SECTION F

METAL EFFECTS ON REPRODUCTION

CHAPTER F1: METALS & REPRODUCTION INTRODUCTION

Ecotoxins act at the molecular, biochemical, and cellular level, and if they are not effectively dealt with at these levels, then effects may present themselves at the level of the organism, the population and the ecosystem (Rice *et al.* 1996). Most of the limited number of studies done on metal contamination in turtles have simply reported tissue concentrations, and although this indicates metal exposure it should also be determined if physiological parameters have been compromised, and this is best done using parameters that are linked to survival and reproduction (Dethloff *et al*. 2001). Metals have a wide variety of reproductive effects in turtles: they may compromise the health and reproductive output of adults, they may directly affect embryonic development, and they may affect the growth and maturation of hatchlings. Direct effects on eggs include eggshell thinning, reduced egg size, and death of embryo or hatchling (e.g. Kime 1995, Colborn & Clement 1992).

Metal accumulation in turtle eggs and metal-associated reproductive effects were compared between turtles from four sites in the highly industrialised area of south-eastern Sydney, and turtles from four sites in national park areas located just to the south of the city.

F1.1 Reproductive Aspects of Turtles

All turtles are oviparous (Congdon & Gibbons 1990b) and, excepting *C. rugosa* (Kennett *et al*. 1993), female turtles leave the water to dig a nest into which the eggs are laid (Georges *et al.* 1993). Although temperature-dependent sex determination occurs in the Australian cryptodire *Carettochelys insculpta* (Webb *et al*. 1986), it is not known from any of the Australian chelids including *E. macquarii* (Thompson 1988) and *Chelodina longicollis* (Georges 1988). Australian chelids exhibit two basic genetically-controlled reproductive patterns – tropical and temperate. *Chelodina longicollis*, *E. macquarii* and *El. latisternum* follow the temperate pattern (Parmenter 1976, Chessman 1978, Legler 1985), laying small eggs in spring and summer, with a short incubation period and nest eruption before winter (Legler 1985). This reproductive pattern is usually controlled by annual light and temperature variations as detected by the pineal gland and regulated endocrinally (Palmer 2000).

Chelodina longicollis mate in September and October (Ernst & Barbour 1989), and ovulation occurs in late October and November (Parmenter 1985, Kennett & Georges 1990). Although *C. longicollis* females may lay up to three clutches in one season (Ehmann 1992), the trait is uncommon (Parmenter 1985), or absent (Vestjens 1969) in some populations. *Emydura macquarii* can also have a low incidence of multiple clutches (< 10%, Thompson 1983b; Spencer 2002), although *E. m. krefftii* is capable of depositing three clutches in a season in the wild (Georges 1983), and four clutches in captivity (Banks 1987). Body size of female turtles is positively related to clutch size and clutch frequency (Iverson 1992). In freshwater turtles follicles for multiple clutches develop at the same time so the second clutch can be ovulated shortly after the first clutch is laid, and laid less than two weeks later (Congdon & Gibbons 1990b). A rapid deposit of multiple clutches must be the case for *C. longicollis* as females are only found in the gravid state over a very short time period (Parmenter 1985).

Yolk production begins with vitellogenin production in the liver, largely due to oestrogen stimulation, and on release from the liver, the vitellogenin is taken up by the oocytes where it is converted to the lipovitellins and phosvitins of egg yolk (Palmer 2000). Vitellogenesis (follicular enlargement) occurs throughout most of the active year (Parmenter 1985), meaning that pollutant uptake at any time can be incorporated into egg yolk. Ovaries (of *C. longicollis*) grow quickly in early spring, and ovulation occurs in late October and November, and peters off in early December (Parmenter 1985, Kennett & Georges 1990). Mature female *C. longicollis* appear to breed every year, and probably continue breeding until death (Parmenter 1985), and over 86% of mature female *E. macquarii* breed each year (Spencer 2002).

The eggs are thought to be fertilised in the anterior oviducts, with subsequent deposition of albumen occurring within the oviducts, and both the fibrous (inner) and calcareous (outer) eggshell layers then deposited by the endometrial glands of the uterus (reviewed in Palmer 2000).

F1.1.1 Nesting

Placing eggs in a nest can reduce the temperature fluctuation experienced at the substrate surface by approximately 30ºC (Georges 1992). Different populations of the same species may vary with respect to the size of clutches, the number of clutches, the

time of nesting, and the incubation period (Georges *et al.* 1993). In cohabited areas, nesting seasons and sites overlap to a large extent for *C. longicollis* and *E. macquarii* (Thompson 1983b). *Chelodina longicollis* nests in the spring and early summer (Parmenter 1976, Chessman 1978), with the very last eggs laid in early January (Canberra, Vestjens 1969; Ernst & Barbour 1989). In coastal NSW, *C. longicollis* nest from mid-October to the end of December, with a peak in November and early December (Kennett & Georges 1990). The eggs of *E. macquarii* are laid from late October through December (Ernst & Barbour 1989). Further north, in their natural distribution, *E. latisternum* nest in September and October (Cann 1978), and into December (Ehmann 1992).

Generally, *C. longicollis* and *E. macquarii* nest at night during or after rain, although they may nest during the day if it is heavily overcast (Goode & Russell 1968, Vestjens 1969, Thompson 1983b). Nest digging takes about an hour, followed by 1-3 hours for egg-laying, with nests then filled and surface tamped down with the plastron (Goode 1965, Vestjens 1969, Beck 1991), before the turtle heads directly back to the water (Stott 1987). Once laid, there is no further parental investment in eggs or hatchlings (Georges *et al.* 1993).

F1.1.2 Eggs

The eggs of most chelonians are ellipsoidal spheroids (Legler & Georges 1993), and may have flexible calcareous (shell units do not interlock, eg. sea turtles) or ridged calcareous (thick, with interlocking units, more resistant to desiccation) eggshells (Congdon & Gibbons 1990b). The eggs of *C. longicollis* and *E. macquarii* are ridged-shelled ellipsoids (Kennett & Georges 1990) and are the smallest of the Australian chelids (Legler & Georges 1993). When laid, the eggshells are translucent but within a day an opaque white patch forms and spreads as a result of drying of the shell and the outer membranes, increasing shell gas exchange capacity (Thompson 1985).

F1.1.3 Hatching

In temperate species, hatchlings emerge from nests in mid- to late summer (Georges 1983), without overwintering in the nest (Vestjens 1969, Parmenter 1985) as some north American species do (Gibbons 1990a). Hatchlings usually leave the nest after rainfall, which softens the overlying soil and the hard nest plug (Vestjens 1969,

Parmenter 1985, Ehmann 1992), and usually at night, heading directly for water (Ehmann 1992). In dry conditions, some hatchlings may remain in the nest cavity for weeks (Vestjens 1969). Feeding begins within a few days of hatching (captives; Cann 1998), and the initially soft shells become firm within a week to 4 months, depending on the species and environmental conditions (Banks 1987, Cann 1998). The rate of Australian chelid hatchling survival is unknown (Georges 1983), although the eggs and hatchlings are both highly vulnerable to predation (Georges *et al.* 1993), with an annual nest mortality generally greater than 80-90% (Section C4.2.3). In contrast, annual adult survivorship approaches 100% (Spencer 2001).

F1.2 Reproductive Toxicity of Metals

F1.2.1 Metals in Eggs

Many more studies on the effects of ecotoxins, including metals, have been conducted on birds than reptiles, and these have shown that developmental effects include impacts on the size and weight of eggs, the thickness of the eggshell, and the size, health and survivorship of hatchlings (Hoffman 1990, Fox *et al.* 1991, Fry 1995, Lundholm 1997, Paveglio *et al.* 1997). Although normal concentrations of metals and the affects of toxic concentrations in turtles are largely unknown, metals are transferred from the female turtle to her eggs, as evidenced by the presence of metals (Cd, Cu, Fe, Hg, Mn, and Zn) in oviductal eggs (*Caretta caretta*, Sakai *et al.* 1995).

The metals in reptile eggs have not often been studied. So far, a total of 18 metals have been measured in the eggs and hatchlings of five species of turtle, three crocodilians, and two species of snake (reviewed in Linder & Grillitsch 2000). Information on reptile egg metals is sparsely scattered over different taxonomic groups, animals at different stages of development, different tissues and different metals (Linder & Grillitsch 2000), meaning that comparisons are hard to make and general trends are not easily elucidated.

Turtle embryos are protected to some extent from environmental xenobiotics by the calcareous eggshell and the amniotic membranes (Palmer 2000). Nonetheless, the reptilian eggshell is porous, and the fine eggshell structure and the nature of the substrate at nesting sites must influence the amount and type of pollutants that are

absorbed by the eggs as they take up water from the environment (Linder & Grillitsch 2000).

F1.2.2 Reproductive Toxicity of Metals on Young

Ecotoxins can reduce a reptile's reproductive capacity (Palmer 2000). Knowledge of the normal state greatly aids the study of abnormal or malfunctional states, yet reproductive endocrinology and physiology of reptiles has been studied in very few species (Palmer 2000).

Hormonal pathways that control reproduction and development are among the most susceptible to xenobiotic perturbation (Palmer 2000). Embryonic development is a sensitive stage for ecotoxicological effects, and both hormone synthesis/metabolism and signal transmission/recognition along the biochemical pathways controlling development of physiological and anatomical systems are disrupted by ecotoxins (Palmer 2000). Sublethal doses cause persistent developmental abnormalities (Diana *et al.* 2001), including anatomical defects and physiological imbalances (Palmer 2000).

F1.2.3 Reproductive Toxicity of Metals on Adults

Pollution-induced alteration of the embryonic and early postnatal development may lead to the premature death of offspring, but often effects, such as loss of fertility, may not be apparent until adulthood (Mason 1996).

