g., tertiary treated sewage effluent, harvested storm effluent) are stored in holding dams that on the East Coast of Australia, at least, typically host resident populations of freshwater turtles (Ryan and Burgin 2007).

These holding dams are different to polishing wetlands used in the treatment of effluent, which are shallow reed beds with no open water (Greenway 2005). In contrast, holding dams used to store treated effluent, as with farm dams, are usually purpose built dams (Ávila et al. 2013). These impoundments are usually characterized by having a large surface area, and limited riparian and emergent aquatic vegetation, a management ‘tool’ to maximize water storage. In addition, to provide habitat for freshwater turtles, these constructed wetlands also provide habitat for a diversity of aquatic species, both macroinvertebrate and vertebrate (Brainwood and Burgin 2009; Greenway 2005; Norris and Burgin 2011b; Rodusky et al. 2008).

There have been numerous studies on the effect of effluent discharge on macroinvertebrate communities in flowing water systems (Alvarez-Cabria et al. 2011; Spanhoff et al. 2007) but fewer in impoundments (Rodusky et al. 2008). These studies have demonstrated that the diversity and abundance of macroinvertebrate assemblages can be affected both positively (Alvarez-Cabria et al. 2011; Brainwood and Burgin 2009; Rodusky et al. 2008) and negatively (Kalcounis-Ruppell et al. 2007; Rodusky
et al. 2008; Spanhoff et al. 2007) by the water quality changes due to effluent discharge.

Studies on recycled sewage and harvested storm effluent on vertebrate assemblages have focused on endocrine disruption in, for example, fishes including *Gambusia holbrooki*, (Edwards et al. 2013; Norris and Burgin 2011a); *Cyprinus carpio* (Lavado et al. 2004; Petrovic et al. 2002), wetland birds including gulls and terns (Tyler et al. 1998; Wainwright et al. 2001), and reptiles including alligators and freshwater turtles (Crain and Guillette 1998; Crews et al. 1995; Sumpter and Johnson 2005).

Freshwater turtles may contribute a large proportion of the biomass to aquatic ecosystems (Thompson 1993), and endocrine disruption was shown to affect *Trachemys scripta elegans*, the red eared slider turtle. In the presence of endocrine disruptors in the effluent where *T. s. elegans* were present the turtle population had a biased sex ratio with more female than male hatchlings. There were also higher levels of abnormalities in embryos than when endocrine disruptors were not detectable in the waters (Bergeron et al. 1994; Crain and Guillette 1998; Lindeman 1996).

Studies that have investigated the impact of water quality on freshwater turtles, have typically focused on endocrine disruption (Bergeron et al. 1994; Crews et al. 1995; Clairardin et al. 2012), rather than the influence of other water quality parameters, wetland characteristics, and/or how these may impact on resident turtle populations. This study investigates the impacts on a resident species of native turtle, *Chelodina longicollis*, of water from three different origins - precipitation and ground water flow; excessive irrigation of agricultural lands, tertiary treated sewage effluent, and stormwater. The study investigates the influence of water quality parameters on population attributes of the population of *C. longicollis*.

4.1.1 - Study Species

*Chelodina longicollis* is the most widespread Australian freshwater turtle. They inhabit a diversity of water bodies throughout Eastern Australia including rivers, lakes, ephemeral wetlands, farm dams (ponds), and even degraded urban and peri-urban water bodies (Burgin 2006; Cann 1998; Chessman 1988a; Rees et al. 2009).
Estimates of their density in impoundments range from 236 ha\(^{-1}\) (Chessman 1978) to 400 ha\(^{-1}\) (Parmenter 1976).

Hatchlings may be as small as 2.1 cm (Burgin and Dalem 1996), and adults may grow to 28 cm. Hatchlings tend to grow rapidly during their first year; however, after this their growth rate typically slows, and is influenced by productivity in the water body in which they reside (Kennett and Georges 1990). Although age in the wild has not been reported, *C. longicollis* has been estimated to have a lifespan of 100 years (Parmenter 1976; Thompson 1993), and in the study area an individual was determined to be in excess of 60 years (Chapter 5). Their diet primarily consists of aquatic macro-invertebrates and carrion (Chessman 1984b). At all stages of their life these turtles are susceptible to predation, although eggs in the nest, hatchlings and juvenile turtles are the most susceptible (Parmenter 1976). Their known predators include *Vulpes vulpes* foxes, *Hydromys chrysogaster* water rats, a variety of species of goannas, domestic cats and dogs, and a variety of water birds (Beck 1991; Thompson 1983). It has also been suggested that large fish species, such as *Cyprinus carpio* carp and *Anguilla reinhardtii* and *Anguilla australis* eels may predate on small turtles (Parmenter 1976). For a fuller description see Chapter 2.

4.1.2 - Study Site

This study was undertaken in peri-urban Western Sydney (Australia) on the Hawkesbury campus of University of Western Sydney. The nearest township was Richmond, approximately 50 km northwest of the Central Business District of Sydney. The area has a temperate climate and during the sampling period for this study (2007-2008), the mean monthly maximum temperature varied between 14.8ºC (June 2007) and 31.6ºC (January 2008). The annual precipitation varied between 1051.2 mm (2007) and 800 mm (2008). Mean monthly precipitation during the period varied between 2.8 mm (May 2008) and 255.6 mm (February 2008) (Bureau of Meteorology 2011).

The peri-urban site had been used for agricultural teaching purposes for more than 120 years. Over this period, dams were constructed to water grazing stock (Burgin 2010). Since the 1960s, tertiary treated sewage effluent from Richmond (population approximately 6000 (Bureau of Meteorology 2013)) has been collected in purpose-
built wetlands to ‘polish’ the water. More recently the scheme has been upgraded and expanded, and it now includes wetlands constructed specifically to harvest stormwater collected from the local township. These effluent systems have a design working volume of 188.5 ML of tertiary treated sewage effluent, and 267.3 ML of urban stormwater runoff (Derry et al. 2005).

This recycled water supplements the non-potable water requirements of the campus, particularly for farm irrigation, and watering gardens and playing fields, and also contributes to environmental flows into a nearby tributary of the Hawkesbury–Nepean River. Other farm dams within the area sampled receive no supplementation of recycled water and their surrounding paddocks are not irrigated, and samples of these were used as control wetlands in this study.

4.2 – Methods

4.2.1 - Data collection
4.2.1.1 - Wetland selection
There are 34 small impoundments on the University of Western Sydney Hawkesbury campus. All of these wetlands receive water from precipitation, overland flow, and groundwater seepage, and these are the only sources of water for farm dams. Wetlands that received no additional supplementation are referred to as ‘farm dams’. A second category received supplementary water from tertiary treated sewage effluent (referred to as ‘treated sewage effluent’). The third set of wetlands sampled received supplementary water from stormwater effluent (referred to as ‘stormwater effluent’). For this Chapter an additional set of wetlands were sampled which received, in addition to the natural sources of water outlined above, runoff from irrigation of adjacent fields with recycled water and are referred to as ‘irrigation wetlands’. Three of each type of dam were randomly chosen from within each wetland category (Figure 4.1).
Figure 4.1 – Examples of wetlands sampled in peri-urban Western Sydney (Australia): a) irrigation, b) sewage, c) stormwater and d) farm dam.
4.2.1.2 - Turtle sampling
Turtles were collected between October 2007 and May 2008, the period of the year when *C. longicollis* are most active (Chessman 1988b). Each dam was sampled over four consecutive days each month. Four dams (one of each dam type) were chosen at random and sampled over a four-day period, after a two day break four new dams were sampled for four consecutive days.

Two fyke nets were used to sample each dam (Burgin et al. 1999). Fyke nets were set in the late afternoon on the first day of sampling in a specific wetland and subsequently cleared in the early morning for each of the next four days before being removed with sampling on the final morning.

Retrieved turtles were, measured (curved carapace length, ±0.1 mm; *cf.* Burgin and Ryan 2008; Burgin, 2006), weighed (±0.1g) using portable field balances (Sartorius portable scales (A&D EK-1200i)), and uniquely marked with shellfish tags (Ryan and Burgin 2007). Turtles with a carapace length under 14.5 cm were classified as juveniles (Roe and Georges 2008a), and turtles over 14.5 cm were sexed by determining if the plastron was concave (male) or convex (female) (*cf.* Chessman 1978; Kennett and Georges 1990). After processing, turtles were released at the point of capture. The species and number of fish caught incidentally in the nets were also recorded.

4.2.1.3 - Water quality sampling
Wetlands were sampled for water quality between October 2007 and May 2008 using a portable ‘Intelligent Water Quality Analyser’ (Model 611, YEO-KAL Electronics Pty Ltd, Brookvale, Australia). In each of the 12 wetlands, replicate samples (30 in October 2007, 10 otherwise) were taken monthly to assess commonly sampled physiochemical parameters (salinity, pH, temperature, dissolved oxygen, and turbidity). Depth was measured with the ‘Intelligent Water Quality Analyser’, surface area was measured with a field tape, and percentage of emergent vegetation cover was assessed visually.

An intensive investigation of water quality was conducted at all wetlands in September 2008. Although only undertaken once due to budget restrictions, for all
wetlands that receive recycled water (effluent, stormwater), the University is required to adhere to the prescribed water quality standards laid down by the regulator (Sydney Water Cooperation) and report to Sydney Water Corporation on a regular basis. Sampling for this study was undertaken in a sub-set of these reuse wetlands, and sampling was undertaken in parallel with sampling for Sydney Water Corporation. It is, therefore, assumed that water quality for the recycled waters is maintained within the range required by Sydney Water Corporation and thus a one-off survey would be indicative of the water quality of the recycled wetlands. Since the farm dams sampled tend to maintain water levels as a result of groundwater flow, the one off sampling of potentially toxic substances that may be present in the local wetlands was assumed to be reflective of water quality over time.

At each dam between 2 and 4 replicate samples were randomly collected from different near-shore locations. Three to four water samples were collected from wetlands with larger surface area, and two samples collected from those with a smaller surface area. Grab samples were collected in decontaminated sample containers provided by a commercial testing laboratory. Samples were collected, immediately chilled, and delivered to the laboratory for analysis within 24 hours of collection. All grab samples were analysed using standard methods (APHA 1998).

Samples were analysed at a commercial NATA (National Associated of Testing Authorities) accredited laboratory (Australian Laboratory Services, Smithfield) for determination of ionic components. This included total alkalinity (divided into bicarbonate, hydroxide and carbonate components) and other dissolved major anions and cations (calcium, sodium, magnesium, potassium, chloride and sulfate). Sulfate, chloride, bicarbonate, hydroxide and carbonate were determined using titration. Cations were measured using inductively coupled plasma atomic absorption spectrometry (ICP-AES). All major ions were reported at the lower detection limit of 1 mg/l. Samples in separate unused containers, using methods consistent with standard methods (APHA 1998) were also tested at the NATA accredited laboratory (Australian Laboratory Services, Smithfield) for determination of total metals (arsenic, cadmium, chromium, copper, lead, nickel and zinc) and total phosphorus, total organic carbon and total kjeldahl nitrogen.
4.2.2 - Data analysis

The sex ratio of the turtles among wetlands was determined using a Goodness of Fit Chi Square analysis. Initially a one-way analysis of variance was undertaken on the carapace length of turtles to investigate the effect of water source on turtle size.

To investigate differences due to size and sex, monthly turtle catches in each wetland were divided into subgroups based on their size (hatchling < 5 cm, small juvenile 5 cm–9.9 cm, large juvenile 10 cm–14.4 cm, small adult male 14.5–17.9 cm, large adult male >18 cm, small adult female 14.5cm–19.9 cm and large adult female > 20 cm (cf. Roe and Georges 2008b). Using PRIMER these data were grouped according to their water source in a non-metric multi-dimensional scaling (nMDS) plot using Bray-Curtis Dissimilarity measure. Analysis of Similarities (ANOSIM) was subsequently undertaken to investigate differences between the size/sex characteristics of turtles to assess whether they varied according to different water source.

ANOVAs were used to determine if there was a difference between water quality parameters and wetland type. Biota and/or environment matching (BIOENV) (Clarke and Ainsworth 1993) was subsequently conducted to determine which physiochemical factor/s (temperature [ºC], pH, dissolved oxygen [% saturation], conductivity [µs/cm], turbidity [ntu], surface area [ha], average depth [m] and emergent vegetation cover [%]) was correlated with the attributes of the turtle population structure in the different wetland types to determine which variables were most highly correlated with turtle population profile in different water types. Similarity percentages (SIMPER) were used to determine the influence of different demographic attributes of turtles from wetlands due to water source.

To extend the investigation of the relationship between environmental parameters and turtle size structure within wetlands, a regression analysis was used. The same analysis was used to compare juvenile turtle numbers (outliers that were more than two standard deviations from the mean were removed from this analysis) and environmental parameters. A regression analysis was also used to test for the relationship between the number of adult females and number of juveniles. Because
data included repeated measures of turtles, the result was considered significant if p < 0.01.

4.3 – Results

4.3.1- Turtle population
A total of 951 turtles (1,147 captures) were netted during this study (208 juveniles, 315 males, 428 females). Overall there was no significant difference in the numbers of turtles captured due to water source ($F_{3, 8} = 4.07, p = 0.45$; Figure 4.2).

![Graph showing the number of individuals captured from different wetland types](image)

Figure 4.2 - The number of individuals captured from the different wetland types sampled in peri-urban Sydney between October 2007 to May 2008.

There was a significant female bias in sex ratio overall ($\chi^2_{1, 0.0001}=25.862$); however, when looking at differences between the water sources the irrigation wetlands ($\chi^2_{1, 0.001}=15.112$) and farm dams ($\chi^2_{1, 0.0001}=23.439$) had a female sex bias, but both the recycled water wetlands (stormwater, treated sewage effluent) did not have a sex ratio bias (stormwater ($\chi^2_{1, 0.9420}=0.005$) and treated sewage effluent ($\chi^2_{1, 0.0843}=0.005$)) (Figure 4.2).
There was a significant difference in the population structure of turtles among water sources (Global $R = 0.9$, $p = 0.001$; Figure 4.2) for both carapace length ($F_{3, 1043} = 2.61$, $p < 0.001$; Figure 4.3) and condition ($F_{3, 1043} = 2.61$, $p = < 0.001$).

Figure 4.3 – nMDS ordination of *Chelodina longicollis* population structure (size and sex) grouped according to wetland type in peri-urban Sydney sampled between October 2007 and May 2008. Green triangle = Irrigation, blue triangle = farm dams, light blue square = Sewage Treatment Plant, and red diamond = stormwater.
A larger number of smaller turtles were captured from irrigation wetlands and farm dams than from treated sewage and stormwater effluent wetlands. There were larger turtles in the recycled water wetlands (Figure 4.4). A PRIMER pairwise test showed that the water types that had the most significant difference in population structure were the wetlands that received water from irrigation seepage and reclaimed storm water ($R =0.153$, $p < 0.01$).

4.3.2 – Water chemistry

With the exception of four water quality parameters there was significant difference in water quality among wetlands (table 4.1). The four that were not significantly different were the metals, cadmium, chromium, nickel and zinc.
Table 4.1 – Factors associated with *Chelodina longicollis* distribution among wetlands sampled in peri-urban Sydney, ANOVA results (*F* values, probabilities and degrees of freedom) and summary statistics (range and mean) from wetlands grouped according to water source (Irrigation, Farm Dam, Stormwater, Sewage).

