THE ECOLOGY OF THE POLYTYPIC FRESHWATER TURTLE SPECIES, *Emydura macquarii macquarii*.

David Judge
Bachelor in Applied Science

Applied Ecology Research Group
Faculty of Applied Science
University of Canberra
ACT

Submitted for the degree of Master of Applied Science,
University of Canberra April 2001

Statement of Originality

This thesis is my original work and has not been submitted, in whole or in part, for a degree at this or at any other university. Nor does it contain to the best of my knowledge and belief, any material published or written by another person, except as acknowledged in the text.

Signed………………………………….. Date………………………..

Copyright

Signed………………………………….. Date……………………….

Acknowledgments

I would like to thank the following people:

Arthur Georges for the valuable written comments on early drafts of this thesis as well as his advice and encouragement throughout the course of this degree.

Angie Jenkinson, Suzanna Podreka, Chris Hall and Scott Thomson for their tireless work in the field.

Tom Goodman for allowing me to use his property as a site for the Hunter River population.

Mani Berghout for reviewing my work as well as helping with the formatting.

Wayne Robinson for his statistical advice

Frank Pelligrini and Jo Beaver for their help and advice in the laboratory.

Finally, I would like to thank Rajani Rai and my parents. Without their love and support, this thesis would not have been possible.

This study was partly funded by the Nepean-Hawkesbury River Management Trust and the Peter Rankin Trust Fund for Herpetology.

Abstract

An ecological study of *Emydura macquarii macquarii* in the south-east region of Australia was conducted between October 1995 and March 1998. *E. m. macquarii* is an abundant and widespread species of short-necked turtle that is highly variable in morphology and related life history attributes. No study in Australia had previously looked at geographic variation in biological traits in freshwater turtles, hence the level of variation in *E. m. macquarii* had been poorly documented. The principal aims of this study were to investigate the plasticity of life history traits across populations of *E. m. macquarii* and to speculate on possible causes. A more intensive study was also conducted on a rare and suspected declining population of *E. m. macquarii* in the Nepean River to determine whether relevant management and conservation measures were required.

The study involved comparing various life history attributes between five populations of *E. m. macquarii* (Brisbane River, Macleay River, Hunter River, Nepean River and Murray River). The populations were specifically chosen to account for the range of variation in body size within this subspecies. Body size (maximum size, size at maturity, growth rates), population structures (sex ratios, age and size structures), reproductive traits (clutch mass, clutch size, egg size, egg content, etc.) and other attributes were collected for each population. Patterns of life history traits, both within and among populations, were explored so that causes of variation could be sought.

Geographic variation in Body Size and other Related Life History Traits

Body size in *E. m. macquarii* differed markedly between populations. Females ranged in maximum sizes (carapace length) of 180 mm in the Macleay River to over 300 mm in the Murray River. *E. m. macquarii* was sexually dimorphic across all populations with females larger than males in all cases. Maximum body size was positively related to the size at which a turtle matures. The size at maturity in turn was positively related to juvenile growth rates. Age was a more important factor for males in terms of timing of maturity whereas in females it was body size. Morphological variation was not only great between populations, but also within populations. Maximum body size was unrelated to latitude; hence it was inferred that habitat productivity had the most important influence on geographic variation in body size. Population structures also differed between populations. Sex ratios did not differ in the Brisbane, Macleay and Murray Rivers. However, a male bias was present in the Nepean River population and a female bias in the Hunter River. Juveniles were scarce in the Brisbane and Macleay Rivers but numerous in the Nepean and Hunter Rivers.

Geographic Variation in Reproduction

There was large variation in reproductive traits across populations of *E. m. macquarii*. Nesting season began as early as mid-September in the Brisbane River and as late as December in the Hunter River, and continued until early January. Populations in the Hunter and Murray Rivers are likely to produce only one clutch per season while populations from the Macleay and Nepean Rivers can produce two, and on some occasions, three clutches annually. The majority of females would appear to reproduce every year.

Clutch mass, clutch size, and egg size varied greatly both within and among populations. A large proportion of variation in reproductive traits was due to the effects of body size. *E. m. macquarii* from large-bodied populations such as in the Brisbane and Murray Rivers produced bigger eggs than small-bodied populations. Within a population, clutch mass, clutch size, and egg size were all correlated with body size, except the Nepean River. The variability of egg size was smaller in large-bodied populations where egg size was more constant.

Not all variation in reproductive traits was due to body size. Some of this variation was due to annual differences within a population. Reproductive traits within a population are relatively plastic, most likely a result of changing environmental conditions. Another source is the trade-off between egg size and clutch size. A negative relationship was found between egg size and clutch size (except the Brisbane River). Reproductive variation was also influenced by latitudinal effects. Turtles at lower latitudes produces more clutches, relatively smaller clutch sizes, clutch mass and larger

eggs than populations at higher latitudes. Annual reproductive output is greater in tropical populations because they can produce more clutches per year in an extended breeding season.

Eggs that were incubated at warmer temperatures hatched faster and produced smaller hatchlings. Incubation temperatures above 30^{o}C increased egg mortality and hatchling deformities, suggesting this is above the optimum developmental temperature for *E. m. macquarii*. Hatchling size was positively related to egg size, hence hatchling sizes was on average larger in the Murray and Brisbane rivers. However, population differences remained in hatchling size after adjustments were made for egg size. For example, hatchlings from the Hunter River were smaller than those from the Macleay River despite the egg size being the same. These differences were most likely due to the shorter incubation periods of hatchlings from the Hunter River.

Nepean River

The Nepean River population of *E. m. macquarii* is at the southern coastal limit of its range. This is a locally rare population, which is believed to be declining. This study aimed at determining the distribution, abundance, and population dynamics to assess whether any conservation management actions were required. *E. m. macquarii* in the Nepean River was mainly concentrated between Penrith and Nortons Basin, although even here it was found at a very low density (10.6 – 12.1 per hectare). The largest male caught was 227 mm while the largest female was 260.4 mm. Males generally mature between 140 – 150 mm in carapace length and at four or five years of age. Females mature at 185 –195 mm and at six to seven years of age.

Compared with other populations of *E. macquarii*, Nepean River turtles grow rapidly, mature quickly, are dominated by juveniles, have a male bias and have a high reproductive output. Far from being a population on the decline, the life history traits suggest a population that is young and expanding. There are considered to be two possible scenarios as to why the Nepean River population is at such a low density when it appears to be thriving. The first scenario is that the distribution of the population on the edge of its range may mean that a small and fluctuating population size may be a natural feature due to sub-optimal environmental conditions. A second scenario is that the population in the Nepean River has only recently become established from dumped pet turtles.

Table of Contents

List of Tables

Chapter 2

Chapter 3

Chapter 4

Chapter 5

List of Figures

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 1

General Introduction

1.1 Introduction

A life history is an organism's lifetime pattern of growth, differentiation, storage and reproduction (Begon *et al.,* 1990). It consists of a suite of coevolved characteristics that directly influence population parameters. Important components of life history include size, rates of growth and development, reproductive output, age at maturity, survivorship and longevity. Natural selection favours those life history attributes that increase an individual's fitness through increased fecundity and survivorship.

Turtles display a relatively narrow range of life history attributes when compared to reptiles such as lizards and snakes. Characteristic of the life history of turtles are longevity, high survivorship, delayed sexual maturity and iteroparity. All species of turtle reproduce by laying eggs in the ground, which are covered up and abandoned. Viviparity or post-ovipositional care has not evolved in turtles. High levels of predation on nests and offspring result in only a small proportion of eggs producing mature adults. Low reproductive success is offset by reduced adult mortality and a relatively long life span. High survivorship is partly due to the turtle's shell, which protects against predators and adverse environmental conditions. The time and energy required to develop a shell large enough to be of protective value may be associated with the delayed maturity and longevity of turtles (Congdon & Gibbons, 1990).

An important life history attribute of turtles is body size. The larger the turtle the more effective its shell as a defensive mechanism. The enhanced survival of turtles would be especially important to nesting females and those species that make regular terrestrial migrations. Body size in many species of turtles has also been strongly correlated with reproductive parameters such as clutch size (Gibbons 1970, 1982; Moll and Legler, 1971; Gibbons *et al.,* 1982; Congdon and Gibbons, 1983, 1985), clutch mass (Plummer, 1977; Gibbons *et al.,* 1982; Congdon and Gibbons, 1983, 1985;

Congdon *et al.,* 1983, 1983a, 1987; Georges, 1985; Congdon and van Loben Sels, 1991; Mitchell and Pague, 1991), and egg size (Congdon and Tinkle, 1982; Congdon and Gibbons, 1983; 1985; Congdon *et al.*, 1983; Congdon and van Loben Sels, 1991). Larger turtles have greater reproductive output, and presumably the higher fitness. An increase in reproductive output may result in more offspring, higher quality offspring or both. To take advantage of increased body size, turtles exhibit indeterminant growth. That is, turtles continue to grow right throughout their lives. Indeterminant growth in turtles selects against senescence by increasing reproductive output and/or quality, later in life (Congdon & Gibbons, 1990).

Life histories of organisms are not fixed, but rather plastic, reflecting the interaction between the genotype and the organism's environment. Research from North American studies have demonstrated that many species of freshwater turtles display large variation in life history attributes across their range (*Chrysemys picta*: Moll, 1973; Christiansen & Moll, 1973; MacCulloch & Secoy, 1983; *Trachemys scripta*: Gibbons *et al.,* 1981; Congdon & Gibbons, 1982; Moll & Moll, 1990; *Chelydra serpentina*: Galbraith *et al.,* 1989; Brown *et al.,* 1994; Iverson *et al.,* 1997). Some of this variation can be explained by latitudinal effects on the population (Moll, 1973; Christiansen & Moll, 1973; Iverson *et al.,* 1993). In general, populations from lower latitudes mature smaller and faster, and reach smaller maximum body sizes than populations from higher latitudes (MacCulloch & Secoy, 1983; Galbraith *et al.,* 1989). Clutch frequency and egg size tends to be larger at lower latitudes (Moll & Moll, 1990), while clutch size is generally greater at higher latitudes (MacCulloch & Secoy, 1983). This relationship has been explained by differences in the length of the breeding season due to the effects of temperature. A short breeding season limits the number of clutches that can be successfully produced and incubated. Because clutch size is often correlated with body size in turtles (see references above), delayed maturity enables a larger maximum size to be obtainerd. This combined with smaller eggs, enables larger clutch sizes to be produced. Delayed maturity is therefore of no disadvantage as reproductive output over a lifetime is maximised by reaching larger body sizes. In tropical populations, where large number of clutches can be produced in a season, the cost of delaying maturity is too great.

Not all variation in life history traits of turtles can be explained by latitudinal effects. Even within a single geographic area, large variation in biological traits has been found (Gibbons and Tinkle, 1969; Congdon and Gibbons, 1983; Brown *et al.*, 1994; Rowe, 1994). Habitat productivity is especially important in determining life history traits in turtles. Populations in more productive environments may grow faster (Gibbons, 1967; Gibbons, 1970; Brown *et al.,* 1994), mature at a larger size (Georges, 1985), mature earlier (Gibbons, 1970; Lindeman, 1996), reach larger maximum body sizes (Gibbons & Tinkle, 1969; Gibbons, 1970; Georges, 1985), produce larger clutch sizes (Gibbons & Tinkle, 1969; Gibbons, 1970; Georges, 1985; Brown *et al.,* 1994) and possibly increase clutch frequency (Gibbons, 1970; Georges, 1985).

Even within a population, life history traits can vary due to fluctuations in environmental conditions. Growth rates and age at maturity for male *C. picta* in a Michigan population was faster and younger in the late 1980s than earlier in the decade (Frazer *et al.,* 1993). This response was to increasing temperatures resulting in a longer growing season. Growth rates, body size and reproductive output in a population of *Pseudemys scripta* increased due to the thermal effluent from a nuclear reactor (Gibbons, 1970). These responses were attributed to the increase in aquatic primary productivity (Gibbons, 1970) at a warmer water temperature (Gibbons *et al.,* 1979).

Life-history traits in turtles are therefore not only a result of long-term evolutionary forces, but also the immediate response to local environmental conditions such as temperature and habitat productivity. Even within a population, these factors are rarely constant, reflecting that life-history attributes in turtles are highly plastic. Therefore, for a true understanding of the life history patterns within a species, it is important that populations are studied over a wide range of environmental conditions.

1.2 Geographic variation in Australian turtles

Our knowledge of the biology for many species of Australian freshwater turtles is relatively poor (Georges, 1993). Therefore, it is of no surprise that geographic variation of life-history traits within a single species is virtually unknown, except for *Chelodina longicollis*. Differences in the size at maturity and maximum body size of *C. longicollis* were described by Chessman (1978) and Kennett (1987). *Chelodina longicollis* from the Murray River site (Chessman, 1978) matured at a larger body size and reached a larger maximum body size than populations from Jervis Bay (Kennett, 1987), Gippsland (Chessman, 1978) and Armidale (Parmenter, 1976). Kennett (1987) ascribed this difference to rapid growth rates of juveniles in the Murray River. Size at maturity and the maximum body size for the other populations were relatively similar, as is data from the Nepean River (Judge, unpubl. data). Therefore, the Murray River aside, geographic variation in maximum body size of *C. longicollis* in south-eastern Australia is not great.

Geographic variation in reproductive traits of *C. longicollis* has also been studied (Chessman, 1978; Kennett, 1987). In contrast to the Northern Hemisphere patterns, Chessman (1978) concluded that smaller clutch sizes were produced at higher latitudes and that clutch frequency was not greater at lower latitudes. At lower latitudes, clutch size is highly variable with little apparent pattern. As many of these localities were at different altitudes, few conclusions regarding latitudinal variation could be drawn (Chessman, 1978). Kennett (1987) concluded from a larger dataset that clutch frequency is greater at lower latitudes, although more data from Northern Australian populations are required. He also found no relationship of clutch size with latitude, although this may be masked by differences in female body size.

Studies have neglected *Emydura macquarii* in this area despite the acknowledged differences in various life history attributes across populations. Georges (1985) compared selected life history parameters of *Emydura macquarii krefftii* between populations from Fraser Island and the mainland. Georges (1985) found that turtles from Fraser Island mature at a smaller size, lay fewer and smaller eggs per clutch, have a much lower reproductive potential and reached smaller maximum sizes. However, the paper was not focused on life history variation, but rather explanations of reduced body size and reproductive output of the Fraser Island population.

Of the 16 species of turtle in Australia (Georges and Adams, 1992), *C. longicollis* and *Em. macquarii* would appear to be the most suitable species in which to study geographic variation in life history traits. Both species are highly abundant and easy to trap. They are also very widespread, distributed from tropical environments in North Queensland down to temperate climates in Victoria. However unlike *C. longicollis*, *Em. macquarii* is unable to migrate between waterbodies, as it is largely restricted to permanent waters (Chessman, 1978, 1984, 1988). Therefore, populations of *Em. macquarii* are reproductively isolated, as movement between drainages is not possible. As a result, *Em. macquarii* is genetically and morphologically more variable than *C. longicollis* over the same range (Georges, 1994). Body size and shape varies considerably from one population to another. This makes *Em. macquarii* an ideal species of turtle in Australia to explore theories of geographic variation in life history traits such as investigated for North American turtles.

While *Em. macquarii macquarii* is a widespread, abundant species that is currently under no threat, the morphological distinctiveness of certain populations along the eastern coast of New South Wales suggests they deserve attention in their own right (Georges and Legler, 1994). However, current knowledge comes soley from brief descriptive studies on a handful of individuals from each river (Cann, 1969; Cann, 1998). Georges (1994) suggests that the morphological and genetic differences between these populations means they could be in the process of undergoing allopatric speciation. To safeguard this process of speciation, it is important that each population be conserved, which entails greater knowledge of its systematics and ecology (Georges, 1994). One population of *Em. m. macquarii* that could be considered under threat is the Nepean River. Although only recently described, this population is found in relatively low densities in an increasingly disturbed habitat.

1.3 Scope and aims of this thesis

In this thesis, I present an account of the population structure and life history attributes of a population of the short-necked Australian chelid turtle, *Emydura macquarii macquarii*, from the Nepean River of the central coast of New South Wales and explore patterns of geographic variation in life history attributes across populations of this species in south-eastern Australia. Specific objectives of the thesis include:

- To determine the level of variation in body size across the range of *Em. m. macquarii*
- To investigate the relationship between growth rate and the timing of maturity on body size of *Em. m. macquarii*
- To determine the effect of body size on the reproductive biology of *Em. m. macquarii*.
- Determine the status of the Sydney Short-necked Turtle, *Em. m. macquarii*, in the Hawkesbury-Nepean drainage and to make relevant management and conservation recommendations.

Chapter 2

Study Animal, Study Sites and General Methods

2.1 Study animal – *Emydura macquarii macquarii*

All Australian freshwater turtles belong to the family Chelidae apart from the pig-nosed turtle *Carettochelys insculpta* (Family: Carettochelydidae). The family Chelidae is restricted to the Australasian and South American continents, and no fossil material has been found outside their present range (Pritchard, 1979). They date back in the fossil record to the Cretaceous (Broin, 1987). A phylogeny for the family has been established using both morphological (Gaffney, 1977) and molecular techniques (Georges and Adams, 1992; Seddon *et al.*, 1997; Georges *et al.*, 1999). Although these phylogenies do not concur entirely, they do agree that the Australian Chelidae contain two major lineages – the long-necked turtles of the genus *Chelodina*, and the short-necked turtles of the genera *Elseya*, *Elusor*, *Emydura*, *Pseudemydura* and *Rheodytes*.

Turtles in the genus *Emydura* are the best studied species of freshwater turtle in Australia (Chessman, 1978; 1984; 1988; Georges, 1982; 1982a; 1983; 1985; Thompson, 1983; 1983a; 1985; 1988), together with *Chelodina longicollis* (Parmenter, 1976; Chessman, 1978; 1984; 1988; Kennett, 1987; Kennett and Georges, 1990). They are the forms considered to have the greatest parallels with unspecialized short-necked forms of the Northern Hemisphere in the Emydidae (Gibbons, 1990).

There are four species of *Emydura* classified in Australia -- *Em. macquarii* (with two sub-species recognised here), *Em. tanybaraga*, *Em. subglobosa*, and *Em. victoriae*. A fifth species, *Em. australis*, has been synonymised with *Em. macquarii* by Cogger *et al.*, (1983), who believe that the holotype of *Em. australis* came from the Macquarie River in NSW.

Emydura macquarii is the only species that is distributed in the south eastern regions of Australia. All other species of *Emydura* are restricted to the northern parts of Australia. *Em. subglobosa* is found in Cape York (far North Queensland) as well as in

Papua New Guinea. *Em. tanybaraga* and *Em. victoriae* inhabit coastal flowing rivers in the northern regions of Western Australia and the Northern Territory.

Emydura macquarii macquarii is distributed throughout the coastal rivers from the Nepean-Hawkesbury drainage in Sydney to the Brisbane River of south-east Queensland, with the exception of the Manning River (Fig. 2.1). It is rarely found in the Bellingen River. The sub-species is also widespread in the Murray-Darling drainage. *Em. m. krefftii* is found in coastal flowing rivers from north of the Brisbane River to the Normanby and Kennedy rivers in North Queensland (Fig. 2.1). Populations of *Emydura* in the Cooper Creek drainage are more closely aligned with *Em. m. krefftii*, and populations in the Barcoo River are of unknown affinity.

Emydura macquarii is a short-necked species of turtle with a carapace that is light brown to black in colour. Body size is highly variable, with reported maximum sizes for females ranging from 180 mm in the Macleay-Hastings rivers (Cann, 1998) to over 320 mm in the Murray River (Chessman, 1978). Similarly, body shape is also variable, both within and amongst populations. Hatchlings have rounded carapaces in shape tending towards a more oval carapace outline as size increases. Unlike *Em. m. krefftii*, megacephaly is not present in populations of *Em. m. macquarii*. More detailed morphological descriptions of *Em. m. macquarii* across its range are given by Cann (1998). A distinctive feature of *Em. m. macquarii* is a white to creamish band from the mouth along the ventro-lateral margin of the neck (Fig. 2.2). Adult males are easily distinguished from adult females by their longer, thicker tail. *Em. macquarii* prefers deep permanent still waterbodies such as rivers and its backwaters (swamps and lagoons) where they are generally the pre-dominant turtle species (Chessman, 1988). The high rate of evaporative water loss under desiccating conditions prevents *Em. macquarii* from migrating overland (Chessman, 1984), thereby restricting them to permanent waterholes.

