CHAPTER A: GENERAL INTRODUCTION

All the world's surface biota are exposed to anthropogenic toxins, with deposition of pollutants to land and water following atmospheric transport occurring in even remote uninhabited areas (Nriagu 1979, de Voogt & Jansson 1993, Pacyna 1996). Two hundred years ago, the main water pollutant produced by the then much smaller and more scattered world population was organic waste, which could be assimilated into the environment, with only minor impacts on a local level. Water pollution became a severe problem with industrialisation and subsequent urbanisation, which coincided with a rapid acceleration of population growth (Mason 1996). Currently, the majority of Australians live in urban rather than rural areas, with the most densely populated 1% of the country accounting for 84% of the population (ABS 2002). Industrial, domestic and vehicular wastes concentrate in these areas. Despite the impact that concentrated human activity has on local flora and fauna, only 0.4% of ecology papers published in 1999 were on urban ecology (Suzuki 2000), and there is no quantitative data on urban impacts on freshwater turtles (Mitchell 1988).

Urban effluents reaching natural waterbodies are diverse in their strength and composition (EPA 1996), and so have a variety of impacts on biota, from sublethal effects which reduce fitness (e.g. Hoffman 1990, Jacobson *et al.* 1991, Müller & Lloyd 1994, Ieradi *et al.* 1996) through to deaths of thousands of animals (Bury 1972) sometimes resulting in the extinction of an entire local population (Erichsen Jones 1964, Boet *et al.* 1999). Sublethal effects include interference with development of reproductive, endocrine, immune and nervous systems, which can have dramatic affects on survival, yet have been little studied in field situations (Mason 1996, Sparling *et al.* 2000). Pollution effects on aquatic vertebrates have mainly been studied in fish, and usually in the laboratory rather than in the field. Freshwater turtles and other aquatic reptiles are frequently exposed to water pollution, but have been little studied in either field or laboratory. Most studies on aquatic and other reptiles merely report tissue contaminant load but not effects of contamination (Beck 1956, Avery *et al.* 1983, Helwig & Hora 1983, Sabourin *et al.* 1984, Davenport & Wrench 1990, Burger 1992, Gordon *et* *al.* 1998, Presti *et al.* 1999, de Solla *et al.* 2001, Kenyon *et al.* 2001). The degradation of eastern Australian waterways is a major threat to aquatic fauna (Cann 1993), yet no studies have been conducted to assess its effects on turtles.

A.1 Chelidae

There are four extant reptilian orders, of which the Chelonia encompasses 257 species (Ernst & Barbour 1989) of marine, freshwater, and terrestrial turtles. The two major lineages, Pleurodira and Cryptodira, are distinguished by morphological features (Ernst & Barbour 1989), the most noticeable being lateral (pleurodiran) versus vertical (cryptodiran) neck retraction. The pleurodires (families Chelidae and Pelomedusidae) are the more primitive, and have a purely Gondwanan origin, with the extant Chelidae found only in Australia/Papua New Guinea and South America (Ernst & Barbour 1989, Cogger 2000). All the Australian freshwater turtles except the cryptodiran *Carettochelys insculpta* belong to the family Chelidae (Ernst & Barbour 1989, Cogger 2000). Chelids are all aquatic or semi-aquatic freshwater turtles (Legler & Georges 1993).

The first complete guide to Australian chelids (Goode 1967) noted that: '*Much more exact information is needed on almost every aspect of tortoise behaviour and on every species in the Chelidae*'. Despite subsequent research (Burbidge 1967, Parmenter 1976, Chessman 1978, Cann 1978 & 1998, Georges 1982a, Thompson 1983a, Legler 1985, Kennett 1994, Spencer 2001), life history details remain scant for most species. Even more surprising, and despite their moderate size, new species continue to be described (e.g. Thomson *et al.* 2000).

Australian chelids are colloquially known as 'shortnecks' or 'longnecks'. Longnecks are all in the genus *Chelodina*, comprising the *Chelodina expansa* and *Chelodina longicollis* sub-generic groups (Legler 1985, Legler & Georges 1993, Cann 1998, Thomson *et al.* 2000). The shortneck genera are *Emydura*, *Elseya*, *Rheodytes*, *Elusor* and *Pseudemydura* (Cogger 2000). Ecological monitoring of Australian aquatic environments has been rare (ANZECC 1992), and general ecological and biological information has not been collected for some Australian freshwater turtle species even though they are threatened, endangered (Cogger *et al*. 1993), or of unknown status (IUCN/SSC 1989).

Three species from three genera occur in Sydney: *Chelodina longicollis* (Cogger 1992), *Emydura macquarii* and *Elseya latisternum* (pers. ob.). The first two are the most studied of any Australian freshwater turtle species.

A.1.1 Morphology

The most distinctive feature of chelonians is their shell, which has remained highly conserved for 200 million years, and is usually composed of about 50 bones with a covering layer of keratinised scutes (Ernst & Barbour 1989). Limbs of the Chelidae are jointed with webbing between the toes for aquatic propulsion, although all are also capable of terrestrial locomotion (Cogger 2000). Australian chelids have acute hearing, highly developed olfaction functional during submersion, and good eyesight (Cann 1998, Alderton 1993), with *Chelodina* species in particular having very good stereoptic vision (Legler & Georges 1993).

In addition to lungs, Australian chelids have highly vascular buccal and pharyngeal mucosae and cloacal bursae for gaseous exchange with water (Legler & Georges 1993). These extrapulmonary respiratory structures can be used concurrently with or independently of lung use (Palmer 2000), and can provide around half of oxygen requirements (Tasker 1991) allowing prolonged submersion. Freshwater turtles are 7 to 25 times more tolerant of anoxia than marine turtles and other reptiles (Spotilla *et al*. 1990), with several days of anoxia tolerated at temperatures of approx 20 ºC, and several months at temperatures below 10 ºC (Ultsch 1985). Although reptilian skin is mostly impermeable because of the scales, significant CO_2 exchange can occur across the skin between the scale hinges (Palmer 2000). The metanephric kidneys of turtles drain to a urinary bladder which is connected to the cloaca via the urethra, with the dilute urine allowing the excretion of ammonia without conversion to less toxic forms (Palmer 2000).