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>F value (p), degrees of freedom</th>
<th>Wetland Type</th>
<th>Biotic indices</th>
<th>Habitat Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Irrigation</td>
<td>Farm Dam</td>
<td>Stormwater</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td><strong>Biotic indices</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juvenile &lt;5cm</td>
<td>3.97 (0.11) 3,68</td>
<td>1.22</td>
<td>(0-9)</td>
<td>0</td>
</tr>
<tr>
<td>Juvenile 5 - 9.9cm</td>
<td>13 (0.000) 3,68</td>
<td>2.055</td>
<td>(0-7)</td>
<td>0.33</td>
</tr>
<tr>
<td>Juvenile 10 - 14.4cm</td>
<td>2.9 (0.03) 3,68</td>
<td>3.11</td>
<td>(0-6)</td>
<td>3.44</td>
</tr>
<tr>
<td>Males 14.5 - 17.9cm</td>
<td>1.6 (0.19) 3,68</td>
<td>2.56</td>
<td>(0-12)</td>
<td>2.06</td>
</tr>
<tr>
<td>Males &gt;18cm</td>
<td>2.06 (0.11) 3,68</td>
<td>1.39</td>
<td>(0-6)</td>
<td>0.72</td>
</tr>
<tr>
<td>Females 14.5 - 19.9cm</td>
<td>0.4 (0.75) 3,68</td>
<td>3.89</td>
<td>(0-12)</td>
<td>5.22</td>
</tr>
<tr>
<td>Females &gt;20cm</td>
<td>4.29 (0.007) 3,68</td>
<td>3.28</td>
<td>(0-13)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Habitat Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface area (ha²)</td>
<td>6.44 (0.015) 3,8</td>
<td>0.11</td>
<td>(0.06-0.17)</td>
<td>0.064</td>
</tr>
<tr>
<td>Average Depth (m)</td>
<td>9.7 (0.004) 3,8</td>
<td>0.8</td>
<td>(0.7-0.9)</td>
<td>0.9</td>
</tr>
<tr>
<td>Emergent vegetation cover (%)</td>
<td>0.67 (0.58) 3,8</td>
<td>8.33</td>
<td>(0-22)</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Mean (Sample SD)</td>
<td>Min (Min Max)</td>
<td>Max (Min Max)</td>
<td>Min (Min Max)</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>------------------</td>
<td>---------------</td>
<td>---------------</td>
<td>---------------</td>
</tr>
<tr>
<td><strong>Water Quality</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Water Temperature</strong>*</td>
<td>46.2 (0.0001)</td>
<td>19.7</td>
<td>(11.8-26.7)</td>
<td>21.3</td>
</tr>
<tr>
<td><strong>pH</strong>*</td>
<td>58.7 (0.0001)</td>
<td>7.03</td>
<td>(6.3-9.7)</td>
<td>7.78</td>
</tr>
<tr>
<td><strong>Dissolved Oxygen %</strong>*</td>
<td>496.9 (0.0001)</td>
<td>18.41</td>
<td>(0.5-124.8)</td>
<td>65.6</td>
</tr>
<tr>
<td><strong>Electrical Conductivity μS/CM</strong>*</td>
<td>2983 (0.0001)</td>
<td>320.2</td>
<td>(154-844)</td>
<td>195</td>
</tr>
<tr>
<td><strong>Turbidity ntu</strong>*</td>
<td>179.5 (0.0001)</td>
<td>133.7</td>
<td>(8.8-600)</td>
<td>281.1</td>
</tr>
<tr>
<td><strong>Total Hardness as CaCO3 mg/l</strong></td>
<td>292.32 (0.001)</td>
<td>34.67</td>
<td>(28-44)</td>
<td>31</td>
</tr>
<tr>
<td><strong>Carbonate Alkalinity as CaCO3 mg/l</strong></td>
<td>10.76 (0.001)</td>
<td>0.5</td>
<td>(0.5-0.5)</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Bicarbonate Alkalinity as CaCO3 mg/l</strong></td>
<td>32.84 (0.001)</td>
<td>104.67</td>
<td>(99-114)</td>
<td>32.42</td>
</tr>
<tr>
<td><strong>Sulphate as SO4 – Turbidimetric mg/l</strong></td>
<td>46.17 (0.001)</td>
<td>2.33</td>
<td>(2.0-3)</td>
<td>18.85</td>
</tr>
<tr>
<td><strong>Chloride mg/l</strong></td>
<td>183.9 (0.001)</td>
<td>37.33</td>
<td>(34-41)</td>
<td>25.85</td>
</tr>
<tr>
<td><strong>Calcium mg/l</strong></td>
<td>46.17 (0.001)</td>
<td>5</td>
<td>(4.0-7)</td>
<td>6.14</td>
</tr>
<tr>
<td><strong>Magnesium mg/l</strong></td>
<td>268.22 (0.001)</td>
<td>6.67</td>
<td>(6.0-8)</td>
<td>3.85</td>
</tr>
<tr>
<td><strong>Sodium mg/l</strong></td>
<td>215.26 (0.001)</td>
<td>36</td>
<td>(34-38)</td>
<td>16.1</td>
</tr>
<tr>
<td><strong>Potassium mg/l</strong></td>
<td>29.41 (0.001)</td>
<td>26.67</td>
<td>(20-34)</td>
<td>12.71</td>
</tr>
<tr>
<td></td>
<td>μs/cm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>-------</td>
<td>-----------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Arsenic</td>
<td>72.02</td>
<td>(3.0-6.0)</td>
<td>0.71</td>
<td>(0.5-1.0)</td>
</tr>
<tr>
<td>Cadmium</td>
<td>1.1</td>
<td>(0.05-7.1)</td>
<td>0.09</td>
<td>(0.05-0.2)</td>
</tr>
<tr>
<td>Chromium</td>
<td>2.8</td>
<td>(2.0-16.0)</td>
<td>6.42</td>
<td>(0.5-17.0)</td>
</tr>
<tr>
<td>Copper</td>
<td>2.3</td>
<td>(0.5-4.0)</td>
<td>3.42</td>
<td>(0.5-7.0)</td>
</tr>
<tr>
<td>Lead</td>
<td>6.3</td>
<td>(1.0-13.0)</td>
<td>6.2</td>
<td>(0.5-11)</td>
</tr>
<tr>
<td>Nickel</td>
<td>0.3</td>
<td>(1.0-7.0)</td>
<td>3.7</td>
<td>(0.5-7)</td>
</tr>
<tr>
<td>Zinc</td>
<td>1.92</td>
<td>(2.5-12)</td>
<td>15.85</td>
<td>(5.0-25)</td>
</tr>
<tr>
<td>Total Kjeldahl Nitrogen as N mg/l</td>
<td>184.7</td>
<td>(6.5-11.8)</td>
<td>1.74</td>
<td>(0.9-2.4)</td>
</tr>
<tr>
<td>Total Phosphorus as P mg/l</td>
<td>8.03</td>
<td>(0.5-1.7)</td>
<td>0.35</td>
<td>(0.07-0.59)</td>
</tr>
<tr>
<td>Total Organic Carbon mg/l</td>
<td>28.0</td>
<td>(19-42)</td>
<td>17.57</td>
<td>(11.0-31)</td>
</tr>
</tbody>
</table>

*Data were collected using Yeo-Kal field meter at each wetland every month*
Variation in salinity (electrical conductivity) was highly significant among water sources (table 4.1). Mean salinity was lowest in farm dams (mean 195 µS/cm) and highest at wetlands that received treated sewage effluent (mean 852.5 µS/cm; Figure 4.5).

![Electrical Conductivity Graph](image)

Figure 4.5 - The mean (plus/minus standard error) salinity (electrical conductivity) of wetlands sampled in peri-urban Sydney measured between October 2007 and May 2008.

Dissolved oxygen (% saturation) varied significantly among water sources (Table 4.1). Mean dissolved oxygen (DO) was lowest within irrigation wetlands (mean 18.41 %) and highest within wetlands that received treated sewage effluent (mean 114.4 %; Figure 4.6).
Water temperature varied significantly among water sources (Table 4.1; Figure 4.7). Mean water temperature was lowest in wetlands that received irrigation runoff (mean 19.66 °C) and highest in those that received treated sewage effluent (mean 22.78° C).
Mean total metals were often in greatest concentrations in the farm dams (chromium; copper; lead and zinc) followed by wetlands that were supplemented with irrigation runoff (highest for arsenic and cadmium) with only one metal (nickel) having the greatest mean concentration at the treated sewage effluent wetlands (Figure 4.8; Table 4.1).

![Bar chart](image)

**Figure 4.8** – The mean metal concentrations in the wetlands sampled in peri-urban Sydney.

All wetlands in this study had enriched levels of nitrogen and phosphorus nutrients. The highest nutrient levels were recorded in the wetlands that received runoff from irrigation with a mean total phosphorus level of 1.2 mg/L (Table 4.1). In addition, these wetlands also recorded the highest mean nitrogen level of 9.1 mg/L (Figure 4.9; Table 4.1).
4.3.3 - Water quality and the turtle population

BIOENV multivariate analysis found that physical water quality variables with the highest correlation to the differences in the size structure of turtles among wetlands were temperature (°C), dissolved oxygen (percentage saturation), surface area (ha), and the percentage of emergent vegetation (correlation = 0.122). There was a significant correlation between dissolved oxygen and the number of juveniles ($R^2 = 0.5318$, $p < 0.01$; Figure 4.10), surface area and the number of juveniles ($R^2 = 0.4128$, $p = 0.014$; Figure 4.11), and percentage of emergent vegetation cover and the number of juveniles ($R^2 = 0.5169$, $p = 0.01$) (Figure 4.12). The lower the dissolved oxygen, the smaller the surface area of the wetland, the higher the percentage of emergent vegetation the greater the number of juveniles. There was not a significant relationship between the number of adult females and the number of juveniles in wetlands ($R^2 = 0.1257$).
Figure 4.10 – Regression of percentage saturation of dissolved oxygen against the number of juveniles captured in each wetland ($y = -0.3547x + 47.015$, $R^2 = 0.53184$, $p < 0.01$) in peri-urban Sydney wetlands.

Figure 4.11 – Regression of surface area (ha) against the number of juveniles ($y = -8.4047\ln(x) + 14.143$, $R^2 = 0.4128$, $p = 0.014$) in peri-urban Sydney wetlands sampled.
Figure 4.12 – Regression analysis of percentage emergent vegetation in each wetland sampled against the number of juveniles captured in each wetland ($y = 1.4344x + 9.4328$, $R^2 = 0.5169$, $p = 0.01$) in peri-urban Sydney wetlands sampled.

Four species of fish were collected as bycatch; *Angullia australis*, *Anguilla reinhardtii*, *Gambusia holbrooki* and *Cyprinus carpio*. At least one species was collected from wetlands with each of the recycled water types and from farm dams. No fish were netted from wetlands supplemented with irrigation seepage water.

4.4 - Discussion

The difference in the size structure of the population of *Chelodina longicollis* among wetlands in this study was influenced by dissolved oxygen levels, the extent of emergent vegetation, and the surface area of the wetlands. The wetlands that received irrigation runoff and farm dams had mean dissolved oxygen levels that were much lower than the recommended by the Australian and New Zealand Environment and Conservation Council (ANZECC) guidelines for the protection of freshwater ecosystems (ANZECC 2000). These guidelines (ANZECC 2000) recommend dissolved oxygen for lakes and wetlands in South Eastern Australia to be in the range of 90-110% saturation. The minimum dissolved oxygen level recorded in the current study was from the wetlands supplemented with irrigation waters (0.5% saturation). These wetlands contained the largest populations of juveniles of any wetlands in the study, and there was an absence of fishes (eels *Anguilla reinhardtii* long finned eel
and *Anguilla australis* short finned eel, and *Cyprinus carpio* common carp) from these wetlands.

All wetlands in this study had enriched levels of nitrogen and phosphorus nutrients above the ANZECC (2000) guidelines for protection of ecosystems. The highest nutrient levels were recorded in the wetlands that were supplemented with irrigation runoff. The highest total phosphorus level was 120 times the ANZECC (2000) guideline. In addition, they also recorded the highest mean nitrogen level, 26 times above the ANZECC (2000) guideline. These wetlands were, therefore, highly eutrophic. The excess nutrients were presumably due to the decay of organic matter, which would also impact on dissolved oxygen levels in these wetlands. This gives air breathing animals, such as turtles, a competitive advantage over other aquatic vertebrates. However, low dissolved oxygen levels do not necessarily explain the absence of *C. carpio*. While *C. longicollis, A. reinhardtii* and *A. australis* move overland (Burrows et al. 2009), and are thus common inhabitants of impoundments within the Sydney Basin, *C. carpio* need to be assisted in their dispersal between wetlands (Petrovic et al. 2002).

Fish and turtles both feed on macroinvertebrates (Georges et al. 1986; Kennett et al. 2009), this overlap in diet may create competition for food between these taxa when it is limiting. The high level of nutrients in the wetlands supplemented by irrigation runoff may directly affect the abundance of macroinvertebrates, providing an abundant food source for turtles in these wetlands. The lack of competition with fish and/or lack of predation in these wetlands may have contributed to the success of the juvenile population. Whilst in the other wetlands there are larger turtles, which may supplement the competition from the other fish species by preying on *G. holbrooki*, which may be too large to be part of the diet of juvenile *C. longicollis*.

The absence of *C. carpio* in wetlands may have a more indirect effect on juvenile success. Whilst feeding on benthic organisms *C. carpio* uproot aquatic vegetation which results in its removal from wetlands (Crivelli 1983), and greatly increases turbidity. This destruction of aquatic vegetation could potentially remove a turtles dietary source by removing macroinvertebrate habitat and breeding grounds, but could also contribute to increased predation on juveniles, which may be another
reason why the wetlands that have *C. carpio* present have low numbers of juveniles. This correlates with Kennett and Georges (1990) who found that there were larger numbers of juveniles present in water bodies with a greater extent of emergent vegetation in these wetlands than elsewhere. This trend has also been reported in an American freshwater turtle species *Emydoidea blandingii* Blanding’s turtle (Brecke and Pappas 1992). A greater percentage coverage of the wetland with emergent vegetation provides hides for juveniles, and thus protects them from predators. Juveniles that reside in wetlands with a substantial coverage of the surface area with aquatic vegetation are able to aquatically bask within emergent vegetation, and are thus less likely to be sighted and predated upon by aerial predators. The results of this study may be attributed to high predation on juveniles in open water (Janzen et al. 2000b). The lack of fishes in the farm dams with the largest number of juveniles could also indicate that large fish species (such as eels and carp) are preying on hatchling and small juvenile *C. longicollis*.

The development of turtles in this peri-urban area may be affected by the high concentrations of heavy metal in all wetlands. The ANZECC (2000) guidelines for protection of 99.0% of species indicate that many of the wetlands had metal levels that were higher than the recommended trigger value for ecosystem protection. For example the irrigation wetlands had a mean cadmium concentration more than 14 times higher the ANZECC (2000) trigger value of 0.06 µg/L. The mean metal levels exceeded the ANZECC (2000) trigger values for lead at the irrigation and farm dam wetlands and the ANZECC (2000) guideline was exceeded for mean copper and zinc concentrations at all wetlands. The level of arsenic was also five times greater in the wetlands that received irrigation runoff than in other wetlands.

Metals may have entered the water system through the recycled water system that has been in place for over 50 years with stormwater being linked to high levels of metals in wetlands (Ávila et al. 2013). However, the majority of the metals that were in higher concentrations were associated with the wetlands that were considered the most ‘natural’ of those sampled; farm dams and those that received irrigation runoff. Whilst the wetlands that receive irrigation runoff would have residual pollutants from the recycled water, the high levels of metals in these wetlands are assumed to have been due to chemical applications to the agricultural lands. The study site has been
used for agricultural teaching purposes for over 120 years (Burgin 2010), and although not documented, pesticides, herbicides and a variety of fertilisers would have been used on the fields used for growing crops, which were adjacent to a number of wetlands sampled. The historic agricultural use of nitrogen fertilisers, and pesticides, such as organochlorins, organophosphorus and carbonate based pesticides, in land surrounding wetlands, may persist and thus impact on the wetlands for decades after initial application due to the metals being ‘trapped’ in the sediments (Marburger et al. 2002; Regueiro et al. 2013) and cause high concentrations of metals in aquatic vertebrates in the wetlands (Baker 1992; Marburger et al. 2002; Smith 1992).

While there are a lack of studies on the impact that heavy metals have on freshwater turtle species, their accumulation in other long-lived aquatic species (e.g., marine turtles and mammals (Sakai et al. 2000a; Sakai et al. 2000b; Storelli and Marcotrigiano 2003)) have been investigated more widely. Metals such as mercury, cadmium, lead and arsenic have been found to be present in Caretta caretta loggerhead turtle livers, kidneys and muscles (Sakai et al. 2000b). In the current study, the highest levels of metals were also the wetlands with the highest numbers of juvenile C. longicollis. With heavy metals accumulating in the liver, it may affect the development of these juvenile turtles as many metabolic processes of importance occur in the liver and may impact on the development of the resident juvenile turtles (Storelli and Marcotrigiano 2003). For example, Sakai et al. (2000a) found that cadmium levels in Chelonia mydas green turtles, changed as the turtles matured and shifted feeding habits from a carnivorous to a herbivorous diet. Cadmium levels were higher in juveniles that had a carnivorous diet. In carnivorous marine turtles, such as the Caretta caretta loggerhead turtle, heavy metal has been found in higher concentration than the herbivorous Chelonia mydas (Storelli and Marcotrigiano 2003). Chelodina longicollis, however, only consume a carnivorous diet and, therefore, it can be assumed that turtles in the wetlands studied would not reduce metal concentrations, such as cadmium, unless they migrated to wetlands with lower concentrations of metals. However, as C. longicollis are known to use a complex of wetlands, and that all wetlands in our study site had heavy metal concentrations above recommended levels, the likelihood that wetlands within the wider peri-urban area would not have heavy metal concentrations would be low.
Other than heavy metals; salinity, turbidity, water temperature, dissolved oxygen, and nutrients (nitrogen and phosphorus) were highly variable among water sources. ANZECC (2000) recommends that salinity for lakes and reservoirs in south eastern Australia should be in the range 20-30 µS/cm. In contrast, salinity was measured in wetlands fed by tertiary treated sewage effluent at levels as high as 934 µS/cm. Turbidity was also substantially outside of the ANZECC guidelines for the protection of freshwater ecosystems. Compared to the ANZECC (2000) guideline of 1-20 NTU, farm dams and wetlands that received irrigation runoff had a maximum turbidity reading (600 NTU) which was off the scale of the measuring instrument. High turbidity can greatly impact on freshwater turtles by reducing the food availability. For example, Cann (1998) reported that over one year, 620 dead turtles were collected from the edge of Lake Mokoan in Victoria. It was found that these turtles had died of starvation caused by high turbidity that killed the plant life in the lake, and subsequently reduced the available food resources for the resident turtles.

Temperature varied among wetlands, although it was highest in the wetlands that were fed by tertiary treated sewage effluent, and by stormwater effluent. The *C. longicollis* in these wetlands tended to be larger turtles. When food resources are not limiting, increased water temperature has been linked to the accelerated growth rates and larger body size in the freshwater turtle *C. s. scripta*. This was attributed to increased water temperature that extended the growth period and increased digestion (Parmenter 1980).

Lindeman (1996) conducted a study of two populations of *Chrysemys picta*: one population was resident of a natural lake and the other resided in a wastewater lagoon. Females that resided in the wastewater lagoon double-clutched in a season. In contrast, no multiple clutching occurred in the natural lake population. However, turtles experienced faster growth rates in the wastewater lagoon and, therefore, reached sexual maturity more rapidly than in the natural lake population. Males in wastewater lagoon matured at approximately 2 years, females 6-7; while males in the natural lake reached sexual maturity at 3 years, females 8-10. This accelerated growth and maturity were not observed in the current study. There were few juveniles in wetlands fed by the two different types of recycled water. It therefore appears that
females do not have greater reproductive output in the recycled water wetlands. They may, however, have a greater reproductive output in the wetlands that have the higher levels of nutrients (i.e., the wetlands that receive irrigation runoff) where a largely greater percentage of the population was juveniles. This has also been found in a population of *Chrysemys picta* where increased nesting was reflected in a population that was skewed towards smaller individuals (Lindeman 1996). However, there was no evidence that females in these wetlands were double clutching so the absence of fish species, *A. australis*, *A. reinhardtii*, and *C. carpio* or greater percentage coverage of emergent vegetation (that has been considered the preferred habitat of juveniles), may play a role in juveniles’ survival.

It is widely accepted that *C. longicollis* live in a wide range of water bodies; however, in this study juveniles were found in relatively large numbers in some wetlands, while in other wetlands, even within 500 m, no juveniles were netted. The storage of recycled water in wetlands and the use of recycled water for irrigation provide a variation in water quality parameters, which appear to influence *C. longicollis* population structure. Water parameters, which may be detrimental to other aquatic vertebrates, may provide a ‘safe haven’ for juvenile turtles by indirectly decreasing competition, reducing predation, and increasing food sources for these highly vulnerable juvenile turtles.
Chapter 5 – Long-term changes in a peri-urban population of 
Chelodina longicollis

A *Chelodina longicollis* in the process of nesting (top) and juvenile *Chelodina longicollis* captured during sampling in peri-urban Sydney (bottom), Australia.
5.1 – Introduction

Most published research on vertebrates is based on short term, ‘snap-shot’ studies, conducted over a period of fewer than two years. This is due to the nature of research, which includes difficulty in obtaining funding for long-term research (Cody and Smallwood 1996), the length of postgraduate study programs, and the briefness of professional careers (Cody and Smallwood 1996; Strayer et al. 1986). However, short-term studies may not provide an accurate picture of the consequences of human-induced changes on animal populations. This is because they may take decades to become apparent, especially in long-lived vertebrates (Cody and Smallwood 1996; Smith et al. 2006).

