CHAPTER B: GENERAL METHODS

All work was carried out under NSW National Parks & Wildlife Service licence #B2071, and Fisheries licence #F95/310, with animal ethics approval from the University of Western Sydney.

B.1 Fieldwork

B.1.1 Trapping

Fyke nets were used for the Sydney Turtle Survey (Section C), fyke nets and yabby traps for the Immune study (Section D), and fyke nets, yabby traps and snorkelling for the Metal Biomonitoring/Reproductive studies (Sections E & F).

B.1.1.1 Fyke Nets

Fyke nets 1.9m in length with five metal hoops and an additional 2.5 m long, 0.8 m deep leader net were used. The mesh size was 20 mm diagonal stretch, and the maximum width of the net entrance was 200 mm. Nets were randomly set with at least one end of the net secured within 2m of the bank in water between 0.5m and 1.1m deep. Some nets at Lake Toolooma (Section E2) were also set in deeper water (>2 m), attached to reeds up to 10m from the bank. An empty plastic bottle with tightly attached screw-topped lid was placed in the holding end of each net to provide an air pocket from which captured turtles could breathe. The only time bait was used in these traps was in the Sydney Turtle Survey (Section C2). See individual sections for number of traps used, trapping period, etc.

B.1.1.2 Yabby Traps

Yabby traps are rectangular and measure 600mm x 440mm with a height of 230mm and mesh size of 12mm^2. Bait consisted of Savings™ sardine cat food (50g/container; 10% crude protein, 0.5% crude fat, 1.0% fibre, 1.0% NaCl) and fresh chopped beef heart, sometimes supplemented with fresh prawns, and was placed in screw-topped plastic containers with 8-10 4.5mm holes drilled in them to allow the dispersion of bait odour

while prohibiting bait consumption. The bait containers were suspended by string in the centre of the trap. A long rope was attached to one end of the trap so that it could be thrown in and retrieved from the bank. Traps were thrown from random points along accessible sections of bank so that their resting point was within 5m of the bank. Nets were checked every 2-3 hours. See individual sections for number of traps used, trapping period, etc.

B.1.1.3 Snorkelling

The only sites with water quality good enough to allow snorkelling were the national park control sites for the Metal Biomonitoring/Reproductive studies (Sections E & F). Effort was concentrated in areas where water depth was 1-2m, although dives were made in areas up to 4m in depth.

B.1.2 Transport & Holding

Turtles were placed individually in calico bags (geology bags; Prospectors Earth Sciences Pty Ltd, Seven Hills NSW) for transport. Bags were placed in ventilated plastic containers with snap lock lids. If turtles could not be processed on the day of capture they were maintained in this way for up to 48 h. During hot weather, bags were partially moistened. On days when the ambient temperature exceeded 30^{o}C, ice was placed in the bottom of containers and covered with a towel with turtles placed on top prior to transport.

B.2 Turtles

B.2.1 Identification

A reptile and amphibian guide (Cogger 1992) was used to identify turtles to species level. Mature *E. macquarii* and *El. latisternum* were sexed based on tail length, and an attempt was made to sex *C. longicollis* using plastron concavity/convexity.

B.2.2 Measurement

Shell measurements (+/- 0.5mm) were made using 150mm and 300mm vernier calipers. Straight carapace length (CL) was measured from the centre top of the carapace (nuchal scute if present) along the mid-line to the distance of the most posterior edge of the marginal scutes. Curved carapace length (CCL) was measured over the dome from the centre top of the carapace along the mid-line to the distance of the most posterior edge of the marginal scutes. Straight plastron length (PL) was measured along the centre line from the most anterior point, to the point corresponding to the most posterior point (PLmax), or to the anal notch (PLmin). Widths were measured at the broadest point of the carapace (CW) and the broadest points of the anterior (PWa) and posterior (PWp) sections of the plastron. Carapace height (CH) was measured from the base of the plastron to the top of the carapace over the greatest vertical distance perpendicular to the plastron.

Mass was measured (+/- 5 g) using a plastic basket attached to a 2 kg spring balance (Salter, Suffolk, England). Overly active turtles were weighed in bags.

B.2.3 Aging

Age is important in pollution studies as it reflects the period over which an organism has potentially been exposed to contaminants. Aging turtles is problematic and existing techniques are inadequate. Analysis of body size, weighing eye lenses, counting growth rings on shell scutes, skeletochronology, and layers in claw sections have all been assessed (reviewed in Thomas *et al.* 1997), but there remains no standard reliable technique.