Although longevity is not known precisely, members of the Chelonia are among the longest-lived vertebrates on earth (reviewed in Harless & Morlock 1979), with Australian species estimated to live for over 50 years (Parmenter 1976, Cann 1978, Georges 1982a).

A.1.2 Habitat

The Chelidae are more dependent on standing water than most other Australian reptiles, as they must be submerged to feed (Ehmann 1992, Legler & Georges 1993). Shortnecks are more restricted to permanent water, while the *C. longicollis* group have a greater propensity for overland travel (Chessman 1978, Stott 1987).

Despite mild winters, chelids in south-eastern Australia may assume an inactive state on land under litter or soil, in aquatic sediments, or in fringing vegetation during the colder months (Stott 1987, Ehmann 1992), or remain semi-active on bottom sediments. Although metabolic rate is lowered in colder areas during winter, animals remaining in the water column may still surface for atmospheric gas exchange (Tasker 1991, Cummings 1997).

A.1.3 The Eastern Longneck Turtle (*Chelodina longicollis*, Shaw 1794)

Chelodina longicollis was collected by Sir Joseph Banks from the east coast of Australia (probably near Botany Bay; Cann 1998) on James Cook's first voyage in 1770 (Legler & Georges 1993), and was the first Australian freshwater turtle to be described (Shaw 1794) and have its natural history documented (Parmenter 1976, Chessman 1978).

A.1.3.1 Distribution & Habitat

Chelodina longicollis is an abundant turtle with a distribution over 1 million km^2 in eastern and south-eastern Australia (Ehmann 1992) (Figure A.1a), and occurs further south than any other Australian chelid (Legler & Georges 1993). Its distribution includes both inland and coastal areas, and encompasses the catchment of the Murray-Darling River system (Cogger 2000). There is a small morphologically distinct disjunct population in the south-west of its range, but this does not reach species status (Beck 1991). *Chelodina longicollis* distribution ranges from the subtropical to cool temperate regions (Ehmann 1992).

Chelodina longicollis shows a preference for still or slow-flowing weedy watercourses (Cann 1998, Cogger 2000) including oxbow lakes, anabranches, ponds, and swamps (Chessman 1978), and rapidly colonises farm dams (Steven Emerton pers. comm.). Within its range, *C. longicollis* usually dominates the turtle fauna in water bodies that are shallow, ephemeral or distant from major rivers (Chessman 1988b).

A.1.3.2 Morphology

Chelodina longicollis is distinguished from other *Chelodina* species by the black margins of the plastral seams (Ehmann 1992, Swan 1995), and the extension of the anterior lobe of the plastron to the midline of the carapacial marginal scutes (Cann 1998). *C. longicollis* grows to a carapace length of 240-275 mm (Parmenter 1985, Cogger 2000, Ehmann 1992, Ernst & Barbour 1989). Males mature sooner (6-7 years) and at a smaller size (150 mm) than females (9-11 years, 170 mm; Parmenter 1976) and also reach a smaller maximum size (200 mm males, 240 mm females; Parmenter 1985).

A.1.3.3 Diet

The *Chelodina* are largely carnivorous (Cogger 2000), their long neck allowing them to lunge at moving prey, which is then ingested by the suck and gape method (Legler & Georges 1993). *Chelodina longicollis,* an obligate carnivore, is a forager as well as a sit-and-wait predator (Legler & Georges 1993). *Chelodina longicollis* displays a broad and opportunistic diet, with little selectivity for particular prey items (Chessman 1984a, Parmenter 1976, Georges *et al.* 1986), eating fish, amphibians, crustaceans, insects, worms, molluscs (Ernst & Barbour 1989), plankton, nekton, benthic macroorganisms, carrion, and terrestrial organisms that fall into the water (Georges *et al.* 1986). Thus, the prevalence of local prey items is likely to be the major influence on dietary composition. There is little change in dietary item size and taxa between males and females, or with turtle size (Georges *et al.* 1986) except that larger turtles eat more carrion, and smaller turtles consume greater numbers of littoral and benthic invertebrates (Chessman 1984a). Feeding is greatly reduced or may cease in the winter months (Chessman 1984a).

A.1.3.4 Migration

Chelodina longicollis may leave a water body to nest, to use terrestrial refuges, or to migrate to a new waterbody, sometimes covering large distances (Goode 1967, Parmenter 1976, Stott 1987). Migrations are often in the latter half of summer and rain is the usual stimulus (Ehmann 1992, Kennett & Georges 1990, Georges *et al.*1993), possibly because high humidity greatly reduces evaporative water loss (Chessman 1984b). Dehydration is also reduced by low cutaneous water loss, and possibly by the large amounts of water carried in the cloacal bursae (Chessman 1984b). Migration to and from permanent water may be annual (Parmenter 1976) or less frequent (Kennett and Georges 1990).

Chelodina longicollis may take refuge in leaf litter, soil or dense vegetation during terrestrial movements (Chessman 1978, Stott 1987). *Chelodina longicollis* shows some navigational capacity, with odour detection, solar guidance (Graham *et al.* 1996) and familiarity with visual landmarks (Stott 1987) as possible cues. Although some authors have found movements restricted to daylight hours (Graham *et al.*1996), others have not (Chessman 1978, Stott 1987).

Their migratory abilities allow *C. longicollis* to move from more competitive environments to remote ephemeral waters, which are usually highly productive with large populations of aquatic invertebrates (Chessman 1988b, Georges *et al.* 1993), before returning to permanent water during drier periods (Chessman 1988b, Kennett & Georges 1990, Legler & Georges 1993). Approximately eight times more food is ingested by *C. longicollis* in waters without fish (Chessman 1984a), and fish are usually absent from ephemeral waters (Chessman 1988b). Other turtles lacking the ability for long distance terrestrial migration may also be excluded, further reducing competition (Georges 1982a).