Environmental contaminants, including metals, have a very broad range of toxic effects, all of which could compromise the health and function of reproductively active adults, even if they were not previously damaged as embryos or hatchlings (Fry 1995). Metal-mediated organ damage will vary with metal species and dose, duration of exposure, and metal interactions (Fowler 1996b).

Proteins (especially those rich in sulfhydryl groups) are the dominant site of damage by metals, with enzymes the most commonly affected (Sanders *et al.* 1996). Toxic metals may interfere with substrate binding, or displace an essential catalytic metal from the enzyme, a situation exacerbated by a deficiency in the essential element (Sanders *et al.* 1996).

Biological (e.g. plasma, mitochondrial, microsomal) **membranes** are major sites of metal toxicity due to their multitude of metal-attracting sulfhydryl (–SH) groups, with metal-binding likely to inhibit membrane transport systems (Clarkson 1979, Chang 1996a).

Toxic concentrations of metals may also modify proteins involved in DNA repair resulting in **genotoxic effects** (Sanders *et al.* 1996). Toxic metals (e.g. Cd) can replace essential metals (e.g. Zn) on enzymes required for the synthesis and repair of nucleic acids, thus resulting in a mutagenic effect (Clarkson 1979). Metals (e.g. Cd, Cu) may also destabilise DNA structure by binding to it directly (Ware 1988, Cohen *et al*. 1996). High concentrations of essential metals can cause teratogenic or embryotoxic effects, probably secondary to maternal toxicity, although the mechanism is uncertain (Keen 1996).

Exposure to excessive metal levels can lead to adverse structural or functional changes to the **nervous system** (Chang 1996b), ranging from subtle lesions and effects to gross physical signs culminating in death (Mailman *et al.* 1996). Metals (e.g. Hg, Pb, Cd, Mn, Al, As) are the most common neurotoxicants (Reuhl & Dey 1996), although no metals are exclusively neurotoxic (Bondy 1996). The central nervous system controls coordination, and the regulation of cardiovascular, neuroendocrine, and immune function (Mailman *et al.* 1996). Toxic metals may also disturb normal behaviour (Reuhl & Dey 1996). Neuronal development is a sensitive target, and malformations can result from relatively small metal-induced changes (Audesirk & Audesirk 1996). The biochemical mechanisms by which metals alter neural function are poorly understood, but it is likely that metals interfere with sites that normally bind calcium ions or with sulfhydryl groups (Audesirk & Audesirk 1996). Some essential metals (e.g. Zn, Cu, Fe) are critical for proper central nervous system function, yet excess levels can be associated with neurotoxicity (e.g. behavioural impairments) (Cory-Slechta 1996).

Kidneys are the main route of excretion for most metals, and have a number of metal-sensitive metabolic processes, making them another major target for metal toxicity (Fowler 1996a). Damage to the proximal tubule is common for acute or chronic exposure to many metals, and capillary damage can also occur (Fowler 1996b).

Direct atmospheric exposure of the **lungs** to metals is significant in urban areas (Bigazzi 1996), with a major contribution coming from vehicle fuel-additive emissions, notably Pb from older vehicles Nriagu 1979, and Cr, Cu, Ni or Mn from newer vehicles (reviewed in Bigazzi 1996). Even at low concentrations these metal exposures can significantly increase the risk of respiratory infection by compromising host defence mechanisms, and can impair mechanical clearance of metal-containing particles, insoluble particles sometimes remaining for months in the lower airways (reviewed in Bigazzi 1996). Although auxiliary respiratory structures in turtles may lessen atmospheric metal exposure, this will not be the case in anoxic waters.

F1.3 Aims

This study will indicate whether metal contamination is affecting reproduction in freshwater turtles in Sydney by determining:

- the transfer of metals from mother to egg
- the effect of maternal metal concentrations on hatchling development

Null hypotheses

1. Metal concentrations in maternal carapace are not correlated with metal concentrations in (a) eggshell or (b) egg contents.

2. Metal concentrations in maternal carapace are not correlated with (a) clutch size, (b) incubation period, (c) egg dimensions, (d) hatchling dimensions or (e) eggshell thickness or mass in either *Chelodina longicollis* or *Emydura macquarii*.

3. Metal concentrations in eggshell are not correlated with eggshell thickness in either *Chelodina longicollis* or *Emydura macquarii*.

CHAPTER F2: METALS & REPRODUCTION METHODS

Field sites (Sections D2.2.1, D2.2.4, E2.2), field work (Sections D2.4, E2.3), tissue preparation for metal analysis (Section E2.5.1), and metal analysis (Section E2.6) have already been described. Eggs were collected and processed as follows.

F2.1 Induction of Oviposition

Twenty gravid females were induced to lay. *Chelodina longicollis* came from Botany Swamps (2), RH.CONT.up (9 collected, 8 successfully induced), and QH.STP.up (4). *Emydura macquarii* came from Botany Swamps (4) and Bicentennial Park (1). *Elseya latisternum* (2) from Botany Swamps was also induced. Within two days of collection, turtles were again palpated and eggs gently pressed. If eggs were pliable turtles were left for a further 3 days before induction.

Turtles were acclimated in a tub (length x width x depth = 38 x 29 x 18 or 46 x 31 x 22) of 32ºC water for 5-10 min. Following the method of Craig Latta (care sheet, pers. comm.), turtles were given an intramuscular injection into the thigh of 0.5 ml calcium gluconate (10%w/v) followed by 0.1 ml oxytocin /kg turtle (10i.u./ml oxytocin for injection; Heriot Agvet Pty Ltd, Rowville, Victoria). Eggs were not laid so the oxytocin concentration was modified to 1 ml/kg (Gabrielle Latta, pers. comm.). After injection, turtles were returned to the tub with a towel provided as a 'hide' and kept in a quiet dark environment. Eggs were laid within 1 h, after which they were retrieved, patted dry with a tissue, and length and width recorded. Six eggs were randomly chosen from all but the heaviest and lightest eggs of the clutch and frozen (in cotton cloth in plastic bags) for later metal analysis. All *E. latisternum* eggs were frozen.

F2.2 Egg Incubation

Remaining eggs were incubated buried in vermiculite with water added until it just started to clump, with the surface of the egg just visible, in plastic containers covered with plastic wrap secured with an elastic band. No constant temperature room was available, and funds were not available for construction of incubators, so eggs were placed in a warm room of fluctuating temperature (median and mean both 29ºC, min

22 ºC, max 36 ºC). All incubations were commenced and completed within the period 7 November 2000 to 6 March 2001. Hatchlings were weighed and measured and examined for external defects, then released at the site of maternal capture.

F2.3 Eggshell Thickness

Two eggs from each clutch were randomly chosen, thawed at room temp, length and width recorded, then a line pencilled around the equator and on a 5 mm radius around each pole. The eggs were then cut with a stainless steel scalpel blade to one side of the equator. The egg contents were discarded and the shell rinsed in tap water and drained on paper towel. Due to curling, flaking and loss of malleability of eggshells after periods of less than an hour in ambient air, each egg was dissected just prior to measurement. The eggshell consists of fibrous shell membranes attached to an overlying calcareous shell, but these are fused and are measured as one (Thompson 1983a). Five measurements were taken around the equator of each egg and five around either pole on one egg (Thompson 1983a), within 5 mm of the pole itself, using a 0-25 mm torque micrometre (to 0.01 mm). The five equatorial values were averaged. After drying for 48 h at 40ºC the dry weight was measured on an Explorer OHAUS digital balance to 0.001 g.

CHAPTER F3: METALS & REPRODUCTION RESULTS

F3.1 Reproductive Parameters

Twenty gravid female turtles were induced to oviposit (Section F2.1). *Chelodina longicollis* came from RH.CONT.up (n = 8, CL = 184-220 mm), and QH.STP.up (n = 4, CL = 177-197 mm). *Emydura macquarii* came from Botany Swamps (n = 5, CL = 203-241 mm) and Bicentennial Park (n = 1, CL = 205 mm). *Elseya latisternum* (n = 2, CL = 226, 237 mm) came from Botany Swamps.

F3.1.1 Clutch Size

Clutch sizes vary within and between the three species (Table F3.1). The clutch size for *C. longicollis* ranged from 6 to 12 with a mode of 9 and a mean of 9.4 (n = 11), with one additional clutch of 4 representing an incomplete oviposit (data from this partial clutch were included in analyses of egg variables, but not clutch size). The clutch size for *E. macquarii* ranged from 12 to 16 with a mode of 12 and and a mean of 13.5. The clutch sizes for the two *El. latisternum* were 11 and 24.

For *C. longicollis* there is a strong relationship between maternal CL and clutch size (r^2 = 0.604, F = 13.717, df = 1,9, p = 0.005). Consequently, for study of relationship between clutch size and maternal metal concentration, residuals of the linear regression of clutch size on CL are used in this species. For *E. macquarii*, there is no significant effect of maternal size (CL, carapace height, or mass) on clutch size (r^2 = 0.304, 0.318, 0.331, respectively; p > 0.232 in all cases), and hence clutch size is used unaltered for comparison with maternal metal concentration for this species.

F3.1.2 Egg Dimensions

Elseya latisternum has the largest eggs, with *Emydura macquarii* and *C. longicollis* having similar sized eggs (Table F3.1). Mean egg length and width are significantly correlated in both *C. longicollis* (Pearson's p = 0.714; P = 0.009) and *E. macquarii* (Pearson's p = 0.900; P = 0.014). There is no effect of maternal size (CL) on either mean egg length or egg width for *C. longicollis* (egg length: r^2 = 0.245, F = 3.239, df = 1, 10, p = 0.102; egg width: r^2 = 0.162, F = 1.927, df = 1, 10, p = 0.195) or *E. macquarii* (egg length: r^2 = 0.048, F = 0.204, df = 1, 4, p = 0.675; egg width: r^2 = 0.039, F = 0.160, df = 1, 4, p = 0.709). Hence, raw values are used in studying the effect of maternal metal concentration on egg dimensions.