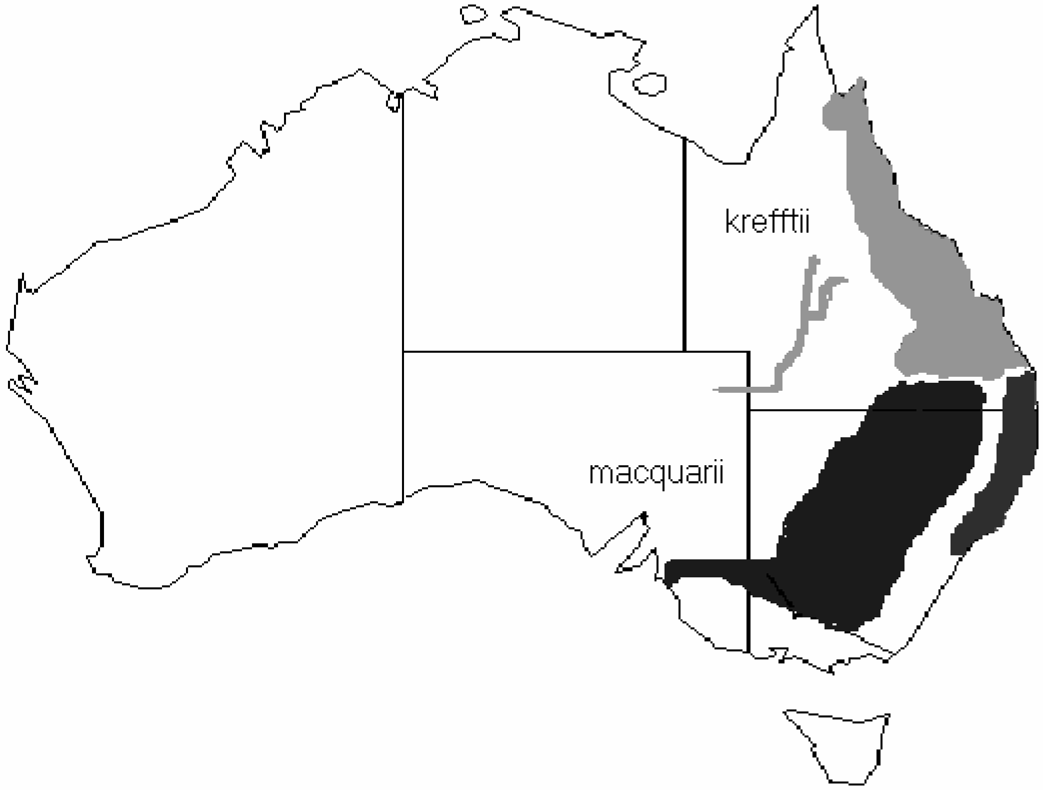


Figure 2.1. Distribution of the two subspecies of *Emydura macquarii* in Australia.

Figure 2.2. A male *Emydura macquarii macquarii* from Nortons Basin on the Nepean River

Emydura macquarii is omnivorous consuming a wide range of food types such as filamentous algae, periphyton, aquatic macrophytes and invertebrates, terrestrial insects and carrion (Georges, 1982b; Chessman, 1986). Juveniles tend to be more carnivorous than adults (Georges, 1982b). *Em. macquarii* may lay one (Murray River – Chessman 1978; Thompson, 1983), three (Fraser Island – Georges, 1983) and even five clutches (North Queensland – Legler and Cann, 1978) during each spring and early summer. Reported clutch sizes have been as small as four eggs (Fraser Island: Georges, 1983) to as large as 34 (Murray River: Thompson, 1983) and are strongly correlated with maternal body size (Georges, 1983). The hard-shelled eggs vary in size from an average weight of 7.4g on Fraser Island (Georges, 1983) to 10.4 g in Murray River populations (Thompson, 1983). Eggs normally hatch in mid to late summer producing hatchlings ranging from a mean weight of 4.6g on Fraser Island (McNicol & Georges, 1980) to 5.1g in the Murray River (Thompson, 1983). The sex of *Em. macquarii* is genetically determined (Thompson, 1983; Thompson, 1988; Bull et al., 1985) rather than by incubation temperature as in many Northern Hemisphere species of freshwater turtles (reviewed by Bull 1980, 1983; Ewert and Nelson, 1990). Males mature at smaller sizes than females and reach smaller maximum sizes (Georges, 1985).

An analysis of species boundaries within the short-necked genera using allozyme electrophoresis revealed that species formerly regarded as *Emydura macquarii*, *Em. krefftii* and *Em. signata* are actually a single taxon, with populations sharing even rare alleles (Georges and Adams, 1992). Georges and Adams recommended that the three species be synonymised. However, the Queensland forms formerly recognised as *Em. krefftii* are regarded in this thesis as being sufficiently distinct to warrant recognition at sub-species level. These Queensland populations are referred to in this thesis as *Em. macquarii krefftii*. Those populations formerly referred to *Em. macquarii* and *Em. signata* are classified as *Em. macquarii macquarii*. The populations studied in this thesis belong to the subspecies *Em. macquarii macquarii*.

To complicate matters further, Cann (1998) recognised a range of sub-species among the populations formerly referred to *Em. signata*. These sub-species are *Em. m.*

dharuk (Sydney Basin), *Em. m. coonanbarra* (Hunter River), *Em. m. dharra* (Macleay River) and *Em. m. binjing* (Clarence River). None of these sub-species are recognised in this thesis, being regarded largely as size variants of a broader taxon *Em. m. macquarii*.

2.2 The study areas

To determine the geographic variation in life history traits, five populations of *Em. m. macquarii* from separate catchments were chosen. Four of these catchments are distributed in coastal flowing drainages along south-eastern Australia while the fifth catchment is located in the large inland Murray-Darling system (Fig. 2.3). Populations were specifically chosen to account for the large variation in body size of this sub-species.

2.2.1 Brisbane River

The most northerly-located population in this study was at Kholo Crossing on the middle reaches of the Brisbane River. The Brisbane River flows eastward through Brisbane, the capital city of Queensland, and as a consequence much of the river is quite heavily disturbed. Farms surround the study site while the populous area of Ipswich is very close by. Kholo Crossing is a popular recreational area for swimming and fishing. The site was in the main river channel itself, where the river was wide (30 – 40 m) and deep, and the flow relatively slow. This region has the warmest climate as well as the greatest rainfall (mainly between October and March) of any of the study sites (Table 2.1). *Em. m. macquarii* can get quite large in the Brisbane River, with reported carapace lengths as great as 260 mm for males and 276 mm for females (Cann 1998). Three other species of freshwater turtle can be found in the Brisbane River – *Elseya latisternum*, *C. longicollis*, and *C. expansa*.

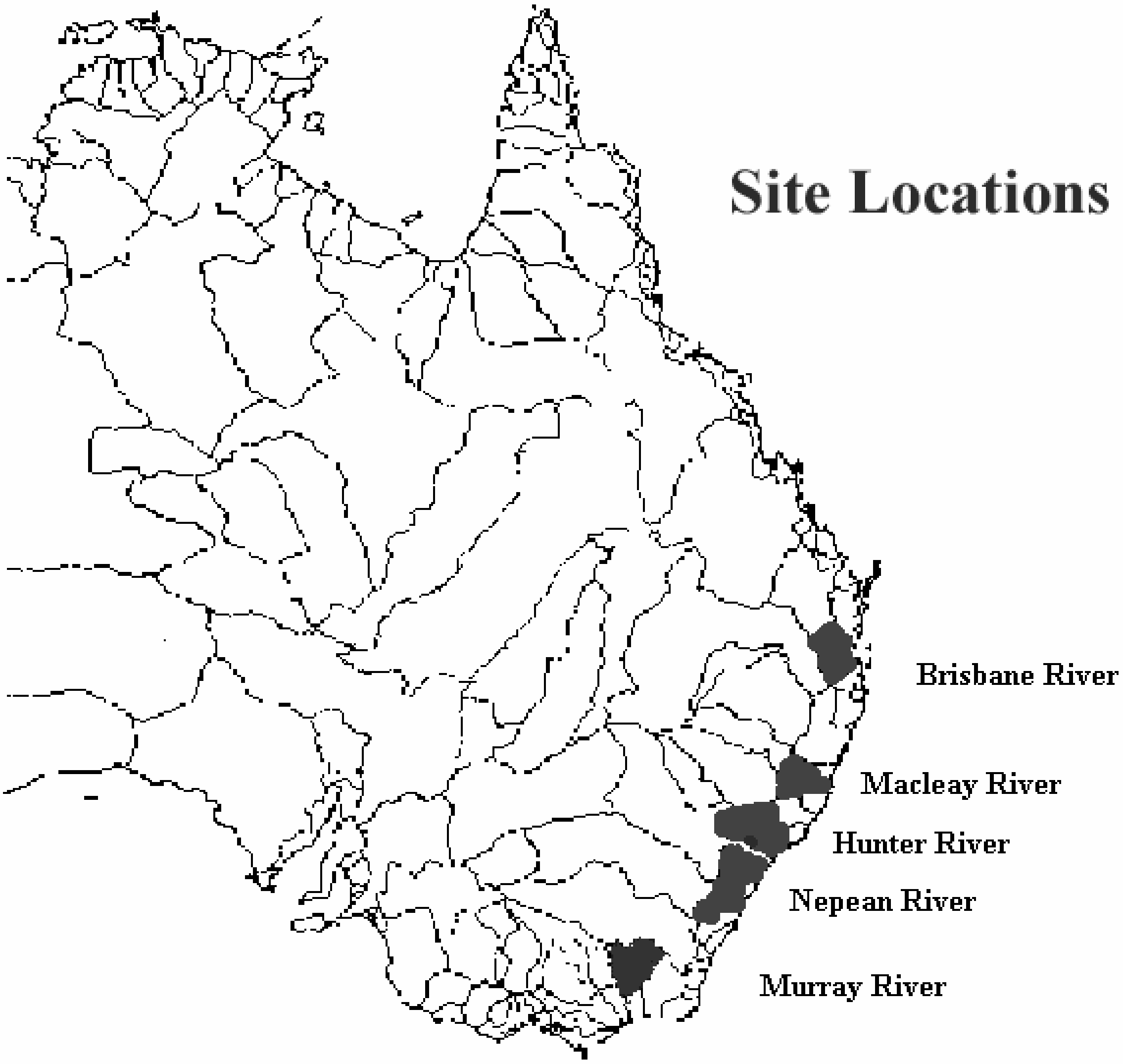


Figure 2.2. Location of the drainages for populations of *Emydura macquarii macquarii* that were studied in this project.

Table 2.1. Location and climate of the Brisbane, Macleay, Hunter, Nepean and Murray River study sites.

River	Latitude	Longitude	Altitude (m)	Mean Minimum Temperature (^{o}C)	Mean Maximum Temperature (^{o}C)	Annual Rainfall (mm)
Brisbane	27^{o} 34’	152^{o} 45’	10	13.7	27.1	877.2
Macleay	30^{o} 45’	152^{o} 11’	160	7.1	20.3	789.0
Hunter	31^{o} 49’	151^{o} 18’	510	9.8	24.9	647.1
Nepean	33^{o} 52’	150^{o} 37’	20	10.8	23.2	790.6
Murray	36^{o} 05’	146^{o} 57’	160	8.4	21.3	784.7

2.2.2 Macleay River

The Macleay River lies about 320 kilometres south of the Brisbane River. The headwaters are on the escarpment near Armidale and the river flows east to the coastal town of Kempsey. The study site was high in the catchment at Georges Crossing, about 60 kilometres south-east of Armidale. The area is surrounded by dairy farms and hobby farms, although natural riparian vegetation can still be found along much of the river. The Oxley Wild Rivers National Park is about 2 kilometres upstream of the study site which is indicative that the river is still in a relatively pristine condition. In this region, the Macleay River is normally very clear and fast flowing with long stretches of a sandy and pebbly substrate.

Turtles were concentrated in still backwaters off the main river channel or in small protected pockets in the river. Turtles were trapped primarily in a small backwater (10m x 50m) at Georges Crossing. Georges Crossing is a recreational reserve for primitive camping, fishing, swimming and boating, although human impact on the river is minimal. *Em. m. macquarii* in the Macleay River is a relatively small-bodied turtle, with reported maximum sizes of around 162 mm for males and 185 mm for females (Cann, 1998*). Chelodina longicollis* is the only one other freshwater turtle in the Macleay River.

2.2.3 Hunter River

The Hunter River is about 180 kilometres south west of the Macleay River site is the Hunter River. The river begins on the western foothills of Barrington Tops National Park, flowing through Muswellbrook and Maitland before reaching the ocean at Newcastle. The study site was high in the catchment (highest altitude of any study site (Table 2.1)), around 50 kilometres north-east of Scone. The site was located on a sheep and cattle property owned by Tom Goodman. Almost all native vegetation has been cleared in the region. Little riparian vegetation remains except the occasional *Eucalyptus sp.* or introduced willow (*Salix sp*.). The river consists of long stretches of rapids, interspersed with deep waterholes. The substrate is almost entirely sand and pebbles, with no aquatic macrophytes present. The water is very clear and extremely cold as the headwaters receive regular snowfalls in winter. *Em. m. macquarii* are concentrated in high densities in the still waterholes all along this region. *Em. m. macquarii* in the Hunter River catchment have been reported to reach maximum sizes as large as 225 mm for males and 257 mm for females (Cann, 1998). *Chelodina longicollis* is the only other turtle in the Hunter River drainage.

2.2.4 Nepean River

The Hawkesbury-Nepean River is the next catchment south of the Hunter River drainage. The river starts out south-west of Sydney, flowing through Penrith and Windsor before entering the ocean at Broken Bay. Located within Australia's largest city, the Nepean-Hawkesbury River is heavily disturbed. The Nepean River site is located at Nortons Basin, which is just downstream from Wallacia and about 20 km upstream of Penrith. The site is surrounded by native vegetation, and remains largely undisturbed relative to other sections of the Nepean River. Sections of the river can be up to 50 m wide and 17m deep. Nortons Basin itself is a large oval waterbody the size of a football ground. The Nepean River is highly productive with an abundance of aquatic macrophytes and fish.

The Nepean River represents the most southerly of the eastern drainages in which *Em. m. macquarii* is found. Unlike the other populations, *Em. m. macquarii* in the Nepean River is relatively uncommon (Cann, 1998). Surprisingly, males reach larger maximum sizes (240 mm) than females (220 mm) (Cann, 1998), although this is based on sparse data. *Chelodina longicollis* is the only other native species of turtle in the Sydney Basin, although several introduced species have been discovered recently (South, pers. comm.).

2.2.5 Murray River

The most southerly population was at Albury on the Murray River. The Murray-Darling drainage is the largest in Australia (in terms of water volume) distributed over four states and one territory. The study site was located at Mungabareena Reserve on the outskirts of Albury. Mungabareena Reserve is a large recreational park popular for picnicking and fishing. The surrounding areas have been cleared, although large river red gums (*Eucalyptus camaldulensis*) line the riparian zone. Turtles were trapped in the oxbow lake adjacent to the main river channel. The oxbow lake was roughly three hectares in surface area and relatively shallow (1m – 1.5m). The oxbow lake connects to the main river only in floods as water is released from the Hume Dam upstream. Water levels are generally high in winter and low during summer. Water in the oxbow lake is highly turbid and visibility is poor. The substrate consists of mud and silt, and large branches and logs litter the water. No aquatic macrophytes were present, although filamentous algae is quite common. The introduced European Carp and Redfin are highly abundant in the lagoon, while native fish such as Murray Cod are also present. Large concentrations of waterfowl were often found in the lagoon. *Em. m. macquarii* in the Murray River can attain very large sizes, with males reported as large as 274 mm and females 324 mm (Chessman, 1978). Two other species of turtle were caught at Mungabareena Reserve, *C. longicollis* and *C. expansa*.

2.3 General methods

Turtles were caught in hoop traps (Legler, 1960) baited with bread and sardines. Hoop traps were unbiased in respect to size and sex in *Em. m. krefftii* (Georges, 1985). Traps were either left totally submerged and checked every 90 to 150 minutes or left partially out of the water overnight.

Each turtle was individually marked by notches cut into the outer carapace scutes with a hacksaw (see Fig. 2.4 for marking system). For example, a turtle with the

ID number 87, would have the 70 and 10 scute (70+10=80) notched, plus the 7 scute. Notches for small juveniles were cut with sharp scissors due to the softness of their shell. Straightline carapace length, plastron length, carapace width, body weight, tail length (plastron to vent), maturity status, sex, capture date and location of capture were all recorded for each turtle before release. Carapace length, plastron length, carapace width, and tail length were measured with calipers (to the nearest 0.1 mm). Body weights were measured with an electronic scale (to the nearest g). The sex *of Em. macquarii* is simple to determine as males have a much longer and thicker tail than females. The scutes and shells of each turtle were inspected for deformities or abnormalities.

2.3.1 Sex and Maturity Status

To determine maturity status for males and females, turtles were examined by a laparoscope (Wood *et al.*, 1983). Laparoscopy offers the advantages over dissection in that no turtles need to be killed, more turtles can be sampled, and repeated analysis can occur on the same individual. Turtles were sexed by visual examination of the gonad and associated ducts via a laparoscope (Olympus A7591). Incisions were closed with plain surgical catgut sutures. Turtles were analysed at the site, kept for a few hours to ensure a full recovery, and then released. Surgeries were conducted with animal ethics approval of University of Canberra.

Mature females were viewed internally to determine if they had recently ovulated by the presence of corpora lutea and/or ovarian scars. Mature females were also inspected for the presence of large vitellogenic preovulatory follicles to derive estimates of the annual clutch frequency. Maturity status for males and females was determined as follows:

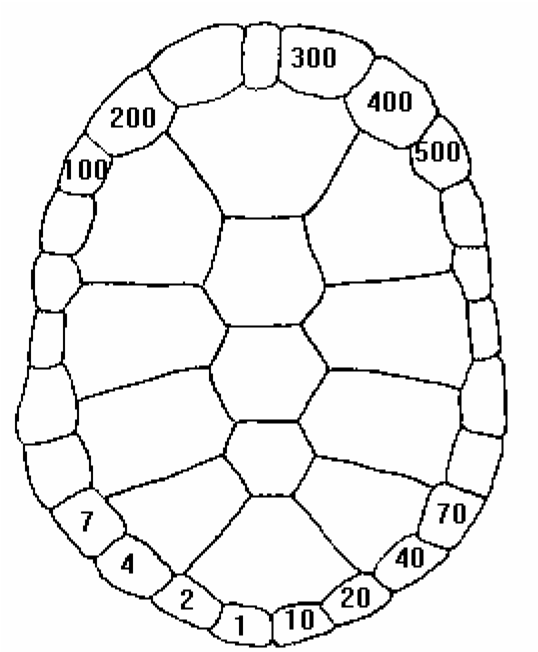


Figure 2.4. The marking system used in this study. Each turtle was given a unique combination of notches to enable future identification.

Males

<u>Immature</u> – The tail is not enlongated with the anterior margin of the cloaca positioned inside the carapace margin or the tail is beginning to lengthen with the anterior margin of cloaca positioned on or just outside the margin of the carapace. The epididymus is a straight tube with no coiling or is coiled but translucent white. The testes is small, compact, with no seminiferous tubules visible on close inspection and is not vascularized.

<u>Mature</u> – Tail fully developed or penis protrudes. The epididymidus is heavily coiled and opaque white. Testes is enlarged and vascularized with seminiferous tubules visible on close inspection.

Females

<u>Immature</u> – The oviduct is a straight and narrow translucent tube with no vascularization. The ovaries are small and discrete with no enlarged follicles or other structures to indicate reproductive condition.

<u>Mature</u> – The oviduct is a well-developed convoluted opaque tube. The ovaries are expanded in a way consistent with enlargement for imminent first breeding, current breeding or post breeding. Maturity in some females were determined by the presence of eggs.

2.3.2 Growth Rates and Size-At-Age

The number and spacing of growth rings on carapace scutes were counted where it was clear and recognisable (mostly in juveniles). This enabled the determination of the size-at-age as well as the annual growth rate (Moll and Legler, 1971). Growth was determined from mark-recapture data and scute annuli (Moll and

Legler, 1971). Growth in carapace length using scute annuli (costal scutes) was constructed from the formula:

$$\mathrm{CL_t} = \left(\frac{\mathrm{CL_o - CL_{now}}}{\mathrm{- D_o + D_{now}}} \times (\mathrm{D_t - D_{now}}) \right) + \mathrm{CL_{now}}$$

where CLt = Initial carapace length, CLo = carapace length at birth, CLnow = carapace length now, Do = scute length at birth, Dnow = scute length now, and Dt = initial size of scute. For construction of the model, see Appendix A.

The annual deposition of growth rings was verified from mark-recapture data. Growth ring data was only used for juveniles and young adults, as rings became intermittent and undetectable in older individuals. Growth rates for large adults, as well as juveniles, were determined from mark-recapture data.

Von Bertalanffy growth models were fitted to the turtle growth data for each sex separately (Schoener and Schoener, 1978). This approach estimates growth parameters enabling age to be predicted from length and growth rates to be compared between sexes and populations. The interval growth models were fitted using the non-linear least-squares regression procedure, PROC NLIN (SAS). The iterative procedure DUD was chosen (Freund and Little, 1991). The model was validated by comparing the predicted asymptotic size with that of the mean adult size. The asymptotic size should be slightly larger than the mean adult size for the model to be meaningful biologically (Frazer *et al.,* 1990).

2.3.3 Clutch Size and Clutch Frequency

Adult female turtles were palpated for the presence of shelled eggs and gravid females were transported to the University of Canberra. Radiographs of gravid females were taken to establish the clutch size, (Gibbons and Greene 1979). Exposures were shot at 30 MA and 60 kVA for 0.07 seconds. Data by Hinton *et al.,* (1997) suggests that x-radiography does not place adults, embryos, or populations in jeopardy. They believe higher radiosensitivity during rapid cell division of embryogenesis is partially negated, as the female is x-rayed while the eggs are in a quiescent developmental period.

Oviposition was induced by intracoelomic injection of synthetic oxytocin at a rate of 1 unit per 100g body mass, with a further dose of 0.5 units per 100g if no eggs were laid within 3 hr. After injection, females were placed in a tub of warm water (30^oC) and the eggs were collected as they were layed. Each egg was then weighed (to the nearest 0.1 g) and measured (to the nearest 0.1 mm) (length and width). Eggs from each clutch were randomly allocated to incubators set at 26^oC, 28^oC, and 30^oC. The eggs were incubated in moist vermiculite (1:1 ratio of water to vermiculite by weight). At hatching, each turtle was weighed (to the nearest 0.1 g) and measured (to the nearest 0.1 mm) - carapace length, carapace width, plastron length, and head width. Each hatchling was inspected for any scute abnormalities or deformities to determine the effect of temperature on development. The date at pipping was also recorded.

All statistical analyses were conducted in the statistical package SAS (SAS Institute, 1986). Means are reported with standard errors unless otherwise specified. Statistical tests set alpha at $P = 0.05$ to reject the null hypothesis.