Migratory tendencies are important in relation to pollution exposure, as contaminant type and concentration may vary between water bodies, as may prey type and hence exposures through the food chain. In a sedentary animal, tissue contaminant loads can more readily be assumed to reflect local conditions.

A.1.4 The Macquarie Turtle (*Emydura macquarii*, Gray 1830)

Emydura macquarii was first collected in 1825 (Legler & Georges 1993), 55 years after *C. longicollis*. Female *E. macquarii* are larger than males, with carapace lengths of up to 300-340 mm (Goode 1967, Ernst & Barbour 1989, Ehmann 1992, Cann 1998, Cogger 2000).

A.1.4.1 Distribution & Habitat

Emydura macquarii has an eastern Australian distribution of up to 1 million km^2 (Ehmann 1992) throughout the Murray-Darling drainage basin and in south-eastern coastal rivers in South Australia, Victoria, and NSW (Goode 1967) (Figure A.1b). *Emydura macquarii* is sympatric with *C. longicollis* over most of its range (Cogger 2000).

Emydura macquarii can have morphological differences between drainages, and Cann (1998) recognises six subspecies including *E. m. dharuk*, a new subspecies described from the Sydney Basin. The original distribution was not thought to include the Sydney Basin (Cogger 1975) and it seems likely that this species has been introduced to the area (Section C1.1.1). *Emydura macquarii* shares rare alleles with *E. signata* and *E. krefftii* (Georges & Adams 1996) with which it should be merged (Cogger *et al.* 1983, Georges & Adams 1996).

Compared to the preference of *C. longicollis* for remote or ephemeral ponds, *E. macquarii* is closely associated with large, deep, permanent rivers and their backwaters (Chessman 1988b), usually only leaving them to bask or nest (Legler & Georges 1993).

A.1.4.2 Diet

Most of Australia's shortnecked chelids are active foragers that slowly cruise the bottom layers and use tactile, visual and olfactory cues to detect prey (Legler & Georges 1993), although they also take dietary items from the water surface (Spencer *et al.* 1998). Species of the genus *Emydura* are typically omnivorous (Chessman 1978, Georges 1982a, Legler & Georges 1993) with up to 95% vegetation in their stomachs (Ehmann 1992), although they may be carnivorous when young (Georges 1982a). *Emydura macquarii* juveniles tend to consume more periphyton than adults, whereas adults consume more plant material and carrion (Chessman 1986).

The main components of the diet of *E. macquarii* are filamentous algae (53-61%), plant debris and fish carrion (Teleostomi) (Murray Valley; Chessman 1978, Spencer *et al.* 1998), although not in all populations (Georges 1982b). A wide range of other items are consumed, including periphyton and sponges (reviewed in Legler & Georges 1993), terrestrial insects from the water surface (Diptera, Hymenoptera, Coleoptera), crustaceans, aquatic dipteran larvae and Hemiptera (Spencer *et al.* 1998), molluscs and tadpoles (Ehmann 1992). Lacking rapid ambush predation, *E. macquarii* generally only take fish and other fast-moving items as carrion (Legler & Georges 1993, Spencer *et al.* 1998). As the diet is broad, food items may vary between locations and seasons (Chessman 1986), although a high preference is shown for fish carrion (Spencer *et al* 1998).

A.1.5 Saw-shelled Turtle (*Elseya latisternum*, Gray 1867)

The genus *Elseya* is paraphyletic with two genera indicated, one, the *El. dentata* group, being more genetically similar to all species of *Emydura* than to *El. latisternum* (Georges & Adams 1992). *Elseya latisternum* naturally occurs throughout north-eastern Australia (but not in Sydney, Figure A.1c), inhabiting rivers, streams and lagoons (Ernst & Barbour 1989, Cogger 2000). Like *C. longicollis, El. latisternum* can occupy seasonally ephemeral waters and migrate overland to new waterbodies (Legler & Georges 1993).