	Egg Length			Egg Width		
Site/Turtle #	**Range**	**Mean (SD)**	**n**	**Range**	**Mean (SD)**	**n**
C. longicollis						
RH/777	25-29	27.1 (1.10)	10	17-18	17.1 (0.32)	10
RH/173	31-33	32.0 (0.45)	11	18-19	18.6 (0.51)	11
RH/776	29-31	30.1 (0.64)	8	18	-	8
RH/782*	30-33	31.9 (0.782)	9	20	-	9
RH/770	30-33	31.2 (0.83)	9	19-20	19.3 (0.50)	9
RH/775	30-32	30.6 (0.73)	9	19-20	19.3 (0.50)	9
RH/171	28-31	30.2 (0.84)	12	18-19	18.9 (0.29)	12
RH/778	28-29	28.5 (0.53)	10	17-18	17.9 (0.32)	10
QH/112	27	-	6	18	-	6
QH/796	29-31	30.0 (0.50)	9	19	-	9
QH/795	29-30	29.7 (0.50)	9	17	-	9
QH/240	30-31	31.2 (0.50)	4	19	-	4
Total	**25-33**	**29.9 (1.87)**	**106**	**17-20**	**18.5 (0.95)**	**106**
E. macquarii						
U2/6	31-33	31.9 (0.54)	14	19	-	14
U2/15	29-34	31.4 (1.88)	12	18-20	19.2 (0.94)	12
U2/21	30-34	32.3 (1.29)	15	19-20	19.6 (0.51)	15
U2/22	30-33	31.4 (1.00)	12	19-21	20 (0.60)	12
U2/23	27-35	28.9 (2.00)	16	17-20	17.8 (0.93)	16
U4/18	27-31	28.7 (1.16)	12	16-17	16.8 (0.39)	12
Total	**27-35**	**30.8 (2.02)**	**81**	**16-21**	**18.7 (1.25)**	**81**
El. latisternum						
U2/7	35-37	35.5 (0.69)	11	21-22	21.8 (0.41)	11
U2/8	32-34	33.6 (0.58)	24	21-24	22.7 (0.76)	24

Table F3.1 Egg length (mm), egg width (mm), and clutch size (n) for three species of turtle. Sites: RH = RH.CONT.up, QH = QH.STP.up, U2 = Botany Swamps, U4 = Bicentennial Park. *One abnormally small egg (16 mm x 10 mm) not included.

F3.1.3 Eggshell Thickness

Within one eggshell, thickness was more variable around one pole and between the two poles than around the equator (data not shown) and hence only eggshell thickness at the equator was used as a measure for comparison. Also, there was little variation in eggshell thickness around the equator for eggs from the same clutch (Table F3.2).

There is no effect of maternal size (CL) on mean clutch equatorial eggshell thickness for either *C. longicollis* (r^2 = 0.007, F = 0.074, df = 1, 10, p = 0.791) or *E. macquarii* (r^2 = 0.391, F = 21.569, df = 1, 4, p = 0.184), and hence eggshell thickness is used unaltered in analyses of the effect of maternal metal concentrations. Similarly, there is no effect of maternal CL on mean clutch eggshell mass (dry) for either *C. longicollis* (r^2 = 0.064, F = 0.681, df = 1, 10, P = 0.428) or *E. macquarii* (r^2 = 0.142, F = 0.661, df = 1, 4, P = 0.462), and eggshell mass is used unaltered in studying the effect of maternal metal concentrations.

F3.1.4 Hatchlings

Incubation periods for individual eggs varied from 78-90 days for *C. longicollis* (n = 21) and from 50-53 days for *E. macquarii* (n = 4). Embryonic mortality was high, especially for *E. macquarii* (Table F3.3). Size and weight of hatchlings varied between species, between clutches, and within clutches (Table F3.3). There were no obvious external deformities on any hatchling, the only adverse variations being death or smaller size. All released hatchlings were viable, being active and readily taking to the water at the release site.

For *C. longicollis*, there is no effect of maternal size (CL) on mean hatchling mass (r^2 = 0.012, F = 0.083, df = 1, 7, p = 0.781) or length (r^2 = 0.118, F = 0.933, df = 1, 7, p = 0.366), and hence these variables are used unaltered in analyses of the effects of maternal metal concentrations. However, egg size does have some effect on hatchling size. There is a significant effect of mean egg length for clutches on mean hatchling mass (r^2 = 0.516, F = 7.449, df = 1, 7, p = 0.029) and on mean hatchling width (r^2 = 0.737, F = 19.575, df = 1, 7, p = 0.003). However, there is no effect of mean egg length (r^2 = 0.416, F = 4.990, df = 1, 7, p = 0.061) or egg width (r^2 = 0.184, F = 1.579, df = 1, 7, p = 0.249) on hatchling length, mean egg width on hatchling mass (r^2 = 0.371, F = 4.125, df = 1, 7, p = 0.082) or mean egg width on hatchling width (r^2 = 0.382, F = 4.329, df = 1, 7, p = 0.076). There were insufficient clutches with hatchlings for *E. macquarii* to determine whether a similar pattern occurred in that species.

	Eggshell Thickness (mm)		Eggshell Mass (mg)	
Site/Turtle #	**Range**	**Mean (SD)**	**Range**	**Mean (SD)**
C. longicollis				
RH/777	0.22	-	455-480	468 (18)
RH/173	0.23-0.25	0.240 (0.0141)	593-628	610 (25)
RH/776	0.22-0.23	0.225 (0.0071)	544-545	545 (01)
RH/782	0.27-0.28	0.275 (0.0071)	725-823	774 (69)
RH/770	0.22-0.23	0.225 (0.0071)	568-584	576 (11)
RH/775	0.23-0.25	0.240 (0.0141)	534-590	562 (40)
RH/171	0.21-0.23	0.220 (0.0141)	517-519	518 (1)
RH/778	0.23-0.24	0.235 (0.0071)	520-527	524 (05)
QH/112	0.24	-	506-531	518 (18)
QH/796	0.23-0.24	0.235 (0.0071)	544-583	563 (28)
QH/795	0.22-0.25	0.235 (0.0212)	505-590	547 (60)
QH/240	0.29	-	730-802	766 (51)
Total (n = 24)	**0.21-0.29**	**0.240 (0.0223)**	**455-823**	**581 (96)**
E. macquarii				
U2/6	0.26-0.27	0.265 (0.0071)	630-690	660 (42)
U2/15	0.21	-	469-548	508 (56)
U2/21	0.22-0.23	0.225 (0.0071)	575-579	577 (03)
U2/22	0.22-0.23	0.225 (0.0071)	575-593	584 (13)
U2/23	0.21	-	409-453	431 (31)
U4/18	0.24-0.25	0.245 (0.0071)	495-537	516 (30)
Total (n = 12)	**0.21-0.27**	**0.230 (0.0209)**	**409-690**	**546 (79)**
El. latisternum				
U2/7	0.24-0.25	0.245 (0.0071)	728-768	748 (28)
U2/8	0.25	-	627-712	669 (60)

Table F3.2 Eggshell characteristics for three species of turtle. Sites: RH = RH.CONT.up, QH = QH.STP.up, U2 = Botany Swamps, U4 = Bicentennial Park. In all cases n = 2 eggs.

Site/Turtle #	% Hatched (# eggs incubated)	Hatchling Mass (g) Range	Mean (SD)	n	Hatchling Length (mm) Range	Mean (SD)	n	Hatchling Width (mm) Range	Mean (SD)	n
C. longicollis										
RH/777	25 (4)	2.7	-	1	20	-	1	18	-	1
RH/173	20 (5)	3.7	-	1	23	-	1	22	-	1
RH/776	50 (2)	3.4	-	1	24	-	1	22	-	1
RH/782	100 (3)	3.9-4.0	3.95 (0.071)	2	26	-	3	22	-	3
RH/770	100 (3)	3.3-3.8	3.55 (0.354)	2	22-25	24 (1.73)	3	20-22	21 (1.00)	3
RH/775	100 (3)	4.3-4.4	4.33 (0.058)	3	22	-	3	22	-	3
RH/171	100 (6)	3.3-3.7	3.57 (0.103)	6	23-26	25 (1.10)	6	20-22	21 (0.82)	6
RH/778	50 (4)	3.4	-	2	21-24	22.5 (2.12)	2	20-21	20.5 (0.71)	2
QH/112	-	-								
QH/796	0 (3)	-								
QH/795	33 (3)	3.8	-	1	25	-	1	21	-	1
QH/240	-	-								
Total	**58 (36)**	**2.7-4.4**	**3.67 (0.404)**	**19**	**20-26**	**24 (1.86)**	**21**	**18-22**	**21 (1.06)**	**21**
E. macquarii										
U2/6	-	-								
U2/15	33 (6)	3.9	-	1	24-25	24.5 (0.71)	2	16-21	18.5 (3.54)	2
U2/21	0 (8)	-								
U2/22	0 (6)	-								
U2/23	0 (8)	-								
U4/18	33 (6)	2.1-2.2	2.15 (0.071)	2	22-23	22.5 (0.71)	2	-		
Total	**12 (34)**	**2.1-3.9**	**2.73 (1.012)**	**3**	**22-25**	**23.5 (1.29)**	**4**	**16-21**	**18.5 (3.54)**	**2**

Table F3.3 Hatchling parameters. For capture sites, RH = RH.CONT.up, QH = QH.STP.up, U2 = Botany Swamps, U4 = Bicentennial Park.

F3.2 Maternal Carapace Metals

The regressions of the concentrations of 13 elements (Ti, Al / Mn, Cu, Se, Zn, Fe / Mg, Ca, Sr, Ba / Na, K) in maternal carapace against reproductive variables (clutch size, incubation period, egg length, egg width, hatchling mass, length and width, and eggshell thickness and eggshell mass) are studied. Se is not analysed for *E. macquarii* because none was detected in any of the females. Carapace metal concentrations are unavailable for one of the six female *E. macquarii*, reducing the sample available for that species to five.