Although Australian freshwater turtles can be active (Beumer *et al.* 1981) and feeding (Chessman 1978) in all months of the year, they still show an annual cycle of growth (Georges 1982a), resulting in the formation of ‘growth rings’ on the scutes of the shell, which can be used to estimate the age of individuals (Cagle 1946). This technique is problematic due to the formation of minor (Moll & Legler 1971) or major (Cagle 1946)

growth rings during the main growing period which are not always distinguishable from yearly rings (Chessman 1978), and the fading of growth rings over time (Cagle 1946). Attempts have been made to use growth rings for aging of Australian turtle species with limited success (Parmenter 1976, Chessman 1978). It appears that the regularity of growth ring deposition must first be ascertained for each population (Stott 1988), and even then the technique may only be applicable in the first year or two of life (Spencer 2002).

In this study, juveniles were rarely caught, and with few exceptions only small juveniles had distinct growth rings (as with most Australian chelids, Goode 1967). With the low juvenile captures of current trapping methods, future development of growth/age curves based on recaptures and growth ring analysis of Sydney Basin turtles is unlikely. Even if sufficient juveniles were caught to construct growth models, accurate age determination of mature adults would not be possible (Spencer 2002). So for want of a reliable alternative, and due to its ease of measurement, carapace length was used to indicate general relative ages in this study, although it must be noted that variable growth rates lessen the reliability of this approach (Georges 1985).

B.2.4 Marking

For all but the Metal Biomonitoring/Reproductive studies (Sections E & F), turtles were uniquely marked using a system of notches (Figure B.1). The notches were approximately 3mm wide and 3-4mm deep and were made in the marginal scutes of the carapace using a 300mm, 32T, low alloy or high speed hacksaw blade (Stanley, Australia). The site and the saw were swabbed with 70% ethanol, two cuts made perpendicular to the edge, then the bone snapped off with a sideways twisting movement of the saw.