Chapter 3

The Sydney Short-Necked Turtle (*Emydura macquarii macquarii*)

3.1 Introduction

The Sydney Short-necked Turtle (*Emydura macquarii macquarii*) is known from the Nepean-Hawkesbury River, Lane Cove River and the Eastlake Swamps, and possibly the Georges River (Cann, 1998). Once overlooked or dismissed as an escapee from hobby collections, the Sydney Short-necked turtle was recognised as comprising natural populations only in the last 15 years (Cann, 1998). The Sydney Short-neck is a relatively uncommon turtle found only in scattered populations (Smith and Smith, 1990; Cann, 1998) and as a distinct form, is of conservation concern. This concern is heightened by suspected declines in recent years (Cann, 1998). The encroaching urbanisation of Sydney's waterways and the concurrent deterioration of riverine habitat are at the heart of these concerns. Another important threat is the release of pet turtles into Sydney's rivers by pet owners and hobbyists. The release of *Em. macquarii* from other populations of Australia threatens the genetic integrity of Sydney's populations, while the release of exotic species creates potential competition for the Sydney Short-neck.

There are counterbalancing views that may explain the low population abundance and restricted distribution of the Sydney Short-necked Turtle. One view is that the Sydney region represents a marginal population at the boundary of the coastal distribution of *Em. m. macquarii*. If so, low population sizes and wide fluctuations in population densities may be a characteristic of this population. A second possibility emerging from this study (see synopsis) is that the Nepean population, and possibly all populations of the Sydney Short-neck, were established from released turtles.

As a population with low numbers and potentially in decline, the Sydney short-neck warrants closer attention by management agencies. It is a high level consumer, and as such may provide a good indicator of river health. The principle aim of this study was to determine the current population status of the Sydney Short-necked Turtle, *Em. m. macquarii* and to gather basic data that will be relevant to its management and conservation. Specific objectives were :

- To determine the current distribution and relative abundance of *Em. m. macquarii* throughout the Nepean drainage for an assessment of its conservation status.
- To gather fundamental data on the population parameters (fecundity, recruitment, growth) of *Em. m. macquarii* as a baseline for future monitoring and assessment.
- To develop specific recommendations on management actions (if any) required to ensure the persistence of *Em. m. macquarii* in the Nepean drainage, and to identify key threatening processes.

3.1.1 Study site

The Hawkesbury-Nepean catchment covers an area slightly less than 22,000 km^2. It supplies 97% of Sydney's water and that of surrounding regions (Rosen 1995). One million people live within the catchment, and it is expected that this will increase by 750,000 by 2011 (Rosen, 1995). The Hawkesbury-Nepean River is heavily impacted by human disturbances such as pesticide contamination, waste discharge, dredging and quarrying, dams, erosion, industrial and urban run-off, domestic animals, fertilisers, increased recreational use and the introduction of feral species. The result has been increased nutrient loads, reduced water flows, blue-green algae blooms, excessive growth of aquatic plants, high turbidity and a large reduction of aquatic life (Sydney Water, 2000).

The primary study site on the Nepean River was between Nortons Basin and Erskine Creek - a distance of 3.8 km. Four reaches are recognised within this study site (Fig. 3.1), with each site separated by a series of rapids. The arrangement restricts movement by turtles between sites. The first area, Nortons Basin, is a large oval waterbody 140m x 110m in size and as deep as 17m. Upstream of Nortons Basin are a waterfall and about 250 m of rapids, so upstream movement of turtles is unlikely. The second area, Pool1 is a small shallow waterbody 100 m downstream of Nortons Basin. The third area, Junction is the confluence of the Nepean and Warragamba Rivers. Two kilometres upstream of the Warragamba River is Warragamba Dam. The fourth area, Below Junction, is the reach downstream of the Nepean-Warragamba River confluence to Erskine Creek. This area is commonly known as Fairlight Gorge. It is a long and wide (50 m) reach for a distance of around 2.5 km.

Most of the site is within the Blue Mountains National Park. Nortons Basin is not within the national park, although it is protected as a recreational reserve and therefore remains relatively undisturbed. This stretch of the Nepean River is surrounded by steep sandstone cliffs and dry sclerophyll forests. Large sandstone boulders line the river providing excellent basking sites for turtles. Water quality in this region is not as impacted as other parts of the catchment (McCotter *et al.*, 1984), although many water quality parameters are still classified as fair to poor (Sydney Water, 2000). Nortons Basin is very popular for recreational activities such as swimming, fishing, boating and bushwalking.

To augment the detailed study site in Nortons Basin, a more extensive survey of the Hawkesbury-Nepean catchment was undertaken (Fig. 3.2).

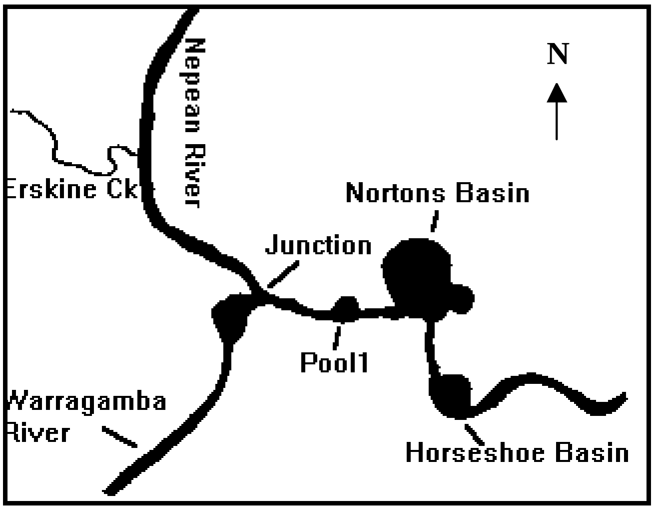


Figure 3.1. Nortons Basin study site. Map not drawn to scale.

3.2 Materials and methods

Data on the population size and density, as well as survivorship, were collected by a mark-recapture study (see general methods) between Nortons Basin and Erskine Creek (Fig. 3.1). The Jolly-Seber method (Jolly, 1982; Seber, 1982) was used to estimate the size and survivorship probabilities for each population. Analyses were conducted using the computer program JOLLY (Pollock *et al.,* 1990) on capture data spanning the period 1995-1998. Because of the problem of low capture rates in any one sampling period, the data was pooled for each year. Therefore the data consisted of three sampling periods (1995/96, 1996/97 and 1997/98), where an individual was only included once for each year, even if it had been recaptured several times. Goodness of fit tests are included to indicate that assumptions of the Jolly-Seber models (see Pollock *et al.,* 1990 for details of assumptions) have not been violated, although a nonsignificant result does not guarantee that all model assumptions have been met (Pollock *et al.,* 1990).

Studies on movements were also determined from mark-recapture data. A movement was considered to have occurred when a turtle had moved between the four study sites.

For information on the methods used in capturing, marking, and measuring turtles, obtaining reproductive data, or determining age and growth rates, see general methods (Chapter 2).

The area of the study site was calculated using distances measured from a topographic map (Penrith, NSW, Australia (9030-111-N), 1:25000). Population density was estimated by dividing the population size (estimated from the Jolly-Seber model) by the number of hectares in the study site. Biomass was estimated from the average turtle size.

3.3 Results

3.3.1 Distribution

The distribution of *Em. m. macquarii* in the Nepean River catchment was mainly limited to the region between Penrith and Wallacia (Fig. 3.2). One adult female was caught in the sand quarry dams below Penrith Weir, while nine individuals were caught at Bents Basin above Wallacia. However, the only place where *Em. m. macquarii* was found in any numbers was between Erskine Creek junction and Nortons Basin on the Nepean River. At this main study site 263 turtles were marked over three years. From here downstream towards Penrith, turtles become increasingly scarce. No *Em. m. macquarii* were caught or sighted downstream of Glenbrook Creek. Despite looking like ideal *Emydura* habitat, only *C. longicollis* were caught in Mulgoa Creek (just above Penrith). No short-neck turtles were seen or caught further down the Nepean River at Yarramundi Crossing or in the upper Nepean River at Camden and Menangle (Fig. 3.2).

Warragamba Dam was constructed in 1960 and has resulted in the isolation of the Warragamba catchment from the Nepean. The Warragamba catchment is located within a wilderness region and the river remains relatively pristine. Even so, no short-neck turtles were seen or caught in the Warragamba catchment (Fig. 3.2). However, *C. longicollis* was found at all sites that were surveyed, with numerous *C. longicollis* shells on the banks of Lake Burragorang near the Nattai River inflow. There were no carcasses of *Em. m. macquarii* here although it appeared to be typical *Emydura* habitat. The water at another Lake Burragorang site near Katoomba was so clear *Em. m. macquarii* would have been easy to detect visually. However, only *C. longicollis* were seen or caught.

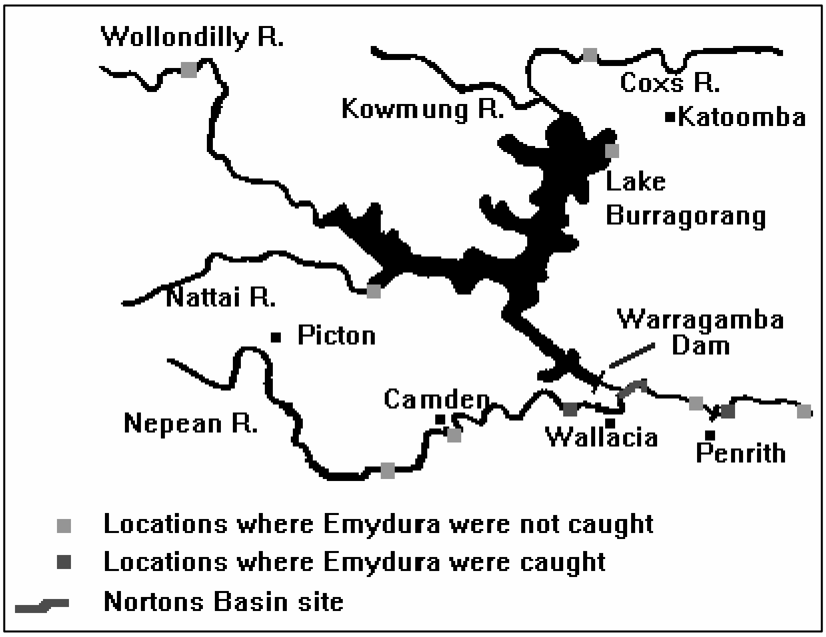


Figure 3.2. Nepean River drainage showing the location of the Nortons Basin site, as well as other locations that were surveyed for *Emydura macquarii macquarii.* Map is not drawn to scale.

Tim Marsden of NSW Fisheries has carried out field research on fish populations in the Wollondilly and Nattai Rivers, and no short-neck turtles were seen down the surveys. Marsden has caught long-neck turtles in his fish traps and it seems likely that if short-neck turtles were present, he would have caught them as well. The owner of Wollondilly River Station (Goodmans Ford) has never seen any short-neck turtles in the Wollondilly River (Fig. 3.2), although he has seen numerous long-necked turtles. This particular location had numerous deep waterholes that looked to be ideal *Emydura* habitat.

The present distribution of *Em. m. macquarii* in the Hawkesbury-Nepean catchment is concentrated primarily between Penrith and Bents Basin on the Nepean River (Fig. 3.2).

3.3.2 Population size

Between Nortons Basin and Erskine Creek on the Nepean River, 263 turtles were marked and released between the period of April 1995 and March 1998. A total of 192 recaptures included 125 turtles being recaught one or more times (Table 3.1).

The Jolly-Seber method estimated the population size of *Em. m. macquarii* between Nortons Basin and Erskine Creek to be 241 ± 51 (Table 3.2). This model provided an adequate fit to the data ($\chi 2=0.86$; d.f.=2; p=0.35). Because juveniles were more likely to be recaptured than adults ($\chi 2=10.18$; d.f.=2; $p<0.005$), population estimates were also made separately for adults and juveniles. The population size estimate was 99.8 ± 40.3 for adults and 111.3 ± 25.6 for juveniles (Table 3.2). The combined total of 211 ± 48.4 is below that found when the combined data were analysed (Table 3.2).

Between Nortons Basin and Erskine Creek the survival rate was 0.47 (Table 3.2). With the standard Jolly-Seber model, the analysis was repeated separately for adults and juveniles. These results suggested that the survival rate for juveniles (0.54

Table 3.1. The number of times each turtle was recaptured.

Times Recaught	Numbers Recaught
0	138
1	81
2	31
3	6
4	5
5	1
6	1

Table 3.2. Jolly-Seber parameter estimates for the total population, adults and juveniles at Nortons Basin. Population Size and Immigration represent total numbers while Survival Rate and Capture Probability are in proportions. These results assume that vital rates are constant across years.

	Parameter Estimate	S.E.	95 % C.L.
Total Population			
Population Size (N)	240.77	51.21	140.39 - 341.15
Survival Rate (PHI)	0.470	0.083	0.302 - 0.638
Immigration (M)	44.18	6.43	31.57 - 56.79
Capture probability (p)	0.430	0.097	0.240 - 0.620
Adults			
Population Size (N)	99.76	40.30	20.77 - 178.76
Survival Rate (PHI)	0.297	0.099	0.059 - 0.535
Immigration (M)	12.47	4.15	4.34 - 20.60
Capture probability (p)	0.481	0.21	0.069 - 0.894
Juveniles			
Population Size (N)	111.27	25.59	61.12 - 161.43
Survival Rate (PHI)	0.544	0.105	0.339 - 0.749
Immigration (M)	29.39	4.31	20.95 - 37.83
Capture probability (p)	0.442	0.112	0.223 - 0.661

(0.34 - 0.75)) was higher than that for adults (0.30 (0.06 - 0.54)), although this was not significant.

The total area sampled was 19.9 ha and 3.8 km in length, yielding a population density of between 10.6 and 12.1 ha^{-1} and a biomass of between 5.0 and 6.3 kg ha^{-1}. The number of turtles per 100 metres of river channel was between 5.6 and 6.3 individuals and the biomass of 2.6-3.0 kg/100m.

3.3.3 Size at maturity / body size

The largest male caught at Nortons Basin was 227.9 mm in carapace length (Fig. 3.3). Several other individual males of similar size were also captured – 227.4 mm, 227.0 mm and 224.1 mm. The 95th percentile of adult male body size was 214.1 mm. Two larger males were caught – one 235.9 mm and the other 257.2 mm – however both were considered to be introductions from other *Em. macquarii* populations. Male sizes at maturity were highly variable. Males laparoscoped at 121.8, 129.1, 132.6, 133.0, 142.3, and 154.4 mm were still immature, while others at 138.7, 148.7, 154.4, and 156.7 mm were mature. Most males in the Nepean River matured between 140 and 150 mm. Results from growth ring data and mark-recapture data suggest that males mature at 4 to 5 yr.

The largest female was 260.6 mm (Fig. 3.3). The next largest was only 237.7 mm and 234.8 mm. The 95th percentile of adult female body size was 237.7 mm. Female size at maturity was also highly variable. Females at 178.3, 178.9, 180.4, 183.1, 188.6, and 191.1 mm were immature, while other females at 178.6, 184.9, 193.8, and 195.2 mm were mature. Females matured between 185 and 195 mm in carapace length. Females generally mature between 6 and 7 yr.

3.3.4 Population structure

There were significantly more males (101) than females (35) at the Nortons Basin site ($\chi2=31.07$; $p<0.0001$) and male bias was consistent across all four years (Table 3.3), although the season of 1996/1997 was marginally insignificant ($p=0.09$). Sex ratios across seasons (1994/1995 excluded due to low sample size) were not significantly different ($\chi2=4.13$; d.f.=2; $p=0.13$).

Relatively even ratios of juveniles to adults were found in all years on the Nepean River, except 1994/95 where the sample size was small (Table 3.4). With 1994/1995 excluded there was no significant difference in the ratios of adults to juveniles between seasons ($\chi2=2.91$, d.f.=2, $p>0.05$).

Few large older individuals (especially females) were recorded in the population (Fig.3.3). Most adults were only recently recruited. Pairwise comparisons between the size distributions among seasons (Kolmogorov-Smirnov test) revealed significant differences between 1995/96 and 1996/97 ($D=0.21$ $p<0.01$) and 1995/96 and 1997/98 ($D=0.24$ $p<0.01$), but not between 1996/97 and 1997/98 ($D=0.06$ $p>0.05$). The major differences come from the high frequency of individuals in the 80-100 and 100-120 mm size classes in 1995/1996 (Fig. 3.4). Also, the 1996/97 and 1997/98 samples had a much higher % frequency of individuals in the 120-140 and 140-160 mm size classes, as well as adult females greater than 180 mm (Fig 3.4). These differences point to a population that is changing in size structure over time from a smaller to an increasingly larger body size distribution.

3.3.5 Growth

Most adults from the Nepean River grew substantially between captures. One adult male (ID 2) grew from 155.4 mm to 204.0 mm in less than three years, while an adult female (ID 149) grew from 214.9 mm to 233.7 mm in 17 months. Growth of juveniles was generally rapid although variability was

Table 3.3. Comparison of adult sex ratios between seasons at the Nepean River.

Season	Male	Female	$\chi 2$	
1994/1995	12	1	7.69	*
1995/1996	32	8	13.23	***
1996/1997	36	22	2.91	NS
1997/1998	54	31	5.69	*

* $p<0.05$
*** $p<0.0005$
NS $p>0.05$

Table 3.4. Number of adults and juveniles caught in each season.

Season	Adults	Juveniles
1994/95	11	2
1995/96	40	62
1996/97	57	56
1997/98	78	87

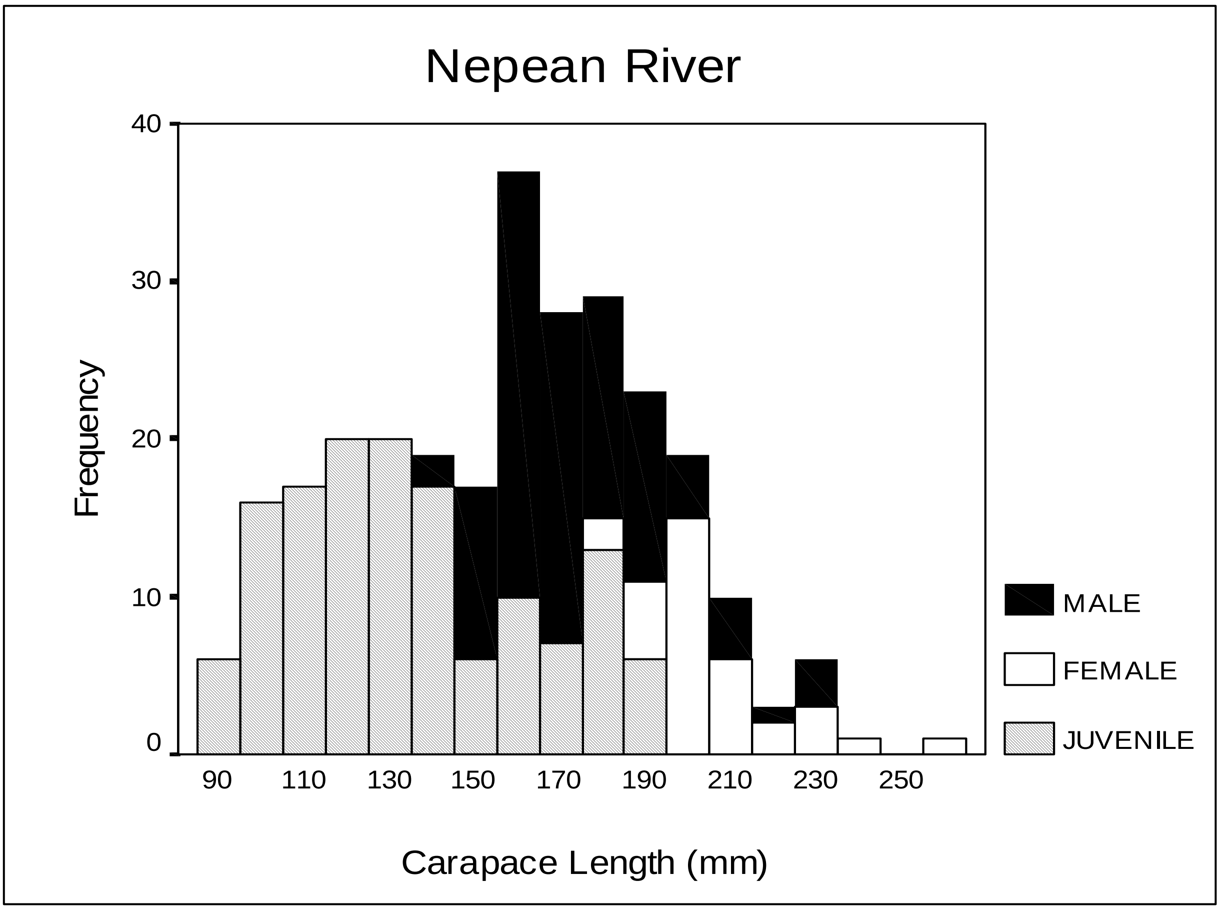


Figure 3.3. Size distribution of *Emydura macquarii macquarii* in the Nepean River. Data represent all individuals caught during the study period from 1995-1997 and over the study area between Penrith and Nortons Basin.