For *C. longicollis*, the species with the larger samples, there are no significant regressions of any maternal carapace metal concentrations on clutch size, incubation period, egg length, egg width, eggshell thickness, hatchling mass, or hatchling length. There is a significant effect of K on eggshell mass ($r^2 = 0.386$, $F = 6.279$, $df = 1, 10$, $p = 0.031$), but no effect of any other metal on this variable.

For *E. macquarii*, there are no significant regressions of maternal carapace metal concentrations on clutch size, egg width, or eggshell mass. There is a significant positive effect of maternal carapace Sr on egg length ($r^2 = 0.801$, $F = 12.054$, $df = 1, 3$, $p = 0.040$), and of Cu ($r^2 = 0.834$, $F = 15.107$, $df = 1, 3$, $p = 0.030$) on eggshell thickness, and a significant negative effect of Ca ($r^2 = 0.899$, $F = 26.725$, $df = 1, 3$, $p = 0.014$) and Mg ($r^2 = 0.873$, $F = 20.661$, $df = 1, 3$, $p = 0.020$) on eggshell thickness, but no effect of other metals on these two variables.

F3.3 Egg Metals

Seventeen metals (Pb, Al, Zr, Cd, As, Sn, V, Sb / Cr, Co, Mo / Be / Li, Cs / La, Th, U) were not detected in either egg contents or eggshell. These are the same 14 metals that were not detected in carapace, plus Pb, Al, and Cr, which were present in carapace. Of the 13 metals detected in egg tissue (Ti, Ni / Mn, Cu, Se, Zn, Fe / Mg, Ca, Sr, Ba / Na, K), only Ni was never detected in maternal carapace (Tables F3.4-F3.8). Where a metal was not detected in a tissue, it is excluded from the summary statistics for that tissue. Hence, for each tissue in each table, the maximum value of n is the number of samples analysed from that site, while lesser n values exclude values of zero.

	eggshell					egg contents					maternal carapace				
	range	mean	SD	median	n	range	mean	SD	median	n	range	mean	SD	median	n
Ti	1911-13529	6063.6	4112.0	4294	8	1612-5442	2692.8	1272.5	2178	8	15840-75130	51794.1	28334.2	70170	8
Ni	686-1343	941.7	351.9	796	3	-	-	-	-	-	-	-	-	-	-
Mn	-	-	-	-	-	499-1374	798.5	288.6	789	8	5769-117791	26470.3	37284.7	13382	8
Cu	2541-22887	5752.9	6982.6	3040	8	3532-5282	4218	619.3	4088	8	264-418	342.7	77.1	346	3
Se	1015-3277	1764.3	664.1	1609.5	8	2180-7631	3645.0	1786.2	3196.5	8	5232-7515	6341.0	1142.9	6276	3
Zn	457-12419	2958.4	4303.8	936	7	58931-87039	68768.3	8857.2	67888	8	74680-97121	86037.6	8447.3	87757.5	8
Fe	-	-	-	-	-	53-77	63.8	9.8	60.5	8	4-224	61.0	91.7	28	5
Mg	514-1123	716.5	188.8	663.5	8	955-1394	1137.1	159.3	1110	8	2488-2962	2696.4	177.6	2631	8
Ca	302579-5475530	3385562.6	2056076.8	3897741	8	772-5716	2291.3	2074.6	1308.5	8	231618-266274	251352.8	11952.6	253000	8
Sr	16964-248390	191040.6	45879.5	199975	8	9899-19358	14708.0	3237.1	14810.5	8	460402-671789	575726.3	78399.9	592894	8
Ba	779-19385	5623.5	5752.7	3874.5	8	7614-23392	14028.8	4519.0	13528	8	134299-452470	211532.6	108097.6	171920	8
Na	2277-3139	2607.9	289.7	2514.5	8	3682-9230	7201.0	1835.8	7757.5	8	5-4663	1563.0	2170.2	17	8
K	133-515	338.0	120.1	351	8	4990-9581	7029.0	1632.0	6851	8	145-1252	458.6	347.7	404.5	8

Table F3.4 Summary statistics for metal concentrations in eggshells, egg contents, and maternal carapace for ***C. longicollis*** **at RH.CONT.up**. Values are in μg/kg except for Fe, Mg, Ca, Na & K where values are in mg/kg.

	eggshell					egg contents					maternal carapace				
	range	mean	SD	median	n	range	mean	SD	median	n	range	mean	SD	median	n
Ti	2127-6837	3517.8	2228.1	2553.5	4	1852-3665	2772.0	906.8	2799	3	70211-77303	75273.0	3383.4	76789	4
Ni	714	714	-	714	1	-	-	-	-	-	-	-	-	-	-
Mn	-	-	-	-	-	1110-1757	1366.0	284.1	1298.5	4	14814-28221	20965.0	6647.9	20412.5	4
Cu	1494-6852	4192.5	2191.6	4212	4	2906-8671	5316.5	2416.4	4844.5	4	-	-	-	-	-
Se	1466-1761	1601.8	121.8	1590	4	2910-4817	3816.3	880.8	3769	4	-	-	-	-	-
Zn	1063-1983	1503.5	407.0	1484	4	61752-84290	70951.5	9565.1	68882	4	80134-137352	109806.3	23858.9	110869.5	4
Fe	-	-	-	-	-	61-85	72.0	12.1	70	3	21-30	25.5	6.4	25.5	2
Mg	417-741	606.3	148.6	633.5	4	997-1375	1093.0	188.0	1000	4	2705-3133	2909.8	187.0	2900.5	4
Ca	344695-4622436	3077054.0	1933485.3	3670542.5	4	948-6159	3281.8	2556.8	3010	4	257536-281425	268852.8	9887.8	268225	4
Sr	106632-233342	185508.0	55263.6	201029	4	9392-17868	14113.8	3856.4	14597.5	4	450346-594191	507667.5	67440.0	493066.5	4
Ba	2541-7546	4072.3	2339.7	3101	4	9084-20067	14161.3	4548.7	13747	4	117657-251730	196307.5	60562.9	207921.5	4
Na	2113-3043	2700.5	407.6	2823	4	6622-9098	7346.8	1171.8	6833.5	4	14-28	20.3	6.9	19.5	4
K	163-721	491.3	245.7	540.5	4	6615-7951	7185.3	557.4	7087.5	4	248-486	357.5	101.9	348	4

Table F3.5 Summary statistics for metal concentrations in eggshells, egg contents, and maternal carapace for ***C. longicollis*** **at QH.STP.up**. Values are in μg/kg except for Fe, Mg, Ca, Na & K where values are in mg/kg.

	eggshell					egg contents					maternal carapace				
	range	mean	SD	median	n	range	mean	SD	median	n	range	mean	SD	median	n
Ti	1911-13529	5215.0	3699.4	3968.5	12	1612-5442	2714.4	1139.9	2194	11	15840-77303	59620.4	25448.9	71526	12
Ni	686-1343	844.8	309.0	755	4	-	-	-	-	-	-	-	-	-	-
Mn	-	-	-	-	-	499-1757	987.7	391.3	912	12	5769-117791	24635.2	30067.2	15336	12
Cu	1494-22887	5232.8	5738.2	3580	12	2906-8671	4584.2	1459.1	4484.5	12	264-418	342.7	77.1	346	3
Se	1015-3277	1710.1	539.5	1590	12	2180-7631	3702.1	1499.6	3466.5	12	5232-7515	6341.0	1142.9	6276	3
Zn	457-12419	2429.4	3420.8	1294	11	58931-87039	69496.0	8719.5	68152.5	12	74680-137352	93960.5	18374.3	90150.5	12
Fe	-	-	-	-	-	53-85	66.0	10.6	63	11	4-224	50.9	76.9	28	7
Mg	417-1123	679.8	177.9	663.5	12	955-1394	1122.4	162.1	1046	12	2488-3133	2767.5	201.6	2757.5	12
Ca	302579-5475530	3282726.0	1932052.0	3814542	12	772-6159	2621.4	2181.7	1397	12	231618-281425	257186.1	13849.9	258603.5	12
Sr	106632-248390	189196.4	46688.8	199975	12	9392-19358	14509.9	3287.8	14810.5	12	450346-671789	553040.0	79213.3	558769	12
Ba	779-19385	5106.4	4810.0	3703.5	12	7614-23392	14072.9	4317.7	13583	12	117657-452470	206457.6	92154.7	183566	12
Na	2113-3139	2638.8	317.5	2654.5	12	3682-9230	7249.6	1588.8	7258.5	12	5-4463	1048.8	1890.5	17.5	12
K	133-721	389.1	177.0	373	12	4990-9581	7081.1	1336.2	7087.5	12	145-1252	424.9	286.8	384.5	12

Table F3.6 Summary statistics for metal concentrations in eggshells, egg contents, and maternal carapace for ***C. longicollis*** **at RH.CONT.up and QH.STP.up combined**. Values are in μg/kg except for Fe, Mg, Ca, Na & K where values are in mg/kg.