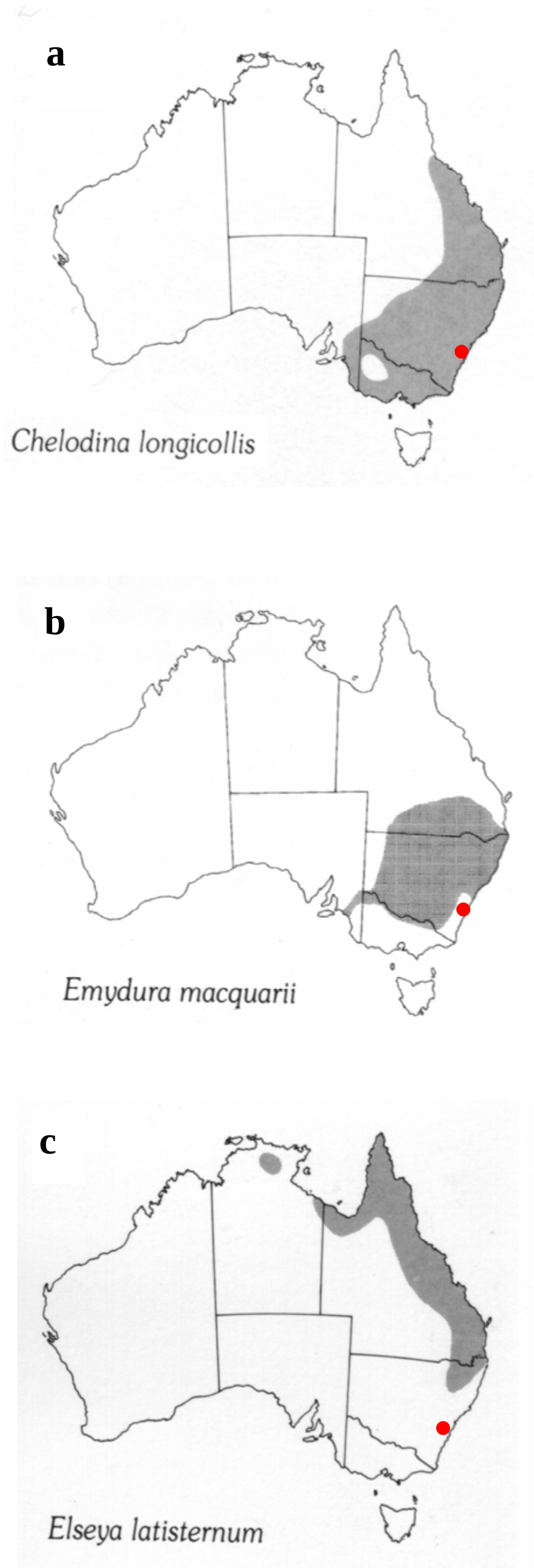

Figure A.1 Natural distributions (Cogger 1992) of the three freshwater turtle species captured in Sydney (●) during this study.

Elseya latisternum is largely carnivorous, taking fish, tadpoles, frogs and insects (Ehmann 1992, Legler & Cann 1980), although omnivory has also been reported (Ernst & Barbour 1989, Cogger 2000). Females are larger than males (Ehmann 1992), growing to a carapace length of 200 - 280 mm (Cogger 2000, Ernst & Barbour 1989).

A.2 Study Area: Sydney Basin

A.2.1 Geography & Waterbodies

Sydney (33º51'S, 151º12'E) is the capital of the state of New South Wales (NSW) and is the largest city in Australia, both in terms of area (ABS 2003) and population, with over 4 million residents (ABS 2002). The greater metropolitan area (Sydney, the surrounding Central Coast, Illawarra and lower Hunter) contains 74% of NSW's population and many areas continue to show rapid growth (ABS 1997). Sydney sits in a natural basin, which is bordered in the west by the minimally populated Blue Mountains (part of the Great Dividing Range). It has several large river systems in five major catchments (Hawkesbury-Nepean, Parramatta, Georges, Cooks and Hacking) which are tidal for at least some of their passage, as well as many smaller tributaries and numerous lakes and lagoons.

Sydney experiences a warm wet summer/autumn and cool drier winter/spring, with January the hottest month and July the coldest (with mean minimum air temperatures of 8ºC on the coast) (Benson & Howell 1990). Sydney's rainfall is highly variable, almost all of it occurring in brief periods of relatively heavy rain on a small number of days, with an annual average rainfall of over 1200 mm on the coast (Benson & Howell 1990). Thus, for long periods of the year, the rivers tend to be stagnant or slowly flowing, with a very low capacity to withstand anthropogenic stresses (Butlin 1976).

A.2.2 History of Urbanisation

The history of urbanisation and pollution is important for several reasons. Firstly, Chelonia are long-lived (Gibbons 1987), so may have been exposed to toxicants that were present many decades previously. Secondly, bioaccumulated toxicants (e.g. lead) may be sequestered in tissues (e.g. bone) for several decades (Gerhardsson & Skerfving 1996), so historically released environmental contaminants may be present in extant individuals. Thirdly, contaminants may remain in sediments for many years (Beijer & Jernelöv 1979a), so historical pollution events can affect current exposure. Finally, the entire history of pollution in an area will have been putting selection pressures on the local biota so that the current population is the result of previous genetic selection.

Sydney was settled by Europeans in 1788, and by the late 1800s agriculture had destroyed much the area's flora and fauna, with increasing population and suburban expansion (Figure A.2) continuing to diminish bushland through the 1900s (Benson & Howell 1990). As with most cities, little natural bushland remains, with remnants only surviving in poorly accessible areas unsuitable for agriculture or housing, and these often invaded by weeds (Benson & Howell 1990, Young 2000).

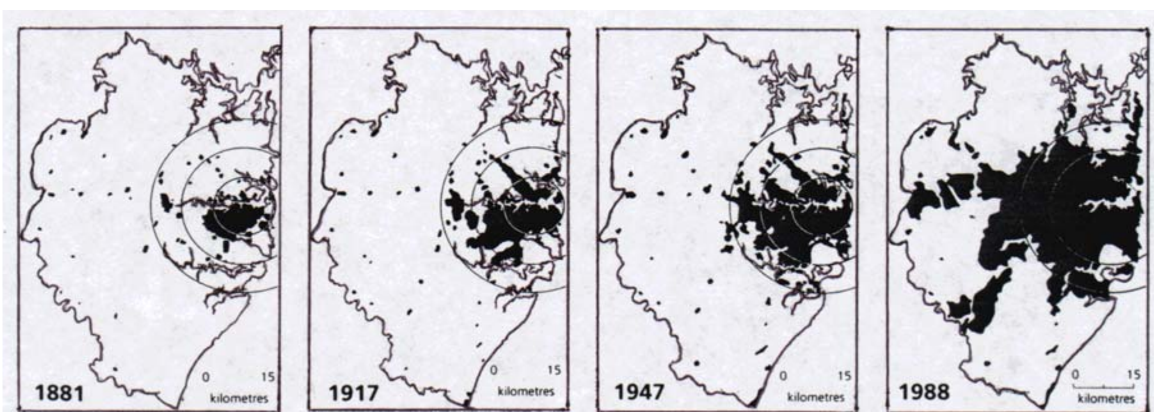

Figure A.2 Sydney Basin's expanding suburbs from 1881 to 1988. The maps are bounded by the coast to the east (at right), and by the Hawkesbury-Nepean River to the west, beyond which lie the Blue Mountains (from government sources in Benson & Howell 1990).

Urbanisation has two devastating environmental effects. First natural areas are cleared, then naturally drained land becomes impermeable after construction of buildings, roads, footpaths and canals (Young 2000). Secondly, massive waste flows are created in restricted areas that have a limited capability to assimilate them (Butlin 1976).