Rather than a ‘snap-shot’ in time, long-term research provides a broader perspective into the understanding of how animal communities cope with year-to-year, and decade-to-decade variations in conditions within their environment (Smith et al. 2006), and may detect processes that unfold slowly that would not be detected in short-term studies (Cody and Smallwood 1996). With environmental research now tending to focus on the effects of climate change, long-term studies are more likely to show how populations adapt under associated increasing pressures imposed by their environment. Climate change and environmental alteration occur in parallel and, therefore, changes in climate patterns can shape biological communities (Roe and Georges 2010). ‘Snap-shot’ studies may, therefore, not identify the consequences that human-induced changes have on community composition and structure of long-lived vertebrates (Cody and Smallwood 1996).

Freshwater turtles are long-lived vertebrates, reported to live to over one hundred years (Gibbons 1987). Those, such as *Chelodina longicollis* eastern longneck turtle, that have a propensity for overland movement (Chessman 1984a; Roe and Georges 2008b; Ryan and Burgin 2007), may be affected by changes to both the terrestrial and aquatic environment. Smith et al. (2006) considered that “understanding the long-term dynamics of freshwater turtle communities is important in understanding fully the impacts of human-induced changes in aquatic and terrestrial habitats inhabited by freshwater turtles”. 
The loss, degradation, and fragmentation of wetland habitats can also result in an abnormal population structure in freshwater turtles (Dodd 1990; Marchand and Litvaitis 2004; Reese and Welsh 1998). However, despite their longevity, there are few published studies that follow a marked population over time.

Longer-term studies undertaken to date have been mostly in North America (Browne and Hecnar 2007; Congdon and Gibbons 1996; Hall et al. 1999; Smith et al. 2006). These have shown that shifts in community composition (Smith et al. 2006), population declines (Hall et al. 1999), and a development, and/or shift in sex ratio bias may occur (Browne and Hecnar 2007; Congdon and Gibbons 1996). In addition to climate change, such changes in populations have been considered to have resulted from an increase in human impacts due to activities such as an increase in local road construction, outdoor recreational activities, and/or wild turtle harvesting (Marchand and Litvaitis 2004; Smith et al. 2006; Williams and Parker 1987).

Urban development and other anthropogenic alteration in the terrestrial landscape surrounding the turtle’s wetland/s have also been shown to affect populations (Smith et al. 2006; Williams and Parker 1987). For example, as a result of urban development and other land use changes, turtles that use multiple habitats within a large area can become trapped during overland migration. With loss of habitat, turtles may become confined within a subset of their home range and, as a result, typically become more densely concentrated. This potentially places additional pressures on the population (Marchand and Litvaitis 2004). A resulting change in population structure may have ecological and conservational implications that may affect community structure (Browne and Hecnar 2007). For example, sex ratio bias could lower recruitment into a population (Congdon and Gibbons 1996).

No published Australian study has considered the changes in population structure of a native freshwater turtle over a decade or more. In this Chapter, a population of the native freshwater turtles, *Chelodina longicollis*, from peri-urban Western Sydney, were sampled over more than a decade after the population was first sampled. The study focused on sex ratio, growth, turnover in the population, changes in population
dynamics, and movement between wetlands. It was hypothesised that because land use had not changed in the intervening period that there would be no change in the population structure over the period. Because the species are known to have a home range that encompasses several wetlands (Roe and Georges 2007), it was also hypothesised that most of the population would be available for recapture.

5.1.1 - The study species

*Chelodina longicollis* is the most widespread Australian freshwater turtle, with a distribution within continental Australia from Eastern Queensland to South-eastern South Australia (Kennett et al. 2009). It inhabits a variety of water bodies including rivers, lakes, ephemeral wetlands, farm dams (ponds), and even degraded urban and peri-urban water bodies (Burgin and Ryan 2008; Cann 1998; Chessman 1988a; Rees et al. 2009). Estimates of its density in impoundments range from 236 ha$^{-1}$ (Chessman 1978) to 400 ha$^{-1}$ (Parmenter 1976).

In addition to having the most extensive range of any native Australian turtle, *C. longicollis* is the most terrestrial and may undertake overland migration at all stages of its lifecycle. Some individuals may move among several wetlands within a single year (Roe and Georges 2007; 2008a). The species’ ability to efficiently migrate is supported by the low rate of evaporative water loss (Chessman 1984a). Individuals are, therefore, able to migrate when conditions become unsuitable, for example, to limit competition with fish and other turtles (Kennett and Georges 1990).

The onset of sexual maturity is dependent on size, and not necessarily age. Larger females commonly have a larger clutch size (Kennett et al. 2009). Hatchlings may be as small as 2.1 cm carapace length (Burgin and Dalem 1996), and adults may grow to 28.0 cm (Chessman 1978; Thompson 1993). Hatchlings tend to grow rapidly during their first year; however, growth rate typically slows beyond this period, and is largely influenced by food availability (Kennett and Georges 1990). For a fuller species description see Chapter 2.

5.2 – Methods
5.2.1 - Study site

Peri-urban Western Sydney (New South Wales [NSW], Australia) is one of the most rapidly urbanising areas of Australia. In 2011, the human population was slightly over 2 million, which was a 1.6% increase over the previous year. The population is forecast to reach 2.9 million by 2036 (DPC 2012). To support the increased population growth of the next 25 years, many peri-urban areas of Western Sydney have been rezoned for urbanisation and will be the focus of future land releases (DPC 2012). This study was undertaken within this area (Hawkesbury campus, University of Western Sydney, adjacent to Richmond), which is approximately 50 km northwest of the Sydney Central Business District.

Land use adjacent to the wetlands sampled had been used for agricultural teaching purposes for over 120 years. At the beginning of this period, land was cleared to create fields for grazing and cropping, and dams were constructed to water the grazing stock. Subsequently, there has been limited change in the landscape. There are 34 wetlands on the campus, 17 supplemented with recycled stormwater or tertiary treated sewage effluent (Burgin 2010).

During the period of the study (1994-2008), the area experienced droughts in 1995, 2000, and 2003-2006. There have also been many days of ‘highest temperatures on record’ in the past 20 years (BoM 2006). From 1996 to 2008 NSW experienced above normal temperatures (Macey 2008), and below average rainfall (Sydney Water 2009). The lack of rainfall was sufficiently severe to implement water restrictions in 2003, and these were subsequently strengthened in 2004 and 2005 as water levels in domestic water supply storage dams were depleted (Sydney Water 2009). During the study period the annual rainfall varied between 490.6 ml/year in 2006 and 1051.2 ml/year in 2007 (Figure 5.1). The highest temperature that occurred during the period (1993-2008) was 44.9°C in 2007 (Figure 5.2).
5.2.2 - Turtle sampling

5.2.2.1 - Sampling for changes in wetland turtle populations

Three dams within 15 m of each other were studied in a capture-mark-recapture study between November 1994 and December 1995 (Burgin unpub. data), and resampled between October 2007 and November 2008. In 1994-1995, four fyke nets (cf. Burgin et al. 1999) were set in each of these dams, and in 2007-2008 two fyke nets/dam were set every 21 days for four consecutive days. On each morning of sampling, turtles were removed and measured (curved carapace length, ±0.1 mm), weighed (±0.1 g),
using a portable field balance (Sartorius portable scales (A&D EK-1200i), and sex determined (based on whether the plastron was concave [male] or convex [female]; (Chessman 1978; Kennett and Georges 1990). Turtles with a carapace length of less than 14.5 cm were classified as juveniles (Roe and Georges 2008a). Each turtle was uniquely marked, processed, and released at their point of capture.

5.2.2.2 - Movement of turtles
To determine the terrestrial movement of turtles, wetland of capture was recorded. Turtles used for this segment of the study were those captured in 1994-1995 and again in 2007-2008. To investigate movement among wetlands, the 2007-2008 sampling included turtles from nine additional wetlands in the immediate area (sampled using the same method as outlined above for the 2007-2008 sampling). The minimum distance travelled between two captures of an individual was measured by determining the minimum distance between wetlands using aerial photographs.

5.2.2.3 - Growth rate of turtles
Turtle growth was measured as the change in carapace length between captures, divided by the fraction of the annual six-month growth period (15 September – 15 March) that had elapsed between initial and final capture. Individuals were only included if the period between their first and last capture spanned at least half of the six month growth period (Roe and Georges 2008a).

Turtles were also sampled in nine dams for two months in 2006 (Ryan 2006). Turtles initially captured in 2006, and recaptured in 2007-2008 were only included (where applicable) in the growth rate analysis.

5.2.3 - Data analysis
To determine changes in the wetland turtle population parameters over time, a Chi Square Goodness of Fit analysis was conducted to determine if there was a difference in sex ratio in the three dams sampled in 1994-1995 and again in 2007-2008. Turtle condition was also compared, and this was determined using the ratio of weight to carapace length (Ryan & Burgin 2007). A Paired Sample t-Test was used to investigate if there had been an overall change in carapace length, and also to
investigate if there was a change in condition of the turtles in the population between 1994-1995 and 2007-2008.

Although it could not be determined that turtles captured only in the latter sampling period were migrants to the wetland, the assumption was made that they were more likely to be immigrants than those captured only in the same wetland in both sampling periods. Condition of turtles recaptured in the same wetland in both sampling periods were; therefore, compared with individuals that were captured for the first time in those wetlands during the latter sampling period. A Paired Sample t-Test was used for this analysis. A Chi Square Goodness of Fit analysis was used to investigate change in the proportion of juveniles to females over time.

A Chi Square Goodness of Fit analysis was undertaken to compare the number of turtles recaptured in only one wetland compared to turtles that were recaptured in at least two wetlands. A Paired Sample t-Test was used to determine if there was a difference in minimal distance travelled by the sexes. Chi Square Goodness of Fit was used to compare the mean number of movements made by the sexes. A regression analysis was used to determine if there was a relationship between initial carapace length and minimum distance travelled. As a surrogate for predation (Castellano et al. 2008), differences in injury rate between the sexes was also investigated using Chi Square Goodness of Fit test.

To investigate differences in the growth rate between the sexes, and between adults and juveniles, a One-Way Analysis of Variance (ANOVA) followed by a Post Hoc Bonferroni Test was used. To determine if males and females differed in growth rate among size classes, a Paired Sample t-Test was used. A Regression Analysis was undertaken to determine the relationship between initial length and the rate of growth. Growth rates were also compared among wetlands using ANCOVA, with wetland used as the independent variable, and Log_{10} carapace growth rate as the dependant variable. The initial carapace length was designated the covariate. An ANOVA was used to investigate differences between the growth rate of turtles that were known to have moved wetlands (i.e., netted in multiple wetlands), and those captured only in one wetland. A Paired Sample t-Test was also used to determine if the turtles that
were recaptured in the same wetlands more than a decade on had a higher rate of growth than turtles that had only been captured in 2007-2008.

5.3 – Results

5.3.1 - Changes in wetland turtle populations
In the initial sample (1994-1995), a total of 174 *Chelodina longicollis* (81 juveniles, 38 adult males, 55 adult females) were captured across the three wetlands sampled. The sex ratio was equivalent to unity ($\chi^2_1 = 3.108, p=0.07$), and there was a ratio of 1.5 :1 juveniles : female. In the sample taken over a decade later (2007-2008), there was a 59.2% increase in captures: 277 *C. longicollis* were captured in the three wetlands. The sample population was made up of 109 juveniles, 66 adult males and 102 adult females. The sex ratio had a female bias ($\chi^2_1 = 7.714, p=0.005$), and a juvenile to female ratio of 1:1. Effectively all sexed turtles, and two juveniles that were trapped in 1994-1995, and recaptured in 2007-2008, were female. From the original sampling, 15% of the turtles were recaptured. With one exception, turtles that were over 20 cm were female (n=48). There were a higher proportion of juveniles to females in 1994-1995 than in 2007-2008, although this increase was not statistically significantly different ($\chi^2_1 = 2.083, p=0.149$) (Figure 5.3).
Figure 5.3 – A comparison of the population structure of *Chelodina longicollis* 1994-1995 and 2007-2008, captured in wetlands in peri-urban Sydney (New South Wales, Australia).

The mean carapace length of *C. longicollis* was greater in 1994-1995 than in 2007-2008 (Table 5.1); however, this difference was not significant ($t_{449} = 1.54, p=0.1219$). There was; however, a significant difference in the condition of turtles. In 1994-1995, *C. longicollis* were in better condition than turtles captured in 2007-2008 ($t_{449} = 6.477, p=0.0001$, Figure 5.4). There was also a significant difference in the condition of females recaptured from the 1994-1995 sampling and those collected only in 2007-2008 ($t_{19} = 4.57, p<0.001$). Those recaptured after a decade were heavier for their size than turtles that had not been previously captured.

Table 5.1 – The mean, standard error, and range of the carapace length, weight, and condition of *Chelodina longicollis* captured from the same wetlands in peri-urban Sydney (New South Wales, Australia) in 1994-1995 and in 2007-2008.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carapace length (cm)</strong></td>
<td>Mean (standard error)</td>
<td>15.13 (0.25)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>3.3 – 22.3</td>
</tr>
<tr>
<td><strong>Weight (g)</strong></td>
<td>Mean (standard error)</td>
<td>466.71 (18.46)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>8-1200</td>
</tr>
<tr>
<td><strong>Condition</strong></td>
<td>Mean (standard error)</td>
<td>28.80 (0.75)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>2.08-55.81</td>
</tr>
</tbody>
</table>
5.3.2 - Movement of turtles

When the study was expanded to 12 wetlands across the study area in 2007-2008, a total of 1,146 (1,453 total captures) *C. longicollis* were captured (33.6% adult males; 42.4% adult females; 24.0% juveniles). Overall, there was a significant female sex bias ($\chi^2 = 9.532$, p=0.002). There was no significant difference in recapture rate between adult females and males ($\chi^2 = 1.866$, p>0.05; 97, [25.2% of total male captures], 158 adult females [32.6% of total female captures] and 52 juveniles [18.8% of total juvenile captures]. Of the recaptures, 76 turtles [males, 28.95%; females, 46.05%; juveniles, 25%] were confirmed to have moved wetland. There was no significant difference between the sexes in the mean minimum distance travelled among wetlands ($t_{51} = 1.3578$, p= 0.1805; Table 5.2), or the mean minimum number of movements made ($\chi^2 = 2.965$, p=0.085). There was also not a significant relationship between the initial carapace length and the minimum distance travelled ($R^2<0.001$, p>0.05).

Table 5.2 – The minimum, maximum, mean, and standard error in metres of the distance travelled by male, female and juvenile *Chelodina longicollis* in wetlands in peri-urban Sydney (New South Wales, Australia).
<table>
<thead>
<tr>
<th></th>
<th>Mean (standard error)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juvenile</td>
<td>444.50 (131.17)</td>
<td>14-1288</td>
</tr>
<tr>
<td>Male</td>
<td>919.52 (130.23)</td>
<td>14-1976</td>
</tr>
<tr>
<td>Female</td>
<td>643.54 (156.91)</td>
<td>14-3131</td>
</tr>
</tbody>
</table>

There was 6.9% (n = 1146) of the population who showed visible injuries. There was no significant difference between the sexes in the number of individuals with visible injuries ($\chi^2 = 0.727, p = 0.39$).

5.3.3 - Turtle growth

There was no significant relationship between the initial length of turtles and growth ($R^2 = 0.004, P = 0.55$). No carapace length growth occurred in 24.2% of the recaptured turtles (size range 4.6–23.0 cm, n = 99). Among the 75 individuals that registered growth, there was no significant difference in the growth rate between males and females, although there was a highly significant difference in growth rate of juveniles compared to adult turtles ($F_{2, 11445} = 2.589, p = 0.0001$) (Table 5.3).

Table 5.3 – The mean, minimum, and maximum growth rates of *Chelodina longicollis* captured in wetlands dams in peri-urban Sydney (New South Wales, Australia).

<table>
<thead>
<tr>
<th></th>
<th>Mean growth (cm/year), standard error</th>
<th>Range (cm/year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>0.22 (0.19)</td>
<td>0 – 1.58</td>
</tr>
<tr>
<td>Female</td>
<td>0.19 (0.02)</td>
<td>0 – 1</td>
</tr>
<tr>
<td>Juvenile</td>
<td>0.79 (0.18)</td>
<td>0 – 3.12</td>
</tr>
</tbody>
</table>

There was no significant difference in the growth rate of larger adult (> 17.0 cm carapace length) males and females ($t_{40} = 4.78, p = 0.635$). There was also no significant difference in growth rate in *C. longicollis* among wetlands ($F_{7, 0.845} = 5.19, p = 0.09$), and no significant difference in the growth rate of turtles that had been captured in more than one wetland (i.e., those known to have undertaken terrestrial migration) and those that had only been captured in a single wetland ($t_{264} = 1.2716, p = 0.2$). Turtles that were recaptured from the 1994-1995 survey also had no significant difference in the growth rate compared to turtles only captured in 2007-2008 ($t_{72} = 1.1486, p = 0.25$).
5.4 – Discussion

Despite a lack of major change in land use in the immediate study area in the intervening period, and the use of the same techniques, although with decreased sampling intensity during the later sampling, the numbers of *Chelodina longicollis* trapped had increased substantially over time. A sex bias towards females had also developed despite (on average) lower recruitment of juveniles per female into the population. In addition, only females were recaptured over a decade on. Factors contributing to the observed differences in population attributes may have been due to 1) size dimorphism (Lovich and Gibbons 1990); different age/size at maturity in males and females (Chessman 1978) which leads to bias in sampling technique (Kennett and Georges 1990); 2) endocrine disruption (Crain and Guillette 1998); 3) differential mortality (Howard 1981); 4) natal site-fidelity (Freedberg et al. 2005); and/or 5) changed environmental conditions (Ezaz et al. 2009).

5.4.1 - Size dimorphism, different age/size at maturity, sampling bias

On average, adult female *C. longicollis* had a longer carapace length than males. Of the 49 adult turtles captured in 2007-2008 that were over 20 cm, only one was male. A lack of ‘larger’ males has been typically observed in populations of *C. longicollis* (Chessman 1978; Parmenter 1976; Wong and Burgin 1997); however, discussion of this population attribute has been limited. As with many reptiles, *C. longicollis* females are under selection pressure to maximise their body cavity size (and, therefore, carapace dimensions) to maximise clutch size (Bowden et al. 2011). *Chelodina longicollis* females typically mature later than males (Parmenter 1976), and in the absence of a dominance hierarchy among males, once they have reached sexual maturity there is presumably not an equivalent evolutionary pressure on males, compared to females, to attain large size (Harless and Morlock 1979; Lovich and Gibbons 1990). The pattern of growth among adult males and females would, therefore, be expected to differ. This was not observed, and has also not previously been reported (Kennett and Georges 1990; Roe et al. 2011). Large *C. longicollis* males and females had an equivalent growth rate. Maximum size and/or different growth rates between the sexes were; therefore, not a reason for the lack of large males in the sample population.
Sex differences in longevity have been recorded to occur in a number of vertebrate species (May 2007). The most efficient way for male turtles to increase their reproductive fitness is to mate with as many partners as possible while the most efficient for females is to maximise their reproductive lifespan and thus the potential number of clutches they produce in their lifetime (Gibbons 1987). *Chelodina longicollis* are long-lived (Kennett et al. 2009; Parmenter 1976). While differences in longevity between the sexes is unknown, since adult males and females have similar growth rates, the preponderance of large females could reflect that, on average, female *C. longicollis* have a longer lifespan than males. Although there is a dearth of information for turtles, such size difference has been reported in a population of bullfrogs - females were significantly larger than males, despite males and females having the same rate of growth throughout their adult life. It was suggested (Howard 1981) that this was due to higher male mortality as a result of male aggression in defending territories and mating effort. There does not appear to be similar data for any turtle species; however, no such aggression has been observed in *C. longicollis* and, therefore, the size difference in the study population would not be due to male aggression.