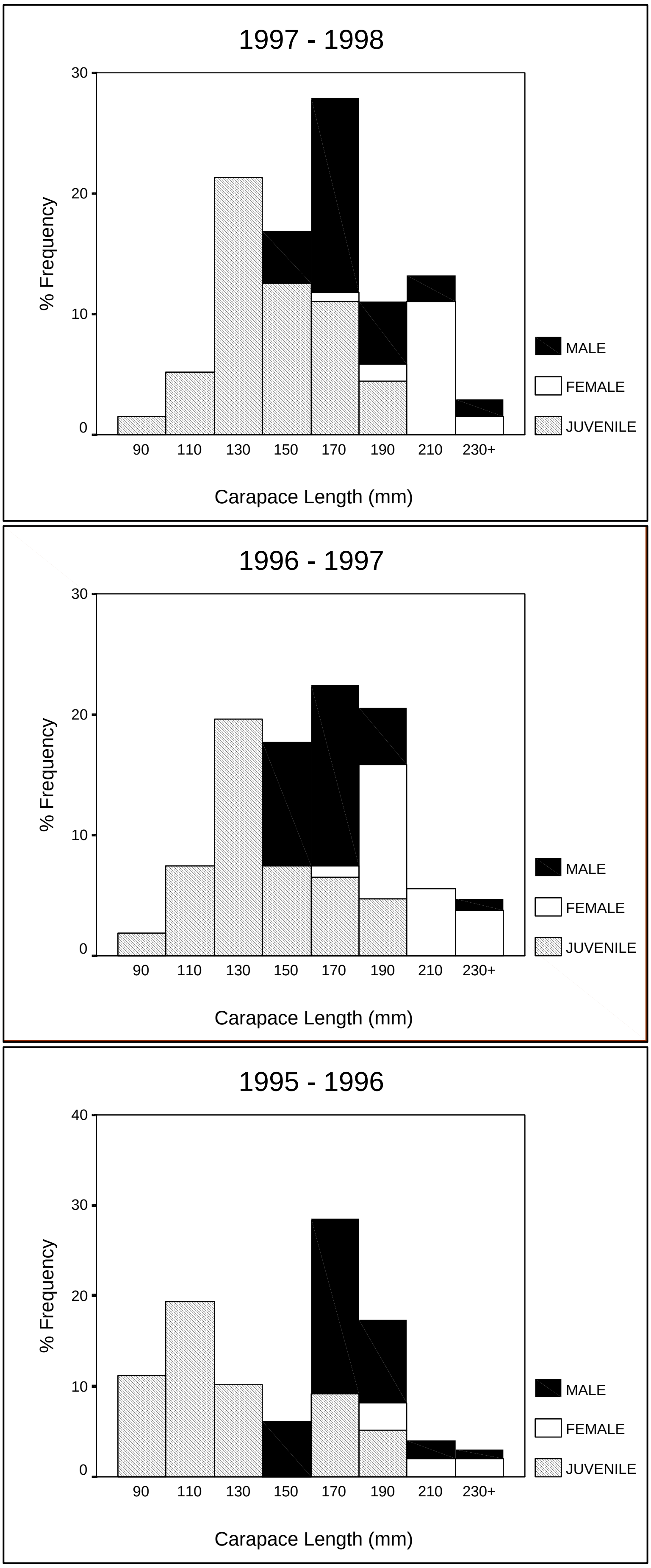


Figure 3.4. Annual variation in the size distribution of *Emydura macquarii macquarii* at Nortons Basin between 1995/96 (n=98), 1996/97 (n=107) and 1997/98 (n=136).

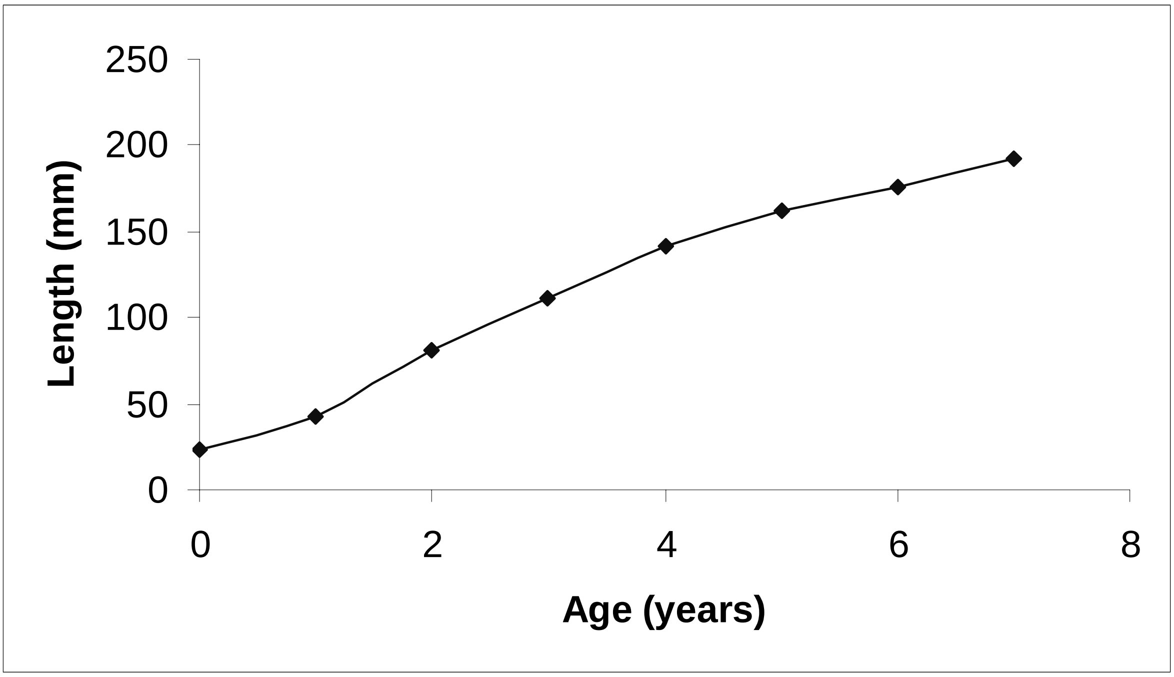


Figure 3.5. Average size for each age class of *Emydura macquarii macquarii* from the Nepean River. Age class determined from scute annuli. Note that the age classes 5.0, 6.0, and 7.0 are related to females as growth in males has slowed due to the onset of maturity.

high. One individual (ID 57) grew from 113.0 to 129.1 mm in 53 days, while another juvenile (ID 141) grew from 105.0 mm to 156.3 mm in less than 15 months.

The rapid growth rates for juveniles are evident in Fig. 3.5, which represents the average body size for each age class. Growth rates for juveniles were also highly variable. For example a four-year-old turtle could be as small as 112 mm or as large as 177 mm. One hatchling that was released in the Nepean River site was recaptured three years later. Its scutes contained three rings, and its carapace measured 124.4 mm in length. This was just above the average size found using growth rings (Figure 3.5). Of 25 turtles recaptured over a period of a year or more, 21 had left annual rings, while four had not. Of these four, one had fewer growth rings than expected, while three had more than one growth ring per year. In the latter case, minor rings have been mistaken for annual rings. Although the use of growth rings to determine age and growth rates was not perfect, it was still reliable enough to give meaningful results.

Von Bertalanffy growth models were applied for each sex using a combination of growth-interval data and size-at-age data. Sexual size dimorphism was evident in growth parameters (asymptote (a1) and intrinsic rate of growth (r)) for males and females (Table 3.6). The models were generally validated with the asymptotic parameter just larger than the average adult body size. Males had a faster intrinsic rate of growth than females. That is, they reach an asymptotic body size faster than females. Growth rates in males and females are similar up to five years of age, after which growth in males rapidly declines (Fig.3.5).

Table 3.6. Von Bertalanffy growth parameters for male and female *Emydura macquarii macquarii* from the Nepean River. Figures in brackets are 95 % confidence limits. Other values are means with SE. Models were based on a combination of growth interval and size-at-age data.

Sex	Asymptote (a1)	r	Average Adult Size
Male (n=61)	186.12 ± 6.31 (173.49 - 198.75)	0.368 ± 0.043 (0.282 - 0.454)	173.2
Female (n=77)	224.82 ± 5.55 (213.76 - 235.88)	0.268 ± 0.021 (0.226 - 0.309)	205.8

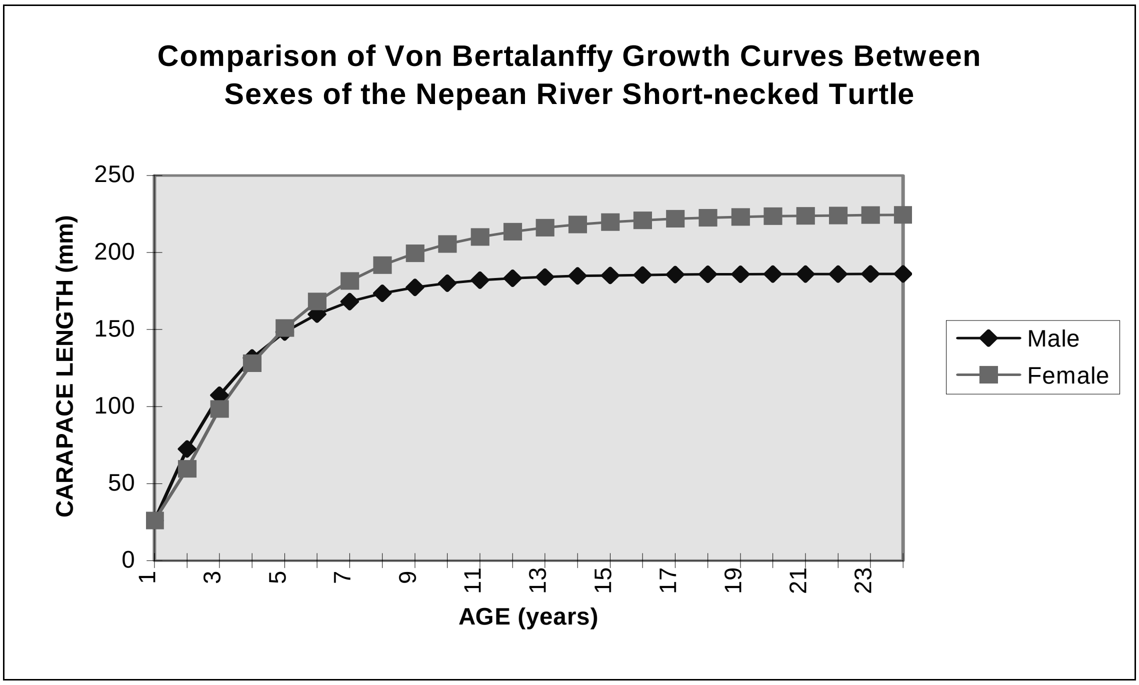


Figure 3.5. Von Bertalanffy growth curves for male and female *Emydura macquarii macquarii* from the Nepean River.

3.3.6 Reproduction

Females were gravid from late October through to early January (Table 3.7). This timespan would potentially allow up to three clutches a season. Laparoscopy detected three sizes of copora lutea within one individual. However, it is likely that most females produce only two clutches per season as few individuals were gravid towards the end of the breeding season.

Mean clutch size was 14.4 eggs, each of average mass 5.1g (Table 3.8). As a consequence, the hatchlings were extremely small with some weighing less than two grams (Table 3.8). Only 5 of 14 clutches that were incubated produced hatchlings. For the viable clutches, the incubation period was dependent on the temperature. Eggs incubated at 30^{o}C took 43 days to hatch while eggs at 26^{o}C took 52 days to hatch (Table 3.9). Neither clutch mass ($F=0.04$; d.f.=1,11; $p=0.84$; $r^2=0.004$), clutch size ($F=2.24$; d.f.=1,11; $p=0.16$; $r^2=0.17$), egg mass ($F=2.19$; d.f.=1,11; $p=0.17$; $r^2=0.17$), egg width ($F=1.49$; d.f.=1,11; $p=0.25$; $r^2=0.12$) nor egg length ($F=3.62$; d.f.=1,11; $p=0.08$; $r^2=0.25$) were related to female body size (Fig. 3.6).

3.3.7 Movements and dispersal

The majority of turtles were recaptured near their initial capture point (Table 3.10). However, the extent of movement was dependent on the sex of the turtle ($\chi2=7.12$, d.f.=2, $p<0.05$). Males were significantly more likely to have moved from the site of initial capture then either females ($\chi2=3.91$, d.f.=1, $p<0.05$) or juveniles ($\chi2=5.61$; d.f.=1, $p<0.05$). Females were as likely to be recaught in the same place of initial capture as juveniles ($\chi2=0.50$, d.f.=1, $p=0.48$).

Table 3.7. Dates in which female *Emydura macquarii macquarii* from the Nepean River were gravid.

Female ID	Date Gravid
52	24th October 1995
41	25th October 1995
44, 45, 1	28th October 1995
422, 423	30th October 1997
439	6th November 1997
440	8th November 1996
52	13th December 1995
56	15th December 1995
156	20th December 1996
495	31st December 1998
176	3rd January 1997

Table 3.8. Summary of reproductive results of *Emydura macquarii macquarii* from the Nepean River. Hatchling data collected from eggs incubated at 26°C and 28°C.

	N	Mean	SE	Range	CV
Clutch Size	14	14.4	0.9	9 – 21	22.7
Clutch Mass (g)	14	73.0	6.3	49.8 – 144.0	32.2
Egg Mass (g)	14	5.1	0.3	3.3 – 7.1	24.0
Egg Length (mm)	14	28.4	0.7	23.8 – 31.5	9.0
Egg Width (mm)	14	17.4	0.4	15.2 – 19.6	8.2
Hatchling Weight (g)	17	2.4	0.1	1.8 – 2.9	14.5
Hatchling Length (mm)	17	23.1	0.3	20.9 – 25.3	5.2
Hatchling Width (mm)	17	22.3	0.4	19.5 – 24.9	7.8

Table 3.9. Incubation period of eggs incubated at temperatures of 26°C, 28°C, and 30°C.

Incubation Temperature	n	Mean Incubation Period (days)	Range
26°C	8	51.6	50 - 54
28°C	9	48.0	47 - 50
30°C	8	43.1	42 - 45

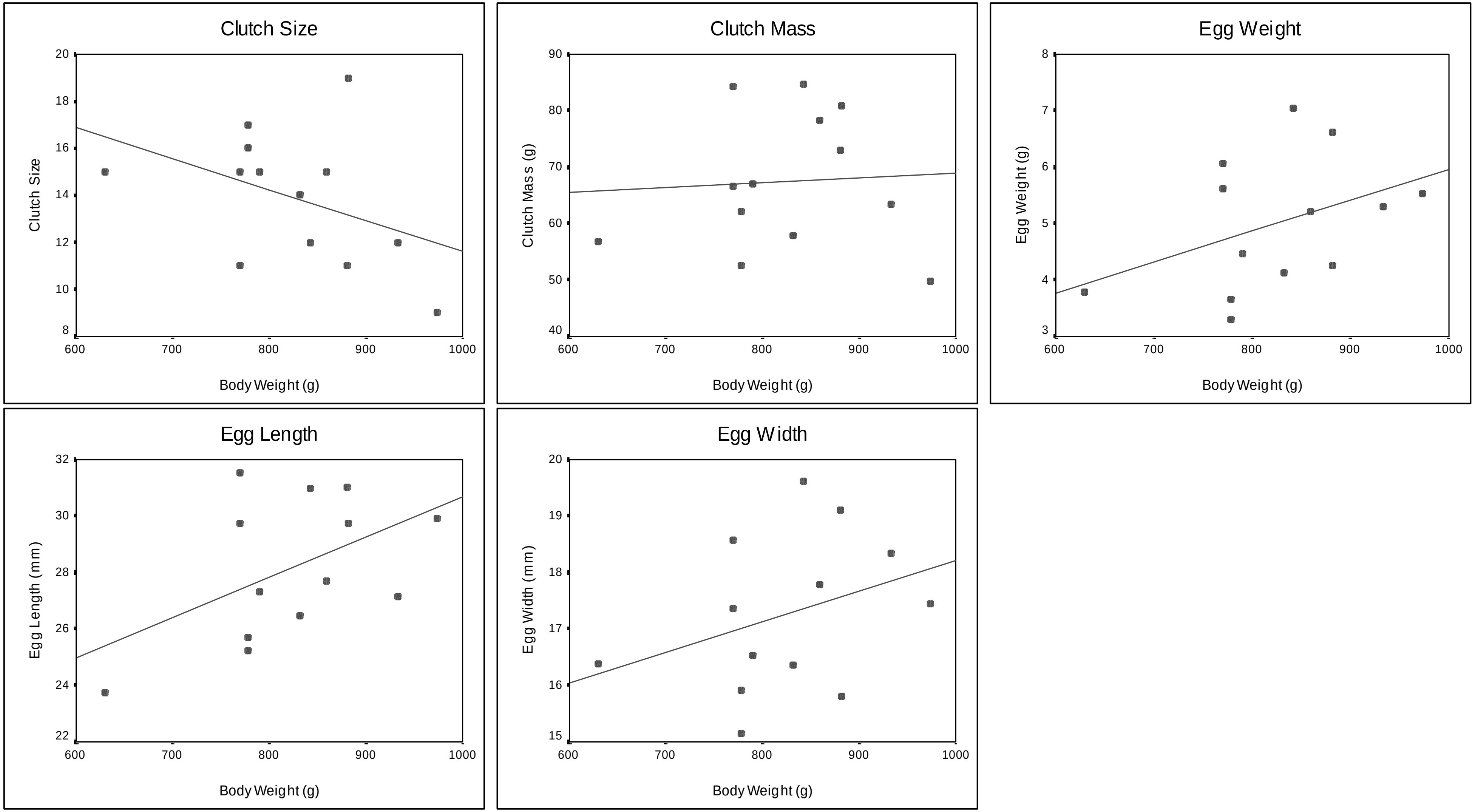


Figure 3.6. Relationship of clutch size, clutch mass, egg weight, egg length, and egg width with maternal body weight. No results were significant.

Table 3.10. Summary of the number of recaptured individuals for each sex that were caught in the same location that they were initially captured as well as those that were not. A movement was defined as a turtle moving between the four study sites.

Sex	Moved since initial capture	Not moved since initial capture
Male	12	21
Female	2	18
Juvenile	16	82

3.3.8 Other turtle species

Chelodina longicollis were not as abundant as *Em. m. macquarii* at Nortons Basin with only 84 individuals caught during the study. However, *C. longicollis* was the only turtle found in other parts of the catchment. No exotic turtles were seen or caught in this study, although three introduced *Em. macquarii* were recorded at Nortons Basin. These included an adult male (238.1 mm) that resembled *Em. macquarii krefftii* from North Queensland , a juvenile male (119.6 mm) that resembled the northern NSW populations (probably from the Hastings or Macleay Rivers) and an adult male (257.2 mm) of unknown provenance. There were several other individuals that resembled the Macleay or Hastings River Short-necks, or possibly hybrids involving these populations.

3.4 Discussion

Emydura macquarii macquarii in the Nepean River catchment is primarily concentrated between Penrith and Bents Basin, where it is found in relatively low densities. Individual growth rates indicate that males mature at 4 – 5 yr and 140 – 150 mm in carapace length. Females mature at 6 – 7 yr and a carapace length between 185 – 195 mm. Males reach a maximum length of more than 225 mm. Females reach carapace lengths of over 260 mm. The population of Nortons Basin is heavily biased towards males, and also contains a high proportion of juveniles. The population appears to be relatively young with few old or large individuals. Females lay between two and three clutches from late October through to early January. Clutch size ranges between 9 and 21 eggs, and eggs weigh on average 5.1 g. Mean hatchling weight was 2.4 g and incubation takes 48 days at 28°C.

The largest recorded male of *Em. m. macquarii* in the Sydney region is 242 mm (Cann, 1998) which is 15 mm bigger than the maximum recorded by this study. Two very large males were caught (including one at 260 mm), although these clearly were released. Cann (1998) suggested that males and females would reach similar maximum sizes However, the present study confirms that *Em. m. macquarii* from the Nepean River are sexually dimorphic with females larger than males.

The largest female *Em. m. macquarii* in this study was 260.6 mm, about 20 mm bigger than previously reported for Sydney Short-necks (Cann, 1998; Burgin, unpubl. data (Lane Cove River)). Its shell morphology head shape did not appear to be that of an older individual, so this turtle might yet grow larger. This would support Cann's (1998) view that the Sydney Short-neck might reach maximum body sizes near that of *Em. m. macquarii* from the Murray River. One adult female grew from 214.9 mm to 233.7 in 16 months, yet was only 8-9 yr. old at recapture. If this turtle was to live for 30-40 years and continue to show large increments in growth, it might approach similar maximum sizes as found in the Murray River. However with so few old turtles in the population, it is difficult to estimate what the maximum body size of the Sydney Short-neck Turtle is.

Growth of the Sydney Short-neck Turtle was rapid for both juveniles and adults. Growth rates slowed somewhat, but were still relatively large and constant. This contrasts with most turtle populations where growth rates after maturity tend to be very slow or intermittent (Bury, 1979). Comparison of the von Bertalanffy growth parameter (r) among turtle species demonstrates that relative to body size, growth rates in *Em. m. macquarii* from the Nepean River are very fast (Table 3.11). Only *Chelodina rugosa* had similar relative growth rates to that of *Em. m. macquarii* from the Nepean River (Table 3.11). Life-history strategies for both these species involve rapid growth and early maturation.

Em. m. macquarii occurred at very low densities in the Nepean River. The density of 10.6 - 12.1 turtles / ha compares poorly with *Em. macquarii krefftii* from Fraser Island (87 turtles/ha; Georges, 1983) and *Em. m. macquarii* from the Murray River (111 turtles/ha; Judge, unpublished data). *Chelodina longicollis*, a species with a similar distribution as that of *Em. macquarii*, also occurs in higher densities. In the dune lakes of Jervis Bay, the density of *C. longicollis* was 163.8 ± 34.2 turtles/ha (Georges *et. al.,* 1986), and in farm dams around Armidale it was as high as 400/ha

(Parmenter, 1976). Chessman (1978) calculated the density of *C. longicollis* in Gippsland ranged at 160/ha in lagoons to 240/ha in farm dams.