Aquatic, terrestrial and atmospheric pollution has increased greatly since 1945 due to rapid technological changes, population increase, and expanding commerce (Butlin 1976). Ecosystems have become contaminated with a huge variety of materials, the major toxic pollutants including metals (e.g. lead), synthesised organic compounds (e.g. organochlorine pesticides, polychlorinated biphenyls), oils and tars, toxic gases (e.g. chlorine, ammonia), anions (e.g. cyanides, fluorides, sulphides), acids and alkalis, and putrescible substances (Mason 1996). These are produced through a range of activities such as domestic and industrial waste disposal, accidental and process spillage, mining, and use of agricultural fertilisers and pesticides.

A.2.2.1 The History of Water Pollution Control in Sydney

Government regulation of industry-based water pollution was introduced with *The Noxious Trades and Cattle Slaughtering Act* in 1894, over 100 years after settlement (Butlin 1976). In the 1930s the Maritime Services Board was responsible for inland waters, but there was little regulation, and only in 1941 were freshwaters finally recognised, with the dumping of sick or dead animals and the discharging from factories, chemical works or slaughterhouses into navigable waters prohibited near populated areas (Butlin 1976). Finally, in 1955, regulations became more comprehensive with the introduction of maximum effluent standards and laboratory testing (including heavy metals) for the assessment of water quality (Butlin 1976).

By the 1960s, wastes were becoming unmanageable within the Sydney metropolitan area, with environmental concerns generally not addressed (Butlin 1976). It was common practice for councils to routinely dump garbage into swamps and when full, convert them into parks or sportsfields, which would leach contaminants into local watercourses (Butlin 1976). With people directly experiencing high levels of water, air, and ground pollution, public concern increased locally and globally through the 1960s (Butlin 1976). In 1968, two Senate Select Committees began pollution investigations and concluded that air and water were used as free garbage dumps and that the matter was of national concern, and in 1969 Sydney's last council tip for industrial liquid waste disposal was closed (Butlin 1976).

From the 1950s until the 1970s, when three-quarters of water intake by manufacturing industries in Sydney was used for carrying liquid wastes (Butlin 1977), approximately half of Sydney's factory wastes were legally discharged directly into inland waterbodies, creating a major environmental stress (Butlin 1976). Controls tightened in 1971, but illicit dumping of highly concentrated or toxic wastes continued, the incentive being avoidance of treatment and disposal costs (Butlin 1976).

The *Environmental Impact Statement*, designed to assess environmental implications of developments and other activities, was introduced in 1972, as was *The Clean Waters Act*, which resulted in the direction of a large proportion of liquid wastes away from stormwater drains and watercourses and into public sewers (Butlin 1976). *The Clean Waters Act* led to the *Trade Waste Policy* in 1988, which encouraged industry to use on-site water pre-treatment to remove domestic-type and chemical (e.g. heavy metal) wastes which would otherwise incur a high sewerage fee (Water Board 1990). Within two years this led to a halving of the amount of industrial and commercial waste going through the sewers (Water Board 1990).

Awareness and regulatory action affecting NSW has continued to increase, with the banning of production and use of noxious organics and metals continuing through the 1990s (EPA 1997). High costs of waste disposal (Water Board 1990), however, will probably mean illegal dumping of industrial liquid waste will always be a problem (Butlin 1976).

A.2.2.2 The History of Air Pollution Control in Sydney

Air pollution started to be monitored in the Sydney Metropolitan area in the mid-1950s and it was found that air pollution quantity and quality had been considerably altered by an enormous increase in industrial, chemical, and vehicle exhaust emissions (Butlin 1976). *The Clean Air Act* was passed in 1961, in 1965 emission standards for scheduled industrial premises came into effect, in 1972 emission standards were introduced for all new motor vehicles, and in 1973 open air burning was generally prohibited including solid wastes at garbage depots (Butlin 1976).

Controls on motor vehicle emissions were minimal until 1986, but cars have subsequently been fitted with catalytic converters to reduce emissions of oxides and reactive organic compounds (EPA 1996). As a result, emissions from individual vehicles have decreased, but suburban expansion has increased total vehicle kilometres travelled (EPA 1996). Emissions have also been reduced through pollution reduction programs for industry and controls on backyard burning (EPA 1996). Most air pollutants are now at lower concentrations than in the 1970s and early 1980s, and lead in air has declined since the introduction of unleaded petrol in 1985 (EPA 1996). Country-wide air quality standards were set by the National Environment Protection Council in 1998 (Young 2000).

A.3 Pollution

Urbanisation leads to large volumes of liquid waste, with pollutants reaching waterways from both point sources and diffuse sources (Butlin 1976, EPA 1996). The main point sources of contaminants are sewage treatment plants and industrial wastes or spills (EPA 1996). Also, industrial process waters carry many contaminants, including metals such as mercury, cadmium, lead, zinc, and copper (Butlin 1977). As for diffuse sources, stormwater in urban areas is problematic due to the covering of extensive areas with impermeable materials, and subsequent increases in the volume and speed of water runoff (EPA 1996). Stormwater contains a mixture of toxicants and contaminants collected from these impermeable surfaces which is then funneled into rivers and creeks (EPA 1996), resulting in severe pollution of waterways, especially in storm conditions after periods of dry weather (Mason 1996). Little is known of the quantity and quality of these waters in Sydney (Butlin 1976), although generally stormwater can carry oils and grease, vehicular wastes from roads, suspended solids, nutrients, and waste from sewage overflows or leaks, as well as leachate from contaminated sites or rubbish tips (EPA 1996). Runoff and seepage may also carry fertilisers, pesticides, herbicides, insecticides and faecal coliforms from residential gardens or agricultural areas (EPA 1996, Butlin 1976).