The average growth rate among juveniles was 0.79 cm/year, with the maximum of 3.12 cm/year, and sexual differentiation could be established at approximately 14.5 cm. At such a growth rate, juveniles may have hatched and become mature breeding adults in less than half the period of the study. If they grew at the fastest growth rate measured, the largest female (23.5 cm carapace length) could be as young as 16 years, and the largest male (21.1 cm carapace length) may have been 8 years old. However, as Kennett and Georges (1990) also observed, turtle growth rate can fluctuate greatly due to environmental conditions. Based on the average growth rate in adults, the largest female captured in our study (23.5 cm carapace length) was estimated to be approximately 65 years old, and the largest male (21.1 cm carapace length) was estimated to be 48 years old. This indicates that male *C. longicollis* may not live as long as females. Turtles do go through non-growth periods (Kennett and Georges 1990), and in this study one individual had no measurable growth between initial capture in 1994-1995 and recapture in 2007-2008. This indicates that, at least, some individuals in the local population may be much older than calculated. It is, therefore,
feasible that *C. longicollis* do live to be over 100 years old as Parmenter (1976) previously suggested. Some turtles in this study may, therefore, have been using these wetlands, and experienced the changes in the surrounding terrestrial environment when agriculture and associated urbanisation were first introduced to the area over 120 years ago (Ryan and Burgin 2007).

Only adult females were recaptured in the same wetland that they were initially collected from more than a decade before. Although length attained and growth rate did not differ, the recaptured females were in better condition (heavier for their size) than the females that had not been previously captured in the wetland. While some of these individuals not previously captured may have been resident and simply eluded capture in the earlier years of the study, and those recaptured within the same wetland more than likely to have been migrants in the intervening period, there does appear to be an advantage to staying in one wetland provided resources are available. A female that did not migrate between wetlands had the opportunity to conserve the energy required in overland movement (Roe and Georges 2008a) and, therefore, had greater potential to maintain condition than those that did undertake migration.

As with all chelonians (Elgar and Heaphy 1989), *C. longicollis* retain their entire clutch until eggs are laid and (as previously commented), clutch size is restricted by the female’s body cavity. Larger turtles are able to produce a larger number of eggs (Congdon and Gibbons 1983; Schwarzkopf and Brooks 1986; Wilkinson and Gibbone 2005), and hatch larger sized hatchlings (Gutzke and Packard 1984; Janzen et al. 2000a; Shine 1988). The larger number of hatchings may offset predation because, once hatched, the risk of predation for an individual hatchling is diluted by numbers (Colbert et al. 2010). Larger hatchlings also have a greater chance of survival than smaller hatchlings (Janzen et al. 2000a). In this study it was observed that turtles that definitely migrated had reduced body condition compared to those that were recaptured from the original sample period in the same wetland. Turtles in poorer condition lay fewer eggs (Harless and Morlock 1979) and/or have smaller hatchlings with an associated decreased chance of survival (Janzen et al 2000). This may have been an influence since females that did migrate were in poorer condition than those that were not observed to migrate, which may have contributed to a lower proportion of juveniles to females at the end of the study. Condition could, therefore, reduce the
future viability of the population in areas of encroaching urbanisation where turtles have to move to find wetlands

In freshwater turtles with chromosomal sex determination, such as *C. longicollis*, that have a sex ratio bias, it has often been attributed to differences in age of maturity between the sexes (Georges et al. 2006). Males typically mature earlier than females (Thompson 1983; 1993). Any assessment of sex ratio among adults would, therefore, include a wider age range of males than females and thus, if a sex bias was present, it would be predicted to be a male bias (Georges et al. 2006; Lovich and Gibbons 1990). Male *C. longicollis* do mature at a smaller size than females (Chessman 1978; Parmenter 1976), and thus a male sex bias may occur among adults; however, a female sex bias was observed.

Sampling throughout the year using the same sampling methods, presumably eliminated any sex ratio bias due to different behaviour of the sexes among seasons (Burgin et al. 1999). There is, therefore, no apparent reason that the bias in sex ratio was due to difference in size at maturity or sampling technique.

5.4.2. - Endocrine disruption

Endocrine disruption compounds (EDCs) found in air and water pollution in the past 30 years has been implicated in affecting the reproductive fitness of the reptile species *Pogona barbata* (Eastern Bearded Dragon) in the study area (Wotherspoon and Burgin in review), and EDCs have been found to have an effect on mosquito fish *Gambusia holbrooki* in some wetlands sampled within the study area (Norris and Burgin 2011a; b). Midgley (2010), however, found that there was a male sex ratio bias in the *G. holbrooki* population in four of the wetlands which were also used in the current study. These results were, therefore, not compatible with the influence of endocrine disruption on the *G. holbrooki* population.

Whilst EDCs have been found to have an effect on the reproductive fitness of the turtle species *Trachemys scripta* (Crain and Guillette 1998), there has been limited investigation of their effects on Australian freshwater turtles. Due to the high propensity for terrestrial migration of *C. longicollis*, and their use of several wetlands throughout their lifecycle, it is not expected that EDCs would have an effect in the
study area. While some individuals would be, at least, intermittently exposed to waters supplemented by tertiary treated sewage or stormwater runoff from an urban area, since there is no major industry in the area it is an unlikely scenario that EDCs are responsible for the lack of males in the population.

5.4.3. - Differential mortality
Kennett and Georges (1990) suggested that the female sex bias that they encountered during drought conditions resulted from male starvation due to severe food limitation. Over the same period that the female bias developed in the study area, there was an increase in the number of juveniles. Since females reduce, or even cease reproduction during times of hardship (Kennett and Georges 1990), it is assumed that food was not a limiting factor, and thus starvation was not an explanation for the lack of adult males in this study.

As previously indicated, hatchings of turtles with chromosomal sex determination typically have 1:1 male : female sex ratio (Georges et al. 2006). In the apparent absence of any published data to the contrary, it was assumed that there is not a difference in the mortality rate between the sexes of juvenile turtles. Based on the bias towards females in the current study, and the lack of larger males compared to females, there may have been differential mortality with higher mortality rate among males. However, there was no indication of substantial predation on adult turtles. Mortality is highest in *C. longicollis* when they are undertaking terrestrial movement (Rees et al. 2009; Roe and Georges 2007). In this study females undertook terrestrial movement more than males with no apparent difference in the overall distance travelled. Previous studies of *C. longicollis* have also reported a lack of sex bias in terrestrial movement (Kennett and Georges 1990; Roe et al. 2009; Roe and Georges 2007). The males were, therefore, presumably at no greater risk of predation than females while undertaking terrestrial activities. There is, therefore, no apparent reason that predation caused the female sex bias.

The number of injuries sustained by a segment of a population has been assumed to be indicative of differential predation in the population and, therefore, mortality rates among turtles (Rand 1954; Schoener 1979). If injuries are assumed to be correlated with death (Lovich and Gibbons 1990), since injury rate was observed to be
equivalent between males and females, predation could also be dismissed as a factor causing the observed sex bias. Another factor that indicates that predation rates had not substantially changed over the study period was that recruitment did not vary substantially. Therefore, there was no evidence of differential mortality found.

5.4.4. - Natal site-fidelity

After 13 years, only females were recaptured in the same wetland as they were captured initially. This may reflect natal site fidelity. Since for evolutionary success, females need to maximise recruitment to the population. Returning to the nesting area that resulted in successful recruitment could prove more beneficial than testing new nesting sites. As discussed previously, the most efficient method for males to increase their reproductive fitness is to maximise their opportunities to mate (Gibbons 1987). Moving among wetlands to seek females would potentially maximise opportunities to mate and maximise the potential for outbreeding. In the absence of attraction to the natal nesting site, they would not have the same drive to return to a specific wetland as females.

Marine turtles have been shown to have a strong natal site-fidelity, in which individuals travel hundreds of kilometres to feed and then return to their natal beach (nest emergence site) to nest (Allard et al. 1994; Hays et al. 2010; Poloczanska et al. 2009). Freshwater turtles have also been observed to have such nest site-fidelity (Congdon et al. 1983; Mitrus 2006; Tucker 2001). However, there are very limited published studies on natal site-fidelity, although the freshwater turtle species *Graptemys kohnii* have been shown to have very strong nest site-fidelity, which was associated with natal site-fidelity (Freedberg et al. 2005). The results of this study indicate that female *C. longicollis* tend to have natal site-fidelity, and this would provide an explanation for the disparity between male and female recapture rates.

5.4.5. - Increased ambient temperature

Sarre et al. (2004) suggested that the transition from genetic sex determination (GSD) to temperature sex determination (TSD) has occurred several times over the evolutionary history of reptiles. It has been suggested that for reptiles GSD and TSD are malleable and that for some species, genotype and environment interact to determine sexual outcomes. These transitions may result from increased or decreased
thermo-sensitivity (Sarre et al. 2011). Temperature has been shown to have an effect on sex determination in some GSD reptile species (Ezaz et al. 2009; Quinn et al. 2009; Radder et al. 2008), for example, nest temperature in the genetically sex determined turtle *Clemmys insculpta*, showed a ‘mild’ effect on sex determination. Nests that attained higher temperatures produced a higher percentage of males (Ewert and Nelson 1991). In contrast, at higher temperatures, the European pond turtle, *Emys orbicularis* (also genetically sex determined), produced more females hatchlings than males (Servan et al. 1989). This may, at least in part, provide an explanation for the apparent development of the female bias.

The current study was undertaken in open agricultural land with shallow wetlands. It has been hypothesised that the most recent evolutionary transition from TSD to GSD in the ancestral lineage of *C. longicollis* has occurred relatively recently (Ezaz et al. 2006). If *C. longicollis* is in transition between GSD and TSD, or if temperature was going to have an effect on sex determination, it is feasible that it is occurring in the study area where climatic extremes appear to be increasing, exacerbated by changes in the landscape since European farming practices were introduced that have resulted in open agricultural fields (rather than woodland habitat), and shallow wetlands within this landscape. Spring and summer temperatures, the time of reproduction, are typically hotter in the study area than elsewhere in the Sydney Region by 2-4 °C (Steffen and Hughes 2012). The wetlands sampled were uniformly shallow, and typically in a cleared agricultural landscape. Females undergoing terrestrial migration typically traverse these agricultural lands. These turtles also nest in open woodland habitat (Spencer and Thompson 2003), and in more open areas on the banks of the wetlands (pers. obs.). Under such conditions, solar radiation is expected to be greater than commonly encountered by the population pre-European intervention, even without climate change. Under such circumstances, nest temperatures may be elevated (Janzen 1994). The hatchlings in this study may, therefore, have been affected by increases in ambient temperature (Macey 2008), below average rainfall (Sydney Water 2009), and many days of ‘highest temperatures on record’ (BoM 2006), which resulted in the shift in sex ratio bias towards females.
Without any land use change in the immediate area for decades, in one decade there were a number of changes in the turtle population. It was deduced that increased predation, a lack of difference in growth rate, differential mortality, and sampling bias, could not account for these population changes. Females recaptured after more than a decade from the same wetland were in better condition than turtles that were known to migrate. If it is assumed that those turtles that were not previously captured had dispersed, this would indicate that turtles that do not undertake terrestrial migration are in better condition than those that move. This presumably would influence reproductive fitness of non-migrating females.

Between the sampling periods, there were unusually hot and dry conditions in the study area. The differences in the population structure may, therefore, be due to increased ambient temperature. The results indicate that in the population of *C. longicollis* in this study show that some individuals have site fidelity. Bull and Charnov (1989) found that natal site fidelity and TSD will bias sex ratios towards females in reptiles. Such a change in sex ratio is likely to be exacerbated by further climate change. If so, this widespread and common species may be impacted, at least on the extremes of its range. The current population of turtles, some of which are estimated to be over 60 years of age, even without consideration of non-growth periods, may have already dealt with major changes in the landscape followed by a long period without substantial change. Extremes in climate (temperature and rainfall) have increased, turtles may, therefore, at least during times of extreme climatic conditions, be exposed to physiological stress (Mahmoud and Licht 1997).
Chapter 6 – Death of *Chelodina longicollis* on roadways in peri-urban Northwest Sydney.

A juvenile *Chelodina longicollis* undertaking terrestrial migration in peri-urban Sydney.
6.1 - Introduction

With the world’s human population continuously increasing, infrastructure to service this growing population, including road networks, is also expanding rapidly. Typically these ‘new’ roads increasingly carry greater volumes of traffic. Australia is tracking the world’s population growth, and road creation/upgrading trajectory. Much of this growth is occurring at the peri-urban fringe of cities. In Australia’s largest city, Sydney, much of the predicted growth will be in the Northwest peri-urban sector where there is already a rapidly growing human population, and an expanding network of roadways to accommodate the needs of this expanding population (NSW Department of Premier and Cabinet 2012). Such roadways often traverse native fauna habitat, and animal mortality from vehicle collision is a common daily occurrence (Klocher et al. 2006). While many Australian studies on vehicle collision involving wildlife have focused on large vertebrates (Jones 2000; Klocher et al. 2006; Lee et al. 2004; Taylor and Goldingay 2004), there are limited published studies that have investigated reptile species and how their populations are impacted by roadways (Wotherspoon and Burgin 2011).

Wetland reptiles, particularly freshwater turtles, are most vulnerable to vehicle collision on roadways when they undertake terrestrial migration during dispersal, seasonal movements associated with reproduction (e.g., seeking mates, nesting), opportunistic movements to exploit recent flooded areas, and to escape wetland drying (Roe and Georges 2008b; Steen et al. 2006). The propensity of some species of freshwater turtles, for example, the long-necked turtle *Chelodina longicollis*, to undertake terrestrial migration exposes individuals to hazards in the terrestrial environment, including vehicle collision. *Chelodina longicollis*, the most terrestrial of the Australian freshwater turtles, is presumably the most vulnerable of the native turtles.

Individuals of the species move within a network of wetlands and thus use the terrestrial buffer zones within their range, and travel corridors between wetlands (Roe and Georges 2007). They navigate through their home range, moving effectively in a straight line towards their destination. They tend to not deviate, even when an
obstacle is placed in their migration path (Stott 1987). Where the turtle’s travel corridor includes a roadway, unless there is a barrier, the turtle will navigate across it.

While hatchling and juvenile freshwater turtles are vulnerable to predation (e.g., predatory birds, fish, canines), adult turtles have few predators. In the terrestrial environment, therefore, road mortality is probably their greatest threat. This is exacerbated by attributes of their life history that include longevity, low recruitment, and delayed sexual maturity (Beaudry et al. 2008). Road mortality can, therefore, affect the demography and the dynamics of a population (Aresco 2005a; Steen et al. 2006), potentially greatly influencing future viability of the population. To date there has been no published research on Australian freshwater turtles that has investigated the impact of vehicle collision and considered this in the context of the long-term viability of a population. This study looked at the impact that vehicle collision may have on *Chelodina longicollis* in one of the most rapidly expanding peri-urban environments in Australia, Northwestern Sydney.

6.1.1 - Study area
Northwest Sydney (New South Wales [NSW], Australia) is one of the most rapidly urbanising areas of Australia. In 2011, the human population was slightly over 2 million, which was a 1.6% increase over the previous year. The population is forecast to reach 2.9 million by 2036 (DPC 2012). To service its growing population, Western Sydney already has a large road network that spans arterial roads, which carry approximately 35,000 cars/day to local roads that have a volume of less than 50 cars/day. The speed on these roadways varies between 40 km/hour on some local roads and 110 km/hour on arterial roads (Roads and Maritime Service 2012).

6.1.2 – Climatic conditions
The temperature during the study period, and including one year before (2006-2011) the study commenced, is presented in Figure 6.1. The mean maximum monthly temperature in Western Sydney varied between 22.8°C and 24.7°C.
Figure 6.1 – The mean maximum temperature (°C) in peri-urban Northwest Sydney between 2006 – 2011 (Bureau of Meteorology 2013).

Total rainfall varied substantially during 2006-2011. Most rain fell in 2007 while 2006 was the driest year (Figure 6.2).

Figure 6.2 – Rainfall (mm) for the years 2006 – 2011 in peri-urban Northwest Sydney (Bureau of Meteorology 2013).
6.2 – Methods

6.2.1 - Turtle collection

Turtles that had suffered vehicle collision, or were observed crossing roadways were collected from the roads of Northwestern Sydney in an area that incorporated the peri-urban study site (see Figure 6.3). An attempt was made to ensure that different road types were sampled with the same intensity, rather than systematically sampling predetermined sections of roads of local arterial, distributor, and local roads. Instead, to maximise the sample size, turtles were intensively collected opportunistically between September 2007 and November 2009 and on an *ad hoc* basis in 2010-2011.

![Figure 6.3 – Peri-urban study area in Northwest Sydney (New South Wales, Australia) highlighted in pink (Source: maps.google.com).](image)

When a turtle was encountered on a roadway, their location was recorded on a Global Positioning System (GPS, Garmin GPSMAP 76CSx), and the animal was placed in a sealed bag, returned to the laboratory, and stored at -17°C until processing. Turtles were then removed from the freezer and any morphometric details that could be retrieved were recorded.

Since turtles collected varied between decomposing individuals with soft tissue previously scavenged by predators to freshly killed, intact animals, the information obtained from animals varied greatly. For example, in some individuals the sex could
not be determined and/or carapace length accurately measured. For those individuals where it was realistic to determine carapace length, curved carapace length ±0.1 mm was measured. When sex could be determined it was by viewing the reproductive organs. Turtles with a carapace length of less than 14.5 cm were classified as juveniles (Roe and Georges 2008a).

6.2.2 - Data Analysis
Because it could not be quantified, it was assumed that turtles collected from roads had travelled from, or were moving to the closest wetland. The distance to the nearest wetland (straight line) provided the minimum distance a turtle would have travelled from the last wetland it had visited. To determine this distance the GIS (Graphical Information System) program ‘ArcMap 10’ was used.