	eggshell					egg contents					maternal carapace				
	range	mean	SD	median	n	range	mean	SD	median	n	range	mean	SD	median	n
Ti	2393-13046	5738.5	4325.6	3722.5	6	2363-6587	3706.2	1665.1	3074	5	21135-29120	23355.0	3282.9	21979	5
Ni	772	772.0	-	772	1	-	-	-	-	-	-	-	-	-	-
Mn	-	-	-	-	-	350-1259	750.5	355.2	624	6	6286-14129	11053.4	3341.3	12882	5
Cu	2492-2999	2719.7	238.3	2700	6	1241-3486	2905.7	841.2	3188	6	296-9731	3273.3	4451.3	1533	4
Se	1413-1908	1649.7	181.8	1665	6	355-1267	944.3	310.8	1032.5	6	-	-	-	-	-
Zn	592-1985	1101.0	564.1	1042	5	57068-86851	76161.8	10515.0	77977	6	101773-216801	140955.8	46360.3	125514	5
Fe	-	-	-	-	-	58-69	62.8	4.1	62	5	5-176	43.6	74.1	14	5
Mg	647-1191	974.8	206.7	984	6	1016-1340	1187.2	121.7	1218.5	6	2538-2829	2661.0	123.9	2689	5
Ca	3648043-6051526	6761535.0	931304.7	4799635	6	2951-7649	3850.3	1866.1	3082.5	6	208129-242645	228586.2	15129.1	233598	5
Sr	202944-316751	255354.3	37366.4	253298.5	6	15648-30943	23444.3	6178.5	24322.5	6	345770-739461	525774.4	146882.4	529308	5
Ba	3038-11718	6635.8	3640.5	6363	6	9464-39620	26427.2	11934.6	28807.5	6	78937-248492	146247.6	66544.1	216075	5
Na	2614-3197	2861.3	225.0	2818	6	4935-9283	7108.5	1751.8	6803	6	4763-5386	5100.8	276.9	5207	5
K	358-705	463.7	123.7	416.5	6	5489-8318	6731.0	1091.8	6782	6	329-874	576.4	259.7	497	5

Table F3.7 Summary statistics for metal concentrations in eggshells, egg contents, and maternal carapace for ***E. macquarii*** **at Botany Swamps and Bicentennial Park combined**. Values are in μg/kg except for Fe, Mg, Ca, Na & K where values are in mg/kg.

metal	eggshell		egg contents		maternal carapace	
	range	n	range	n	range	n
Ti	3049-5861	2	4192	1	12263-29157	2
Ni	627	1	-	-	-	-
Mn	-	-	931	1	2127-7015	2
Cu	2362-2747	2	1270-4735	2	2192	1
Se	509-1604	2	907-5684	2	4575	1
Zn	588-3662	2	62216-62360	2	91170-91940	2
Fe	-	-	53	1	25-36	2
Mg	1166-1378	2	991-1066	2	2316-2327	2
Ca	285059-4040040	2	5930-7204	2	206069-213092	2
Sr	221311-360987	2	15055-19892	2	886000-1179983	2
Ba	1216-4910	2	6893-12350	2	130130-146004	2
Na	2992-3400	2	4524-6429	2	3121-5561	2
K	325-705	2	6240-6458	2	322-508	2

Table F3.8 Summary statistics for metal concentrations in eggshells, egg contents, and maternal carapace for ***Elseya latisternum*** **at Botany Swamps**. Values are in μg/kg except for Fe, Mg, Ca, Na & K where values are in mg/kg.

Because of the highly skewed distributions for many metal concentrations, comparisons among species and sites use Kruskal-Wallis non-parametric tests. There are no significant differences (Kruskal-Wallis test statistic = 0.219-5.825, df = 2, P = 0.054-0.896) among the three species for the following tissues and metals:

- eggshell Ti, Cu, Se, Ca, Ba, Na, K
- egg content Zn, Mg, Ca, Na, K
- carapace Ti, Fe, Mg, Sr, Ba, K.

There are no significant differences (Mann-Whitney U statistic = 3.5-47.0, 1 df, P = 0.157-1.0) between *C. longicollis* and *E. macquarii* (data for *El. latisternum* insufficient for inclusion in analysis) for:

- egg content Ti, Fe, Mg
- carapace Cu, Al.

There are significant differences among the three species in:

- eggshell Mg (Kruskal-Wallis test statistic = 10.055, 2 df, P = 0.007)
- eggshell Sr (Kruskal-Wallis test statistic = 9.055, 2 df, P = 0.011)
- egg content Cu (Kruskal-Wallis test statistic = 8.400, 2 df, P = 0.015)
- egg content Se (Kruskal-Wallis test statistic = 9.779, 2 df, P = 0.008)
- egg content Sr (Kruskal-Wallis test statistic = 7.226, 2 df, P = 0.027)
- egg content Ba (Kruskal-Wallis test statistic = 6.683, 2 df, P = 0.035)
- carapace Mn (Kruskal-Wallis test statistic = 6.381, 2 df, P = 0.041)
- carapace Zn (Kruskal-Wallis test statistic = 7.222, 2 df, P = 0.027)
- carapace Ca (Kruskal-Wallis test statistic = 10.430, 2 df, P = 0.005)
- carapace Na (Kruskal-Wallis test statistic = 10.975, 2 df, P = 0.004).

Bonferroni-adjusted post-hoc pair-wise comparisons of ANOVA suggest that significant differences occur:

For:	**In:**	**Between:**
Mg	eggshell	*C. longicollis* and the two shortneck species
Sr	eggshell	*C. longicollis* and the two shortneck species
Cu	egg content	*C. longicollis* and *E. macquarii*
Se	egg content	*C. longicollis* and *E. macquarii*
Sr	egg content	*C. longicollis* and *E. macquarii*
Ba	egg content	*E. macquarii* and each of *C. longicollis* and *El. latisternum*
Mn	carapace	*C. longicollis* and *El. latisternum*
Zn	carapace	*C. longicollis* and *E. macquarii*
Ca	carapace	*C. longicollis* and the two shortneck species
Na	carapace	*C. longicollis* and *E. macquarii*.

As the data for *C. longicollis* are from a different site to the two shortneck species, it is not clear whether the 'species differences' relate to species or to sites. However, it is possible to compare data over two sites (QH.STP.up, RH.CONT.up) for *C. longicollis*. In these comparisons there are no significant differences between the two sites in 28 metal/tissue combinations, and significant differences in only 4 metal/tissue combinations:

- egg content Mn (Mann-Whitney U statistic = 30.0, n = 4,8, P = 0.017)
- carapace Ti (Mann-Whitney U statistic = 28.0, n = 4,8, P = 0.042)
- carapace Al (Mann-Whitney U statistic = 1.500, n = 4,6, P = 0.025)
- carapace Ca (Mann-Whitney U statistic = 28.0, n = 4,8, P = 0.042).

Correlations between metal concentrations in eggshell, egg contents, and maternal carapace are made with pooled data across all sites and species to maximise sample size. There are no significant correlations between the three tissues for Ti, Cu, Se, Zn, Mg, Na or K, or between egg contents and maternal carapace (not detected in eggshell) for Mn or Fe (P = 0.217-0.933). Significant correlations between the three tissues are only found for the three alkaline earth metals:
Ca (Pearson's p = -0.189 to -0.661; Bartlett chi-square statistic = 13.698, df = 3, P = 0.003), with a significant negative correlation between eggshell and egg contents (Bonferroni adjustment P = 0.006).
Sr (Pearson's p = 0.129-0.566; Bartlett chi-square statistic = 13.493, df = 3, P = 0.004), with significant correlations between eggshell and egg contents, and eggshell and maternal carapace (Bonferroni adjustment P = 0.034-0.040),
Ba (Pearson's p = 0.511-0.800; Bartlett chi-square statistic = 23.454, df = 3, P < 0.001), with significant correlations between eggshell and egg contents, and eggshell and maternal carapace (Bonferroni adjustment P = <0.001-0.025).

There are no correlations between Sr, Ba, or Ca concentrations in eggshell. In egg contents (Pearson's p = 0.689; Bartlett chi-square statistic = 12.181, df = 3, P = 0.007), there is a significant correlation between Sr and Ba (Bonferroni adjustment P = 0.002) but not between Ca and either Sr or Ba. For maternal carapace, as found previously with a smaller sample (Section E3.4), there are no correlations between Sr, Ba, or Ca concentrations.

There are no significant correlations between eggshell thickness and eggshell metal concentration for Ti, Cu, Se, Zn, Ca, Sr, Ba, Na, or K, either with the three species pooled (n = 20, Pearson's p = -0.347 - 0.189; P = 0.134-0.946), for *C. longicollis* alone (n = 12; Pearson's p = -0.433 - 0.404; P = 0.159-0.800), or for *E. macquarii* alone (n = 6; Pearson's p = -0.810 - 0.779 ; P = 0.053-0.966). There is a significant

negative correlation between eggshell thickness and eggshell Mg concentration both for the pooled data (n = 20; Pearson's p = -0.488, P = 0.047) and for *C. longicollis* alone (n = 12; Pearson's p = -0.643, P = 0.024), but not for *E. macquarii* alone (n = 6; Pearson's p = -0.777, P = 0.072), although the lack of significance for the latter probably reflects the small sample size, as the correlation coefficient is higher than for the pooled data and for *C. longicollis*.

CHAPTER F4: METALS & REPRODUCTION DISCUSSION

F4.1 Clutch Parameters

F4.1.1 Clutch Size

Clutch mass usually increases with increasing maternal size, and although this may result from increasing egg size (Tucker *et al*. 1998), it usually results from an increase in egg number (reviewed in Thompson 1983a), although in some cases both egg number and egg size increase (e.g. *T. scripta*, Congdon & Gibbons 1990a). In this study, maternal size did not correlate with egg size (mean length or width) for either *C. longicollis* or *E. macquarii*, although previously *C. longicollis* maternal size has correlated with mean egg width (but not mean egg length, Kennett & Georges 1990).