A.3.1 Metal Pollution

The term 'metals' is used to encompass the heavy metals, the alkali and alkaline earth metals, the lanthanides and actinides, the lighter metals (e.g. Al), and the metalloids (e.g. Se, As). Over 80 of the 106 elements are metals (Vouk 1979), and some are essential for the functioning, growth and survival of animals (Keen 1996). Metals are among the aquatic contaminants which commonly accumulate in organisms and sediments (EPA 1996), and whether or not they are essential, all metals are toxic above a certain concentration (Linder & Grillitsch 2000), with outcomes of chronic environmental pollution including reproductive failure and suppression of the immune system (Aguirre *et al.* 1994).

There is constant movement of metals between the geosphere, atmosphere and hydrosphere, and Australian freshwater turtles may be exposed directly through intake of sediment, air or water, as well as through consumption of dietary items. In freshwater turtles, pollutants can be absorbed from the atmosphere (pulmonary exposure via inhalation), from dietary items or sediment (oral ingestion with gastrointestinal absorption), or directly from water (oral ingestion, cloacal uptake for breathing/water balance, pharyngeal uptake, transdermal uptake). Uptake of metals can occur through all surface epithelia but mainly occurs through those with high metabolic transport, high diffusion activity, increased surface area, and dense vascularisation, i.e. through the intestinal tissues and respiratory surfaces of aquatic vertebrates (Linder & Grillitsch 2000, Palmer 2000). Degrees of metal uptake via different respiratory structures will vary in aquatic reptiles, and as the use of each varies with oxygen availability, anoxic water may influence absorption of ecotoxins (Palmer 2000).

Unlike many other chemical pollutants, metals are naturally present in all aquatic systems, and aquatic organisms are adapted to natural fluctuations in metal concentrations in food and water (Beijer & Jernelöv 1979b). However, naturally-occuring nonessential metals are rarely bioavailable, being rock-bound or poorly soluble, so there has been little evolutionary pressure favouring macromolecules that bind essential but exclude non-essential metals (Reuhl & Dey 1996, Pacyna 1996). The result is a significant susceptibility of the biota to metals mobilised as a consequence of industrialisation and urbanisation (Reuhl & Dey 1996).

Metals in **freshwaters** originate from terrestrial sources (surface run-off, leaching, bank erosion), atmospheric sources (wet and dry deposition), and from erosion of rocky substrate (Beijer & Jernelöv 1979a). Industrial and agricultural activities are considered the primary sources of anthropogenic environmental metal pollution, with aquatic metal concentrations steadily increasing since the industrial revolution (Beijer & Jernelöv 1979a, Camner *et al* 1979). Major contributors include the combustion of fossil fuels, mining and smelting, processing and manufacturing industries, waste disposal (dumping, domestic and industrial sewage, scrap metal), agriculture through fertilisers, pesticides and land clearing through increased aquatic inputs via soil erosion (Beijer & Jernelöv 1979a).

In aquatic systems, **sediments** may bind contaminants leading to much higher concentration than in the overlying water (Suedel *et al*. 1994). Metals sequestered in sediments may become bioavailable after disturbance by physical factors (e.g. storms, dredging) or by chemical factors (e.g. changes in pH or redox potential) (Beijer & Jernelöv 1979a, Suedel *et al*. 1994). Also in sediments, metals may be transformed biologically or chemically into organometallic compounds which may show increased bioabsorption and toxicity compared to the inorganic forms, and which may decompose rapidly or be highly persistent environmentally (Beijer & Jernelöv 1979a, Clarkson 1979).

Sydney has a limited range of rock types, but soils show considerable variation because of influencing local factors such as climate, slope, and vegetation, although almost all are acidic (Benson & Howell 1990). Sydney has significant areas contaminated by metals, with polluted sediments due to historical as well as continuing pollution events (EPA 1996). Anthropogenic contamination of sediments is considerable in Sydney, with most of the metal contamination likely to have resulted from past industrial practices originating with early settlement, and from urban run-off (EPA 1996).

Air pollution can affect biota by being inhaled directly, or deposited in surface waters by natural particle settlement (dry deposition) or rainfall flushing (wet deposition), thus having a significant impact on urban water quality (Pacyna 1996, Beijer & Jernelöv 1979a). Atmospheric metals originate from a variety of sources including land erosion, incinerators, landfills, hazardous waste sites, sewage treatment plants, industries, and road traffic (Zillioux *et al.* 1993, Young 2000). Sydney's air pollutants include lead and other metals such as copper, iron, and cadmium (Butlin 1976). Meteorological conditions (winds, precipitation), topography, and particle size all affect the dispersion and dilution of air-borne pollutants, thus influencing their affect on air quality (Beijer & Jernelöv 1979a, EPA 1996).

In NSW, the population is concentrated in the greater metropolitan region and this is where polluting activities (vehicular, industrial, commercial, domestic) have the greatest effect on air quality (EPA 1996). The air quality in Sydney and other cities is greatly affected by energy use for transport, electricity generation and industrial processes, which involves the combustion of petroleum products, coal and gas, and their contaminants and additives (EPA 1996). Due to atmospheric inversions (temperature increases with height), which are common in Sydney, atmospheric metals may accumulate to high levels before precipitation arrives to flush the air (Carras & Johnson 1983, Commonwealth of Australia 2001). This means semi-aquatic animals may be exposed to elevated atmospheric concentrations, before then receiving a spike in aquatic concentrations. It is hard to quantify the exact contribution of atmospheric metals to the metal load of aquatic biota (e.g. Zillioux *et al.* 1993).

A.3.2 Bioaccumulation

Bioaccumulation is the net accumulation of an environmental substance by a tissue or whole organism as the result of uptake by all exposure routes, including inhalation and absorption, although it is primarily through ingestion of food and water (Beijer & Jernelöv 1979b, Linder & Grillitsch 2000). Accumulation occurs when the rate of uptake exceeds that of biotransformation, dilution and/or excretion, and may vary among tissues (Beijer & Jernelöv 1979b, Linder & Grillitsch 2000). As with other vertebrates, metals usually have an organ-specific distribution in reptiles, although this may vary with duration and concentration of exposure (reviewed in Linder & Grillitsch 2000), with metals usually initially concentrating in rapid metal-binding capacity tissues from where they may be redistributed via the blood to their final storage site (Linder & Grillitsch 2000).