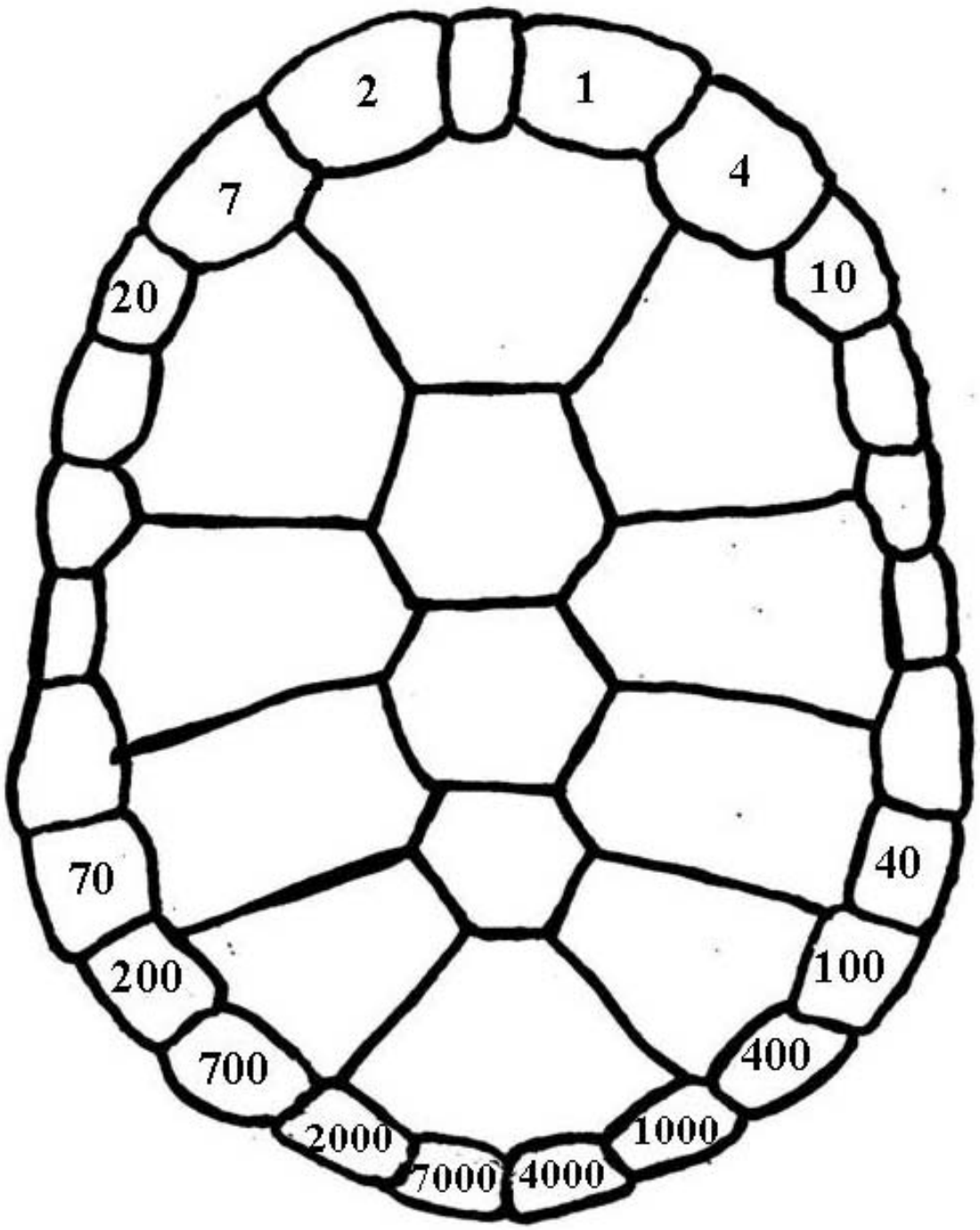

Figure B.1 Turtle carapace numbering system (adapted from Parmenter 1976, Thompson 1982) used for marking turtles with a unique series of notches.

No antiseptic treatment was given to turtles captured in the Sydney Survey (Section C) or in the initial Summer 1 sample in the Immune study (Section D) as no ill effect had been seen from notching in numerous other studies of freshwater turtles in Sydney (Shelley Burgin pers. comm.), and the only reference to infections from marginal notches in the literature was one case of peritonitis in a sea turtle (Glazebrook & Campbell 1990). However, some recaptured animals in the Post-Winter sample of the Immune study (Section D) showed signs of infection around the notches. In these cases the notches were debrided and treated with EDP antiseptic powder (Boots Health Care, North Ryde, NSW, Australia; povidone-iodine powder containing povidone-iodine 14.5% w/w, equivalent to iodine 1.4% w/w). The area was then covered with a spray-on bandage (Leucoplast, 4 Khartoum Rd, North Ryde NSW) to temporarily prevent water access to the site. This EDP/spray bandage technique, with swabbing of the area with 70% ethanol prior to excision, was also used to treat the exposed bone of all subsequent notches in the Immune study and all bone samplings in the Metal Biomonitoring/Reproductive studies (Sections E & F).

B.2.5 Tissue Sampling

B.2.5.1 Carapacial Bone

Carapacial bone for element analysis (Sections E & F) was sampled using a hacksaw (as for notching) from the larger rear marginal scutes on both sides of the shell (three consecutive bone chips were taken from each side). These were the 4th up from the anal notch for *C. longicollis* and the 3rd up from the anal notch for *El. latisternum* (Eastlakes only) and *E. macquarii*. At least 200mg of carapace was sampled, placed in an eppendorf on ice, then frozen as soon as possible (-20C), and stored frozen until analysis.

B.2.5.2 Blood

Blood was taken from turtles for the Immune (Section D) and Biomonitoring (Section E) studies. The neck was first swabbed with 70% ethanol, then blood taken from the jugular vein using a sterilised (gamma radiation) 25 gauge needle (adults) or 26 gauge needle (juveniles) attached to a 1 ml tuberculin syringe (Terumo, Elkton, MD, USA). Universal

1cc disposable sterile 25 gauge needle tuberculin syringes were also used, but the needle was not sharp enough to puncture the skin easily, and the rubber seal in the syringe allowed air in, so their use was discontinued. Blood was removed while the head and neck were retracted, which could be done by a single operator and caused less distress than extending the neck outwards (Mader 1996). The vein lies ventral to the midlateral plane of the neck. If the puncture site bled, pressure was applied.

The maximum blood sampling volume was calculated on the basis that reptile blood constitutes 5-8% of body weight (Jacobsen 1993) and a loss of 10% of blood volume can be tolerated (Campbell 1996). No more than 1ml was ever taken. For the Immune study, the needle was first primed with 10µl heparin (porcine mucous heparin sodium 1000 I.U./ml; David Bull Laboratories, Mulgrave, Victoria), heparin being the preferred anticoagulant for haematologic studies (reviewed in Moon & Foerster 2001). For the Reproductive study, blood was placed in 1.5ml acid-washed eppendorf tubes (Section E2.6.1).