The rainfall measure that was used in this study was rainfall in the previous 24 hours to collection, as studies have shown that most animal carcasses remained on the road for 24 hours or less (Santos et al. 2011). For temperature, the maximum daily temperature was used, as preliminary research had indicated that more turtles would be captured in spring and summer and, therefore, more likely affected by maximum than minimum temperature.

Statistical analyses were performed using SPSS (version 20). To examine the differences between environmental variables, a multivariate analysis of variance (MANOVA) was used with distance from nearest wetland, rainfall, and maximum temperature used as dependent variables while moon phase, road type and season were used as independent variables. When variables were not shown to have a significant effect on turtle distribution, a series of Mann-Whitney U tests, One-Way Analysis of Variance (ANOVA), and regression analyses were used as appropriate to the data.

To investigate turtle sex ratio bias among seasons, and the effect of moon phase on numbers of turtles collected, Chi Square Goodness of Fit Analysis was conducted. A Paired Sample t-Test was used to investigate male and female size.
For analysis of distance to the nearest wetland of juveniles, males and females a series of Mann-Whitney U tests were used. An ANOVA was used to investigate distance from nearest wetland, and vehicle collision and moon phase.

A regression analysis was undertaken to investigate the relationship between carapace length and distance to nearest wetland; rainfall and the number of turtles collected; the distance to nearest wetland and rainfall; maximum daily temperature and the number of turtles collected; and the maximum daily temperature and the distance from the nearest wetland. Statistical significance was accepted at the $\alpha \leq 0.05$.

6.3 - Results

Between 2007 and 2009, a total of 120 (119 *Chelodina longicollis* and one *Emydura macquarii*) freshwater turtles were collected from roads in peri-urban Western Sydney. Sixteen of these turtles were alive and released in a safe location, and 104 were found dead or badly injured as a result of vehicle collision and those that were badly injured were euthanized.

Of these turtles, 52 could be sexed - 10 juveniles, 20 females, and 22 males. There was no sex ratio bias in the adults of this sample ($\chi^2 = 0.095, p = 0.7576$). There was a significant difference in carapace length between males and females. Females had a mean longer carapace length than males ($t_{36} = 3.5301, p = 0.001$) (Table 6.1).

Table 6.1 – The carapace length of *Chelodina longicollis* collected on roads in peri-urban Northwest Sydney.

<table>
<thead>
<tr>
<th></th>
<th>mean carapace length (cm) (SD)</th>
<th>minimum carapace length (cm)</th>
<th>median</th>
<th>maximum carapace length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juvenile</td>
<td>12.59 (1.4)</td>
<td>9.8</td>
<td>12.6</td>
<td>14.3</td>
</tr>
<tr>
<td>Female</td>
<td>19.88 (1.08)</td>
<td>17.5</td>
<td>20.2</td>
<td>21.3</td>
</tr>
<tr>
<td>Male</td>
<td>17.66 (2.4)</td>
<td>14.5</td>
<td>17.2</td>
<td>24.3</td>
</tr>
</tbody>
</table>

Overall, the mean distance that a turtle was collected from the nearest wetland was 152.3 m (SD = 153.86). There was no significant difference in the distance from
nearest wetland for juveniles, males and females (Table 6.2); however, the maximum distance a juvenile travelled from a wetland was further than the maximum distance travelled by males (Table 6.3). There was no relationship between carapace length and the distance to nearest wetland ($R^2=0.002$).

Table 6.2 – The Mann-Whitney U test results on the distance from road to nearest wetland that *Chelodina longicollis* were collected in peri-urban Northwest Sydney.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mann-Whitney U tests (U)</th>
<th>Significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juvenile - Female</td>
<td>92.000</td>
<td>0.746</td>
</tr>
<tr>
<td>Juvenile - Male</td>
<td>94.000</td>
<td>0.535</td>
</tr>
<tr>
<td>Female - Male</td>
<td>144.5</td>
<td>0.057</td>
</tr>
</tbody>
</table>

Table 6.3 – The distance from road to nearest wetland that *Chelodina longicollis* were collected in peri-urban Northwest Sydney

<table>
<thead>
<tr>
<th>Group</th>
<th>mean distance (meters (m)) (SD)</th>
<th>minimum distance (m)</th>
<th>median distance (m)</th>
<th>maximum distance (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juvenile</td>
<td>228.7 (249.8)</td>
<td>18.0</td>
<td>119.0</td>
<td>773.0</td>
</tr>
<tr>
<td>Female</td>
<td>183.3 (168.0)</td>
<td>19.0</td>
<td>141.0</td>
<td>837.0</td>
</tr>
<tr>
<td>Male</td>
<td>112.1 (66.0)</td>
<td>14.0</td>
<td>108.0</td>
<td>304.0</td>
</tr>
</tbody>
</table>

Most turtles were collected in spring (n=81) while the numbers obtained in summer were less than half (n=34), and there were only five turtles collected in the cooler months across autumn and winter combined (Figure 6.4).
Figure 6.4 – The number of *Chelodina longicollis* collected from roadways in peri-urban Northwest Sydney by season.

There was no sex ratio bias due to season, spring ($\chi^2_1 = 1.38, p>0.05$) and summer ($\chi^2_1 = 1.33, p>0.05$); however, there were four times as many females captured in spring than in summer (Figure 6.5).

Figure 6.5 – The number of *Chelodina longicollis* juveniles, females and males captured in each season in peri-urban Northwest Sydney.

There was no significant relationship between rainfall in the previous 24 hours and the number of turtles collected on roadways ($R^2 = 0.00926$) (Figure 6.6), or between male and female movement due to rainfall in the previous 24 hours ($F_{2,48} = 0.32, p =$
There was also no relationship between the distances from nearest wetland from where an animal was collected, and the rainfall in the previous 24 hours for adults ($R^2 = 0.004$) or juveniles ($R^2 = 0.22$) (Figure 6.7).

Figure 6.6 – The number of *Chelodina longicollis* collected on roads and the amount of rainfall in the previous 24 hours in peri-urban Northwest Sydney.

Figure 6.7 – The distance *Chelodina longicollis* were collected from the nearest wetlands and the amount of rainfall in the previous 24 hours in peri-urban Northwest Sydney.
There was not a statistically significant relationship between temperature and the number of turtles collected ($R^2 = 0.049$); however, more turtles were observed on roads when temperature was between 20°C and 30°C (Figure 6.8). There was no relationship between temperature and the distance to nearest wetland $R^2 = 0.00369$ (Figure 6.9).

Figure 6.8 - The number of *Chelodina longicollis* collected and the maximum temperature reached that day in peri-urban Northwest Sydney.

Figure 6.9 - The distance *Chelodina longicollis* travelled from wetlands and the maximum temperature reached that day in peri-urban Northwest Sydney.
Although most turtles were collected from roadways during the ‘new moon’ phase there was no significant difference in numbers of turtles and moon phase ($\chi^2 = 2.63$, $p>0.05$) (Figure 6.10). There was also no significant difference in the distance from the closest wetland and moon phase ($F_{3, 115} = 0.29$, $p = 0.8$).

Figure 6.10 – The number of *Chelodina longicollis* collected on roadways in each moon phase in peri-urban Northwest Sydney.

There was not a significant difference in the number of turtles collected from arterial roads (major connectors between freeways, highways, major centres), distributor roads (major traffic movement between roads of higher order or local streets), and local roads (sealed or improved unsealed road providing property access) ($\chi^2 = 3.65$, $p = 0.161$). However, arterial roads had a higher number of turtles collected (53) than distributor roads (34) and local roads (30). Only one turtle was collected from each of the other areas searched (motorway, track, path).

**6.4 - Discussion**

In contrast to many other studies, there were equivalent numbers of male and female *Chelodina longicollis* collected from the roads in peri-urban Northwestern Sydney. Particularly in North America (e.g., *Chrysemys picta* - (Marchand and Litvaitis 2004); *Chelydra serpentina* - (Steen et al. 2006); *Emydoidea blandingii* - (McGuire et al. 2013), female turtles make up a higher percentage of roadkill than males. This is assumed to be because of their need to leave the water to nest, and thus more female than male turtles are active in undertaking terrestrial movement.
However, whilst in this study equivalent numbers of males and females were collected, females tended to be collected further from wetlands than males. This indicated that they undertook migration over longer distances. No such gender difference has previously been observed in *C. longicollis* during terrestrial movement (Kennett and Georges 1990; Roe et al. 2009; Roe and Georges 2007). For example, based on radio-telemetry tracking of *C. longicollis*, previous researchers (Roe et al. 2009; Roe and Georges 2008b) found no gender difference in the terrestrial movement.

Movement of *C. longicollis* among wetlands has been demonstrated to be non-random. Turtles orientate in the direction of their intended destination, and move effectively in a straight line between wetlands (Graham et al. 1996; Stott 1987) or, presumably from the wetland to the nesting site when the female leaves the water to lay her eggs. The average distance from the nearest wetland that *C. longicollis* were collected was similar to that observed by previous researchers; however, these researchers were tracking animals using tracking devices (radio or spool) and so knew were the animal originated from, the route taken, and the exact distance that individual turtles had travelled (Roe et al. 2009; Stott 1987). In this study; however, the minimum distance a turtle could have travelled was based on the distance it was found from the nearest wetland. If the turtle was not travelling to the wetland nearest to its point of collection, the distance travelled by *C. longicollis* in the peri-urban study site, is further than previous estimates and, therefore, the turtles in this landscape are assumed to be moving much further than previously reported in this species.

This peri-urban area would currently have many more wetlands (usually in the form of farm dams which *C. longicollis* inhabit) than at European settlement. This is because much of the landscape has been cleared for agriculture and, in the process farm dams were created to water stock. Turtles would not, therefore, be travelling further distances between wetlands than they would in areas that had not been cleared for agriculture and lacked the extensive farm dam network. However, with the increasing human population in the study area, and associated infrastructure that includes barriers to movement (e.g., fences, buildings, steeper engineered slopes)
turtles may become ‘lost’ more frequently in this peri-urban landscape than in more natural settings because of changes to cues for navigation. They may thus spend more time in terrestrial navigation between destinations in the current study area, as opposed to nature reserves or rural land where other studies have been undertaken. Although there does not appear to have been a previous comparison between an urbanising landscape and natural areas, the observations of Limpus et al. (2003) on the marine turtle, *Chelonia mydas*, on Raine Island in Queensland (Australia) demonstrates that changes in landscape may result in individuals orientation becoming disrupted. This may result in animals moving greater distances than when they have clear site-lines. The elevation and profile of the Raine Island beach platform can cause females to become disoriented and wander, sometimes, the entire length of the island beach before they either find a cue that orientates them to the sea, or they become dehydrated and die. Barriers built between the wetland a turtle is navigating from and their destination would be expected to result in such disorientation among *C. longicollis* in an urbanising landscape.

Roe et al. (2009) observed that larger turtles moved further than smaller *C. longicollis*. In the current study there was no relationship between carapace length and the distance from the nearest wetland where the turtle was collected. It was observed; however, that juveniles tended to travel further than adult males. They were encountered over 800 m from the nearest wetland, and thus would have needed to travel a minimum 1.6 km overland even to move to the site of vehicle collision and back to the same wetland. It is, therefore, likely that they moved much farther than previously recorded. In the current study area, juveniles may be moving greater distances and dispersing further. This could result in them being more vulnerable to vehicle collision than larger animals because their small body size would make them more difficult to be observed by the driver and thus increase their chance of being involved in a collision with a vehicle.

The impact on juveniles may also be greater than observed through collection of turtles on roads. This is because their smaller body size would make them more vulnerable to consumption by scavengers than larger animals with, by comparison, more impenetrable shells. As a consequence, the larger animals are more likely to have any exposed softer tissue of their anatomy removed and the shell remains left on
the road. The chance of finding the carcases of a juvenile on a road is, therefore, less likely than for adults (Janzén et al. 2000a). It is assumed, therefore, that determining numbers of turtle deaths by collecting vehicle collision victims would provide an underestimate of juvenile death due to such collisions.

More *C. longicollis* females were collected from roads in spring than in summer when the numbers were approximately 25% of those found in spring. In this species reproductive activity commences with mating early in spring, and females nest in late spring and/or early summer. Some individuals also double clutch (Kennett et al. 2009). While the loss of turtles to vehicle collision at any phase of the lifecycle has longer-term implications for viability of the population, the loss of gravid females migrating to their nest site would have a direct affect on recruitment. Some females that were captured in spring were found in a condition that allowed for observation of the oviducts and determination of whether eggs were present. In addition to the loss of the individual reproductively active females to the population, a female carrying eggs would not contribute further offspring to the population.

A study of *C. longicollis* in urban Sydney (Burgin 2006) showed that *C. longicollis* clutch size was 6-14 eggs. In inland areas of Eastern Australia, females have been found to carry up to 23 eggs per clutch (Kennett et al. 2009). Based on the observation that there was no gender bias in vehicle collision deaths, that clutch size varied between 6 and 23, and assuming that the four females collected in summer were seeking to lay a second clutch; the numbers of known females killed in spring was 16 and in summer 4, the potential loss of offspring was between 330 and 1,219, in addition to the 55 females collected.

The ‘reproductive strategies hypothesis’ (Morreale et al. 1984) proposes that differential movements and activity levels between the sexes are different due to reproductive strategies modifying movement and activity. For freshwater turtles this hypothesis predicts that males are more active and travel further distances during the breeding season than females to maximise the number of encounters with females. The increase in female activity during nesting season is due to movement to nesting sites. Whilst this has been demonstrated in some species (e.g., *Emydoidea blandingii*, (Millar and Blouin-Demers 2011) this was not observed in this study. Females did
move more in the nesting season than otherwise; however, there was no indication that more males moved during breeding season (early spring). *Chelodina longicollis*, therefore, do not appear to comply with the hypothesis.

It is a common perception that turtles are more active in association with rain (Burke et al. 1994). If this ‘urban myth’ were correct; then provided other factors were equal, a large proportion of *C. longicollis* would be expected to be collected in association with rain. In addition, with the associated lowered visibility that occurs, at least, with some rainfall events, vehicle collision would be expected to be greater during rainfall than otherwise. The observation that rainfall was not associated with an increase in the number of *C. longicollis* vehicle collisions was, therefore, counter intuitive. It was also observed that turtles, that were a victim of vehicle collision, were found in closer proximity to a wetland during heavy rain than during other climatic conditions. The observation that they are not more active at such times supports the findings of Graham et al. (1996) that *C. longicollis* navigate by sight. Turtles may, therefore, avoid travel during periods of heavy rainfall because they have greater difficulty navigating under such conditions than in fine weather. The ability of *C. longicollis* to undertake overland migration during fine conditions, and not be dependent on wet periods, would be enhanced by their ability to store water in their cloacal bursae and thus slow the rate of desiccation compared to other species of Australian freshwater turtles (Chessman 1984a).

Most turtles were collected on roadways during the new moon phase - the phase in which least light is radiated (Jensen and Das 2008). In contrast, Graham et al. (1996) reported that *C. longicollis* moved under sunny conditions, and in this study it was observed that they did not move during periods of heavy rainfall (when essentially there is less light). In Borneo, Jensen and Das (2008) observed that freshwater turtles (*Amyda cartilaginea, Heosemys spinosa, Cuora amboinensis, Cyclemya dentate*) were captured most frequently during the full moon phase – the period when the moon throws most light. The turtles in the current study, therefore, did not show the same pattern of movement as observed in other moon phase studies. However, since the study population of *C. longicollis* have been exposed to anthropogenic light from the urban surroundings for decades, they may take advantage of the local light pollution, and have thus become less reliant on natural light than populations away from the
influences of anthropogenic light. As a consequence, they may move more in the moon phases where there is low light simply because they have anthropogenic light to support navigation during their overland migration (Longcore and Rich 2004).

Predator avoidance has been shown to be a reason for animals moving less during a full moon. This was found to occur in the Australian species of file snake Acrochordus arafurae (Houston and Shine 1994). File snakes were observed to move less during the full moon than at other moon phases and it was assumed that this was to avoid predators. However, another strategy to reduce predation may be for individuals of a population not to concentrate movement into a specific timeframe. Turtles that do not move at a specific moon phase but rather move more unpredictably, may reduce predation pressure on the population overall. With this strategy, fewer turtles would be moving at any particular time with more sporadic movement and, when numbers get below some specific level, it becomes unproductive for the predator to continue focusing on that species (Ringelman 2014).

Overall, turtles were more likely to have been involved in traffic collision when mean maximum daily temperature was between 20-30°C. Graham et al. (1996) reported that C. longicollis moved under sunny conditions, which are most common in Spring and Summer when there is the most movement; however, the ‘optimum’ temperature range for C. longicollis to undertake terrestrial migration was between 20-30°C. The mean maximum daily temperature for spring and summer for the years 2007 and 2008 were in the mid-twenties (Bureau of Meteorology 2013). However, these results suggest that when temperatures are beyond that range, fewer turtles undertake terrestrial migration. In contrast to the findings of this study, Roe and Georges (2008b) did not observe that there was a relationship between movement and temperature in C. longicollis. Likewise, a population of painted turtles Chrysemys picta marginata in Michigan (US) did not appear to have an optimum temperature for terrestrial movement. Ambient temperature did not affect movement (Rowe 2003). Since the population of C. longicollis sampled in this study rely on wetlands in an open agricultural landscape with limited cover, the extremes of temperature recorded may be much higher in these habitats than the recorded ambient temperature and, therefore, higher temperatures may be experienced during migration than the metrological station broadcasted. The need to move through open spaces, exposed to
the sun, may be a factor in inhibiting movement of the study population at high temperatures.

In the current study more turtles suffered vehicle collision while crossing arterial roads than other road types. These roads have the highest traffic volumes and speed limits, and they have more lanes than other roads in the Region. At these speeds there would be less opportunity for drivers to see a turtle and take evasive action to avoid hitting the animal or, as has been observed locally, to stop and remove the turtle from the road.

Aresco (2005b) estimated that 98% of turtles that attempted to cross a four-lane highway would be unsuccessful. He found that 95% that had suffered vehicle collision were within the outside lane on a four-lane highway. The remaining 5% were found in the second lane from the side of the road. This indicates that turtles would have difficulty surviving migration across a four lane highway. With the growth of urbanisation in peri-urban Sydney, it is expected that there will be further development of arterial roads in the near future due to the continual urban development within the area (NSW Department of Premier and Cabinet 2012), in turn, this is expected to have a greater impact on the local population of *C. longicollis*.

*Chelodina longicollis* have a high propensity for terrestrial movement. In their habitat in peri-urban Northwestern Sydney, continual changes to the landscape have been occurring due to human population growth and associated urbanisation, which has resulted in loss of agriculture and, therefore, wetland habitat for *C. longicollis*. As a result, these turtles will be required to travel further, and deal with more vehicles during their movement than they have had to do in the past. This will make them increasingly susceptible to vehicle collision. However, vehicle collision is impacting on all stages of the population’s lifecycle and a long-lived species with delayed sexual maturity, such as *C. longicollis*, are particularly vulnerable to increases in adult mortality (Steen et al. 2006).