Bioconcentration by aquatic organisms is bioaccumulation resulting from the uptake of substances directly from water, and is independent of trophic level (Sparling *et al.* 2000a). Concentrations in biota may not reflect environmental levels, with very high concentrations sometimes accumulated in fauna from very low concentrations in water (e.g. uptake of metals by fish and invertebrates) (Mason 1996). Bioconcentration is not common for bioaccumulants with low water solubilities, which generally transfer into aquatic vertebrates through dietary pathways rather than direct water uptake (Suedel *et al*. 1994).

Internal factors including physiological condition can influence bioconcentration. For example, the concentration of lipophilic compounds in fish tissue may be related to fat content, and fish with higher metabolic rates accumulate contaminants faster (Mason 1996), although periods of fast growth are associated with a reduction in the contaminant tissue concentrations (Mance 1987). Age, sex and the presence of competing pollutants in the water may also influence accumulation rates (Mason 1996).

For highly soluble metals, bioconcentration may dominate in the transfer of metals to aquatic animals, whereas bioaccumulation by dietary exposure in aquatic food chains may be dominant for poorly soluble metals or metal complexes (Linder & Grillitsch 2000).

Biomagnification is the increase in tissue concentration (usually by two or more orders of magnitude) of a poorly depurated trace substance up successively higher trophic levels, primarily through dietary accumulation (Beijer & Jernelöv 1979b, Linder & Grillitsch 2000, Sparling *et al*. 2000a). If uptake has exceeded depuration in dietary items, biomagnification may progress as each successive trophic level receives higher concentrations of contaminant (Suedel *et al*. 1994). Thus predatory wildlife are particularly vulnerable to substances that biomagnify, and environmental levels that are safe for fish and invertebrates are not necessarily safe for those that consume them (ANZECC 1992).

Biomagnification generally manifests itself when organisms accumulate chemicals via multiple routes of exposure and is classically described in aquatic or wetland systems where the primary routes of exposure are water-contact respiratory surfaces and food ingestion (Linder & Grillitsch 2000). Food-web biomagnification of contaminants in freshwater ecosystems has not been thoroughly investigated (Suedel *et al*. 1994), although generally carnivores contain higher concentrations than herbivores, which contain more than plants (Mason 1996), although some plants hyperaccumulate select substances (Baker 1981). Organochlorine pesticides often biomagnify, but biomagnification is unusual for metals (Mance 1987), except when in organometallic form (Suedel *et al*. 1994).

A.3.3 Biomonitoring

In aquatic systems, pollution can be intermittent, can disperse rapidly, and concentrations may vary widely over short distances, making it hard to quantify without constant monitoring over a wide area (Root 1990). Accumulation of contaminants in animal tissues can give a measure of pollution integrated over a period of time and space, as well as a more relevant measure of potential biological effects than simple chemical analysis, as bioavailability, biomagnification, and duration of exposure are integrated (Root 1990).

Commonly used biomonitors of environmental contamination are invertebrates, fish, birds and mammals (Root 1990), but reptiles are also important components of freshwater ecosystems and should be included in future studies (Campbell & Campbell 2001). Additionally, reptiles can be used as bioindicators for monitoring general impacts on the biota if population decline or tissue contaminant concentration is correlated to adverse effects in other species (Sparling *et al*. 2000b). In toxicological studies, valuable reptilian characteristics include longevity, high trophic level, small home ranges (Delaney *et al*. 1988), bioaccumulation concentrations comparable to or higher than other vertebrates (sometimes from low environmental concentrations of extremely toxic substances), fat deposits that accumulate lipophilic contaminants (Olafsson *et al*. 1983), and non-genetic sex determination useful for assessing estrogenic xenobiotics (Crain & Guillette 1998).

Turtles are useful biomonitors as they are long-lived even when exposed to environmental contaminants (Sparling *et al*. 2000b) and may be the only vertebrate present in degraded waters, e.g. in anoxic waterways which may have greatly reduced species diversity and an absence of many other indicator organisms or in waterbodies isolated from fish stocks (pers. ob.). Their longevity means that trends in local pollution may be obtained by comparing concentrations of the pollutant to the age of the turtle (Sparling *et al*. 2000b). Turtle eggs are also very useful as they are easily collected and large clutches allow measurement of contaminant concentrations and hatching and deformity rates within one nest (Bryan *et al*. 1987, Bishop *et al*. 1991, Sahoo *et al*. 1996, de Solla *et al*. 2001).

A.3.3.1 Comparison with **Chelydra serpentina**

The majority of tissue contaminant studies in freshwater turtles have involved the north American snapping turtle (*Chelydra serpentina*). *Chelydra serpentina* share characteristics with *C. longicollis* which make them good biomonitors: they are common, widespread, large enough to allow tissue sampling (Bonin *et al*. 1995), and may be abundant in polluted waters (Bishop *et al*. 1995), although this is variable (Bonin *et al*. 1995). *C. longicollis* has an advantage over the omnivorous *C. serpentina*, as scavenging and carnivory are often associated with higher concentrations of tissue contaminants (Suedel *et al*. 1994), although the latter is able to accumulate very high concentrations of organochlorines in fat tissue (Olafsson *et al*. 1983). The sedentary nature of *C. serpentina* may be preferable to the itinerant nature of *C. longicollis* as the area of possible contaminant origin is limited. Elevated contaminants are associated with death and deformity of *Chelydra serpentina* embryos (Bishop *et al*. 1991).