Few studies have examined the relationships between maternal size and clutch size in Australian freshwater turtles (reviewed in Booth 1998), but the strong positive relationship between maternal size and clutch size found for *C. longicollis* in this study is usual for turtles (Georges *et al.* 1993), including *C. longicollis* (Kennett & Georges 1990). Although a positive correlation exists between maternal size and clutch size in Murray Valley *E. macquarii* (Spencer 2002) and Fraser Island *E. m. krefftii* (Georges 1985), no correlation was found for *E. macquarii* in the current study. This may be due to the small sample size (6) and a maximum difference in carapace lengths of < 40 mm. Year, season, and habitat may also influence clutch size (*T. scripta*, Gibbons & Greene 1990; *C. expansa*, Booth 1998), but these were not factors for *E. macquarii* in this study. In other species, maternal size and clutch size are correlated in some populations, but not others (*Chrysemys picta*, Mitchell 1988). As reproduction is competing for energy investment with other life history traits such as maintenance, growth, and storage, it will likewise vary with differences in food availability (reviewed in Niewiarowski 2000), and diet may even play a greater role than maternal size in determining clutch size (Gibbons & Tinkle 1969). Thus, maximum clutch volume is limited by maternal size, but the maximum clutch size and frequency can only be achieved when optimal resources are available (Gibbons & Greene 1990), so a small clutch may result from inadequate resources. However, variation in clutch size or other maternal size effects (egg or hatchling mass or size) does not affect survival of hatchlings (*C. serpentina*, Kolbe & Janzen 2001). Dry mass of egg contents gives the best indication of maternal energy investment in the clutch

(Booth 1998), and in the future the effect of pollutants on this parameter could be tested.

A broad range of clutch sizes is common in Australian chelids (e.g. 9-23 for *C. expansa*, Booth 1998). The clutch size for *C. longicollis* of 6-12 (with a mode and mean of 9) does not even overlap the range of 13-24 (n = 15, mode = 19) for more southern *C. longicollis* (near Canberra, Vestjens 1969), and is at the low end of the range for more northern *C. longicollis* (6-23, Armidale, Parmenter 1985), but the mean is similar to that found for Murray Valley *C. longicollis* (10.7 eggs, Goode & Russell 1968). A reduction in clutch size may be used to reduce reproductive output in poorly productive habitats (*E. m. krefftii*, Fraser Island Georges 1985). The habitats studied in Sydney do not appear to fit into that category, and it remains possible that pollution is involved in the small clutch size in *C. longicollis*.

The clutch size for *E. macquarii* of 12-16 (with a mode of 12 and mean of 14) intersects the low end of the range found in some Murray Valley *E. macquarii* (13-36 eggs, Spencer 2002; 15-25 eggs, Cann 1998; 13-25 eggs, Ehmann 1992), although the mean is similar to that found for other Murray Valley *E. macquarii* (15.7 eggs, Goode & Russell 1968). There is geographic variation in body size of freshwater turtles (reviewed in Mitchell 1988), and the lower clutch size in Sydney may be due to smaller size of animals in this area (Cann 1998), and especially the lack of the 'giant' animals that are found in the Murray Valley and in Cooper Creek (some over 4 kg, Michael Thompson pers. comm.). Other populations also have small clutch sizes. The entire range found for Sydney *E. macquarii* is higher than that found for Fraser Island *E. m. krefftii*, which produce 4-10 eggs per clutch (Georges 1985). Reptiles distributed over broad environmental gradients frequently display large variations in life-history traits, and clutch sizes vary for *E. macquarii* from different drainages, with the smaller clutches having 6-9 eggs (Macleay/Hastings River), 7-18 eggs (Hunter River), and 9-18 eggs (Sydney Basin, Cann 1998). Most *E. macquarii* on the Murray River lay one clutch per year (Spencer 2002), but Fraser Island *E. m. krefftii* can lay 3 clutches per year (Georges 1985), with internesting periods as short as 30 days (captive *E. m. krefftii*, Banks 1987). It is not known whether the turtles in the current study were multiple clutching, and this would have to be assessed to gauge annual reproductive output.

The clutch size of one of the *El. latisternum* (24) was outside the previously recorded clutch size range (9-17, Ehmann 1992), indicating that resources are not limiting reproductive investment in this translocated species in Sydney.

F4.1.2 Egg Size

The eggs of *E. macquarii* from coastal southeastern Australia and the eggs of *C. longicollis* are the smallest of the Australian Chelidae (Legler & Georges 1993). It is normal for Australian chelid egg sizes to vary greatly between individuals (by up to 10 mm, Goode 1967), as well as within a single clutch (Cann 1998), with eggs from a single *C. longicollis* clutch varying by up to 6.7 mm in length (and by 1.1g, Kennett & Georges 1990).

Within a clutch, egg length is generally much more variable than egg width (Legler 1985), although natural variation in both dimensions is high for *C. longicollis* (20-42 x 12-29 mm, Ernst & Barbour 1989). The largest *C. longicollis* egg dimensions from this study (33 x 20 mm) are similar to those found for some populations (34 x 20 mm, Cann 1998; 34 mm x 21 mm, Canberra, Vestjens 1969), but smaller than the maximum sizes in other populations (37 x 21 mm, Jervis Bay, Kennett & Georges 1990), although mean dimensions are generally the same (30 x 19 mm, Kennett & Georges 1990), or similar (30 x 20 mm, Vestjens 1969). From this there is no indication that urban living is havng an effect on egg size in this species.

The average *E. macquarii* egg size (31 x 19 mm) is slightly smaller than that found for other *E. macquarii* (33 x 23 mm, Ernst & Barbour 1989), and the *El. latisternum* egg dimensions (32-37 x 21-24 mm) are in the bottom half of the range previously found for the species (33-41 x 21-26 mm, reviewed in Ernst & Barbour 1989). Reduction of egg size can be a response to poorly productive habitats (*E. m. krefftii*, Fraser Island Georges 1985), but whether the tendency to smaller egg sizes is due to some impact related to urbanisation or other effects of translocation (e.g. different climate), or merely due to natural variation is not known.

F4.2 Hatchlings

F4.2.1 Egg Incubation

Although eggs are incubated at a constant temperature in most laboratory settings, natural turtle nests can experience a wide temperature range, with a temperature difference of several degrees even experienced by eggs in the same nest at the same time (differing by 3.5 ºC, *Carettochelys insculpta*, Georges 1992). Also, nests laid later in the season will experience colder soil temperatures, and take longer to develop (*C. longicollis*, Vestjens 1969).

Eggs were incubated at a median and mean temperature of 29 ºC, within the range 22-36 ºC (Section E2.4.2). The maximum temperature is less than that recorded in viable natural nests (*C. insculpta*, max core temperature 38.7 ºC, Georges 1992), although the temperature range (14 ºC) is greater than the natural maximum daily variation for *C. insculpta* (9.2 ºC, Georges 1992), and for *C. longicollis* and *E. macquarii* (8.5 ºC, Goode & Russell 1968). Nevertheless, it is similar to the total incubation period temperature variation (15 ºC, natural nests) which produces normal hatchlings (Goode & Russell 1968). It is possible for extreme environmental heat to kill the embryos at the top of a nest, but this is extremely unusual (Thompson 1983a) and, unless this unknown critical temperature is reached, eggs continue to show more rapid development with increasing incubation temperature (Goode & Russell 1968).

Egg incubation period for *C. longicollis* ranged from 78-90 days, which was much shorter than that found for naturally incubated eggs (131-145 days, Goode & Russell 1968; 118-143 days, Vestjens 1969; 105-123 days, Parmenter 1985; 130-168 days, Ernst & Barbour 1989). It was longer than for some *C. longicollis* eggs artificially incubated at 30 ºC (57-67, Kennett & Georges 1990), but not others (average 138 days, Goode & Russell 1968). Incubation duration was midway between that for *C. longicollis* eggs incubated at 24 ºC (108 days) and those incubated at 32 ºC (68 days, Georges 1988).

During natural incubation at the same location, *C. longicollis* eggs take about 1.8x as long to hatch as *E. macquarii* eggs (Goode & Russell 1968). In this study they took 1.5-1.8x as long to hatch, with the egg incubation period for *E. macquarii* varying from 50-53 days, which is considerably less than previously reported for natural nests

(66-85 days Goode & Russell 1968), or from eggs incubated at 30 ºC (average 75 days, Goode & Russell 1968).

F4.2.2 Hatchlings

Although both species were exposed to the same incubation conditions, full-term embryonic survival was much lower for *E. macquarii* (12%) than *C. longicollis* (58%) (Table E3.25), despite *E. macquarii* usually showing higher survival (90%) than *C. longicollis* (75%) during artificial incubation of eggs (Gerry Swan, pers. comm.) This may indicate less tolerance to temperature variation during incubation, or that Sydney *E. macquarii* have poorly viable eggs, or be a consequence of premature induction of oviposition by oxytocin. Monitoring of natural nests in the field would be necessary to determine the true viability of eggs from animals captured in the area. Survival to hatching in *C. longicollis* was lower than in natural nests (72%, Parmenter 1985). Most nests contain some apparently infertile eggs (Thompson 1983a), although inviability may result from early embryo mortality rather than infertility (Parmenter 1985). Although the percent of eggs in a clutch that are non-viable may increase in later season clutches (Vestjens 1969), this was not a factor in this instance, as all *C. longicollis* eggs were laid within a fortnight each other.

For *C. longicollis*, hatchling mass was 2.7-4.4 g (mean 3.7 g, Table E3.25), which is lower than that found previously for the species (4.6 g, Legler & Georges 1993; 3.0-5.2 g, mean 4.2 g, Jervis Bay, Kennett & Georges 1990; 4.2-4.5 g, Botany Bay, Cann 1998). For *C. longicollis*, hatchling length was 20-26 mm (mean 24 mm, Table E3.25), which is less than the general average length (30 mm, Legler & Georges 1993), and the length previously found for Botany Bay *C. longicollis* (26-29 mm, Cann 1998). However, it is within the broad range given by other authors (15-30 mm, Ernst & Barbour 1989). The small hatchlings found in this study are not surprising due to the smaller size of the eggs.