The majority of females were killed in spring, which has the potential to reduce the reproductive potential of this species, by lowering recruitment to the population. In just this one study, over a 26 month period, in a sample of turtles killed on the road
from just one area in extensive distribution of *C. longicollis*, showed that there was a potential loss of offspring of over 1000 turtles. This does not take into account the potential contribution that these offspring may have made to the adult population of future generations.
Chapter 7 – Comparison of pressures on urban, suburban and peri-urban *Chelodina longicollis* populations.

*Trachemys scripta elegans* captured during sampling at Eagle Vale, Sydney, Australia.
7.1 – Introduction

In parallel with the continuous increase in the human population worldwide, urbanisation has increased, together with increased infrastructure and other forms of anthropogenic land use change to support urbanisation. The associated anthropogenic modifications have resulted in changes to natural habitat. In Australia, since European settlement, there has been ‘huge’ growth in the human population, and associated demand for housing and infrastructure that has led to the modification, isolation, degradation, and removal of native habitat (Forrester and Machlis 1996; Hilty et al. 2006). Sydney, the city with the largest population in Australia, has undergone substantial environmental modification and currently has a variety of urban, suburban and peri-urban areas (NSW Department of Premier and Cabinet 2012).

Urban and suburban wildlife have different pressures or, at least, a different intensity of pressures on wildlife compared to wildlife in peri-urban areas, which include loss (Conner et al. 2005) and fragmentation of habitat (Baldwin et al. 2004; Hitchen et al. 2011a; Marchand and Litvaitis 2004), introduced barriers to movement such as roadways (Aresco 2005a), competition from exotic fauna (Lowe et al. 2000; McKinney 2006; Perez-Santigosa et al. 2008), anthropogenic light (Perry et al. 2008; Vasquez 1994), supplementary feeding (O'Leary and Jones 2006; Robb et al. 2008) and an increase/change in predation (Baldwin et al. 2004; Marchand and Litvaitis 2004). These pressures have had, and typically continue to have, an impact on native populations in urban, suburban and peri-urban areas.

The ability of a population to persist in urbanised environments depends, in part, on their tolerance of habitat change, and the ability to adapt to these changing environments (Rees et al. 2009). Whilst these pressures affect all fauna, freshwater turtles, such as *Chelodina longicollis*, which are the most terrestrial of the Australian freshwater turtles, are one group that are impacted by both changes to the terrestrial and aquatic environment. The life history traits of freshwater turtles, such as delayed sexual maturity and limited fecundity makes them vulnerable to increased mortality caused by changes associated with human habitation (Baldwin et al. 2004; Marchand and Litvaitis 2004).
In Sydney’s urban, suburban, and peri-urban areas, wetlands, once connected by drainage lines or chain-of-ponds may have been modified or removed with many of the remaining wetlands now isolated, sometimes with kilometres of urbanisation between individual wetlands. Animals in smaller, more isolated remnants of habitat are typically most vulnerable. For example, Burgin and Wotherspoon (2009) found that in a small (< 2.5 ha) remnant of highly fragmented natural vegetation in urban Sydney there was only 21% of the predicted herpetofaunal diversity on site. Whilst urban wetlands may provide a high quality habitat refuges for freshwater turtles (Roe et al. 2011), the pressures placed on these populations can affect the population dynamics and the future viability of these populations (Conner et al. 2005; De Lathouder et al. 2009; Souza and Abe 2000).

Whilst previous research on *C. longicollis* has been undertaken in urban (Burgin 2006; Burgin and Ryan 2008; Robey et al. 2011), suburban (Rees et al. 2009; Roe et al. 2011), and peri-urban areas (Burgin et al. 1999; Ryan and Burgin 2007), there has been no published studies that have compared population attributes of any Australian freshwater turtle species in these areas of different human population densities.

In this chapter, three areas of Sydney, one within the Central Business District (CBD) of the city and completely surrounded by urbanisation, a suburban area which by comparison to the urban area is less densely populated and typically has free-standing homes with gardens, and a peri-urban site within an agricultural landscape will be compared.

### 7.1.2 - Study Site

Across the Sydney Basin, three sites were sampled, one urban site (Centennial Park), one suburban site (Eagle Vale), and one peri-urban site (Richmond) (Figure 7.1).
Figure 7.1 – The Sydney Basin and the location of the three sample sites for freshwater turtles, Centennial Park, Eagle Vale and Richmond and the Sydney Central Business District (Source: maps.google.com).

Centennial Park (the urban site) is located 4.0 kilometres southeast from the centre of the city, within the Central Business District (CBD) of Sydney. It is an urban park that occupies 220 ha and contains seven wetlands. Prior to European settlement, the area had a drainage line, Lachlan Swamp, running through it. During the 1900s this drainage line was modified into seven discrete wetlands.

The Park is a popular recreational area, with walkways, sporting fields, and picnic areas which attract more than 20 million visits annually (Centennial Park and Moore Park Trust 2011) (Figure 7.2). The nearest wetlands to Centennial Park are 2.5 km away in Eastlakes Golf Course through the heavily populated CBD of Sydney with an extensive network of roadways, and heavy traffic (Cann 1998).
Eagle Vale (the suburban site) is located 56 km southwest of the Sydney CBD. This isolated suburban wetland is located in a park behind a ‘village’ shopping centre and library. This wetland was created in the 1970s when the suburb was first developed. The closest wetland is located 1.6 km away, across a suburban landscape. The wetland has walking tracks around it, and the park surrounding the wetland is a popular recreation area (Figure 7.3).
Richmond (the peri-urban site) is located 50 km northwest from Sydney’s CBD (Figure 7.4). The wetlands included in this study are in peri urban (the interface between urban and rural) agricultural lands of the University of Western Sydney. Land use adjacent to the wetlands sampled had been used for agricultural teaching purposes for over 120 years. At early European settlement the land was cleared to create fields for grazing stock and cropping. The dams sampled were constructed to water grazing stock. Subsequently, there has been limited change in the landscape (Burgin 2010).

Figure 7.4 – Richmond (peri-urban site), New South Wales, Australia (Source: maps.google.com).

7.1.3 - Study Species

*Chelodina longicollis* (long-necked turtle) is the most widespread Australian freshwater turtle, with a distribution within continental Australia from Eastern Queensland to South-eastern South Australia (Kennett et al. 2009). It inhabits a variety of water bodies including rivers, lakes, ephemeral wetlands, farm dams (*cf.* ponds), and even degraded urban and peri-urban water bodies (Burgin and Ryan 2008; Cann 1998; Chessman 1988a; Rees et al. 2009). Estimates of this species’
density in impoundments range from 236 ha\(^{-1}\) (Chessman 1978) to 400 ha\(^{-1}\) (Parmenter 1976).

In addition to having the most extensive range of any native Australian turtle, \(C.\ longicollis\) is also the most terrestrial, and may undertake overland migration at all stages of its lifecycle. Some individuals may move among several wetlands within a single year (Roe and Georges 2007; 2008a). The species’ ability to efficiently migrate is supported by the low rate of evaporative water loss, and thus reduce the chance of desiccation during terrestrial activities (Chessman 1984a). Individuals are, therefore, readily able to migrate between wetlands, for example, for a range of reasons including to avoid competition with fish and other turtles (Kennett and Georges 1990). Their diet primarily consists of aquatic macro-invertebrates and carrion (Chessman 1984b). For a fuller species description see Chapter 2

\(Emydura\ macquarii\) (Murray River turtle) is a short neck turtle species with a distribution that extends throughout Southeast Australia, and is particularly well established in the Murray-Darling Basin and its drainages (Judge 2001; Thompson 1993). There are a number of recognised sub-species of \(E.\ macquarii\). The subspecies recognised include \(Emydura\ macquarii\ dharra\) (Macleay River turtle), \(Emydura\ macquarii\ gunabarra\) (Hunter River turtle) and \(Emydura\ macquarii\ dharuk\) (Sydney Basin turtle) (Cann 1998). The preferred habitat of \(E.\ macquarii\) are large permanent deep flowing waters, that is rivers and their major drainages (Chessman 1988a). Compared to \(C.\ longicollis\), \(E.\ macquarii\) become dehydrated more rapidly out of water and, therefore, migrate less efficiently. This species is omnivorous, commonly consuming fish, carrion, aquatic plants, and filamentous algae (Spencer et al. 1998).

The onset of sexual maturity in \(C.\ longicollis\) and \(E.\ macquarii\) is dependent on size and not age. Larger females commonly have a larger clutch size (Kennett et al. 2009). Both species nest in late spring and early summer; however, \(E.\ macquarii\) often have more than one clutch annually (Judge 2001; Thompson 1983) whereas \(C.\ longicollis\) more commonly produce a single clutch, and double clutch less frequently than \(E.\ macquarii\). Hatchlings may be as small as 2.1 cm (\(C.\ longicollis\)) (Burgin and Dalem 1996) and 1.9 cm (\(E.\ macquarii\)) carapace length (Cann 1998), and adults may grow to 28.0 cm (\(C.\ longicollis\)) (Chessman 1978), and 31.2 cm (\(E.\ macquarii\)) (Thompson 1999).
Hatchlings of both species tend to grow rapidly during their first year; however, growth rate typically slows beyond this period, and is largely influenced by food availability (Kennett and Georges 1990). Both species are long lived (Cann 1998).

7.2 – Methods

7.2.1 - Turtle Collection

Turtles were collected across three sites in the Sydney Region (table 7.1). For this study it was not possible to find equivalent replicate sites. Sampling was, therefore, restricted to three sites, but where practical multiple wetlands were sampled. In the urban site (Centennial Park) four wetlands were sampled. One wetland was sampled in the suburban area; and in the peri-urban site 12 wetlands were sampled. However data was analysed by site.

Due to large differences in the size of these wetlands, particularly among wetlands in the urban area, the surface area was measured as a surrogate for wetland size.

Table 7.1 – The sampling frequency and methods used to trap turtles in three sites across Sydney, urban (Centennial Park), suburban (Eagle Vale) and peri-urban (Richmond).

<table>
<thead>
<tr>
<th>Site</th>
<th>Month and year</th>
<th>Number of wetlands sampled</th>
<th>Number of fyke nets per wetland</th>
<th>Number of cathedral traps per wetland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban</td>
<td>1 – 5 December 2009</td>
<td>4</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Suburban</td>
<td>October and November 2011</td>
<td>1</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Peri-urban</td>
<td>October and November 2007</td>
<td>12</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Whilst turtles were sampled in different years, sampling was carried out in the same season which was expected to eliminate sex ratio bias due to any seasonal differences in catchability (Burgin et al. 1999).
The intensity of sampling also differed, although the sampling method did not. Fyke nets and cathedral traps were used to capture turtles (Table 7.1). The capture rate of both *E. macquarii* and *C. longicollis* between fyke nets and cathedral traps is equivalent (Burgin 2006; De Lathouder et al. 2009). On each morning of sampling, turtles were removed from the nets and measured (curved carapace length, ±0.1 mm), weighed (±0.1g) using a portable field balance (Sartorius portable scales (A&D EK-1200i)), and sex was determined based on whether the plastron was concave (male) or convex (female) (Chessman 1978; Kennett and Georges 1990). Turtles were uniquely marked, processed, and released at their point of capture. Turtles with a carapace length of less than 14.5 cm were classified as juveniles (Roe and Georges 2008a).

7.2.2 - *Data Analysis*

SPSS (version 20) was used to analyse the data. At sites where more than one species were collected, a Chi Square Goodness of Fit analysis was used to determine the difference in numbers of turtles captured. To determine if differences were related to wetland surface area, a Regression Analysis was used to determine the relationship between wetland surface area and species present.

To determine if there was a difference in the number of injuries among sites, a Chi Square Goodness of Fit analysis was used. This analysis was also used to investigate sex ratio bias, differences in the number of juveniles among sites, and the proportion of females to juveniles.

Where appropriate, a One-Way Analysis of Variance (ANOVA) followed by a Post Hoc Bonferroni Test was used to investigate differences found in intra-species carapace length, juvenile carapace length, and condition of female *C. longicollis* among the three sites. When a Chi Squared Goodness of Fit Analysis was undertaken, and if 20% or more of the expected values were less than five, a Yates Correction was used (Yates 1934). Statistical significance was accepted at $\alpha \leq 0.05$.

7.3 – Results
Overall 606 turtles were netted across the sites sampled (Centennial Park [urban], Eagle Vale [suburban] and Richmond [peri-urban]). At the suburban and peri urban sites the most common turtle netted was *Chelodina longicollis* (Table 7.2). In the urban site more than half of the freshwater turtles captured (55.6%) were *Emydura macquarii*. One female non-native species *Trachemys scripta elegans* red-eared slider was captured in the urban site, and one at the suburban site.

Table 7.2 – The species of turtles captured, number of individuals, and the percentage of the total catch across the three sites, urban, suburban and peri-urban in Sydney.

<table>
<thead>
<tr>
<th></th>
<th>Urban (percentage of total catch)</th>
<th>Suburban (percentage of total catch)</th>
<th>Peri-urban (percentage of total catch)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Emydura macquarii</em></td>
<td>55.6% (79)</td>
<td>14.1% (18)</td>
<td>0.6% (2)</td>
</tr>
<tr>
<td><em>Chelodina longicollis</em></td>
<td>43.7% (62)</td>
<td>85.12% (109)</td>
<td>99.4% (334)</td>
</tr>
<tr>
<td><em>Trachemys scripta elegans</em></td>
<td>0.7% (1)</td>
<td>0.78% (1)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total number captured</strong></td>
<td>142</td>
<td>128</td>
<td>336</td>
</tr>
</tbody>
</table>

Unlike the other two sites where *E. macquarii* were in relatively low numbers, at the urban site, there was no significant difference ($\chi^2 = 2.05, \ p = 0.152$) in the numbers of *E. macquarii* (n=79) and *C. longicollis* (n=62) captured. There was a significant relationship between species and size of wetland with *E. macquarii* more likely to be captured in wetlands with a larger surface area ($R^2 = 0.9976, \ p < 0.01$). *Chelodina longicollis* were captured in higher numbers in smaller wetlands ($R^2 = 0.808, \ p < 0.01$) (Figure 7.5).
Figure 7.5 – The number of *Emydura macquarii* and *Chelodina longicollis* captured in each of the four wetlands sampled at the urban site and the wetland surface area of each wetland.

Whilst there was a higher percentage of *C. longicollis* carrying injury (12.0%, n = 109) at the suburban site than observed at the peri-urban site (7.14%, n = 334) (injuries were not recorded at the urban site), this difference was not significant ($\chi^2_{1} = 2.046, p = 0.153$). In both sites injuries included chips and cracks to the carapace, and scaring consistent with dog or fox attack. In addition, at the peri-urban site three turtles had a limb missing, and at the suburban site two turtles had drill holes in their carapace consistent with tether holes, which are commonly a sign that the turtle had been kept as a pet at some stage in its life. The only *E. macquarii* recorded with an injury was one at the suburban site. This male had a fishhook caught in its throat with fishing line attached.

The *C. longicollis* population at the suburban site was significantly biased towards males ($\chi^2_{1} = 5.343, p=0.0208$). In contrast the populations from the urban ($\chi^2_{1} = 7.805, p<0.005$) and peri-urban sites ($\chi^2_{1} = 38.206, p<0.0001$) had a highly significant female bias (Figure 7.6).
The suburban wetland was the only one sampled that contained juvenile *E. macquarii*. There was a significant difference among sites in the number of juvenile *C. longicollis* netted ($\chi^2 = 12.044, p < 0.002$; Figure 7.6). The highest percentage of juvenile *C. longicollis* was captured in the peri-urban wetlands (20.5%, $n = 334$), followed by the suburban site (7.5%, $n = 109$) and the lowest percentage of juveniles (4.8%, $n = 62$) was collected from urban wetlands. When the comparison was made between juveniles and females, there was a significant difference in the proportion of juveniles to females at the peri-urban site compared to the suburban and urban site. The highest proportion of juvenile : females was at Richmond ($\chi^2 = 9.516, p = 0.009$).

Overall there was a significant difference in the carapace length of *C. longicollis* among sites ($F_{2,505} = 31.5, p < 0.01$). The urban population was significantly larger in
size than the other two sites, and there was no significant difference in the carapace length between the suburban and peri-urban population (p = 0.058) (Table 7.3).

Table 7.3 – The mean, minimum and maximum carapace length (cm) of *Chelodina longicollis* captured in urban, suburban and peri-urban wetlands.

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>mean carapace length (cm) (s.d.)</th>
<th>minimum carapace length (cm)</th>
<th>maximum carapace length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban</td>
<td>20.3 (2.7)</td>
<td>11.9</td>
<td>25.3</td>
</tr>
<tr>
<td>Suburban</td>
<td>17.6 (2.2)</td>
<td>13.8</td>
<td>23.8</td>
</tr>
<tr>
<td>Peri-urban</td>
<td>16.8 (3.4)</td>
<td>4</td>
<td>23.6</td>
</tr>
</tbody>
</table>

There was no significant difference between the carapace length of *C. longicollis* juveniles at the urban site and the other two sites (p > 0.05). There was a significant difference in the carapace length of *C. longicollis* juveniles between the peri-urban and suburban sites (peri-urban – mean 11.55 cm, standard deviation 2.53, n = 69; suburban - mean 14.10 cm, standard deviation 0.18, n = 8; $F_{2, 79}=5.250$, p=0.007). Juveniles from the peri-urban wetlands had a wider range of carapace length than those from the suburban wetland. All juveniles captured at the suburban site were approaching the size at which they could be reliably sexed (Figure 7.7).

![Carapace length (cm) and weight (g) of juvenile *Chelodina longicollis*, captured in the urban, suburban and peri-urban wetlands of Sydney.](image)

Figure 7.7 – Carapace length (cm) and weight (g) of juvenile *Chelodina longicollis*, captured in the urban, suburban and peri-urban wetlands of Sydney.
Overall there was a significant difference in the condition of female *C. longicollis* across the three sites ($F_{2,261} = 23, p < 0.01$; Figure 7.8). The urban population was significantly different from the other two sites with females in better condition. There was no significant difference in the condition of female *C. longicollis* between the suburban and peri-urban sites ($p > 0.05$).