A.3.4 Metal Effects

Pollutant effects on biological processes can be acute (Bury 1972) or chronic (Lundholm 1997, and can be minor, e.g. dermatitis (Norseth & Piscator 1979), or major, e.g. loss of reproductive capability (Pattee 1984, Hoffman 1990, Ragan & Mast 1990, Fox *et al*. 1991, Bezel *et al*. 1998) or death (Bury 1972, Janiga & Zemberyova 1998, Boet *et al.* 1999). Elevated environmental metal concentrations cause deleterious effects in aquatic organisms (Sorensen 1991), potentially affecting all areas of the body, and all biological functioning from molecules, cells, tissues and organs to organisms and communities with a wide range of potential effects (Clarkson 1979, Linder & Grillitsch 2000). Sublethal effects of metals may be expected more commonly than lethal effects at concentrations found in nature (Scheuhammer 1987). Essential trace metals are less likely than xenobiotic metals to reach toxic tissue concentrations as they are under homeostatic control, but elevated environmental levels may lead to controls being overwhelmed or bypassed, (Clarkson 1979). Essential metal deficiencies may also cause malfunctions, morbidity, or even death (Keen 1996).

Detection of elevated metal tissue concentrations indicates increased metal exposure but it is also necessary to determine if biological functioning has been damaged. The most useful parameters to measure are those indicating alterations to an individual's survival or reproduction (Dethloff *et al*. 2001). It is important that populations be examined for underlying effects on immunity and reproduction (Bailey & Guyer 1998).In the current study reproduction will be studied along with effects on the haemopoietic system, the latter being easy to measure, and damage to the system having severe implications for survival. Also, haematological and immunological changes are among the first detectable responses of vertebrates to environmental contaminants such as metals (reviewed in Dethloff *et al*. 2001), and may be present as a result of metal exposure, despite animals appearing otherwise healthy (Redig *et al*. 1991, Khangarot *et al.* 1999, Dethloff *et al*. 2001). Suppression of the immune system by metals and other environmental contaminants is characteristically associated with increased susceptibility to infectious disease (Gupta *et al.* 2002), so this aspect was also included in the study.

A.3.4.1 Metals & Reptiles

Fishes, birds and mammals have been surveyed extensively in relation to environmental contaminants (Linder & Grillitsch 2000). In contrast, while interest in the ecology and ecotoxicology of amphibians and reptiles (27.2% of vertebrate species) increased during the 1990s, they still account for only 3.5% of vertebrate ecotoxicology studies (Sparling *et al.* 2000a). There are also few examples of non-lethal sampling or long-term studies on reptiles with the exception of some marine turtles (Portelli & Bishop 2000), and little information on pollution effects or the ways reptiles affect ecotoxicological processes (e.g. dietary or migrational transfer of contaminants) (Niewiarowski 2000). However, within reptiles, the Chelonia (excepting land tortoises) are the most included in toxicological research, with the focus on snapping turtles (*Chelydra serpentina*) and sea turtles (Cheloniidae, Dermochelyidae) (Jacobson 1994, Sparling *et al.* 2000a).

Aquatic reptiles such as turtles, water snakes and crocodiles are particularly susceptible to water-borne contaminants, with the dominant routes of exposure being direct uptake from water and the ingestion of contaminants in sediments or food items (Sparling *et al.*

2000a). However, although epidermal phospholipids (but not the keratin scales) make the skin of reptiles relatively impermeable, this varies among species and skin may be a significant route of exposure (Palmer 2000).

Metals are highly persistent in environmental systems (Gardner & Zelikoff 1996) and can cause death and deformity in vertebrates (Kime 1995, Eeva & Lehikoinen 1996), yet little is known of their toxic effect on reptiles (Sparling *et al.* 2000b). Urban freshwater turtles are likely to be more susceptible than terrestrial taxa because of their prolonged immersion in polluted aquatic environments. Despite this, very little work has been done on metal accumulation in freshwater turtles, with no studies on any of the Australian species. Many of Australia's endemic turtles are endangered or threatened, so susceptibility to metal pollution may be an important conservation concern.

A.4 Aims

Knowledge of bioaccumulation of metals and its effects in reptiles needs to be expanded in regards to the:

> 'magnitude of tissue concentrations in relation to their natural and anthropogenic concentrations; the magnitude of tissue concentrations in relation to endogenous biotic factors; the balance between uptake, transformation, and excretion; significance of maternal transfer of metals to eggs regarding maternal & offspring levels; the relative distribution of metals among different egg compartments, and eggs and embryos as biomonitors for the assessment of both exposure and effect; relative distribution of metals among organs; biomagnification for indication of contamination via the food web.'
>
> Linder & Grillitsch 2000

This thesis makes a considerable contribution to filling this knowledge gap by conducting the first studies of pollution effects on freshwater turtles in Australia.

The aim of this thesis is to study anthropogenic impacts on turtle populations, with an emphasis on metal pollution. The focus is on urban impacts, which in Sydney also include peri-urban impacts from agriculture in addition to domestic, commercial and industrial sources.

Initially, a random survey of turtles over the more densely populated area of the Sydney Basin (1600 km^2) was conducted to assess the distribution, numbers, population structure and species of turtle in this urban environment (Section C). The initial survey tested hypotheses relating to heterogeneity of turtle populations in urban environments, and developed baseline data for future assessment and management of turtle populations in the Sydney region.

The potential impact of pollutants on the immune system of turtles was then examined (Section D). This section used sewage treatment plant outfalls in north-western Sydney as a source of pollutants, and tested hypotheses relating to the effects of sewage outfalls on turtle numbers, body condition, blood profiles and parasite loads, including the potential for changes in these variables with season and year.

Studies of the potential for metal accumulation in urban turtles are presented in Sections E and F. Section E identifies the patterns of tissue distribution of metals, testing whether easily sampled tissues (blood and carapace) can be used for biomonitoring, and compares metal concentrations in turtles from urban and non-urban sites, testing the general hypothesis that turtles from urban environments have higher metal loads than those from non-urban environments. This study further tests whether there are species or sex differences in metal concentrations.

Section F extends the studies of metal accumulation, comparing metal concentrations in gravid female turtles with those in their eggs, testing hypotheses that maternal metals are transferred to the offspring, and that maternal metal concentrations have an effect on eggs and hatching.