B.3 Study Sites

Australian Map Grid (AMG) references are provided for all sites and, except for Marley Lagoon, each site in this study may be accurately located from a single reference (Gregory's Publishing Company 2001) using site descriptions and AMG coordinates. Sydney is within AMG Zone 56. An overview of site locations is provided here (Figure B.2).

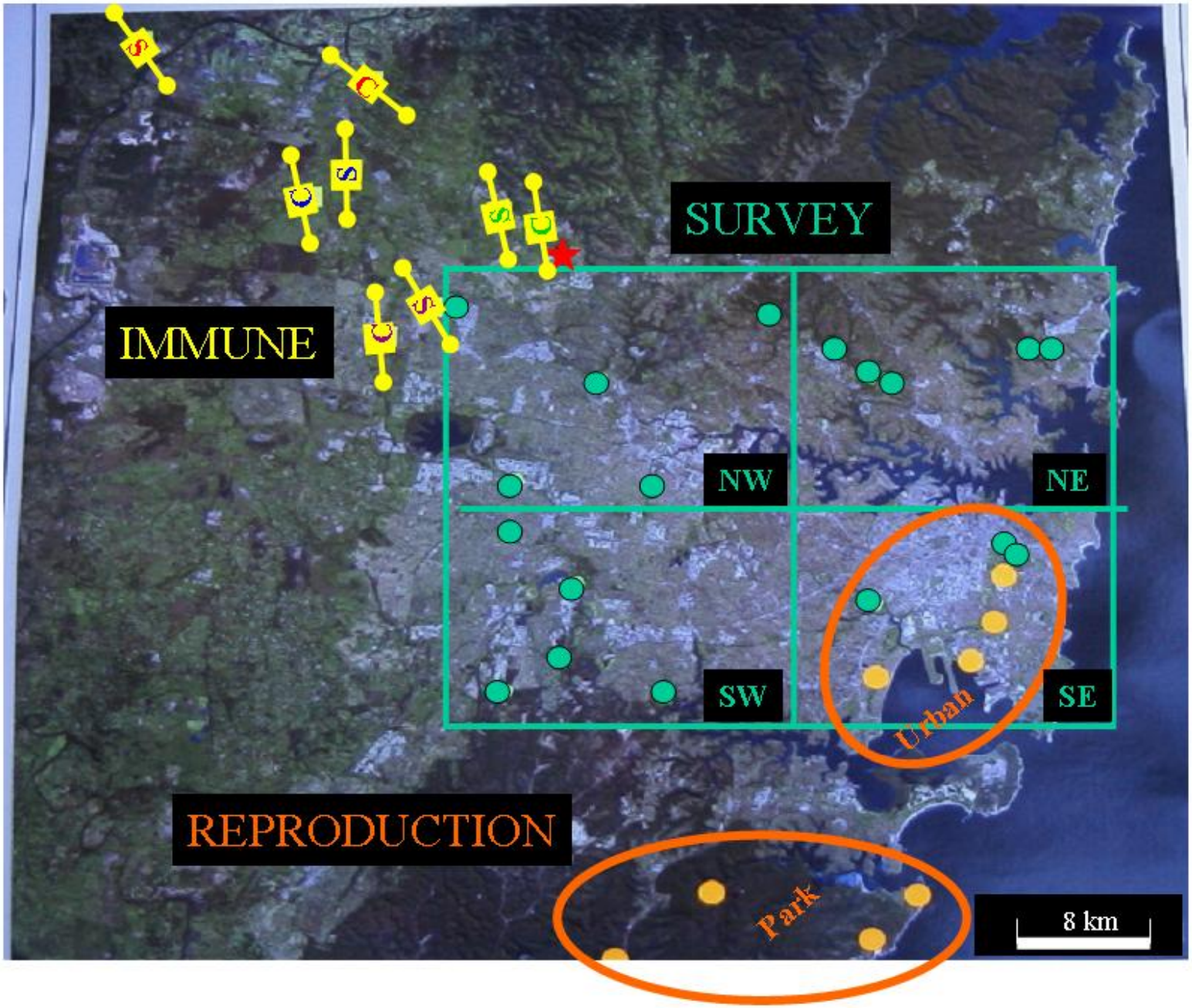

Figure B.2 Satellite photo of Sydney (Spot Imaging Services, Sydney) showing the location of sites for the:

Sydney Survey (surveys A & B; green; Section C);

Immune Study (yellow; S = STP outfall creek, C = control creek; Section D);

Biomonitoring/Reproductive Study (orange; Sections E & F);

Sludge Lagoon (red star; Sections D and E).