Several assumptions of the Jolly-Seber model were violated and these will create bias in population estimates. The assumption that every individual in the population has the same probability of capture (Pollock *et al.*, 1990) was unlikely as adults were less likely to be recaptured than juveniles. However the capture probability was quite high in this study (0.4 to 0.5), so bias from unequal catchability may be minimal (Pollock *et al.*, 1990). Any bias due to trap avoidance would be negative, hence resulting in an overestimation of the population size (Pollock *et al.*, 1990). Violations of the assumption that emigration from the study area is permanent can be serious (Pollock *et al.*, 1990). In this study, individuals were moving in and out of the sampling region continuously. However sampling occurred over a wide spatial area during the turtles active period, hence each individual had a reasonable probability of being trapped or recaptured. Therefore any bias due to temporary emigration may be minimal.

Estimates of survival rates are not affected by violations of these assumptions, although their precision may be reduced (Pollock *et al.*, 1990). Survival rates were extremely low (0.30 - 0.54) compared to that from other studies - 0.91 - 0.94 for *Elseya dentata* and 0.62 for *Chelodina rugosa* (Kennett, 1994). However the Jolly-Seber model cannot distinguish between deaths and emigration (Pollock *et al.*, 1990),

Table 3.11. Comparison of the two von Bertalanffy parameters - asymptotic body size (a1) and intrinsic rate of growth (r) – and age at maturity between species of turtles. *Emydura macquarii krefftii* (Tucker et al., pers. comm.), *Chelodina longicollis* (Parmenter 1976), *Chelodina rugosa*, *Elseya dentata* (Kennett 1994), *Sternotherus minor* (Cox *et al.*, 1991), *Chrysemys picta* (Frazer *et al.*, 1991), *Trachemys scripta* (Frazer *et al.*, 1990).

Species (Location)	a1		r		Age at maturity	
	Male	Female	Male	Female	Male	Female
Emydura macquarii macquarii (Nepean River, NSW)	186.1	224.8	0.37	0.27	4-5	6-7
Emydura macquarii krefftii North Queensland	168.8	184.6	0.41	0.32	-	-
Chelodina longicollis (Armidale, NSW)	321.6		0.15		7	10-11
Chelodina rugosa (Darwin, NT)	197.5	246.2	0.41	0.29	3.9	6.2
Sterotherus minor (North Florida)	112.8	110.4	0.10	0.14	5.6	8.0
Chrysemy picta (SW Michigan, USA)	111.8	152.2	0.18	0.13	-	-
Trachemys scripta (S. Caroliona, USA)	-	202.7	-	0.20	-	-

therefore the low survivorship recorded does not necessarily mean there is a high mortality rate if individuals simply departed the sampling area.

Cann (1998) suggested that the Sydney Short-necked Turtle was declining in abundance. However, the population structure of short-neck turtles from Nortons Basin suggests instead that this is a young population that is currently expanding. The population is dominated by juveniles and young adults while very few old turtles were present. This structure contrasts with stable turtle populations that are predominantly adult and sustained by a low and intermittent recruitment (Bury, 1979). Assuming there is not a sex-difference in survival, the sexual bias in favour of adult males in the Nepean River further demonstrates a population that is expanding. Males mature at half the age of females, therefore in a growing population males will initially dominate. While ever there is a continuous recruitment of juveniles into the population, a male sex bias will continue (Gibbons, 1990; Lovich and Gibbons, 1990). Expansion of the population is also consistent with anecdotal evidence from local fisherman, who have noticed an increase in abundance of *Em. m. macquarii* in recent times.

Emydura macquarii macquarii appear to thrive on the eutrophic waters of the Nepean River. Growth rates are rapid, maturity is reached relatively quickly, reproductive output is high and juvenile recruitment is large. The interesting question is why the Nepean River population is at such a low density in the first place?

Predators can cause serious and rapid declines in turtle populations (Brooks et. al., 1990). However, there are no major predators of adult turtles in the Nepean River, so this is unlikely to be the cause. Cann (1998) believed that the pollution of Sydney’s waterways were largely responsible for the species decline. Still the Nepean River is no cleaner now then in the past, yet turtles are currently thriving. In any case, turtle species such as *Em. m. macquarii* are largely unaffected by human disturbance as their survival is independent of aqueous oxygen levels and they have a broad and flexible diet (ability to eat whatever the river provides). Turbid waters protect against and potentially reduce the number of visually-orientated predators. It is likely that

Em. m. macquarii is unaffected by human disturbance in the Nepean River and may in fact even be advantaged by it.

There are no records of *Em. m. macquarii* in coastal drainages south of the Nepean River. The marginal distribution may explain its local rarity since the Nepean River is at the latitudinal limit of *Em. m. macquarii* for short coastal streams. Sub-optimal environmental conditions would clearly influence the population dynamics resulting in a population that fluctuates in local abundance and continuity of distribution. Thus, a small fluctuating population may be characteristic of *Em. m. macquarii* in the Nepean River. The life history traits of *Em. m. macquarii* from the Nepean River are well-suited to sub-optimal conditions. Rapid growth rates, early maturation, and large clutch sizes enable a population to expand quickly when environmental conditions are favourable.

Another hypothesis is that the population is not native but a recent introduction of abandoned pets. Short-neck turtles are a popular pet, and it is likely that pet turtles would escape or be released illegally into local rivers. Some turtles caught during this study were clearly pets that had been dumped. There have been other introductions of turtles in waterbodies around Sydney including seven non-native species in Centennial Park lakes alone (Stepherson, 1986). The slider turtle (*Trachemys scripta elegans*) is now firmly established in the Sydney region, including the Nepean River. The introduction scenario is dealt with in more detail within the synopsis after the data from chapters four and five are presented and integrated.

Chapter 4

Geographic Variation in Body Size and Related Traits of *Emydura macquarii macquarii*

4.1 Introduction

An important issue in evolutionary ecology is the geographic variation in life histories within species. Many freshwater turtle species display plasticity in life history traits across their range (Gibbons *et al.*, 1981; Galbraith *et al.,* 1989; Ernst ,1990; St. Clair *et al.*, 1994). One of the most influential life history traits is body size. Body size is an important factor in determining life history traits such as clutch size (Gibbons, 1970, 1982; Moll and Legler, 1971; Gibbons *et al.,* 1982; Congdon and Gibbons, 1983, 1985), clutch frequency (Moll and Moll, 1990; Vogt, 1990), and egg size (Thompson, 1983; Congdon and Gibbons, 1985; Mitchell and Pague, 1990; Vogt, 1990; Kennett, 1994). Therefore, body size may be subject to selection driven by benefits across a wide range of life history attributes.

Important determinants of body size are the age and size at which a turtle matures (Shine and Iverson, 1995). After a turtle matures, energy is directed towards reproduction at the expense of growth. Therefore, early maturity may result in a smaller maximum body size but offers the advantage of starting reproduction sooner to balance the risk of not reproducing at all. On the other hand, delayed maturity contributes to increased fecundity, enhanced survival of offspring, and/or a diminished cost of reproduction (Congdon and Gibbons, 1990). Age at maturity is quite variable across the range of some species of turtle (Tinkle, 1961; Moll and Legler, 1971; Christiansen and Moll, 1973; Moll, 1973; Gibbons et. al., 1981; Congdon and van Loben Sels, 1991), suggesting genetic selection for age at maturity in response to a gradient of environmental conditions.

The relative importance of age versus size on maturity is still poorly understood for turtles. Age influences maturity in some populations (Gibbons *et al.,* 1981; Mitchell, 1985; St. Clair *et al.,* 1994), while body size has been considered more important elsewhere (Moll, 1973; Christiansen and Moll, 1973; Bury, 1979; Gibbons *et al.,* 1981; MacCulloch and Secoy, 1983; Wilbur and Morin, 1988; Galbraithe *et al.,* 1989; Brooks *et al.,* 1992; Frazer *et al.,* 1993; St. Clair *et al.,* 1994). Sexual size dimorphism (Berry and Shine, 1980) and variable age at maturity (Bury, 1979; Dunham and Gibbons, 1990; St. Clair et. al., 1994) allow the evolution of separate adaptive strategies between sexes. Several studies concur that maturity in females is determined by body size, although age is more important in determining maturity in males (Gibbons *et al.,* 1981; St. Clair *et al.,* 1994). Sexually divergent patterns for the relationship of age or size at maturity are consistent not only among species, but within species (eg. *Chrysemys picta*: MacCulloch and Secoy, 1983; Mitchell, 1985; Frazer *et al.,* 1993; Christiansen and Moll, 1973; Moll, 1973; St. Clair *et al.,* 1994).

The rate of juvenile growth is the most important factor that determines size at maturity, and hence maximum body size, is (Gibbons *et al.,* 1981; MacCulloch and Secoy, 1983; Congdon and van Loben Sels, 1991). Variation in growth rates is primarily determined by temperature and resource availability. Turtles from highly productive habitats tend to grow more rapidly, mature at a larger body size and reach larger body sizes than turtles from unproductive habitats (Quinn and Christiansen, 1972; MacCulloch and Secoy, 1983; Georges ,1985; Brown *et al.,* 1994). *Em. m. krefftii* in the oligotrophic lakes of Fraser Island matured at smaller sizes and reached smaller maximum sizes than populations in the more productive mainland rivers (Georges, 1985). Turtles from thermally enhanced habitats will grow quicker and reach larger maximum sizes than surrounding populations (Gibbons, 1970; Parmenter, 1980; Gibbons *et al.,* 1981; Thornhill, 1982). However, the results maybe a joint effect of higher temperatures and differences in diet quality (high protein versus mainly aquatic vegetation) (Gibbons, 1970; Parmenter, 1980; Thornhill, 1982).

Two mechanisms are proposed that link growth rates and size at maturity to variation in temperature and resource availability (Atkinson and Sibly, 1997). With temperature held constant, growth rates and size at maturity correspond to food availability. When resources are constant or superabundant, initial growth rates are a function of thermal conditions, enabling the size at maturity to decrease. Therefore, low temperatures or low food quality depress growth rates, but lead to an opposing effect on body size; that is, delayed maturity at a larger body size. In North America, temperature manifests a latitudinal gradient in body size (Christiansen and Moll, 1973; Moll 1973; MacCulloch and Secoy, 1983; Wilbur and Morin, 1988; Galbraithe *et al.*, 1989). This relationship is attributed to the degree-day differences in the length of the activity season. Colder climates dictate that only one clutch can be laid, so it is more advantageous to delay maturity until a larger body size is attained and that larger clutches can be laid (St. Clair *et al.*, 1994).

Another population parameter that varies substantially in turtles is the ratio of males to females. Theory predicts that sex ratios are stabilised at 1:1 as parents that produce offspring of the rarer sex are more likely to leave more grandchildren than offspring of the more common sex (Fisherian hypothesis). Many freshwater turtle populations have a sex ratio of 1:1 (Bury, 1979), but there are numerous exceptions (Galbraith *et al.*, 1988; Gibbons, 1990; Vogt, 1990; Kennett, 1994; Jones and Hartfield, 1995; Lindeman, 1996; Edmonds and Brooks, 1996; van Loben Sels *et al.*, 1997). Variability in sex ratios can be great even within a region for the same species (Gibbons, 1990). Sex ratio differences may be attributed to sampling bias (Gibbons, 1990; Lovich and Gibbons, 1990). Differential behavioural patterns can cause a female bias at nesting season (Gibbons, 1970; Gibbons, 1990) or male bias at mating periods (Parker, 1984; Gibbons, 1990). Captures of turtles in aquatic traps are often biased towards males (Ream and Ream, 1966; Gibbons, 1990)). Differences in the size at maturity can provide difficulties in calculating the sex ratio correctly (Gibbons, 1970; Parmenter,1976; Gibbons, 1990).

Assuming there is no trapping bias and the sex ratio has been determined correctly, differences in adult sex ratios of turtles have been put down to five different possibilities (Gibbons, 1990; Lovich and Gibbons, 1990; Lovich, 1996) :

1. Differences between sexes in mortality (e.g. females leaving water to lay eggs are more vulnerable to predation).

2. Differences between sexes in immigration and emigration (males in many species travel further than females).

3. Temperature-Dependent Sex Determination or TSD (in turtles where sex is not genetically determined) can result in differences in sex ratios since the sex of the turtle is totally determined by the temperature of the nest.
4. Differences between sexes at maturity can influence adult sex ratios. The sex that matures the earliest would be expected to be the sex that dominates.
5. Differences due to sexual dimorphism (smaller sex dominates the larger sex).

Incubation studies confirm that the sex of *Em. macquarii* is genetically determined rather than temperature dependent (Thompson, 1983; 1988; Bull *et al.,* 1985). Therefore, TSD cannot explain any population sex bias in *Em. macquarii.*

Em. macquarii is an abundant species that is widely distributed throughout eastern Australia. Life history data are only available from the Murray River (Chessman, 1978; Thompson, 1983), Fraser Island (Georges, 1983), and to a lesser extent, Coopers Creek (Doddridge, 1992). In this chapter, life-history data from another five populations (Murray, Brisbane, Macleay, Hunter, and Nepean Rivers) are reported, providing the opportunity to explore the significance of geographic variation in their biological traits. Among Australian turtles, the short-necked turtle *Em. macquarii* (sensu Georges and Adams, 1996) displays large geographic variation in maximum body size, even though this trait has been poorly documented in the literature.

This chapter tests three hypotheses predicted for patterns of geographic variation in body size and related life history attributes of turtles. If *Em. m. macquarii* mirrors the predictions found by Northern Hemisphere studies (Bury, 1979; Shine & Iverson, 1995), then (1) females will delay sexual maturity, mature larger and reach greater maximum sizes then males, (2) larger bodied populations will result from either delayed maturity or faster juvenile growth rates, and (3) populations from higher latitudes will mature later at larger body sizes.

4.2 Materials and methods

The methods used in capturing, marking, sexing and measuring turtles, and determining age, maturity status (laparoscopy) and growth rates (including von Bertalanffy growth models) are covered in the general methods (Chapter 2).

The 95th percentile of adult body size was used as the maximum size for each sex, to avoid the outlier bias from unusually large individuals. Sexual dimorphism index was determined by dividing the 95th percentile carapace length of the larger sex (females) by that of the smaller sex (males) (Lovich and Gibbons, 1992).

Differences in adult sex ratios were determined by a chi-square analysis against an expected ratio of 1:1. A Yates correction factor of 0.5 was applied. A chi-square analysis of proportions test, followed up with a Tukey-type multiple comparison test (Zar, 1984; p400), was used to compare the proportion of juveniles between rivers.

4.3 Results

4.3.1 Body size

Maximum body size (95th percentile) of *Em. m. macquarii* varied considerably across drainage basins (Table 4.1) with the most dramatic variation occurring in males. The largest turtles came from Murray River and the smallest came from the Macleay River. Males, compared to the largest race from the Murray River, from the Brisbane River were 7% smaller, Hunter and Nepean River males were 26% smaller, and Macleay River males were 79% smaller.

Females, compared to the largest body sizes of females from the Murray River, from the Brisbane River were 8% smaller, the Hunter and Nepean River females were 24% smaller, and the Macleay River females were 64% smaller. Turtles from the Nepean and Hunter rivers reached similar sizes when the 95th percentile of adult carapace length is compared, although the larger individuals were caught in the Nepean River (Table 4.1).

Females attained larger body sizes than males in all rivers (Table 4.1). Sexual size dimorphism in the Murray, Brisbane, Nepean, and Hunter Rivers was relatively similar with females on average 10.7 % larger than males (S.D. Index = 1.09 – 1.13, Table 4.1). Females from the Macleay River were 21.0 % larger (S.D. Index = 1.21) than males.

4.3.2 Age and size at maturity

Males matured at a younger age than females in all rivers (Table 4.1). Turtles from the Nepean River matured at approximately half the age of turtles from other rivers (Table 4.1). Male turtles from the Murray, Macleay, and Hunter rivers matured at a similar age, albeit with some variation (Table 4.1). Female turtles from the

Table 4.1. Population parameters for *Emydura macquarii* from the Macleay, Hunter, Nepean, Brisbane, and Murray rivers. SD Index (see methods) after Lovich and Gibbons (1992). CL = straight line carapace length in mm. Proportional CL at maturity was determine by dividing the 95 percentile of CL by the midpoint of the CL at maturity. Values in parenthesis represent percentages.

		Macleay	Hunter	Nepean	Brisbane	Murray
No. turtles caught		207	350	274	169	263
Maximum CL	Male	153.4	212.4	227.9	259.2	278.6
	Female	185.4	242.6	260.6	280.4	303.5
95 percentile of Adult CL	Male	148.5	210	214.1	248.4	266.6
	Female	179	236.6	237.7	271.2	293.8
SD Index		1.21	1.13	1.11	1.09	1.10
CL at Maturity	Male	105.5 - 113.3	156.1 - 166.1	138.7 - 160.4	170.8 - 195.0	172.2 - 195.5
	Female	136.8 - 146.2	187.0 - 195.5	178.6 - 191.1	211.8 - 215.9	222.5 – 230.4
Age at Maturity (Years)	Male	7 - 8	7 – 9	3 - 5		6 - 8
	Female	9 - 11	11 - 13	5 - 7		8 - 10
Proportional CL at Maturation (%)	Male	73.7	76.7	70.0	73.6	69.0
	Female	79.0	80.8	77.8	78.9	77.1
Sex Ratio (Adults)	Male	82 (51)	77 (36)	101 (74)	68 (53)	94 (51)
	Female	78 (49)	136 (64)	35 (26)	60 (47)	91 (49)
Adult/Juvenile Ratio	Adults	160 (77)	213 (61)	136 (50)	128 (76)	177 (67)
	Juveniles	47 (23)	137 (39)	138 (50)	41 (24)	86 (33)

Murray River matured at a younger age than turtles from the Macleay and Hunter Rivers. Age at maturity for *Em. m. macquarii* in the Brisbane River was impossible to determine, as growth rings were lost at an early age.

Size at maturity was highly variable within a population, especially for males (Table 4.1). In the Murray River, the smallest adult male was 172.2 mm and the largest immature was 195.5 mm. Males from the Murray River mature anywhere between 177.2 and 195.5 mm, although most are likely to mature closer to 195.5 mm. In the Brisbane River the smallest adult male was 170.8 mm and the largest immature was 195.0 mm. However, most males mature between 175 mm and 190 mm. In the Hunter River, most males mature between 155 and 165 mm in length. Males in the Nepean River matured between 140 and 150 mm. Most males in the Macleay River were mature between 110 and 113 mm in carapace length although the smallest adult male was 105.5 mm.

The smallest adult female in the Macleay River was 136.8 mm and the largest immature was 143 mm. In the Murray River, most females do not mature until at least 230 mm although the smallest adult female was gravid at 222.5 mm. Females in the Hunter and Nepean rivers matured between 185 and 195 mm, although the smallest gravid female in the Nepean River was 178.6 mm. In the Brisbane River, most females matured between 210 and 220 mm.

The maximum body size follows from the size at maturity (Table 4.1). In general, larger-bodied populations matured at a larger size. However turtles from the Nepean River tend to mature smaller than turtles from the Hunter River yet reached a similar or slightly larger maximum body size.

4.3.3 Population structure

There was a female bias in the Hunter River ($\chi2=15.79$; d.f.=1; $p<0.0001$) (Table 4.2) and a male bias in the Nepean River (($\chi2=31.07$; d.f.=1; $p<0.0001$)). There was no significant difference from a 1:1 sex ratio in the Brisbane ($\chi2=0.38$;

d.f.=1; p=0.54), Macleay ($\chi2=0.06$; d.f.=1; p=0.81) or Murray ($\chi2=0.02$; d.f.=1; p=0.89) Rivers.

The sex ratios for collecting trips in the Hunter River (Table 4.3) were significantly different ($\chi2=25.41$; d.f.=4; $p<0.001$). Females were proportionally more abundant on the 28 November 1996 and on 22 October 1996; the sex ratio was equal or in favour of males on other trips (Table 4.3). Adult sex ratios for the Nepean River show a male bias in all four years (Table 4.4), although the year of 1996/1997 was marginally insignificant (p=0.09). Sex ratios across seasons (1994/1995 excluded) were not significantly different ($\chi2=4.13$; d.f.=2; p=0.13).

The majority of turtles from the Macleay, Murray, and Hunter Rivers were older than 7 yr (Figure 4.1). In the Macleay River 88.3 % of the population is ≥ 8 yr, while in the Murray it was 68.4 %. However, in the Nepean River only 21.7 % of the population were over 7 yr, with a relatively even spread across the younger age classes (Figure 4.1). In fact, 22.8 % of the population were four years of age. No age distribution could be determined for the Brisbane River as growth rings on the carapace scutes were lost at an early age.

Table 4.2. Comparison of adult sex ratios between rivers.

River	Male	Female	χ2
Hunter	77	136	15.79 ***
Nepean	101	35	31.07 ***
Macleay	82	78	0.06
Brisbane	68	60	0.38
Murray		94	91
0.02			

*** p<0.0001

Table 4.3. Comparison of adult sex ratios between field trips in the Hunter River.