E. macquarii hatchling mass was 2.1-2.2 g from the first clutch and 3.9 g from the second (Table E3.25), which is considerably less than the general weight previously given for *E. macquarii* hatchlings (5.4 g, Legler & Georges 1993). The mean *E. macquarii* hatchling length of 24 mm (range 22-25 mm), is much lower than the average for the species (30 mm, Legler & Georges 1993), or the range for the species

(24-33 mm, Goode 1967). It has been noted that Sydney *E. macquarii* are comparatively small (Cann 1998), but it is not known if this is due to resource availability, genetic variation, or pollution effects. In birds, probably due to low dietary intake of Ca and high dietary intake of other metals, offspring weigh less in populations breeding near a metal-polluting factory (Eeva & Lehikoinen 1996), and metals could be having an effect on hatchling mass in Sydney chelids.

The eggs in this study suffered unusually high mortality. Although incubation temperature variation or extremes may have contributed to this, pollutants or other urban factors may also play a role. More work is required to determine the natural range of hatchling length and weight and how this may vary between different populations. The impact of pollutants can then be more accurately guaged. Future studies should also compare the merit of assessing hatchlings at birth versus assessing them months later, as hatchling size differences become more apparent after 6 months (captive *E. krefftii*, Banks 1987), and hatchling survival differences after low or high Pb exposure are apparent by 4 months (Burger *et al*. 1998).

None of the chelid hatchlings had any overt abnormalities, a situation also found for offspring of turtles living at coal-ash-polluted sites, even though eggs incubated in coal-ash-contaminated soil had higher embryo mortality (*T. scripta*, Nagle *et al*. 2001). It may be that turtles generally have a high pollution tolerance, and that when adverse effects do occur, mortality may quickly follow.

F4.3 Egg Metals

Most of the work on environmental contaminants in freshwater turtles has been done in North America on the common snapping turtle (*Chelydra serpentina*), and most of the work looking at reproductive effects in this species has examined PCBs, organochlorine pesticides, and other organic chemicals (Bryan *et al*. 1987, Olafsson *et al*. 1987, Bishop *et al*. 1991, Hebert *et al*. 1993, Struger *et al*. 1993, Bishop *et al*. 1994, Bishop *et al*. 1995, Bonin *et al*. 1995, Bishop *et al*. 1996, Bishop *et al*. 1998, Portelli *et al*. 1999, de Solla *et al*. 2001). Work on other North American species such as *Trachemys scripta* and *Graptemys flavimaculata* have also looked at organic contaminants with reference to reproductive tissues or outcomes (Holcomb & Parker

1979, Palmer & Palmer 1995, reviewed in Crain & Guillette 1998, Willingham & Crews 1999, Kannan *et al*. 2000). Examination of links between metals and reproductive tissues or outcomes in the Chelonia are few, and have usually involved sea turtles. Even at a place of prolific freshwater turtle research (the Savannah River Site, South Carolina), the effects of metals on turtles have only just started to be addressed (Gibbons *et al*. 1997, Burger *et al*. 1998).

The maternal tissue or tissues from which egg metals originate remains obscure. Femur differs from egg tissues by containing Pb, but not Ti, Cu, or Fe (Table E3.5), and carapace differs from egg tissues by containing Pb, Al, Zr, and Cr, but not Ni (Section E3.5). This suggests that neither femur nor carapace is solely used for metal mobilisation for egg formation. Further substantiating this, differences in egg tissue metals do not reflect differences in carapacial metals between species, with the four metals that differ in carapace concentration between *C. longicollis* and one or both shortnecks (Mn, Zn, Ca, Na), being different from metals that differed between the species in either eggshell (Mg, Sr) or egg contents (Cu, Se, Sr). Birds search for Ca-rich dietary items during the breeding season to meet requirements for egg formation (Eeva & Lehikoinen 1996) and encorporation of metals directly into eggs from current environmental intakes may help explain the presence of some metals in egg tissues.

Apart from the difference between *E. macquarii* and *El. latisternum* egg content Ba, all significant differences in egg metal concentrations were between *C. longicollis* and one or both of the shortneck species. This, however, may represent a site rather than a taxonomic variation, as was most likely for variations in carapace metal concentrations (Sections E3.5.3, E3.5.4). The fact that *C. longicollis* from two different sites differed in only one egg tissue metal concentration (egg content Mn), whereas *C. longicollis* differed from *E. macquarii* (and sometimes *El. latisternum*) in six egg tissue metal concentrations (eggshell Mg, Sr; egg content Cu, Se, Sr, Ba), shows that a taxonomic difference in metal physiology cannot yet be discounted. However, the contributing factors to metal variation may still be site dependent, as the two *C. longicollis* populations were within a similar geographic region, but distant to the shortneck populations. A laboratory experiment with controlled exposure of these turtle species to defined metal loads would be required to resolve variations in bone

and egg tissue metal concentrations. The lack of significant correlations between maternal carapace metal concentration and those in eggshell or egg contents for those metals with known toxic effects does not reject Hypothesis 1 (Section F1.3), and hence that there is no support for the view that high metal concentrations in maternal carapace are transferred to the offspring.

Similarly, for most metals, the lack of significant relationships between offspring variables (clutch size, incubation period, egg length, egg width or hatchling mass or length) and metal concentrations in maternal carapace for either *C. longicollis* or *E. macquarii* does not allow rejection of Hypothesis 2 (Section F1.3), and hence there is no support for the view that metal concentrations in maternal carapace affect eggs or hatchlings at the gross morphological level.

F4.3.1 Eggshell Thickness

No maternal carapace metal concentrations are related to eggshell thickness in *C. longicollis* (non-rejection of Hypothesis 2e; Section F1.3), but for *E. macquarii* there is a significant positive effect of maternal Cu on eggshell thickness, and a significant negative effect of maternal Ca and Mg on eggshell thickness (rejection of Hypothesis 2e). The significance of the Cu result is unclear, but the result for the alkaline earth metals, if confirmed with larger sample sizes in the future, indicates that there is indeed mobilisation of Ca and Mg from the carapace for eggshell formation. The lack of this finding in *C. longicollis* could simply reflect higher stores of these metals in the thicker carapace of this species.

There is a negative correlation between eggshell thickness and eggshell Mg for *C. longicollis*, with the same trend apparent in *E. macquarii* (Hypothesis 3 rejected for Mg, Section F1.3). Mg could become proportionately higher if inadequate Ca was available for eggshell formation (also resulting in thinner shells), but this does not account for the lack of relationship between eggshell thickness and other metals (Hypothesis 3 not rejected; Section F1.3). The result may be an artefact of early laying and incomplete eggshell calcification due to oxytocin induction.

The lack of correlation between eggshell thickness and eggshell metals other than Mg suggests that ecotoxic metals such as Al, which if elevated in the diet cause defective mineralisation of eggshells in birds (Nyholm 1981), may not produce overt effects in chelonian eggs, or may not be reaching toxic levels. Also, despite being fed Pb in the diet and having elevated Pb levels in bone and liver, there were no adverse effects on survival, fertility, egg laying or eggshell thickness in birds, and previous reports of Pb being associated with reduced eggshell thickness may end up being discounted (kestrels, Pattee 1984). Thus, the effect of metals may be limited, although methylHg remains problematic in regards to eggshell thinning and other reproductive outcomes (Lundholm 1997).

Reproductive parameters such as egg production and offspring survival can also be reduced indirectly by pollutants, as a result of reduced food supply of prey items, (Hornfeldt & Nyholm 1996, Eeva *et al.* 1997), and male reproductive processes are affected (sperm absent from the epididymes) in a population of emaciated *C. longicollis* (Kennett & Georges 1990). Thus elevated environmental metals could still play a role in affecting turtle reproduction through this indirect route. Pollutants may have a double-effect, both on diet, and secondarily on bioaccumulation, which is determined in part by the nutritional status of the animal (Mason 1996). Exposure to organic environmental pollutants (DDE, DDT, PCB, dioxins) can lead to reduced egg production, eggshell thinning, decreased fertility and hatchability, malformations, and decreased survival of young (reviewed in Mason 1996, Lundholm 1997), and this is probably the contaminant group that reproductive research on urban turtles should focus on in the future.

F4.3.2 Egg Metal Concentrations

Most variations in egg tissue metal concentration between *C. longicollis* and *E. macquarii* were for the alkaline earth metals (*E. macquarii* higher for eggshell Mg and Sr, and egg contents Sr and Ba), and thus not likely to be of direct toxicological importance (although they may be related indirectly). However, in egg contents, *C. longicollis* had higher concentrations (mg/kg) of **Cu** (mean 4.6, range 2.9-8.7) and **Se** (mean 3.7, range 2.2-7.6) than *E. macquarii* (Cu mean 2.9, range 1.2-3.5; Se mean 0.94, range 0.36-1.3), with the ranges for Se not even intersecting. These two essential metals are of high ecotoxicological relevance (Freedman 1995). Se can be especially

problematic as it is readily absorbed from environmental sources, and essential requirements and toxic concentrations may be only narrowly separated (Section E4.4.1). In contrast to non-essential elements, essential elements in sea turtle eggs do not generally vary significantly across sites (*C. caretta*, Stoneburner *et al.* 1980), so the result may represent a true species difference, unless the metal burdens are high enough to overload homeostatic control mechanisms at *C. longicollis* sites. If this is occuring, higher Cu concentrations may be reflecting raised Cu concentrations in maternal organs (kidney, *C. caretta*; Sakai *et al.* 1995).