![Figure 7.8 – Carapace length (cm) and weight (g) of *Chelodina longicollis*, captured in wetlands at the urban, suburban and peri-urban sites.](image)

### 7.4 – Discussion

There were differences in a range of population characteristics in the different areas (urban, suburban, peri-urban) of Sydney. There was a substantial difference in the distribution between *Emydura macquarii* and *Chelodina longicollis* among sites. In the urban site, *E. macquarii* made up a higher percentage of the total turtle population, while in the peri-urban site they represented only a small percentage of the total population. If it was assumed that catchability was equivalent for both *C. longicollis* and *E. macquarii* in these wetlands as it was found in other relic habitats of Sydney (Burgin and Ryan 2008), based upon previous estimates of population size (159 – 400ha$^{-1}$) (Chessman 1978; Parmenter 1976), some 328 – 827 *E. macquarii* may have been present in the urban site, 57 – 146 may be present in the suburban site and only 12 – 31 *E. macquarii* were estimated to be present in the peri-urban site.

Both *E. macquarii* and *C. longicollis* live in sympatry throughout their southern range, including rivers and creeks of Sydney, for example, in the Parramatta River
catchment (Burgin and Ryan 2008), the Georges River catchment (Burgin 2006; Cann 1998; Robey et al. 2011), and the Nepean River (Cann 1998; Judge 2001). However, impoundments, such as those sampled in the peri-urban area of this study are not preferred habitat for *E. macquarii*. Despite Judge (2001) having collected *E. macquarii* within the Hawkesbury-Nepean River, within the catchment of the peri-urban study site, they do not inhabit the local farm dams. However, even though *E. macquarii* is a relatively poor disperser compared to *C. longicollis*, the peri-urban wetlands sampled would be potentially accessible from the river in times of flood. With thousands of turtles captured from these wetlands in the last 15 years (e.g., Burgin unpubl., Dalem 1998, Ryan 2006, this study), there have been few (<5) *E. macquarii* netted. All of the wetlands of this site are shallow relative to most of the other wetlands sampled.

In the four wetlands sampled in the urban site, *C. longicollis* and *E. macquarii* tended to separate into different wetlands. This was despite these wetlands being less than 230 m apart, all contained within the parklands, and without evident barriers to turtle dispersal, at least, under appropriate weather conditions. Despite such proximity and ease of dispersal, the two largest, deeper wetlands sampled contained a higher proportion of *E. macquarii*, and the two shallower, smaller wetlands contained a higher proportion of *C. longicollis*. This indicated that when provided with the opportunity, the two species tend to select different and separate habitat, with *E. macquarii* preferring deeper wetlands which has previously been reported (Chessman 1988a).

Whilst urban and suburban wetlands provide habitat for many animals (Rees et al. 2009), both native and feral, they are also used as a ‘dumping ground’ for ‘escaped pets’ (McKinney 2006; Spinks et al. 2003). Pre-European settlement (>200 years ago), the urban site, Centennial Park, contained a drainage line of unbroken flowing streams. Increasingly dense urbanisation in the surrounding area, and the development of Centennial Park created seven discrete impoundments within the park (Cann 1998). Due to habitat preference, *E. macquarii* are typically not present in enclosed waters unless the wetland is large (e.g., oxbow lakes; Chessman 1988a). Due to their apparent reluctance to undertake overland migration (Chessman 1988a), and the distance to appropriate habitat in the CBD of Sydney, it is assumed that the *E.*
*E. macquarii* found in the urban area are a relic population, descended from individuals present before European anthropogenic modification of the flowing waterway. This was found in another site within urban Sydney. Burgin and Ryan (2008) observed that in the highly modified streams of the Upper Parramatta Catchment (Western Sydney), *E. macquarii* also appeared to be a relict population, originating within the Upper Parramatta River before the catchment became heavily urbanised and the River highly modified. The urban population may also have been supplemented (or derived) from escaped pets.

In contrast, the suburban site of this study was not developed from free flowing waters. With the reluctance of *E. macquarii* to undertake terrestrial migration (Chessman 1988a), and their preference for deep-flowing waters (Chessman 1988a), it is possible, but less likely, that the population was descended from individuals that migrated into this isolated water body. It would appear to be a more likely scenario that the population originates from escaped pets. This is supported by the observation that in the suburban site *C. longicollis* were captured that had holes in their carapace, typical of those used to tether individuals in captivity (Cann 1998). The capture of the exotic turtle *T. s. elegans* at both the urban and suburban sites indicated that escaped pet turtles have entered these wetlands. Worldwide, populations of *T. s. elegans* have become established in wetlands as a result of escaped individuals (Perez-Santigosa et al. 2008). Once released they can establish populations and, typically, outcompete native turtle populations (O'Keefe 2005; Perez-Santigosa et al. 2008; Teillac-Deschamps et al. 2009). Theoretically, at least, *T. s. elegans*, can become established from a single female because they may produce fertile offspring for up to five years after insemination, and can have multiple clutches per season (Burgin 2006; Perez-Santigosa et al. 2008). Their widespread successful establishment has resulted in them being listed as one of the ‘100 most invasive alien species worldwide’ (Lowe et al. 2000). During this study, two *T. s. elegans* were captured in two different sites, the urban and suburban sites. If it is assumed that they have equivalent catchability as the other species, the numbers currently present in urban and suburban sites are low and, thus there has not been a substantial effect on the native turtle populations. Burgin (2006) recorded low numbers of *T. s. elegans* at a site in Southern Sydney where native *E. macquarii* and *C. longicollis* were present. This site was resampled 12 years later, and there had been no apparent impact on native turtles (Robey et al. 2011).
This observation indicates that, despite their apparent low numbers in the urban and suburban sites, *T. s. elegans*, may have been present in the sites for many years.

Evidence from elsewhere in New South Wales, including the Bellinger River drainage (Georges et al., 2011), Nortons Basin, and Hawkesbury-Nepean River (Judge, 2001), has suggested that feral populations of *E. macquarii* have been derived from released animals. For example, in the Bellinger River drainage, it has been genetically determined that *E. macquarii* resulted from multiple independent releases (Georges et al. 2011). Judge (2001) found that the Nortons Basin population was dominated by juvenile *E. macquarii*, which, he concluded indicated that this population was recently established, and he confirmed that three *E. macquarii* were introduced to the area. Some of the resident individuals were identified as Queensland sub-species. The origin of another individual was not identified, and several individuals were thought to have been hybrids of individuals from the Macleay or Hasting River, or hybrids of these two subspecies (Judge 2001). Compared to the urban and suburban sites in the current study, these sites where *E. macquarii* had been introduced, were remote from the relatively dense human populations of even the suburban site; however, they are popular recreational areas. Based on evidence from these other introduced populations found in New South Wales, the lack of wetlands close to either the urban or suburban site that would be sufficiently close to assume that *E. macquarii* had immigrated into the suburban wetland, together with the observation that despite *E. macquarii* being resident in the Hawkesbury-Nepean River adjacent to the peri-urban site of this study but are not resident of the site, it is assumed that the *E. macquarii* in the suburban site have been introduced as a result of pet releases and presumably the urban site has been at least supplemented by released animals.

Aquatic animals, such as the freshwater turtles, are a popular attraction in both the urban and suburban sites, evidenced by people visiting the wetlands to feed the animals. However, the wetlands in the peri-urban site are within the farmlands on a University campus in areas with restricted access. While *E. macquarii* was clearly established in both the urban and suburban sites they were not in the peri-urban site. In intermittent sampling over more than 15 years (e.g., Burgin unpubl.; Dalem 1998, Ryan 2006, this study), there were no captures of *T. s. elegans* and fewer than five *E. macquarii*, all from the only wetland that was close to the main road that bisects the
campus. This dearth of *E. macquarii* is despite the wetlands being approximately 1 km from a tributary of the Hawkesbury-Nepean River, a river where Judge (2001) collected the species. Observations of translocation, and lack of *E. macquarii* within close proximity of the source population, strengthen the proposal that *E. macquarii* have been released into the urban and suburban sites sampled. However the only evidence that populations of *C. longicollis* in the urban and suburban sites may have originated from individuals released into the wetlands is the observation of tether holes on a small number of individuals. Since their preferred habitat is smaller wetlands rather than riverine habitat, and they have a greater propensity for overland travel compared to *E. macquarii* while released animals have clearly been added to the population, the population is very likely derived from animals that have migrated into the wetlands.

Although size is not directly related to age in reptiles (Gibbons and Lovich 1990), since the mean size of *C. longicollis* was larger in the urban wetlands than in suburban or peri-urban wetlands, the population appears to be aging. There was also limited recruitment observed. Hitchen et al., (2011b) observed that there was a dearth of large animals in the urban population of *Amphibolurus muricatus* Jacky dragons. They proposed that migration within the adjacent suburban landscape was acting as a sink for emigrants without a commensurate source of immigrants to offset the loss. Despite *C. longicollis* propensity to migrate between wetlands (Roe and Georges 2007), based on the concentration of human population and associated hazards for migration within the urban and suburban areas, the potential for their successful migration within these areas may be assumed to be low, which may be the reason why there were large *C. longicollis* in the urban environment. They are unable to successfully migrate. Of the three sites sampled, the urban site had the highest human population (>129,000 people) (Australian Bureau of Statistics 2013) and the surrounding catchment was the most densely populated of the sites with associated higher concentration of roads, vehicles, and potential domestic predators. Since the closest wetland habitat was 2.5 kilometres from this site, there would have been a greater likelihood of vehicle collision than in less densely populated areas of the other sites studied (Aresco 2005b).
A ‘sustained trickle’ of young, recruited annually, results in equal numbers of juveniles and adults in the population (Georges et al. 1986). Alternatively when recruitment is intermittent there is a relatively large number of a particular age cohort dominant (Georges et al. 1986). In the *C. longicollis* populations of the urban and suburban areas there were only large (> 12 cm) juveniles netted. This indicated that there was intermittent recruitment into these populations, as opposed to the peri-urban site where there was a range of different sized juveniles in the population. Based on growth rate (Chapter 5), it was estimated that the juveniles in the urban and suburban sites were approximately 12 years old and, therefore, there has been a lack of successful recruitment of juveniles for at least 12 years. The population structure of the urban and suburban population indicated that these populations may be poised for decline as older individuals are lost from the population.

One alternative explanation for the lack of *C. longicollis* juveniles in the suburban site, despite a proportionately larger percentage of juvenile *E. macquarii* may be gleaned from the previous study of Kennett and Georges (1990). They observed that *C. longicollis* failed to breed during drought. They also observed that there was a lack of juveniles, which they assumed to be due to limited recruitment, and females were in poor condition. Based on these observations, they concluded that food was limited. However, despite the lack of *C. longicollis* recruitment in the suburban site, females had equivalent body condition to those from the peri-urban site. The difference in successful recruitment was not, therefore, due to poor female body condition. However, a factor that may have influenced the difference in population recruitment by the two species in the suburban site could be supplementary feeding.

Daily, residents in the suburban site fed bread to the local aquatic wildlife. The turtles consumed these offerings. In suburban Brisbane and Melbourne (Australia) up to 57% of residents reported that they fed wildlife (e.g., birds, kangaroos, possums) in their local area (Rollinson et al. 2003). Supplementary feeding has been attributed to increased reproductive output in many species of birds (Elliott et al. 2001; O'Leary and Jones 2006; Schoech et al. 2007), and worldwide environmental managers provide appropriate supplementary provisions to support the increase in populations of threatened and endangered bird species. For example; the threatened American bird species *Aphelocoma coerulescens* Florida scrub-jay (Schoech et al. 2007) and the
New Zealand species *Strigops habroptilus* kakapo (Elliott et al. 2001) have been provided with supplementary food as part of their management to enhance populations. The foodstuffs fed by wildlife managers are carefully formulated to provide appropriate nutrition to the target species. However, supplementary feeding by local residents in the suburban site tended to be household scraps, and in the urban site sampled for turtles, the provisioning appeared to be restricted to bread. *Emydura macquarii* are omnivorous (Spencer et al. 1998), and may metabolise bread more readily than the carnivorous *C. longicollis* (Chessman 1984b). As observed with birds (e.g., Australian Magpie *Cracticus tibicen*, (O'Leary and Jones 2006), supplementary feeding for *E. macquarii* could result in more offspring than without such supplementation and may be contributing to the number of juvenile *E. macquarii* in the suburban population.

Bigger, more aggressive, dietary generalist species may achieve larger size, be more dominant, more territorial and have larger clutch sizes whilst smaller, subordinate species’ numbers tend to decline (Pitt et al. 2005; Robb et al. 2008; Schoech et al. 2007). This has occurred in *Strepera graculina* the Australian pied currawong. Individuals become more aggressive towards smaller bird species within their habitat, and subsequently, the smaller species are in decline (Parsons et al. 2006). In the presence of supplementary feeding, *E. macquarii* may have a competitive advantage over *C. longicollis*, as they attain larger size, are heavier for their size, and have a more generalist diet. This outcome is supported by the findings of Spinks et al. (2003), who observed that limited recruitment in an urban population of *Emys marmorata* the western pond turtle in California (US) was due, in part, to the competition for food from larger, non-native turtles. This could be indicative of what is happening in the urban and suburban population of *C. longicollis* sampled. The possible reason for a lack of recruitment in these populations may be a result of competition with a more aggressive species (*E. macquarii*).

In the urban site only three *C. longicollis* juveniles and no *E. macquarii* juveniles were netted. This may be because nest predation was higher than in other sites sampled. In Australia, a major impact on recruitment of freshwater turtles is nest predation (Schoener 1979; Spencer and Thompson 2003; Thompson 1983). For example, Thompson (1983) estimated that in the Murray River drainage, foxes
destroyed approximately 93% of turtle nests. Predation may also be higher in urban sites than the other two sites due to higher levels of artificial light. Higher illumination levels enhance a predator’s chance of finding prey (Houston and Shine 1994; Vasquez 1994) including nesting turtles and nesting sites. Assuming that injuries are correlated with death (Castellano et al. 2008), and injuries consistent with dog and/or fox attack where observed, predation was evident in both the suburban and peri-urban sites. Since the frequency of such injury was equivalent in both areas, both species nest in the same general location (Cann 1998), and juvenile *E. macquarii* were present in the suburban site, it is assumed that predation on the nests (and hatchlings) of both species would be equivalent. The lack of variation in size of juvenile *C. longicollis* compared to *E. macquarii* indicated that some other factor has resulted in *E. macquarii* having a reproductive advantage over *C. longicollis*.

Burgin and Ryan (Burgin and Ryan 2008) found that in the tributaries and creeks of the Upper Parramatta River (also in suburban Sydney) there was no indication that there was a difference in the recruitment between *E. macquarii* and *C. longicollis*. This indicated that *C. longicollis* did not cease breeding when *E. macquarii* was present. However, *E. macquarii* have a higher reproductive rate than *C. longicollis*, with some females laying up to three clutches annually (Judge 2001). *Emydura macquarii* also have the potential for greater hatchling fitness as they can attain larger body size than *C. longicollis* and thus have a larger body cavity to hold a larger number of eggs and/or larger sized eggs which lead to larger hatchlings. As a result *E. macquarii* have a better chance of survival than hatchlings from the smaller eggs and smaller hatchlings (Janzen et al. 2000a; Sakai and Harada 2001). Hatching is also synchronous in *E. macquarii*, and the young emerge from the nest as a pod. This is assumed to reduce the predation risk by ‘swamping’ potential predators (McGinley 1989; McGlashan et al. 2011; Spencer 2002). It also minimises activity around the nest and it has been proposed that this may reduce the risk of attracting nest predators (Spencer 2012). *Chelodina longicollis* has been recorded to over-winter in the nest and emerge as a pod (Burgin and Dalem 1996), although Spencer (2012) did not find that they hatched synchronously. Differences in emergence attributes between the two species, differences in egg characteristics, and/or the potential for one to produce more clutches within a year could contribute to the population differences in recruitment between species at the suburban site. This would be expected to provide
*E. macquarii* with a reproductive advantage over *C. longicollis* and could account for higher recruitment rates in *E. macquarii* than in *C. longicollis*.

The urban, suburban and peri-urban wetlands sampled all provided habitat for native freshwater turtles. There was a difference in the species composition at each site, with a lack of juvenile turtles in the urban and suburban sites. Although the densities of *C. longicollis* in urban areas superficially suggested healthy populations, examination across sites showed that recruitment into the urban and suburban populations may be below the levels required to sustain the population of both species of native turtles in the urban area and *C. longicollis* in the suburban population. Due to the long life span and delayed sexual maturity of these turtles, the decline in the species may remain unnoticed for many years (Spinks et al. 2003). The lack of juveniles in the urban and suburban populations compared to the peri-urban population, hints that turtles in the urban and suburban wetlands are under greater pressures, for example, because of sub-optimal and/or isolated habitat, the presence of escaped turtles, predation, and supplementary feeding.
Chapter 8 – Discussion

*Chelodina longicollis* captured at Richmond New South Wales
There are few studies worldwide that have reported on the impact of urbanisation on freshwater turtles, although there are exceptions (e.g., (Burgin 2006; Burgin and Ryan 2008; Conner et al. 2005; De Lathouder et al. 2009; Eskew et al. 2010; Kalcounis-Ruppell et al. 2007; Robey et al. 2011; Spinks et al. 2003). No previous study has focused on the impacts of urbanisation on the most terrestrial of the Australian freshwater turtles, *Chelodina longicollis*, and thus potentially the most vulnerable to human impacts in the terrestrial environment (e.g., urbanisation; vehicle collision) and anthropogenic impacts associated with the aquatic environment (e.g., water quality, enhanced competition). *Chelodina longicollis* within the Sydney Basin, the largest urban area in Australia (Australian Bureau of Statistics 2013), has been exposed to substantial change to their environment, both terrestrial and aquatic, post-European settlement (Burgin 2010; Cann 1998). Despite the associated long-term challenges, *C. longicollis* continues to inhabit the highly modified/constructed wetlands of the Region where impoundments mimic their natural environment (see Chapter 4.1.2).

Based on current rates of growth in the peri-urban population (Chapter 5.3.3) and assuming (erroneously) that turtles do not have indeterminate growth, some turtles netted were more than 60 years old. However, it is well known that turtles do have indeterminate growth and may go through long periods without measurable growth (Kennett and Georges 1990). Lack of growth for extended periods was also observed in the current study in the peri-urban wetlands (i.e., where data were available for over a decade). One individual had no measurable growth in the 13 years between its initial capture and final recapture, despite being in good condition and showing no signs of injury. Other *C. longicollis* that spanned a range of sizes also showed no measurable growth between captures (see Chapter 5.3.3). Kennett and Georges (1990) found that individuals of this species displayed slower growth and lost condition during a period of drought in which they sampled. It is, therefore, probable that *C. longicollis* individuals may have multiple periods within their lifetime without growth, probably for varying lengths of time. Based on quantifiable data collected from this study, it is feasible that in the Sydney Basin individuals of this species could live to more than 100 years or at least approaching 100 years. Some individual *C. longicollis* in the Sydney Basin would, therefore, have been present before the major expansion and increased density of the human population in the area that has occurred since the Second World War (Australian Bureau of Statistics 2013), and certainly
before the urban wetlands were modified into a series of wetlands from a drainage line in the urban wetland site of this study, and before the suburban wetland sampled was constructed during development of the surrounding suburb (Chapter 7.1.2).