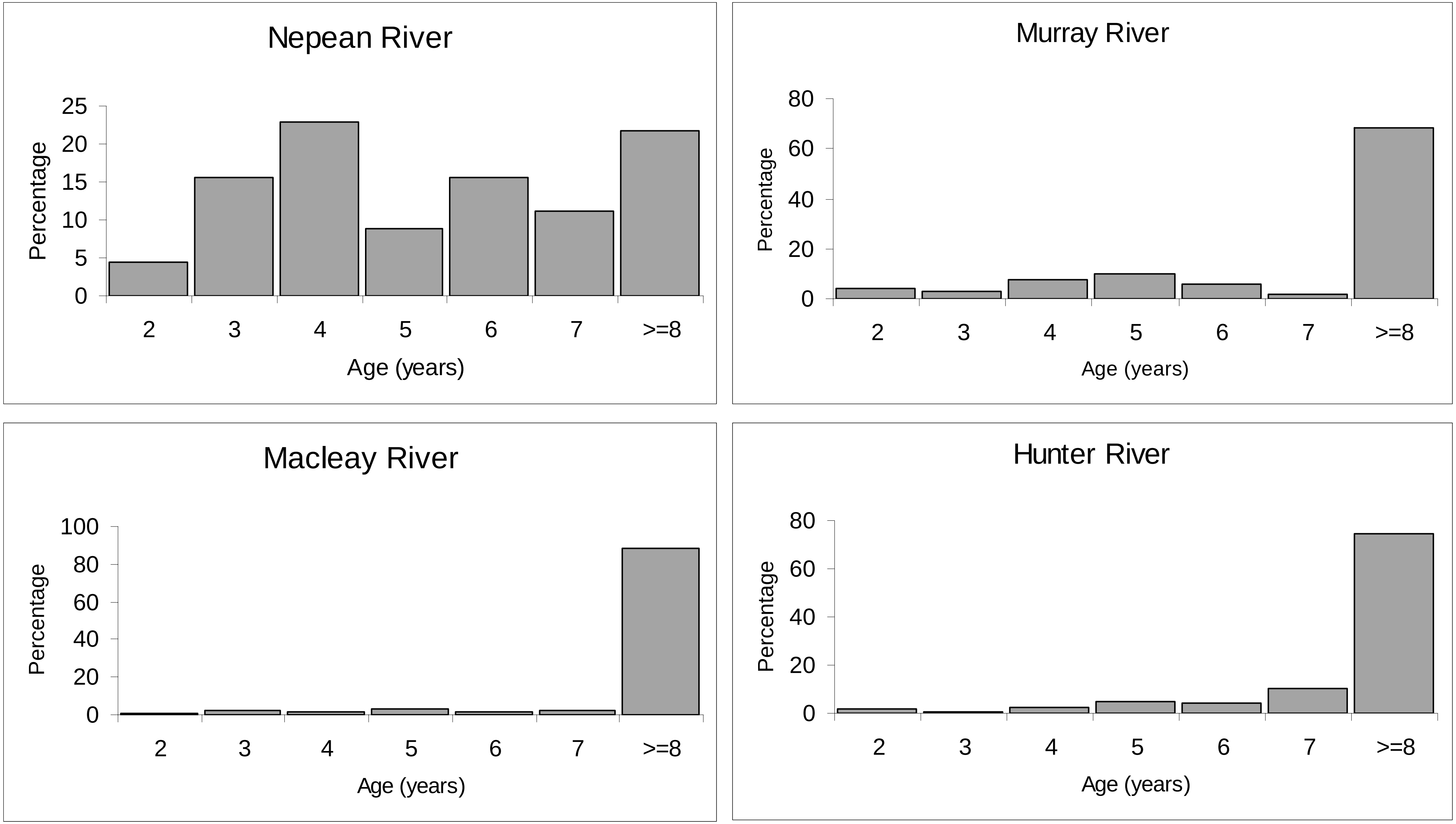
Date	Male	Female	χ2
8th December 1994	11	11	0.00
28th April 1996	16	8	2.04
22nd October 1996	11	23	3.56
28th November 1996	37	108	33.79 ***
4th February 1997	8	3	1.45

*** p<0.0005

Table 4.4. Comparison of adult sex ratios between years at the Nepean River.

Season	Male	Female	χ2
1994/1995	12	1	7.69 *
1995/1996	32	8	13.23 ***
1996/1997	36	22	2.91
1997/1998	54	31	5.69 *

* p<0.05
*** p<0.0005



Nepean River

Murray River

Macleay River

Hunter River

Figure 4.1. Comparison of age distribution between the Nepean, Murray, Macleay, and Hunter rivers.

Populations from the Macleay, Brisbane, Murray and Hunter Rivers were predominated by adults (Figure 4.2). Both the Macleay and Brisbane Rivers contained very few small individuals (Figure 4.2). However, in the Nepean River juveniles made up a large proportion of the population, while few large individuals were present (Figure 4.2). There was a significant difference in the proportion of juveniles among rivers ($\chi 2=53.46$; d.f.= 4; $p<0.0001$). The Nepean River had a significantly higher proportion of juveniles (50%) than the Hunter River (39%), which in turn had a significant higher proportion than the Murray River (33%). The Brisbane (24%) and Macleay (23%) rivers had the lowest proportion of juveniles.

Comparison of densities among rivers was limited, as mark-recapture data were only available for the Nepean and Murray Rivers. Despite this acknowledged limitation, *Em. m. macquarii* from the Nepean River were obviously at a much lower density than found in other rivers. In the Nepean River 274 turtles were marked during three years of intensive trapping over a distance of 3.8 km. However, similar numbers of turtles were caught in the Brisbane (169), Macleay (207), Hunter (351) and Murray (289) Rivers from a much lower sampling intensity in a much smaller area. Turtle density in the Nepean River was at 10.6 – 12.1 individuals/ha (Chapter 3) compared to 111.3 individuals/ha in the Murray River (Appendix B).

Comparison of turtle densities among the Macleay, Hunter, Brisbane and Murray Rivers was prohibitive without a more intensive mark-recapture study at each location. However, consideration of catch per unit effort indicated that the highest densities of turtles were in the Hunter and Macleay Rivers. Almost all of the 207 turtles marked in the Macleay River were caught in a backwater off the main channel that was no bigger than 80 x 15 m. Similarly in the Hunter River, turtles were found in small deep pools in between long stretches of rapids. Relative to the size of these pools the number of turtles caught was extremely high. In contrast, the waterbodies from the Brisbane and Murray River sites were much larger and hence the population of *Em. m. macquarii* less concentrated.

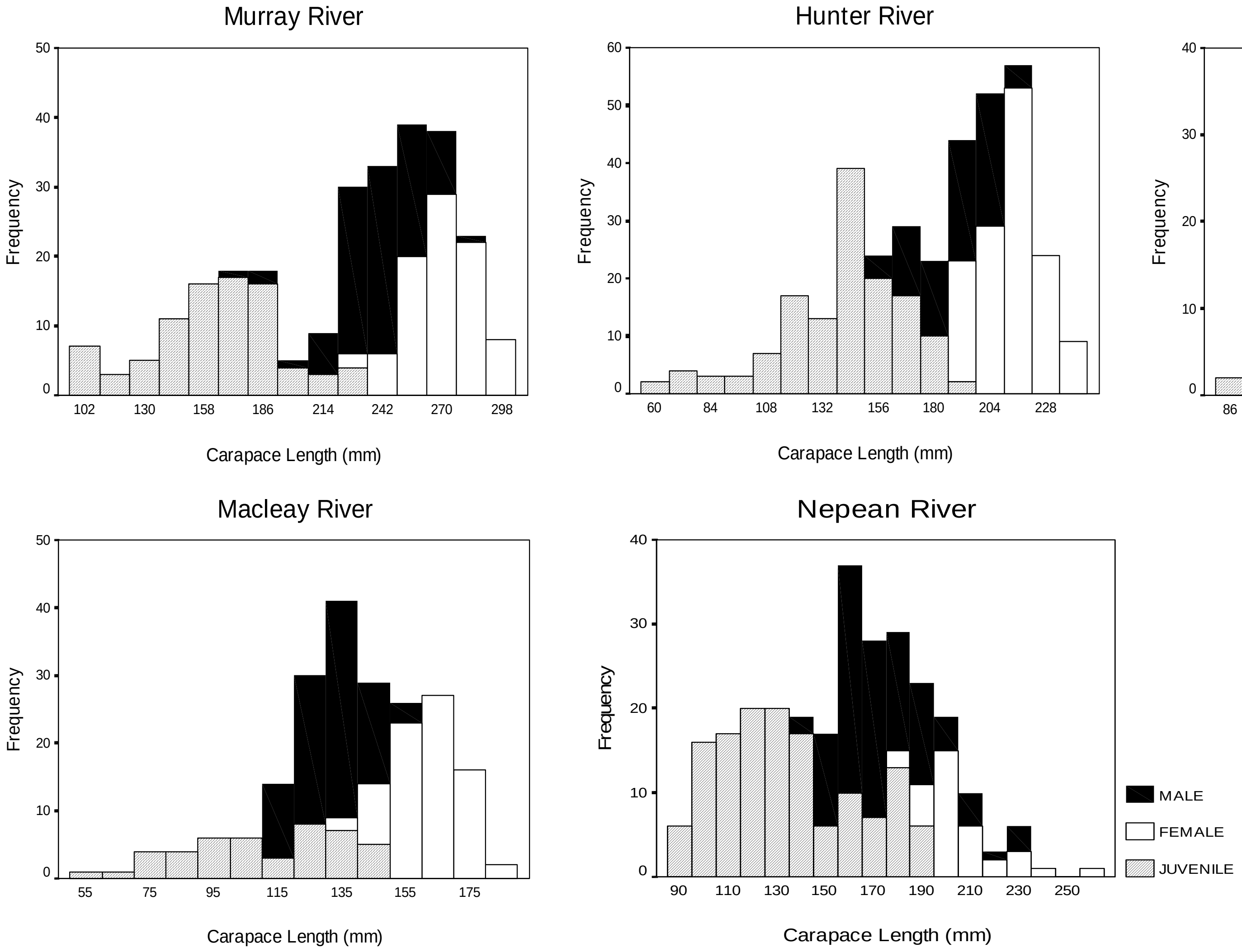


Figure 4.2. Comparison of the size distribution between the Hunter, Murray, Brisbane, Macleay, and Nepean rivers. The distribution is broken up into adult males, adult females, and juveniles. Smaller adults were distinguished from juveniles by laparoscopy.

4.3.4 Growth

Mark recapture growth data were available for the Macleay, Hunter, Nepean and Murray River sites. The Brisbane River site was only visited once, so no recapture data were obtained. Growth of adults in the Macleay, Hunter and Murray Rivers was slow and intermittent (Table 4.5 and 4.6). Most adults showed no growth between years, while those that did only grew fractionally. In contrast, almost all adults from the Nepean River showed some level of growth between captures, with many growing appreciably (Table 4.5 and 4.6).

Juvenile growth rates were estimated using annual growth rings from scutes on the carapace. The juvenile growth rates for the Murray River population in this research was almost identical to another Murray River population studied by Chessman (1978) using mark-recapture data (Table 4.7). This provides confidence in the techniques used to estimate growth rates in this study. The Nepean and Murray River populations had the fastest juvenile growth rates, while the Macleay River population had the slowest (Fig. 4.3). The Hunter River had a juvenile growth rate intermediate to the Murray/Nepean and Macleay Rivers (Fig. 4.3).

Von Bertalanffy growth models were applied to each river (Brisbane River excepted) using the combined mark-recapture and growth ring data. Table 4.8 presents the results of the two Von Bertalanffy growth parameters (asymptote (a1) and intrinsic rate of growth (r)) for females from each river. The models were generally validated by the agreement between asymptotic estimate and average adult size, although the asymptotic size in the Murray River population was slightly smaller than the mean adult size (Table 4.8). The Nepean and Hunter River populations had a similar predicted asymptotic body size, but a vastly different intrinsic rate of growth. Females reached the asymptotic body size much faster in the Nepean River than other rivers (Fig. 4.4). The Murray River population also had a high intrinsic rate of growth. Growth in the Macleay and Hunter River populations was extremely slow.

Table 4.5. Comparison of adult growth rates of *Emydura macquarii macquarii* from the Macleay, Murray, Nepean, and Hunter Rivers. The table contains the individuals with the five fastest growth rates for each river.

River	ID	Initial Carapace Length (mm)	Final Carapace Length (mm)	Growth – mm/year
Macleay	553	161.8	164.5	2.7
	504	170.5	172.3	1.8
	501	167	168.7	1.7
	218	163.1	163.9	0.8
	563	159.7	160.4	0.7
Murray	69	237.5	240.9	1.7
	21	238.3	239.5	1.2
	117	266.8	268.8	1
	128	290.7	292.2	0.75
	200	284.7	285.3	0.6
Nepean	2	155.4	200.9	20.7
	149	214.9	233.7	12.5
	89	121.8	144.7	11.5
	121	155.7	166.8	11.1
	132	208.5	217.1	8.6
Hunter	2	203.4	207.8	4.4
	26	185	187.6	2.6
	33	197.6	199.4	1.8
	25	214.9	215.8	0.9
	29	211.5	212.3	0.8

Table 4.6. The proportion of adults with annual growth rates greater than 1.0 mm for *Emydura macquarii macquarii* from the Macleay, Murray, Hunter and Nepean Rivers.

River	Proportion of Adults with growth > 1.0 mm/year	N
Macleay	0.12	25
Murray	0.09	32
Hunter	0.43	7
Nepean	0.95	37

Table 4.7. Comparison of the size (carapace length mm) at each age group of Murray River populations of *E. macquarii* from Chessman (1978) and this study.

Age	Murray (This Study)	Murray (Chessman 1978)
0	30.0	30.0
1	55.7	71.0
2	98.2	100.0
3	129.3	124.0
4	148.2	145.0
5	164.2	163.0
6	176.8	180.0
7	194.8	195.0

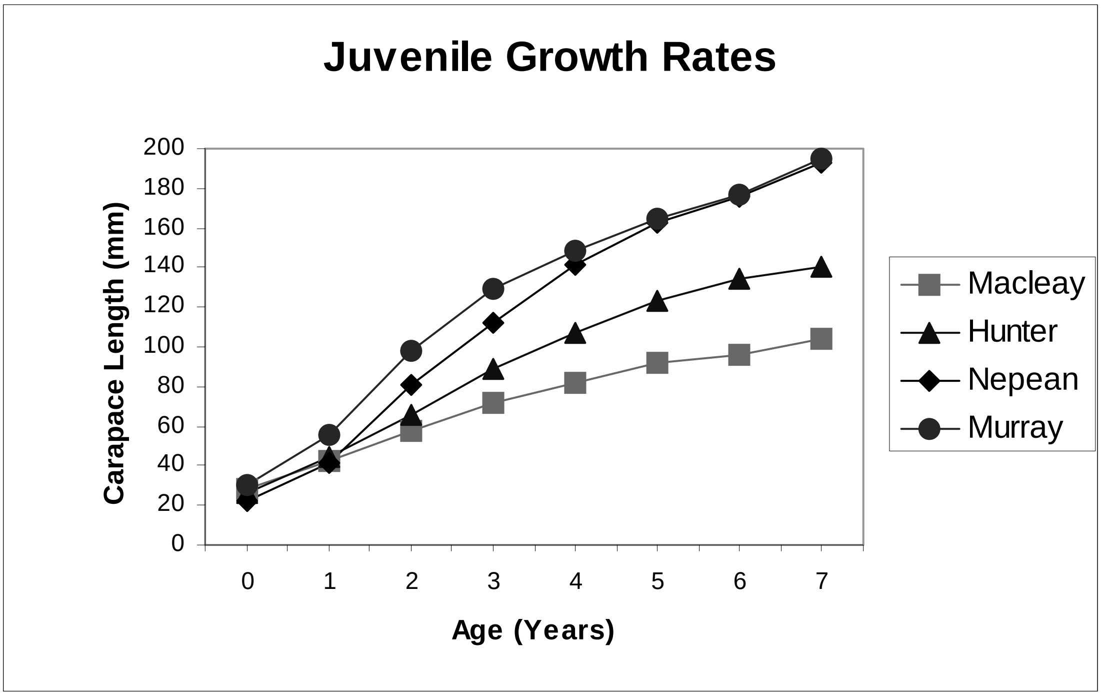
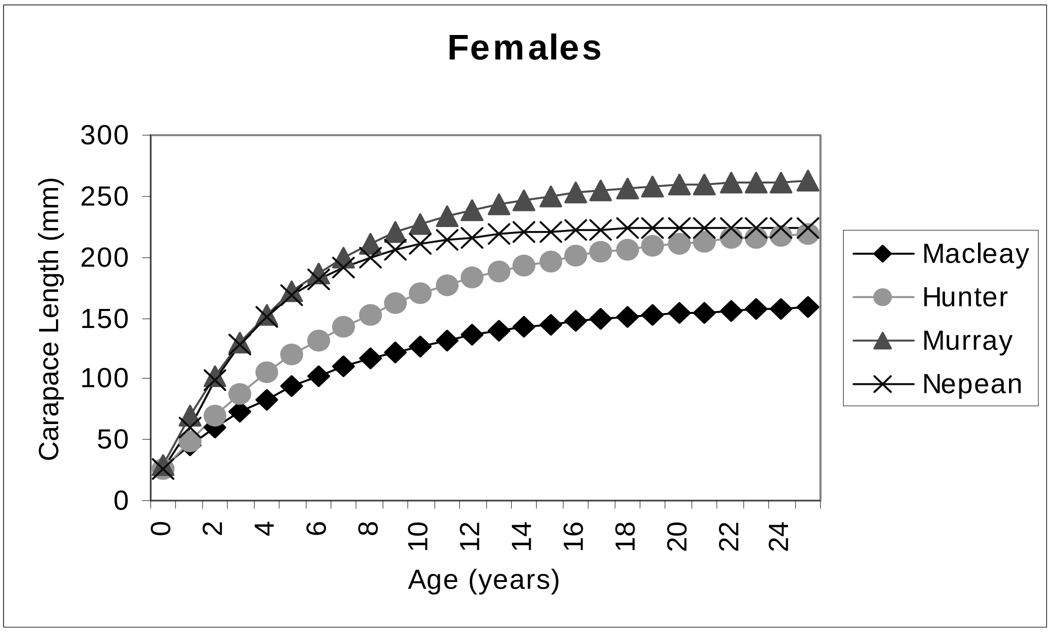


Figure 4.3. Comparison of the average body size for each age group (0-7 years) between the Macleay, Hunter, Nepean and Murray Rivers. These data were determined from annual growth rings.

Table 4.8. Von Bertalanffy growth parameters for female *E. macquarii*. Figures in brackets are 95 % confidence limits. Other values are means with SE. The asymptote and average adult size represent carapace length in mm.

River	Asymptote (a1) (mm)	r	Average Adult Size (mm)
Macleay (n=82)	163.27 ± 8.29 (146.76 - 179.78)	0.132 ± 0.013 (0.106 - 0.158)	161.4
Hunter (n=323)	227.84 ± 7.69 (212.71 - 242.98)	0.125 ± 0.008 (0.110 - 0.140)	213.3
Nepean (n=77)	224.82 ± 5.55 (213.76 - 235.88)	0.268 ± 0.021 (0.226 - 0.309)	205.8
Murray (n=172)	264.66 ± 8.61 (247.65 - 281.66)	0.184 ± 0.012 (0.161 - 0.207)	270.8



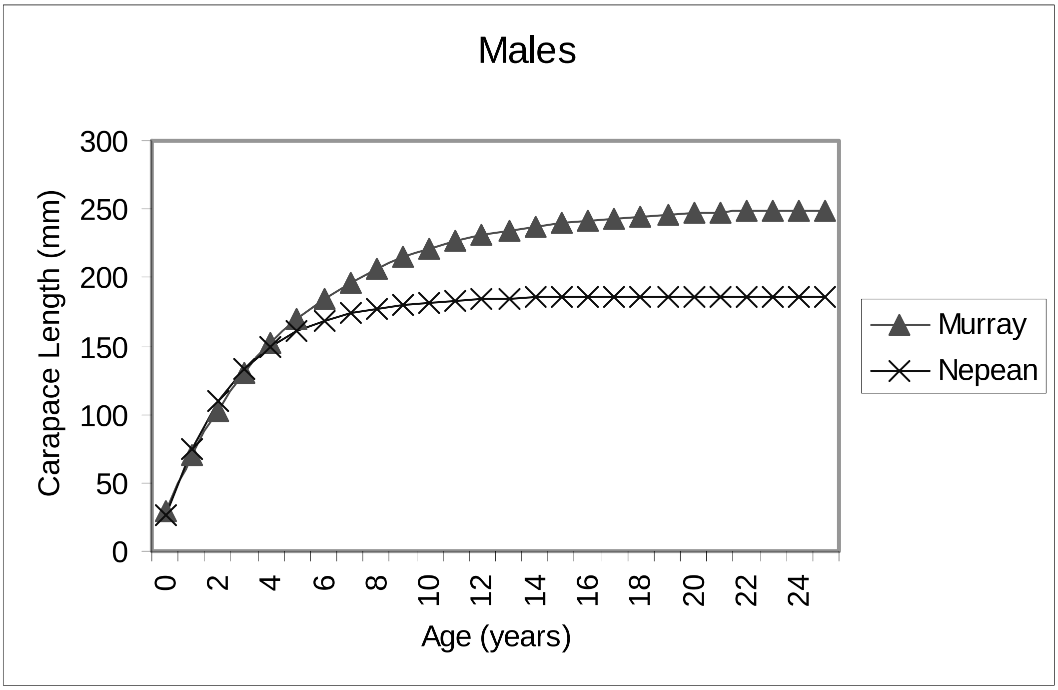


Figure 4.4. Comparison of Von Bertalanffy growth curves between the Murray, Nepean, Hunter, and Macleay rivers.

Females from the Murray and Nepean rivers followed the same growth trajectory for the first six years, after which growth in the Nepean River population quickly tapered off (Fig. 4.4).

Von Bertalanffy growth curves for males could not be calculated for the Hunter and Macleay Rivers owing to insufficient recapture data. Results for the Nepean and Murray River populations were validated, as the asymptotic size was comparable with the average adult size (Table 4.9). The intrinsic rate of growth for males in the Nepean River was almost double that of the Murray River (Table 4.9). Males in the Murray River took much longer to reach the maximum body size. Males from the Murray and Nepean Rivers followed the same growth trajectory for the first four years, after which growth in the Nepean River population declined rapidly (Fig. 4.4).