Compared to *C. longicollis*, Cu concentration in egg contents was similar (6.2 mg/kg) and those in eggshell over three times as high (17 mg/kg) in another aquatic reptile (*Crocodylus acutus*, Stoneburner & Kushlan 1984), suggesting Cu may normally show this degree of species variation. Egg content metals were not measured, but average Cu concentration in spent sea turtle eggshells was 9 mg/kg (*D. coriacea*, Vazquez *et al.* 1997), which falls within the range found for *C. longicollis* eggshell (1.5-23 mg/kg), again suggesting concentrations may not be greatly elevated, although the wide range may be abnormal. Cu concentration in hatchling *T. scripta* was approximately the same as those in *C. longicollis* egg contents (5 mg/kg), yet the variance was much less, supporting the suggestion that the range for *C. longicollis* may be unusual. Although the average egg content Se concentration for *T. scripta* (0.417 mg/kg) is less than that found for *E. macquarii*, the maxiumum *T. scripta* value (1.041 mg/kg, Burger & Gibbons 1998) is within the *E. macquarii* range, suggesting that *C. longicollis* may have the unusual concentrations. In contrast, average Se eggshell concentrations were similar for *C. longicollis* (1.7 mg/kg) and *E. macquarii* (1.6 mg/kg), yet much lower in *T. scripta* (0.04, max 0.2 mg/kg; Burger & Gibbons 1998). Unlike many other metals, elevated Se concentrations in offspring may directly reflect elevated maternal liver concentrations (*T. scripta*, Nagle *et al*. 2001).

Combining results for the three Sydney turtle species (*C. longicollis*, *E. macquarii*, *E. latisternum*) for non-essential elements that were found in egg, **Ti** concentration was similar in eggshell (1.9-14 mg/kg) and egg contents (1.6-7 mg/kg), whereas **Ni** was sometimes present in eggshell (0-1.3 mg/kg), but never detected in egg contents. There are no other turtle studies with which to compare the results for Ti. The developing embryo could be exposed to the Ni as Ca is mobilised from the eggshell to

meet the requirements of embryonic osteogenesis (Miller & Jones 1990). Ni was not detected in the oviductal eggs of sea turtle (*C. caretta, C. mydas*; Sakai *et al*. 2000b), but has been found in the eggshell (22 mg/kg) and egg contents (2.4 mg/kg) of other aquatic reptiles (*Crocodylus acutus*, Stoneburner & Kushlan 1984). Ni has been found in spent sea turtle eggshells at a higher average concentration (8 mg/kg, *D. coriacea*, Vazquez *et al.* 1997) than the maximum concentration found in the chelids, and was also found in sea turtle egg yolk (average 0-2.3 mg/kg, *C. caretta*, Stoneburner *et al.* 1980), suggesting that the metal may be more prolific, and hence of more concern, in pelagic chelonians.

For all three Sydney turtle species combined, the essential metals **Mn** (0.4-1.8 mg/kg) and **Fe** (53-85 mg/kg) were present in egg contents, but not eggshell, and the essential metal **Zn** was much higher in egg contents (57-87 mg/kg) than eggshell (0-12 mg/kg). Mn, Fe, and Zn concentrations are higher in egg contents than in eggshells for a variety of reptiles (Linder & Grillitsch 2000), including sea turtles (*C. caretta, C. mydas*; Sakai *et al.* 1995). The difference is due to high concentrations in the yolk rather than in the albumen (*C. caretta, C. mydas*; Sakai *et al.* 2000b), and is thought to be due to the metabolic requirements of the embryo (Meyers-Schöne & Walton 1994). Mn concentration is higher in the egg contents of *T. scripta* (average 4.5 mg/kg) than that of the chelids, and Mn is detected at only slightly lower concentrations in the eggshell (average 3.5 mg/kg, Burger & Gibbons 1998), indicating that essential egg metals may be quite variable within freshwater turtles. The use of eggs for biomonitoring of metal expsosure may be suited to use for Zn, as concentrations in egg yolk reflects those in the female liver and kidney (*C. caretta*, Sakai *et al.* 1995). Also, egg content Mn concentrations are correlated with eggshell concentrations in *T. scripta*, suggesting that spent shells could be used for monitoring embryonic exposures (Burger & Gibbons 1998), but this is not applicable to the Sydney chelids as no Mn was detected in eggshell, and is not broadly applicable as the more ecotoxicologically relevant metals (Pb, Hg, Cd, Se, Cr) are not correlated.

The absence of **Pb** detection in eggs, despite its presence in both endo- and exoskeleton and some soft tissue stores (*C. longicollis*, Table E3.5), could be due to selective uptake of metals for egg formation, although Pb usually simply distributes as an analog of Ca (Camner *et al.* 1979). Selective uptake of metals has also been

suggested as an explanation for the low concentration of Cd in sea turtle eggs (Sakai *et al*. 2000b) and, in addition to Cd, there seems to be little maternal transfer of As or Cr to avian eggs (reviewed in Nagle *et al*. 2001). Pb was also not detected in sea turtle hatchlings, eggshells (*Chelonia mydas*, Aguirre *et al.* 1994), and oviductal eggs (*C. caretta*, *C. mydas*; Sakai *et al*. 1995, Sakai *et al*. 2000b), although other studies have found Pb in sea turtle eggshell (*D. coriacea*, Vazquez *et al.* 1997), egg contents (*C. caretta*, Stoneburner *et al.* 1980; unhatched *C. caretta* and *C. mydas* eggs, Godley *et al.* 1999), or both (*Lepidochelys olivacea*, Sahoo *et al.* 1996). Pb has also been found in the eggshell (up to 1.4 mg/kg) and egg contents (up to 1.9 mg/kg) of freshwater turtles (*T. scripta*, Burger & Gibbons 1998), and in the eggshell and egg contents of non-chelonian aquatic reptiles (*Crocodylus acutus*, Stoneburner & Kushlan 1984). The reason for the variation in egg Pb presence between taxa is unknown, but it does not appear to directly reflect environmental exposure.

Most turtles die from predation as eggs or hatchlings (Cann 1998), and the exclusion of toxic metals such as Pb from eggs could also benefit predator food chains. Predators of Australian chelid eggs and hatchlings include water rats (*Hydromys chrysogaster*), Australian ravens (*Corvus coronoides*), kookaburras (*Dacelo gigas*), herons (*Ardea* sp.), cormorants (*Phalacrocorax* sp.), goannas (*Varanus* sp.), red-bellied black snakes (*Pseudechis porphyriacus*), tiger snakes (*Notechis scutatus*), king brown snakes (*Pseudechis australis*), crocodiles (*Crocodylus* spp.), freshwater eels (*Anguilla* sp.), and catfish (Anonymous 1974, Thompson 1983a, Beck 1991, reviewed in Cann 1998), as well as freshwater turtles of the same or different species (captive *C. longicollis*, *E. macquarii*, or *El. latisternum* adults, Weigel 1988, Craig Latta pers. comm.), and introduced species such as foxes (Thompson 1983a,b, Spencer 2001), cats, dogs and pigs (Parmenter 1985, Ehmann 1992). Thus, maternal metals transferred into turtle eggs have the potential to migrate through many food chains, and exclusion of toxic metals may help prevent food chain contamination.

Chelodina longicollis and *E. macquarii* nest in a broad range of soils (Goode & Russell 1968), so uptake of dissolved environmental metals during water absorption by eggs (Thompson 1981) could be expected to vary considerably. However, there was no relationship between metal-polluted or control egg incubation substrates and metal contamination of or physical alterations to offspring for *T. scripta*, suggesting

the eggshell or membrane provides a barrier to metal uptake (Nagle *et al*. 2001). If this also holds for the chelids it bestows a further benefit for survival in urbanised areas.

Of the factors that govern lifetime reproductive output (Fox 1995), two may be positively impacted in Sydney populations: a reduced egg incubation period (Section F4.2.1) and a reduced size at maturity (Section C4.3.1). Others that were not assessed (increased death rate, shorter reproductive periods, reduced fecundity or fertility) may be negatively impacted, however varying population numbers and juvenile components indicate reproductive effects do not manifest uniformly across the city. Also, with contaminant stresses exerting strong evolutionary pressures (Fox 1995), urbanisation may have produced a variety of isolated genetically distinct populations. A genetic advantage for surviving populations of a declining frog species (*Litoria aurea*) in Sydney has been suggested (Greer & Byrne 1995), and it is possible that Sydney chelids have developed higher tolerances to metal contaminants than non-urban populations.

F4.4 Summary

There are significant differences in egg metal concentrations between *C. longicollis* and *E. macquarii*, mainly for the alkaline earth metals, but also for two ecotoxicologically relevent metals, Cu and Se, which are significantly higher in egg contents in *C. longicollis*. Further study is required to determine if the results simply reflect natural taxonomic variation or if varying behaviours underlie the difference. Se is notable as it was the only element detected in turtles that was not detected in lagoon sediments.

The only metal whose eggshell concentration correlated with eggshell thickness was Mg, indicating that ecotoxic metals previously associated with eggshell thinning are not problematic in the Sydney chelids. The only non-essential metals found in chelid turtle eggs were the nontoxic alkaline earth metals Sr and Ba, as well as Ni and Ti. Ni was not present in egg contents, and only variably present in eggshell, and it appears that this metal is likely to be of more toxicological concern in sea turtles. The absence of Pb from eggs, despite its presence in many maternal tissues, suggests that selective

metal uptake into eggs may be protective of toxic elements, rather than eggs serving as a maternal method of toxic metal elimination as has been previously suggested. The lack of toxic metal detection in eggs also renders them unlikely tissues for biomonitoring. Ti is considered to be poorly absorbed and generally inert within tissues. This metal was found in reasonably high concentrations in a variety of tissues including liver, kidney and egg. Its high concentration in carapace, and absence from femur, suggests that it may actually be utilised in the carapace, possibly adding a lightweight strength element.

Hatching rates of incubated eggs were poor for both species, but especially for *E. macquarii*, and hatchling mass was generally less for both species than that found in other populations. The few surviving *E. macquarii* hatchlings were particularly small. Results from natural nests are required to confirm low hatching rates and low hatchling mass. Also, eggs for this species and *E. latisternum* were generally smaller than those found for other populations, indicating a possible reproductive impact of urbanisation in Sydney shortnecks. Further research is required to determine the natural range of hatchling length and weight, how this may vary between different populations, and what role may be played by pollutants. As has been found for North American turtles living at polluted sites, none of the chelid hatchlings had any overt abnormalities. Reproductive impacts may not include outwardly-apparent morphological changes as they do in other taxa.