In Sydney, one pressure on *C. longicollis* that was evident in both the urban and suburban areas, but not the peri-urban area where the wetlands were shallower than most wetlands elsewhere, was the presence of the riverine species *Emydura macquarii*. Although *C. longicollis* and *E. macquarii* are found to overlap within their range, they prefer different habitat (Chessman 1988a). The deeper waters of the river system that *E. macquarii* prefer would have been reduced with increasing urbanisation within the Sydney Basin. The species has, therefore, become restricted to, or introduced to, impoundments and thus forced into greater competition with *C. longicollis* than would occur naturally. The difference in habitat preference of these two species is reflected in their preference for different wetlands within the urban study site. Despite the proximity of the four wetlands, and without physical barriers to dispersal, the two larger, deeper wetlands sampled contained a higher proportion of *E. macquarii*, and the two shallower, smaller wetlands contained a higher proportion of *C. longicollis*. This indicated that when provided with the opportunity, the two species select different habitats (Chapter 7.3).

In wetlands, such as the suburban one of this study, there is limited ability for even *C. longicollis*, more prone to terrestrial migration than *E. macquarii*, to move to alternative wetlands. In such situations, both species are effectively trapped within the wetland because of surrounding urbanisation and lack of alternative wetlands. It is, therefore, expected that there will be greater competition for resources between the two species. For example, Chessman (1988b) reported that although both species have their own dietary preferences even in their natural environment they do compete for the live component of their diet. The competition is expected to be greater within the small enclosed wetlands sampled in this study.

In addition to competition for live food, supplementary feeding of bread at both the urban and suburban site could result in more offspring in the omnivorous *E. macquarii* but not *C. longicollis* (Chapter 7.4), as seen in the suburban site. For example, the greater size attained by *E. macquarii* than *C. longicollis*, and greater
reproductive ability (e.g., larger egg and clutch size, more frequent double clutching annually, Chapter 7.4), would be expected to result in *C. longicollis* being outcompeted in a situation of limited resources. In already highly modified suburban and urban areas, where alternative habitat is not available, it is expected that the future viability of *C. longicollis* will be challenged.

Unlike the urban site sampled, the suburban wetland was developed as an urban wetland during construction of the surrounding suburb, and was not previously associated with a local waterway, and neither is there one within the area from which *E. macquarii* would readily migrate. The most reasonable explanation for the presence of this species within the suburban wetland is that the population is based on released pets (Chapter 7.4).

The sale of *C. longicollis* and *E. macquarii* in New South Wales pet shops is now legal (Parker 2013). It is, therefore, assumed that more turtles would be kept as pets, and thus there is a greater potential for larger numbers of these turtles to escape or be released into local wetlands. This would not just potentially increase competition for resources but also, for example, could potentially, introduce disease and/or genetic material that are foreign to the local population, and thus interfere with the genetic integrity of native turtles of the Region.

The feral turtle *Trachemys scripta elegans* red eared slider, are also present in the impoundments sampled in urban and suburban Sydney (Chapter 7.3). With the establishment of this feral turtle species, competition for resources within wetland/s isolated with surrounding and increasingly extensive and dense urbanisation will increase. Whilst currently the proportion of the population of non-native turtles within the urban and suburban wetlands is small (Chapter 7.3), the impact is probably negligible. However, there is the potential for their numbers to increase. This is because the local climatic conditions are within their tolerance range and, therefore, they could successfully reproduce and become established (Ramsay et al. 2007). Although Robey et al. (2011) found that the numbers of *T. s. elegans* remained low a decade after first discovery in a Sydney wetland, in South East Queensland there are established populations of *T. s. elegans* that have apparently excluded *C. longicollis* from wetlands due to competition (O’Keefe 2005). Another vertebrate species of
concern within the wetland habitats sampled is Cyprinus carpio carp. The feeding habits of this fish species results in the loss of rooted vegetation, and increases turbidity, which directly impacts on food availability for resident turtles (Chapter 4.4).

Wetlands that had the highest concentrations of cadmium and arsenic were those that received irrigation runoff and were not supplemented directly with recycled water. The most likely source of these elevated pollutants is from chemicals used in the agricultural activities on the agricultural lands surrounding the wetlands. Despite being highly polluted, these wetlands had the largest number of juveniles (Chapter 4.3.3), and also included wetlands where, for 13 years or more, some females had included at least one of these wetlands in their home range (Chapter 5.3.1). The indication of site fidelity of some C. longicollis females, and the observation that there was apparently successful breeding (i.e., relatively large numbers of juveniles compared to other wetlands within the area), suggested that the heavy metal pollution in these wetlands has not substantially affected viability of the local population.

Overall there was; however, a female sex bias in the peri-urban wetlands and in the urban site for C. longicollis although the species showed a male sex ratio bias in the suburban site. In the past decade elevated temperatures and a steep increase in the number of hottest days on record have occurred in the Sydney Basin, maybe causing a transition between genetic sex determination (GSD) and temperature sex determination (TSD), which appears to have shifted towards a female bias in the peri-urban area (Chapter 4.3.1). Based on growth rates (Chapter 5.3.3) some turtles in the urban and suburban sites would have been more than a decade old. However, there is a lack of more recent successful reproduction at these sites (Chapter 7.3). The data at these two sites do not, therefore, support such a hypothesis due to the lack of recruitment.

The urban wetlands are isolated from other wetlands, and migration would necessarily require navigating through a densely populated urban landscape. Based on the study of Aresco (2005b), it would be doubtful that successful migration would occur in such areas since he found that it would be unlikely that a turtle could successfully cross a roadway that had more lanes than a suburban street. Even the population of C. longicollis, a species known for its penchant for overland migration, would preclude
migrants entering the ecosystem. The female *C. longicollis* in these urban wetlands were in better condition than those at other sites (Chapter 7.3). In the peri-urban site, females netted in the same wetland after 13 years were heavier for their size than turtles that had only been captured for the first time (Chapter 5.3.1). This provides some hint that when *C. longicollis* have strong site fidelity (due to choice or because of wetland isolation), there is a greater potential for individuals to maintain condition than those females who undertake migration. If resources used in migration are diverted to growth and reproduction, the future viability of these individuals would be greater than those who more frequently moved among wetlands or, as discussed in Chapter 6.4, become ‘lost’ in the urbanising landscape. This is because, as discussed previously (Chapter 5.4.1), larger females tend to have more eggs/clutch, larger size hatchlings, and potentially less predation pressure, and thus an increased chance of survival. However, unlike the peri-urban site (Chapter 4.3.1, 5.3.1, 7.3) there was a lack of successful recruitment in the urban site (Chapter 7.3), which indicated that some other factor (e.g., predation) inhibited recruitment into the urban site. For example, predation by domestic dogs and cats may be higher in the urban and suburban areas than in the peri-urban area. Predation by *Vulpes vulpes* foxes, may also be higher in all of these areas due, in part, to a lack of safe and fox-specific management options (Hegglin et al. 2004). Marks and Bloomfield (1999) found *V. vulpes* populations in urban Melbourne at densities as high as 16 foxes/km². Thompson (1983) observed that in a rural area of the Murray River *V. vulpes* take up to 93% of the turtle eggs laid. The combined predation pressure from these different sources may actually be at least as high in the urban and suburban sites as was found in the Murray River Basin.

In the peri-urban population, it would be expected that nest predation was equal throughout the site; however, recruitment was variable with relatively large numbers in a few wetlands (Chapter 4.3.1). This indicated that nest predation was not the only factor that inhibited recruitment to a wetland. The wetlands with the highest number of juveniles also had low dissolved oxygen, higher emergent vegetation cover, and no predatory fish present. In the suburban site, there were very limited *C. longicollis* recruitment, despite recruitment of *E. macquarii* (Chapter 7.3) although both had substantial numbers of large *A. reinhardtii* and *A. australis* eels and *C. carpio* carp, present, compared to other sites, in part, due to supplementary feeding by local
residents. However, the lack of recruitment of *C. longicollis* into this wetland may indicate that these large fish species predate on small juvenile *C. longicollis* but may not be able to cope with the larger juvenile *E. macquarii* with their jagged carapace shell rather than the smooth-edged shell of *C. longicollis*.

Individuals of all stages of the lifecycle undertake terrestrial migration (Chapter 5.3.2, 6.3). However, while there are several accounts of adult *C. longicollis* undertaking overland migration, only one previous study mentioned juvenile movement, although without detail (Roe et al. 2009). In the peri-urban site, juveniles were found to travel at least 1.2 km overland (Chapter 6.3). Individuals were also netted in more than one wetland (Chapter 5.3.2). This indicated that, even at a small size, juveniles may have substantial navigation skills in the terrestrial environment. In one situation in this study a juvenile was at an even greater distance than any adult male was found from the nearest wetland (adult females tend to move further over land) (Chapter 5.3.2, 6.3).

One reason adult females move further than other segments of the population, is to access suitable nesting sites. With females nesting further from wetlands in Northwest Sydney than in other areas (Chapter 6.4), hatchlings emerging from these nests need to have good navigation skills from birth in order to survive. Those that successfully navigate to a wetland could have superior navigational skills than equivalent aged juveniles in other areas. Juvenile turtles moving through the landscape provide easy prey for predatory birds (e.g., *Dacelo novaeguineae* kookaburra, *Falco cenchroides* falcon, *Accipiter fasciatus* goshawk, *Vulpes vulpes* fox, *Varanus varius* goanna) and domestic pets (Kennett et al. 2009). When substantially higher numbers of juvenile turtles are taken whilst undertaking terrestrial migration than under natural conditions, it has the potential to reduce gene flow and places pressure on the species in subsequent generations due to lack of recruitment.

Roe et al. (2009) found that *C. longicollis* use a complex of wetlands annually; however, this movement decreased with increasing distance between wetlands. They found connectivity in wetlands of up to 1.2 km was most beneficial. In the peri-urban site of this study there were 34 wetlands within a 2 km radius (Chapter 4.2.1.1). Based on the proportion of recaptures over the 13 years of the study, there was a large
percentage of adult turtles that were not recaptured, and were presumed lost to the system (Chapter 5.3.1). The longer distances turtles travel in Northwestern Sydney than recorded elsewhere (Chapter 6.3) may indicate that *C. longicollis* navigational skills are not as precise as previously considered. However, due to the urbanising landscape of the Region, once navigational cues are altered (e.g., changed slope, new buildings, and/or removal of vegetation) turtles ultimately need to find an alternative route and, in doing so, may encounter a ‘new’ wetland complex. In Western Sydney more turtles may become lost than elsewhere in the species natural range due to the rapidly changing landscape.

Vehicle collision may have exacerbated the loss of animals moving through their home range in an urbanising environment. In just the peri-urban study area, in only two years, there were 104 turtles that were lost due to a direct result of vehicle collision. This was calculated to equate to a further potential offspring loss of over 1,200 individuals based on the number of females killed during nesting season (Chapter 6.3).

Some turtles in the peri-urban wetlands were conservatively estimated to be over 60 years of age (Chapter 5.4.3). These older turtles have had the potential to recruit hundreds of turtles to the ecosystem. It was also observed that some female turtles had nest site fidelity (Chapter 5.3.1) and that they are travelling greater distances within the landscape than previously recorded (Chapter 5.3.2, 6.3), presumably to nest. When new or expanded roadways are built near nesting sites, or on the terrestrial migration routes, it is expected that increasingly these large, successfully reproducing turtles will be the victims of vehicle collision including during the breeding season. Such loss would be expected to exacerbate pressure on the long-term viability of the population.

### 8.1 Future management requirements

At all sites sampled in the Sydney Basin, superficially at least, it appears that *C. longicollis* are common. However, although Rees et al. (2009) observed that *C. longicollis* have the ability to adapt to low-intensity development, turtles sampled in the Sydney Basin are being increasingly challenged by urban development - both by its density and extent. At the very least, the population structure of *C. longicollis* in
this urbanising Region is under increasing pressure with the longer-term viability of the species being increasingly challenged. This has occurred in other turtle populations. For example, a crash in a population of *Clemmys insculpta* (US) happened when low level human impacts (recreational hiking and fishing) were introduced to an area (Garber and Burger 1995). A crash in another turtle population, *Graptemys flavimaculata* (US), also occurred when human disturbance caused females to abandon nesting sites (Moore and Seigel 2006). In Sydney’s urban and suburban areas, there would be much higher rates of human disturbance than in either of these situations, both directly and indirectly, than the human impacts that induced a population crash in these two populations (*C. insculpta*; *G. flavimaculata*). The lack of recruitment to the *C. longicollis* population in the urban and suburban areas indicates that the species is, at least, on the brink of decline. However, *C. longicollis* are long lived and lack substantial levels of predation beyond the hatchling stage of the lifecycle. Such species require high adult survivorship to maintain viable populations. This is because such animals have evolved life history strategies such that a long life span is required to offset the cost of delayed reproduction, and low nest survival (Eskew et al. 2010). Based on the findings of this study, continued major impacts in peri-urban, suburban and urban areas, on *C. longicollis* indicated that there is a very low chance of long-term viability.

Continued expansion of urbanisation in the Sydney Region, will increase the impediments to movement, but particularly in association with roadways for this highly mobile species, and may also result in increased predation, competition, and loss of wetlands. These cumulative challenges will result in reduced numbers of *C. longicollis*. The urban and suburban populations already have limited or no recruitment. At some future time, be it a decade, or several decades, there will be a rapid decline and then a loss of *C. longicollis* from wetlands in urbanised/urbanising areas of the Sydney Region. While there will probably be a lag time in the peri-urban populations, with the continued expansion of urban development into these areas, together with an associated increase in traffic, loss of wetlands with the transition from farming to urbanisation, and (potential) increased predation by domestic pets, the peri-urban population will also be placed under increasingly greater pressure. Whilst there is currently relatively higher recruitment into this population than into the urban and suburban sites, as pressures of urbanisation increase the population will
be challenged by the urbanising processes. In time, the peri-urban population will become increasingly more similar to the suburban and urban populations with an eventual decline in the population.

Currently no sites in the Sydney Region are managed for the turtles. To maintain urban populations of *C. longicollis* will require management. For example, the loss of turtles was substantial within the peri-urban study area, which represented a small segment of *C. longicollis* distribution, and did not particularly focus on hotspots for vehicle collision. To reduce vehicle collision would require identification of potential road mortality hotspots, and mitigation measures aimed at a reduction in road deaths. Since the death of even a single individual due to vehicle collision, typically also impacts on scavengers (e.g., Burgin and Brainwood 2008), death of even a single individual *C. longicollis* is likely to impact on multiple species. Due to this potential carnage, there is the potential that the creation of roadways, especially arterial roads close to wetland areas, could become identified as a ‘key threatening process’ or equivalent, and thus require consideration in their future developments.

A ‘key threatening process’ may be listed under the Threatened Species Conservation Act 1995 if it has the potential to cause species, populations, or ecological communities that are not currently threatened to become threatened (New South Wales Government 1995). Populations of *C. longicollis* within the Sydney Region could be examined under such consideration.

There were no road mitigation measures in place for turtles in the Region when this study was undertaken; however, such mitigation measures have been implemented throughout the world to reduce the impact of roads on turtle populations. For example, under road ‘box’ culverts may allow turtles to migrate without having to cross roads. For example, Rees et al. (2009) found that in a suburban Canberra (Australian Capital Territory), *C. longicollis* used ‘box’ culverts to move under roads along drainage lines. This allowed this suburban population to migrate between wetlands without contending with crossing roads. These observations highlight the need to consider implementation of such mitigation measures in developing suburban and urban areas within the range of the highly mobile *C. longicollis*.
Barrier fencing may also benefit turtle populations. Aresco (2005b) found that the installation of an inexpensive drift fence (vinyl erosion control fencing) greatly reduced freshwater turtle vehicle collision mortality. Barrier fencing has also been used to protect the endangered frog, *Litoria aurea* green and golden bell frog in Sydney. To mitigate vehicle collision, 11 road underpasses and six kilometres of fencing were installed around suburban Homebush (Department of Environment and Climate Change 2008). The numbers of *C. longicollis* collected in close proximity to wetlands indicated that such measures could also potentially divert *C. longicollis* from roadways and reduce vehicle collision.

An increase in exotic aquatic vertebrates such as *T. s. elegans* and *C. carpio* will impact on native turtle populations. In Southern Queensland, substantial funds have been expended in an attempt to eradicate established populations of *T. s. elegans* (O'Keefe 2005). Eradication of the small populations of this feral species within Sydney would protect native turtles, and potentially save State Government funds in the longer term when feral turtle populations increase in size (and number).

Other methods of reducing aquatic exotic pests impacts is to increase the level of public education associated with the impacts of releasing pet animals, feeding native animals, and controlling domestic pets, particularly cats and dogs, on native species.

In urban and suburban areas where there is, at best, limited recruitment occurring in native turtle populations, predation should be managed. The creation of safe areas where turtles can nest (e.g. fenced off areas, an island in the middle of large wetlands), and more attention to the eradication of feral populations (e.g., foxes, cats) may serve to reduce the impact of predation on eggs and/or hatchling turtles and thus enhance recruitment where it is currently failing.

Whilst elevated heavy metal concentrations were not demonstrated to impact on *C. longicollis* viability (Chapter 4.3.3), it has been observed that the high concentrations of heavy metals in marine turtles results in high concentrations in the individual’s eggs (Sakai et al. 1995). It is relatively difficult to follow the fate of marine turtles due to their long-range movements. Studies on heavy metal levels in *C. longicollis* could, therefore, provide valuable information on the longer-term viability of
populations, and also provide a convenient model for assessing the impacts of heavy metal on marine turtles.

Biodiversity loss is an issue worldwide and is typically a major issue within rapidly urbanising areas such as within the Sydney Region. In a relatively affluent country such as Australia, urban planners and environmental managers are better placed to consider the needs of individual species, for example, the freshwater turtle *C. longicollis*, and provide models of how to manage such species in an increasingly densely populated and extensive urbanising environment than in many other countries. If native turtle populations are to survive major changes in their environment, their management must become a higher profile issue in the community and with those (e.g., town planners, environmental managers, developers) whose actions affect such populations, and may ultimately result in local populations becoming extinct. The creation of different wetlands, connectivity of wetlands, road mitigation measures, and vegetation in the wetlands all need to be designed and implemented with the needs of the turtles and the associated biodiversity considered. The commonness of the freshwater turtle, *C. longicollis*, in the most populated areas of Australia, and its penchant for overland migration, provides an ideal model for the management of aquatic and terrestrial species within an urbanising environment.
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