4.4 Discussion

4.4.1 Body size

The maximum body size of *Emydura macquarii* is highly variable across its range. In this study, maximum female body sizes ranged from 180 mm in the Macleay River to over 300 mm in the Murray River. In Coopers Creek some individuals have been known to reach carapace lengths of up to 450 mm (Ehmann, 1992). Most of the variation in size has been reported from northern N.S.W. (Cann, 1969), although preliminary data for populations along the East Coast of Queensland suggests a similar level of variation (Georges pers. comm., Table 4.10).

Table 4.9. Von Bertalanffy growth parameters for male *E. macquarii*. Figures in brackets are 95 % confidence limits. Other values are means with SE. The asymptote and average adult size represent carapace length in mm.

River	Asymptote (a1) (mm)	r	Average Body Size(mm)
Nepean	186.12 ± 6.31	0.368 ± 0.043	173.2
(n=61)	(173.49 - 198.75)	(0.282 - 0.454)	
Murray	250.86 ± 17.29	0.201 ± 0.023	239.8
(n=136)	(216.66 - 285.06)	(0.155 - 0.247)	

Table 4.10. Comparison of life-history traits from other populations of *Emydura macquarii*.

Life-History Trait	Sex	Fraser Is	Burnett	Murray[A]	Murray[B]	Coopers	Greenvale
State		QLD	QLD	NSW	NSW	SA	QLD
Carapace Length	Males	197	277	272	-	281	227.6
(mm)	Females	246	281	324	-	335	235.4
Size at Maturity	Males	110-117	180-190	200-210	-	185 [C]	150 - 165
(mm)	Females	150-155	-	210-	-	220 ? [C]	-
Age at Maturity	Males	8-10	-	6	-	-	-
	Females	7-8	-	8	-	-	-
Sex Ratio	Males	153	-	239	69	114	-
	Females	142	-	280	125	147	-
Adults		283	92	519	130	261	6
Juveniles		378	19	158	51	251	38

[C] Size at maturity determined by me

Fraser Island (Georges, 1983)

Burnett River (Georges and Legler, unpublished data)

Murray[A] (Chessman, 1978)

Murray[B] (Thompson, 1983)

Coopers Creek (Doddridge, 1992)

Greenvale (Georges, unpublished data)

Em. macquarii is sexually dimorphic throughout its range and over all body sizes, with females larger than males in all cases (Tables 4.1 and 4.10). This supports other studies on aquatic turtles where females were larger than males in most species (Berry and Shine, 1980). As there is no combat between males, large-scale migration, or high levels of predation of adult *Em. m. macquarii*, there is no selection for males being large. Selection would favour males maturing at smaller sizes as they would start their reproductive life earlier. In this study, age was more important than size in determining maturity in male *Em. m. macquarii.* Apart from the Nepean River, the age at maturity was similar for males among rivers, despite large differences in the size at maturity. There was also great variation in body size at maturity within rivers. Therefore, males attain maturity as fast as possible rather than reach a certain body size. The Nepean River population, whose rapid growth rate enables it to mature very quickly, also supports this. A difference in age at maturity between the Nepean River and other populations suggests that the minimum size at which males mature is still important. Males that mature at too small a size relative to that of females may reproduce less effectively.

Size at maturity was a more important factor than age in determining maturity in females. Apart from the Nepean River, age at maturity was inversely related to maximum body size. That is, the smaller the maximum body size, the longer it took to mature. A female reaches a minimum body size before it matures, hence the large variation in age at maturity among rivers. The relationship between body size and reproductive output (see Chapter 5) enables larger turtles to produce larger clutch sizes as well as larger eggs. Therefore, delayed sexual maturity may increase fecundity and/or increase the survival of offspring (Congdon and Gibbons, 1990). However, the large variation in size at maturity among rivers suggests that females do not mature at a fixed size but along an age-size trajectory (Stearns and Koella, 1986).

Males were smaller than females since males matured at a younger age and hence a smaller size. As growth after maturity is slow and intermittent, females will maintain a larger body size. This may also explain why the smallest-bodied population (Macleay River), was more dimorphic than populations from other rivers. As juvenile growth rates in the Macleay River was so slow, maturity for males was at the smallest size possible. However, females must attain a large enough size to increase reproductive output, thereby increasing dimorphism between the sexes.

In general, the results parallel findings for turtles from the Northern Hemisphere. Large-bodied populations such as the Murray River had rapid juvenile growth, matured at a larger size and reached a larger maximum size. Small-bodied populations such as the Macleay River had very slow juvenile growth, matured at smaller sizes and reached a smaller maximum size. Growth rates prior to maturation, rather than indeterminate growth, were the determinants of body size variation. Variation in juvenile growth rates was not only great among populations, but also within populations, and undoubtedly explains why there was such large variance in size at maturity within a population.

The Nepean River was in stark contrast to the general patterns discovered in Australian and North American studies. The Nepean River featured large numbers of juveniles, rapid growth rates, early maturation, a male bias and few old turtles. Growth rates were not only rapid for juveniles, but also for adults. Hence, turtles reached very large maximum body sizes relative to the size at which they mature. Turtles from the Nepean River reached a maximum body size similar to that of the Hunter River population, yet followed a juvenile growth pattern closer to that of the much larger-bodied Murray River population. However, unlike turtles from the Murray River, body size in the Nepean River population was sacrificed in favour of earlier maturity.

Many of the reported life history strategies of turtles in other studies are probably influenced by density-dependent factors. Populations at high density may have greater competition for resources. Growth rates will decline, especially after maturity, where upon growth tends to occur intermittently. Under such conditions, maturity has to be delayed in order to reach larger maximum body sizes. In the Nepean River, *Em. m. macquarii* was at such low density unlike other rivers, the population may not be constrained by density-dependent factors such as food availability. Turtles in the Nepean River grow appreciably even after maturity to reach a relatively large maximum body size, yet with the advantage of breeding earlier. Instead of delaying maturity, turtles in the Nepean River are maturing rapidly.

Variation in body size among rivers may be influenced by differences in habitat productivity. Although primary productivity was not measured in this study, anecdotal evidence would suggest there were large differences in productivity between populations. The Macleay and Hunter River sites werer more characteristic of an upland river, which are generally deemed heterotrophic with low levels of primary production (Lake, 1994). Few macrophytes were present in either river, and the scarcity of fish would provide minimal amounts of carrion. Productivity in the Nepean, Brisbane, and Murray River sites would appear to be higher. All three sites were lowland rivers high in primary productivity and strongly autotrophic (Lake, 1994). Macrophytes and periphytic algae were abundant, and large numbers of fish would provide a steady supply of carrion. This last point is an important distinction as turtles grow more rapidly on a carnivorous diet than one based mainly on aquatic vegetation (Congdon, 1989; Mayeaux *et al.*, 1996).

Temperature may be an important factor in limiting the body size of *Em. m. macquarii* in the Hunter River. The Hunter River site was high up in the catchment just below Barrington Tops, a place that regularly receives snowfall during winter. Consequently, the water was extremely cold year-round. In December the temperature of the water was only 17^{o}C, which is around 10^{o}C cooler than that found in the Nepean River at the same time. This has resulted in a delayed breeding season and a likely reduced growing season.

Unlike North American studies, there appears to be no gradient of body size with latitude in *Em. macquarii.* In fact, there is a random scatter of maximum body sizes in *Em. macquarii* throughout Australia, with the largest populations occurring in both the south (Murray River) and the north (*Em. m. krefftii*). As an increase in growth rate results in a larger size at maturity for *Em. macquarii*, it is likely that resource availability is relatively more important than latitudinal patterns in explaining variation in body size (Atkinson and Sibly, 1997). In Australia where resource availability is limited and less uniform, and temperatures are less extreme, temperature has little effect on body size. This is not suggesting that temperature has no effect on body size of turtles in Australia, but that any variation due to temperature is overwhelmed by differences in productivity.

4.4.2 Population structure

Only two of the five populations contained a biased adult sex ratio. The Nepean River population was heavily male-biased in favour of males. The extensive trapping regime over three years and over a wide area, and the fact that a male bias was found in every year suggests that the bias is real rather than an artefact of sampling error. The most likely explanation for the bias is differences in the age at maturity between sexes. Males in the Nepean River can mature as early as 3 yr while females take 6 - 7 yr to mature. An age-related bias would be magnified in a population that appears to be young and expanding, for as males mature quickly, they would initially pre-dominate. If other potential factors of sexual bias are minimal, the sex that matures earlier will only dominate where there is a regular recruitment of juveniles (Gibbons, 1990; Lovich and Gibbons, 1990). In more stable populations such as the Macleay, Brisbane and Murray Rivers where adults dominate and juvenile recruitment is presumed low, differences in age at maturity between the sexes has less of an influence on adult sex ratios. This may explain the lack of male bias in other populations of *Em. macquarii* (Tables 4.1 and 4.10) even though males always mature earlier.

The Hunter River with a relatively high proportion of juvenile turtles had a female bias. Thompson (1983) found a female bias for *Em. m. macquarii* in the Murray River, although this was possibly an incorrect calculation of the adult sex ratio because of sexual differences in the size at maturity. It is uncertain if the female bias in the Hunter River is real or an artefact of sampling bias. Sex ratios were heterogeneous across sampling trips, although the sample size was very small in some cases. Short-term sampling, especially in a particular season or habitat, may result in a strong bias (Mitchell, 1988; Gibbons, 1990; 1990a). A majority of turtles were caught during one field trip and may have resulted in differences due to seasonal effects. This trip coincided with the breeding season and the large number of sandy banks around the study site may well have been a popular breeding ground for *Em. m. macquarii.*

Additional field trips over the full season, as well as annually, need to be conducted to determine if a female bias in the Hunter River is correct.

Comparison of turtle densities among rivers is rather subjective, as only the Murray River and Nepean River populations were quantified. This was further compounded by the different habitat types within rivers. Even so, the unproductive Macleay and Hunter Rivers had the highest densities of turtles. Many sections of rapid and shallow flowing water in these rivers are unsuitable for habitation, resulting in turtles that congregate in still backwaters and deep pools. A "dwarfed" form of *Em. macquarii krefftii* from Fraser Island was also found in high densities (87 individuals/ha) in an oligotrophic lake (Georges, 1983). Therefore, an unproductive habitat appears more to reduce the size turtles can reach rather than reduce the number of turtles it can support. Results from commercial turtle farms have found growth is inversely related to stocking densities through increased food-competition hierarchies and behaviours, as well as less efficient feed and protein conversion (Mayeaux *et al.*, 1996). Therefore, the high densities of turtles in the Macleay and Hunter Rivers may be another factor that limits the maximum body size they can reach.

Substantial variation exists in the population structure of *Em. m. macquarii* throughout its range (Tables 4.1 and 4.10). In populations such as Fraser Island (Georges, 1985), Greenvale, Coopers Creek and the Nepean River, juveniles dominated the population. In the Burnett, Brisbane, Macleay and Murray Rivers, juveniles were scarce. Sampling bias may affect the profile of population structure as some studies have shown habitat segregation of juveniles and adults (Pappas and Brecke, 1992; Congdon, et. al. 1992). However, differences in localised factors such as predation rates, survival rates, age at maturity, growth rates, body size, reproductive output and population densities would also be important in determining population structures.

Several studies use the proportion of juveniles to index the health of a population of *Em. macquarii* (Thompson, 1983a; Doddridge, 1992). Thompson (1983a) believed that the sparse number of juveniles in the Murray River relative to that found in Coopers Creek were a result of heavy fox predation, and this would eventually result in their decline. This study demonstrates that the wide variation in population structure stems from complex localised factors, even when *Em. macquarii* are highly abundant. Therefore, the use of Coopers Creek as a reference site may be unjustified. Comparisons of the proportion of juveniles from the Murray River with other populations suggests nothing unusual. In fact, the Murray River has a higher proportion of juveniles than many other similarly abundant populations (Tables 4.1 and 4.10). However, the preferred method for determining population status is to monitor it over time rather than make comparisons with other populations under different selection pressures.

4.4.3 Summary

Maximum body size in turtles is greatly influenced by temperature and habitat productivity. Anecdotal evidence in this study suggests that habitat productivity is a more important determinant of body size in *Em. m. macquarii*. In productive environments *Em. m. macquarii* grows faster, mature larger and reach greater maximum sizes. *Em. m. macquarii* is a generalist turtle able to survive in a wide range of habitats. As a consequence, the species displays enormous variation in body size across its range. This variation in body size not only occurs among populations but also within populations. Juvenile growth rates and size at maturity differ markedly between individuals within a population, most likely as a result of spatial and temporal variation in resources. The variation recorded for maximum body size suggests that body size in *Em. m. macquarii* is a highly plastic trait responsive to changes in environmental conditions.

Chapter 5

Geographic Variation in Reproduction

5.1 Introduction

Most studies on turtles have demonstrated large intraspecific variation in reproductive traits both within (Congdon and Gibbons, 1983; Mitchell and Pague, 1990) and among populations (Wilbur and Morin, 1988; Rowe, 1992; Iverson and Smith, 1993). Much of this variation is associated with differences in body size. In many turtles, body size has been found to be correlated with clutch mass (Plummer, 1977; Gibbons *et al.*, 1982; Congdon and Gibbons, 1983; 1985; Congdon *et al.*, 1983; 1983a; 1987; Georges, 1985; Congdon and van Loben Sels, 1991; Mitchell and Pague, 1991), clutch size (Gibbons, 1970, 1982; Moll and Legler, 1971; Gibbons *et al.*, 1982; Congdon and Gibbons, 1983, 1985), egg size (Congdon and Tinkle, 1982; Congdon and Gibbons, 1983; 1985; Congdon *et al.*, 1983; Congdon and van Loben Sels, 1991), and sometimes clutch frequency (Gibbons, 1970; Plummer, 1977). Therefore, body size plays a significant role in the reproductive strategies employed by turtles.

However, not all variation in reproductive traits can be explained by differences in body size. A latitudinal relationship can be found in many reproductive traits both within (Christiansen and Moll, 1973; Moll, 1973; Iverson, 1992, 1993) and across species (Iverson, 1992, 1993; Moll, 1979). Turtles at higher latitudes generally have larger clutch sizes, produce smaller eggs and have less clutches per year than those turtles from lower latitudes (Wilbur and Morin, 1988). Turtles at higher latitudes compensate for a shorter nesting period by maximising reproductive output in each clutch. As a result, reproductive output is much the same as that of populations from tropical regions despite a lower clutch frequency (Iverson, 1992).

An important factor in terms of reproductive variability may be the trade-off between egg size and clutch size. In the optimal egg size theory, selection is believed to favour females that produce the greatest number of offspring, and females that produce the largest offspring where this improves fitness (Smith and Fretwell, 1974; Brockelman, 1975). Larger eggs may hatch more successfully (Gutze and Packard, 1984; Janzen, 1990), or produce larger hatchlings with increased survival rates (Janzen, 1993; Bobyn and Brooks, 1994). However, larger eggs obviously reduce the number of offspring that can be produced. The optimum egg size is the trade-off point where the benefit of producing larger eggs is offset by producing fewer offspring. Many studies have failed to find a trade-off between egg size and clutch size in turtles (Congdon and Tinkle, 1982; Congdon *et al.,* 1983; Thompson, 1983; Congdon and Gibbons, 1985; Schwarzkopf and Brooks, 1986; Vogt, 1990; Kennett, 1994; Roosenburg and Dunham, 1997). The high variability of egg size within a population also contradicts optimal egg size theory that assumes egg size is optimised by natural selection, and that increased reproductive output associated with a larger body size would be made by increasing egg number rather than egg size (Smith and Fretwell, 1974; Brockelman, 1975). It has been proposed that turtles are in fact exceptions to optimal egg size models (Congdon and Tinkle, 1982; Congdon *et al.,* 1983; Congdon and Gibbons, 1985).

Variation in reproductive output is not only associated with egg size and egg number, but also egg quality. As turtles show no parental care, the eggs of turtles represent the total reproductive investment in offspring by females. Energy stored in the yolk is used to fuel the development of the embryo (PIE) and early period of hatchling independence (PIC) (Congdon and Gibbons, 1990). The energy allocated to each egg requires a quantity in excess of what is required for embryogenesis. Excess yolk (at least 50 %) may be used for hatchling maintenance, overwintering in the nest, emergence from the nest, movement to feeding grounds and possibly growth (Congdon and Gibbons, 1990). The greater the quantity of excess yolk, the higher the level of parental investment, and the better the chance of each offspring's survival. In contrast to post-ovulatory parental investment, PIE has a direct influence on the size of the egg (Congdon and Gibbons, 1985), and so offspring number may be sacrificed for increased offspring care. The levels of pre-ovulatory parental investment may be related to the posthatching environment, where competition might be an important factor, or juvenile mortality may be high and unpredictable (Congdon and Tinkle, 1982, Congdon and Gibbons, 1985).

Studies on large-scale geographic variation in reproductive traits within turtles have mainly been restricted to North America (*Chrysemys picta*: Christiansen and Moll, 1973, Moll, 1973; *Trachemys scripta*: Gibbons, 1970, Mitchell and Pague, 1991; *Emydoidea blandingii*: Rowe, 1992). Within Australia, few studies have investigated the relationship between egg size, clutch size and body size in freshwater turtles (Georges *et al.,* 1993; Legler and Georges, 1993). Currently the only published paper on reproductive variability in Australian turtles (Legler, 1985) concentrated on differences in egg size, incubation period, and the nesting season in wide-ranging taxa at the generic level.

Emydura macquarii is a widespread species of freshwater turtle distributed from tropical climates in Queensland down to colder temperate regions in Victoria. Because body size in *Em. macquarii* is extremely variable across its range (see Chapter 4), it is an ideal species in which to explore patterns in reproductive variation. Research on the reproduction of *Em. macquarii* is limited to populations from Fraser Island (Georges, 1982a; Georges, 1983) and the Murray River (Chessman, 1978; Thompson, 1983a; 1985; 1988). The reproductive data collected thus far suggests that variability is comparable to that found in Northern Hemisphere studies. A small-bodied form of *E. m. krefftii* in the unproductive sand dune lakes of Fraser Island produced smaller eggs, smaller clutch sizes and fewer clutches per season than those mainland populations (Georges, 1985). Egg sizes and clutch sizes reported for *Em. m. macquarii* from the Murray River are larger (Chessman, 1978; Thompson, 1983a) than *Em. m. krefftii* (Georges, 1985).

Determination of the levels, patterns and causes of reproductive variability in *Em. macquarii* is limited by the lack of data across the species range. In this chapter, reproductive data including egg size, clutch size, clutch mass, hatchling size and egg contents are reported for five populations (Murray, Brisbane, Macleay, Hunter, and Nepean Rivers) of *Em. m. macquarii*. The objective is to derive an insight into the causes and relationships of various reproductive attributes in *Em. m. macquarii* by comparing these traits among widely separated populations. Specific aims include to:

- Examine the degree of reproductive variation both within and among populations of *Em. m. macquarii*
- Determine the effect of body size on the reproduction of *Em. m. macquarii*
- Determine whether patterns of reproduction in *Em. m. macquarii* are consistent with optimal egg size theories (Smith and Fretwell 1974; Brockelman 1975)
- Determine the egg content of *Em. m. macquarii*
- Determine the effect of egg size and incubation temperature on hatchling size.

5.2 Materials and methods

Banks (1987) found an internesting period of 30 – 51 days in captive kept Krefft's turtles (*Em. m. krefftii*). This is the only published data on internesting periods in Australian chelids. Therefore, an internesting period of 30 – 51 days was used as a guide to estimate clutch frequency in this study.

The volume of eggs was estimated from egg length and egg width by the bicone formula (Iverson and Ewert, 1991):

$$V = (\pi LW^2/6000)[1 + (2/5)c_2 + (3/35)c_2^2]$$

where V = volume in cm^3, L = length of egg in mm, W = width of egg in mm, and c_2 = is negative for pointed eggs and positive for those eggs with blunt ends. The parameter c_2 was estimated by the following formula (Iverson and Ewert 1991) :

$$c_2 = 0.196E - 0.216$$

where E = egg elongation (length/width).

Three to four eggs from each clutch were frozen at –80°C for later component analysis. The frozen eggs were separated into the eggshell and yolk/albumin, and then weighed to the nearest 0.01 g. The eggshell was dried in an oven at 65°C and weighed to 0.01g. To determine the inorganic material, the dried eggshell was ashed in a furnace oven for four hours at 500^{o}C and then weighed (0.01g). The egg yolk was freeze-dried until completely dry and weighed (0.01g). The egg content was homogenised by grinding with a mortor and pestle, and subsamples were analysed for lipids and protein. The protein content was determined by the colorimetric method (Lowry's *et al.,* 1951). Total lipid content was determined by analysing a 0.15-0.40g subsample with the methanol-chloroform extraction method (Freeman *et al.,* 1957). A subsample was placed in 20ml of methanol-chloroform (1:2 v/v) and vibrated for 5 minutes. The solution was left for 24 hours and then centrifuged for 20 minutes. The lipids were then extracted using a rotary evaporator. The residual subsample was extracted a second time with 10ml of methanol-chloroform.

The constants, 9.45 kcal/g lipid and 5.65 kcal/g protein, were used to convert organic matter into caloric units. This can be converted into kilojoules (kJ) by the energetic equivalent of 1 kcal for each 4.184 kJ. The total energy per gram was then obtained by adding the two components. The kJ of energy of eggs were determined from the following equation (Linley and Mushinsky, 1994):

Energy in kJ (ash free) = (dry mass of the component in g) * (ash free % of organic constituent /100) * (energy in kJ/g of organic constituent).

5.2.1 Analysis

A significant point of interest in this study was whether differences in various reproductive traits between populations were due to factors other than body size. Therefore, a multiple regression analysis was used on the complete data set to adjust each reproductive trait for differences in carapace length and body weight (Barron, 1997). Each reproductive trait (dependent variable) was analysed separately against both carapace length and body weight (independent variables forced into the regression model). The residuals from the multiple regression analysis were then analysed by a single factor ANOVA to determine whether there were differences in residuals among rivers. A significant result suggested that other factors apart from body size were also responsible for the variation in specific reproductive traits between populations.

A stepwise multiple regression analysis was used for each river to determine the relationship of clutch mass with that of clutch size, egg width and egg length. To eliminate multi-collinearity, egg weight was not included in the analysis as it was highly correlated (r>0.8) with both egg length and egg width. It was considered that the size of the egg was of more interest than the weight. To determine whether egg length was more variable than egg width, a paired t-test was used, with the coefficient of variation as the measurement variable. The coefficient of variation was calculated for both egg length and egg width within each clutch. While the distribution of the coefficient of variation is generally not normal, the difference between the two measurements is likely to be, while the sample sizes were large.

5.3 Results

5.3.1 Date of reproduction and clutch frequency

Gravid females were caught in the Brisbane River on the 1st October 1996. Almost half of the adult females caught were breeding (n=60, 42%), although some evidence may have been missed as the presence of eggs were difficult to determine from palpating.

Almost all females from the Macleay River were gravid on the 5th October 1995 (n=34, 92%) and 10th October 1996 (n=16, 94%), although eggs at this time were still not fully developed. A small proportion of females was gravid again on the 5th December 1995, which was a second or possibly a third clutch. Laparoscopy suggested that no more clutches would have been laid after this period. Therefore, the maximum number of clutches per year that could be produced by Macleay River turtles is three, although most individuals probably only produce twice. A large number of females were caught in successive years. Of the 17 females recaptured, 15 bred in both years. Therefore, it can be concluded that most females breed annually.

The earliest date on which gravid Nepean River turtles were caught was on the 25th October 1995, 25th October 1996, and 30th October 1997. The latest a female was caught gravid was on the 31st December 1998 and the 3rd January 1997. This evidence suggests that up to three clutches are produced by *Em. m. macquarii* from the Nepean River. This is further supported by laparoscopy where three sizes of copora lutea were determined. It is likely only two clutches are produced by most females. One individual female was gravid in successive years suggesting that reproduction is annual.

A high proportion of female turtles from the Murray River was gravid on the 13th November 1995 (N=56, 64%), as well as a year later on 14th November 1996 (N=29, 79%). A field trip on the 17th November 1997 found only a few gravid females which suggested that the majority had already laid. The late start to the breeding season would mean at the very most only two clutches could be produced in a year, while many females may only produce one. Eight out of 15 recaptured turtles bred in successive years, while one individual was gravid in three successive years. It is quite likely that those individuals that were not gravid had already laid. Therefore, the majority of females reproduce annually in the Murray River.

A field trip on the 22nd October 1996 to the Hunter River found no gravid females indicating that the breeding season had not yet begun. Several weeks later on the 28th November 1996 just under about half of females carried eggs (N=95, 45%). The late start to the breeding season suggests it is likely that only one clutch could be produced per year.

In general, the commencement of the breeding season was related to the latitude of the population (Table 5.1). Populations from lower latitudes began reproducing earlier than populations from higher latitudes. The exception to this was the Hunter River, which was the last population to begin breeding despite occurring at a lower latitude than both the Nepean and Murray Rivers.

Table 5.1. Comparison of the commencement of the breeding season among populations of *Emydura macquarii macquarii*. Rivers are ordered from lowest latitude to highest latitude. +++ represents the period in the month in which the breeding season began.

River	October	November	December
Brisbane	+++		
Macleay	+++		
Hunter		++	+
Nepean	+++		
Murray		+++	

5.3.2 Comparison of clutch size, clutch mass and egg size between rivers

Clutch sizes differed significantly among rivers (F=311.11; d.f.=4,199; p<0.0001)(Table 5.2). *Em. m. macquarii* from the Murray River produced the largest clutch sizes, while the Macleay River females had the smallest. There was no significant difference in clutch size among the Brisbane, Hunter or Nepean Rivers. After adjusting for maternal body size as a covariate, there was still a significant difference in clutch size among rivers (F=31.44; d.f.=4,195; p<0.0001). Relative to their body size, the Nepean River females produced the largest clutch size while

Brisbane River females had the smallest. Relative clutch size was not significantly different between the Hunter and Murray Rivers or the Murray and Macleay Rivers. Turtles from the Hunter River however had significantly greater relative clutch sizes than those from the Macleay River.

Clutch mass differed significantly among rivers (F=287.00; d.f.=4,189; p<0.0001)(Table 5.2). Clutch mass was heaviest in females from the Murray River, followed by the Brisbane, Hunter, Nepean and Macleay Rivers. After adjusting for maternal body size as a covariate, there was still a significant difference in clutch mass among rivers (F=19.59; d.f.=4,192; p<0.0001). Relative to body size, Brisbane River females had the lightest clutch mass. Relative clutch mass did not differ between the Macleay, Hunter, or Nepean Rivers. Relative clutch mass was heaviest in the Murray River although not significantly different from the Macleay River.

Egg wet mass differed significantly among rivers (F=84.05; d.f.=4,192; p<0.0001) (Table 5.2). *Em. m. macquarii* from the Murray River produced the heaviest eggs, followed by the Brisbane River, while turtles from the Nepean River had the lightest eggs. Eggs from the Hunter and Macleay Rivers were not significantly different in weight. After adjusting for female body size as a covariate, there was still a significant difference in egg wet mass among rivers (F=69.48; d.f.=4,194; p<0.0001). Relative to body size, turtles from the Macleay, Murray, and Brisbane Rivers had the heaviest eggs

Table 5.2. Reproductive traits of *Emydura macquarii* from the Macleay, Hunter, Nepean, Brisbane, and Murray Rivers.

River	Clutch Frequency	Clutch Size	Clutch Mass (g)	Egg Mass (g)	Egg Length (mm)	Egg Width (mm)
		Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
		Range	Range	Range	Range	Range
		CV	CV	CV	CV	CV
Macleay (n=64)	2-3	6.8 ± 1	49.6 ± 1.2	7.34 ± 0.10	33.8 ± 0.2	19.0 ± 0.1
		4 – 9	24.8 – 67.3	5.60 – 8.93	30.5 – 37.8	17.5 – 20.6
		16.2	18.5	10.7	5.1	3.8
Hunter (n=42)	1-2	13.1 ± 0.3	95.8 ± 2.6	7.31 ± 0.11	31.9 ± 0.2	19.6 ± 0.1
		9 – 18	59.3 ± 130.8	5.81 – 8.83	29.1 – 35.3	18.3 – 21.0
		14.3	17.8	10.2	4.3	3.6
Nepean (n=14)	2-3	14.4 ± 0.9	73.0 ± 6.3	5.13 ± 0.33	28.4 ± 0.7	17.4 ± 0.4
		9 – 21	49.8 – 144.0	3.27 – 7.05	23.8 – 31.5	15.2 – 19.6
		22.7	32.2	24.0	9.0	8.2
Brisbane (n=24)	3-4	13.2 ± 0.4	113.5 ± 4.4	8.61 ± 0.13	33.8 ± 0.2	20.7 ± 0.1
		9 – 17	67.7 – 147.5	7.53 – 9.89	31.3 – 35.3	19.1 – 22.0
		16.1	18.8	7.3	3.2	3.4
Murray (n=62)	1-2	20.1 ± 0.5	190.4 ± 5.5	9.44 ± 0.13	35.3 ± 0.2	21.1 ± 0.1
		11 – 30	97.4 – 265.1	5.39 – 11.89	30.0 – 37.8	17.6 – 23.1
		19.1	22.0	11.0	4.4	4.3

followed by the Hunter River, while females from the Nepean River produced the lightest eggs.

Egg length differed among rivers (F=59.83; d.f.=4,197; p<0.0001) (Table 5.2). Turtles from the Murray River had the longest eggs, followed by the Brisbane and Macleay Rivers. The Hunter River followed next, while *Em. m. macquarii* in the Nepean River produced the shortest eggs. After adjusting for female body size as a covariate, there was still a significant difference in egg length among rivers (F=43.15; d.f.=4,198; p<0.0001). Relative to body size, turtles from the Macleay, Murray, and Brisbane Rivers produced the longest eggs, followed by the Hunter River, while *Em. m. macquarii* from the Nepean River produced the shortest eggs.

Egg width differed among rivers (F=93.29; d.f.=4,199; p<0.0001) (Table 5.2). Egg width differed between all populations except the Murray and Brisbane Rivers. Turtles from the Murray and Brisbane Rivers had the widest eggs, followed by the Hunter, Macleay and Nepean Rivers. After adjusting for differences in maternal body size as a covariate there was still a significant difference in egg width among rivers (F=42.12; d.f.=4,193; p<0.0001). Relative to body size, turtles from the Nepean River had the narrowest eggs, while *Em. m. macquarii* from the Macleay, Brisbane, and Murray Rivers had the widest. The width of eggs from the Hunter River was significantly different from all other rivers except the Murray River.

Egg volume differed among rivers (F=103.34; d.f.=4,197; p<0.0001). Turtles from the Murray River produced eggs with the largest volume followed by the Brisbane River. Egg volume was not different among the Hunter and Macleay Rivers, while egg volume was smallest in the Nepean River. After adjusting for maternal body size there was still a significant difference in egg volume among rivers (F=61.23; d.f.=4,195; p<0.0001). *Em. m. macquarii* from the Macleay, Murray, and Brisbane Rivers produced the largest egg volumes followed by the Hunter River, while the Nepean River had the smallest egg volume.

5.3.3 Comparison in reproductive traits between years

Seven females captured from the Murray River were gravid in both 1995 and 1996, which enabled inter-annual comparisons in reproductive traits (Appendix C). There was no significant difference in clutch mass, egg mass, egg length, egg width or clutch size between 1995 and 1996 (Table 5.3), although in six of the seven females clutch mass declined in 1996 (Appendix C). Twelve females from the Macleay River were gravid in both 1995 and 1996 (Appendix C). There was no significant difference in clutch mass, egg width or clutch size between years, although egg length and egg mass were significantly greater in 1996 than in 1995 (Table 5.4).

5.3.4 Egg shape

Egg length was correlated with egg width in the Hunter (r=0.43; n=509; p<0.0001), Macleay (r=0.27; n=438; p<0.0001), Murray (r=0.30; n=1190; p<0.0001) and Nepean (r=0.73; n=167; p<0.0001) rivers. There was no correlation between egg length and egg width in the Brisbane River (r=0.04; n=296; p=0.54). Egg length was more variable than egg width in the Brisbane (T=3.03; d.f.=23; p<0.01), Hunter (T=5.86; d.f.=41; p<0.0001), Macleay (T=7.02; d.f.=63; p<0.0001), Murray (T=7.62; d.f.=58; p<0.0001) and Nepean (T=4.81; d.f.=12; p<0.0005) Rivers.

Residuals from the regression of egg length and egg width give a measure of egg shape. Relatively ellipsoid eggs have negative residuals while relatively spherical eggs have positive residuals. Egg shape differed significantly among rivers (F= 40.64; d.f.= 4,201; p<0.0001). Females from the Brisbane (mean residual = +0.81), Murray (+0.57) and Hunter (+0.36) Rivers produced egg shapes that were significantly more spherical than females from the Nepean (-0.67) and Macleay (-0.95) Rivers. There was a positive correlation between carapace length and egg shape (r=0.66; n=203; p<0.0001), indicating that larger turtles produced more spherical eggs. There was also a positive correlation between egg shape and clutch size (r=0.53; n=203; p<0.0001), yet a negative relationship was found after the removal of the effects of body size (carapace length and body weight) (r=-0.16; n=203; p<0.05). Therefore, while larger

Table 5.3. Results from a paired t-test analysis testing differences in reproductive traits between 1995 and 1996 for *Emydura macquarii* from the Murray River. No tests were significant.

Variable	Mean Difference	T	Prob>|T|	N
Clutch Mass	-11.5400000	-1.9442098	0.0999	7
Egg Mass	-0.1314286	-0.7659224	0.4728	7
Egg Length	-0.0471429	-0.1698086	0.8707	7
Egg Width	-0.0628571	-0.5155463	0.6246	7
Clutch Size	-0.8571429	-1.2163273	0.2695	7

Table 5.4. Results from a paired t-test analysis testing differences in reproductive traits between 1995 and 1996 for *Emydura macquarii* from the Macleay River. * represents those tests which were significant.

Variable	Mean Difference	T	Prob>|T|	N
Clutch Mass	0.4192308	0.2890724	0.7775	13
Egg Mass	0.4700000	3.1543554	0.0076 *	14
Egg Length	1.6464286	3.3382610	0.0053 *	14
Egg Width	0.0714286	0.5960151	0.5614	14
Clutch Size	-0.3846154	-1.3284223	0.2087	13

turtles produce bigger clutch sizes of spherical eggs, relatively larger clutch sizes in fact produce eggs that are more ellipsoid in shape.

5.3.5 Relationship of reproductive parameters with body size

There was a significant linear relationship between clutch mass and body weight of female turtles in all rivers except the Nepean River (Table 5.5). In rivers where the result was significant, the relationship was strong in all cases except for the Brisbane River. There was also a significant linear relationship between clutch size, egg mass and egg width with female body weight in all rivers except the Nepean (Table 5.5). Egg length was linearly related to body weight in only the Macleay and Murray rivers, and even here the relationship was only weak (Table 5.5). The relationship between reproductive traits and body size was generally weaker in the Brisbane River compared to other rivers. There was no relationship of any reproductive traits with body size in the Nepean River (Table 5.5).

5.3.6 Relationship of clutch mass to clutch size, egg width and egg length

In a stepwise multiple regression, variation in clutch mass was explained by all three variables (clutch size, egg width, and egg length) in all rivers (Table 5.6). For the Brisbane, Hunter, Macleay, and Murray Rivers, clutch size explained the majority of variation (67% - 86%), while egg width contributed most of that remaining (10% - 25%). Egg length explained only a small fraction of remaining variation in clutch mass (3% - 7%). However for the Nepean River, all three variables (clutch size, egg length and egg width) explained significant levels of variability in clutch mass (Table 5.6). Unlike other rivers, egg length was an important contributor of differences in clutch mass (36 %).

For two large-bodied populations (Brisbane and Murray Rivers), an increase in clutch mass was mostly due to an increase in clutch size (Table 5.6). For the Brisbane River, only 13 % of variation in clutch mass was related to the size of the egg. For the

Table 5.5. Relationships of reproductive traits with female body weight for the Macleay, Hunter, Nepean, Brisbane and Murray Rivers.

River	Reproductive Trait	Intercept	Slope	r^2	F	P	Df
Macleay	Clutch Size	3.54	0.0064	0.26	21.66	<0.0001	1,62
	Clutch Mass	6.59	0.0858	0.66	116.41	<0.0001	1,61
	Egg Mass	4.59	0.0055	0.37	35.90	<0.0001	1,62
	Egg Width	16.18	0.0056	0.46	52.72	<0.0001	1,62
	Egg Length	30.80	0.0059	0.09	6.05	<0.05	1,62
Hunter	Clutch Size	3.58	0.0085	0.41	28.26	<0.0001	1,40
	Clutch Mass	-11.75	0.0954	0.62	64.41	<0.0001	1,40
	Egg Mass	3.86	0.0031	0.33	20.00	<0.0001	1,40
	Egg Width	15.68	0.0035	0.47	36.14	<0.0001	1,40
	Egg Length	29.72	0.0019	0.04	1.62	=0.21	1,40
Nepean	Clutch Size	24.72	-0.0131	0.17	2.24	=0.16	1,11
	Clutch Mass	60.53	0.0084	0.004	0.04	=0.84	1,11
	Egg Mass	0.47	0.0055	0.17	2.19	=0.17	1,11
	Egg Width	12.77	0.0054	0.12	1.49	=0.25	1,11
	Egg Length	16.48	0.0142	0.25	3.62	=0.08	1,11
Brisbane	Clutch Size	6.13	0.0044	0.28	8.45	<0.01	1,22
	Clutch Mass	32.64	0.0509	0.38	13.28	<0.005	1,22
	Egg Mass	6.80	0.0012	0.22	6.28	<0.05	1,22
	Egg Width	18.69	0.0013	0.22	6.06	<0.05	1,22
	Egg Length	32.75	0.0006	0.02	0.52	=0.48	1,22
Murray	Clutch Size	8.67	0.0052	0.41	37.65	<0.0001	1,54
	Clutch Mass	18.88	0.0796	0.73	142.74	<0.0001	1,53
	Egg Mass	6.68	0.0013	0.40	36.96	<0.0001	1,56
	Egg Width	18.67	0.0011	0.34	29.48	<0.0001	1,57
	Egg Length	33.36	0.0010	0.07	4.08	<0.05	1,56

Table 5.6 Summary of stepwise multiple regression analysis for the dependent variable clutch mass. Independent variables (clutch size, egg width and egg weight) were included in the model if significant at the 0.15 level.

Brisbane River

F=890.24 d.f.=3,20 $p<0.0001$ $r^2=0.9926$

Mass=9.817*Width+3.593*Length+8.284*Clutch-320.181

Step	Variable	Partial r^2	F	p<
1	Clutch	0.8589	133.92	0.0001
2	Width	0.1010	52.86	0.0001
3	Length	0.0327	87.94	0.0001

Hunter River

F=1100.64 d.f.=3,38 $p<0.0001$ $r^2=0.9886$

Mass=8.485*Width+3.250*Length+7.332*Clutch-270.594

Step	Variable	Partial r^2	F	p<
1	Clutch	0.6953	91.26	0.0001
2	Width	0.2493	175.23	0.0001
3	Length	0.0441	147.28	0.0001

Macleay River

F=1624.65 d.f.=3,60 $p<0.0001$ $r^2=0.9878$

Mass=5.110*Width+1.588*Length+6.902*Clutch-147.655

Step	Variable	Partial r^2	F	p<
1	Clutch	0.6669	124.14	0.0001
2	Width	0.2497	182.65	0.0001
3	Length	0.0712	351.43	0.0001

Murray River

F=2010.29 d.f.=3,55 $p<0.0001$ $r^2=0.9910$

Mass=17.032*Width+5.137*Length+9.229*Clutch-535.283

Step	Variable	Partial r^2	F	p<
1	Clutch	0.7583	178.86	0.0001
2	Width	0.1961	241.10	0.0001
3	Length	0.0365	222.22	0.0001

Nepean River

F=100.90 d.f.=3,9 $p<0.0001$ $r^2=0.9711$

Mass=8.325*Width+2.264*Length+4.772*Clutch-206.462

Step	Variable	Partial r^2	F	p<
1	Length	0.3985	7.29	0.05
2	Clutch	0.2685	8.06	0.05
3	Width	0.3042	94.82	0.0001

small-bodied populations (Macleay, Hunter, and Nepean Rivers), egg size was the main difference in clutch mass. That is, an increase in the clutch mass was due to larger eggs (especially egg width) as well as larger clutch sizes. For the Nepean River, 70 % of the variation in clutch mass was explained by differences in egg size.

5.3.7 Trade-off between reproductive traits and clutch size

As female body size was correlated with many reproductive parameters, I controlled for maternal body size through a partial correlation analysis to test for a trade-off between various reproductive traits and clutch size. With female carapace length and body weight held constant, clutch size was found to have a significant negative relationship with egg length and egg weight in the Hunter, Macleay and Murray Rivers, but not in the Brisbane and Nepean Rivers (Table 5.7). Apart from the Murray River there was no significant relationship between egg width and clutch size (Table 5.7). While there was generally no relationship found between various reproductive traits and clutch size for the Nepean River, the correlation coefficients were high and the probability values low ($p=0.06$ for egg weight and egg width). Therefore, the lack of significance is most likely a result of the small sample sizes. Hatchling weights in the Macleay, and Murray Rivers were negatively correlated with clutch size, while there was no such relationship in the Brisbane and Hunter rivers (Table 5.7). Clutch mass in all rivers was positively correlated with clutch size (Table 5.7). When all rivers were combined, egg length, egg width, egg weight and hatchling weight were all negatively related with clutch size while clutch mass was positively related (Table 5.7).

5.3.8 Egg component analysis

A breakdown of egg components can be seen from Figure 5.1. Water makes up the dominant proportion of an egg ranging from 72.7% in the Macleay River to 75.8% in the Nepean River (Fig. 5.1). Water content in egg yolk was negatively correlated with egg size in the Nepean ($F=16.55$; d.f.=1,15; $p<0.001$; $r^2=0.52$), Murray ($F=4.97$; d.f.=1,39; $p<0.05$; $r^2=0.11$) and Macleay ($F=6.49$;d.f.=1,38;

Table 5.7 Partial Correlation matrix of total clutch mass, egg length, egg width, egg mass and hatchling mass correlated with clutch size. Female carapace length and body weight held constant.

River	Clutch Mass	Egg Length	Egg Width	Egg Weight	Hatchling (n)
Brisbane (n=24)	0.92 ***	-0.07	-0.02	-0.14	-0.20 (23)
Hunter (n=42)	0.75 ***	-0.40 *	-0.30	-0.42 *	-0.32 (36)
Macleay (n=64)	0.78 ***	-0.50 ***	-0.25	-0.52 ***	-0.58 (47) ***
Murray (n=54)	0.82 ***	-0.34 *	-0.39 **	-0.48 ***	-0.51 (33) **
Nepean (n=14)	0.29	-0.16	-0.53	-0.50	-
Rivers Combined (n=201) (143)	0.74 ***	-0.45 ***	-0.50 ***	-0.55 ***	-0.49 ***

* p<0.05

** p<0.005

*** p<0.0005

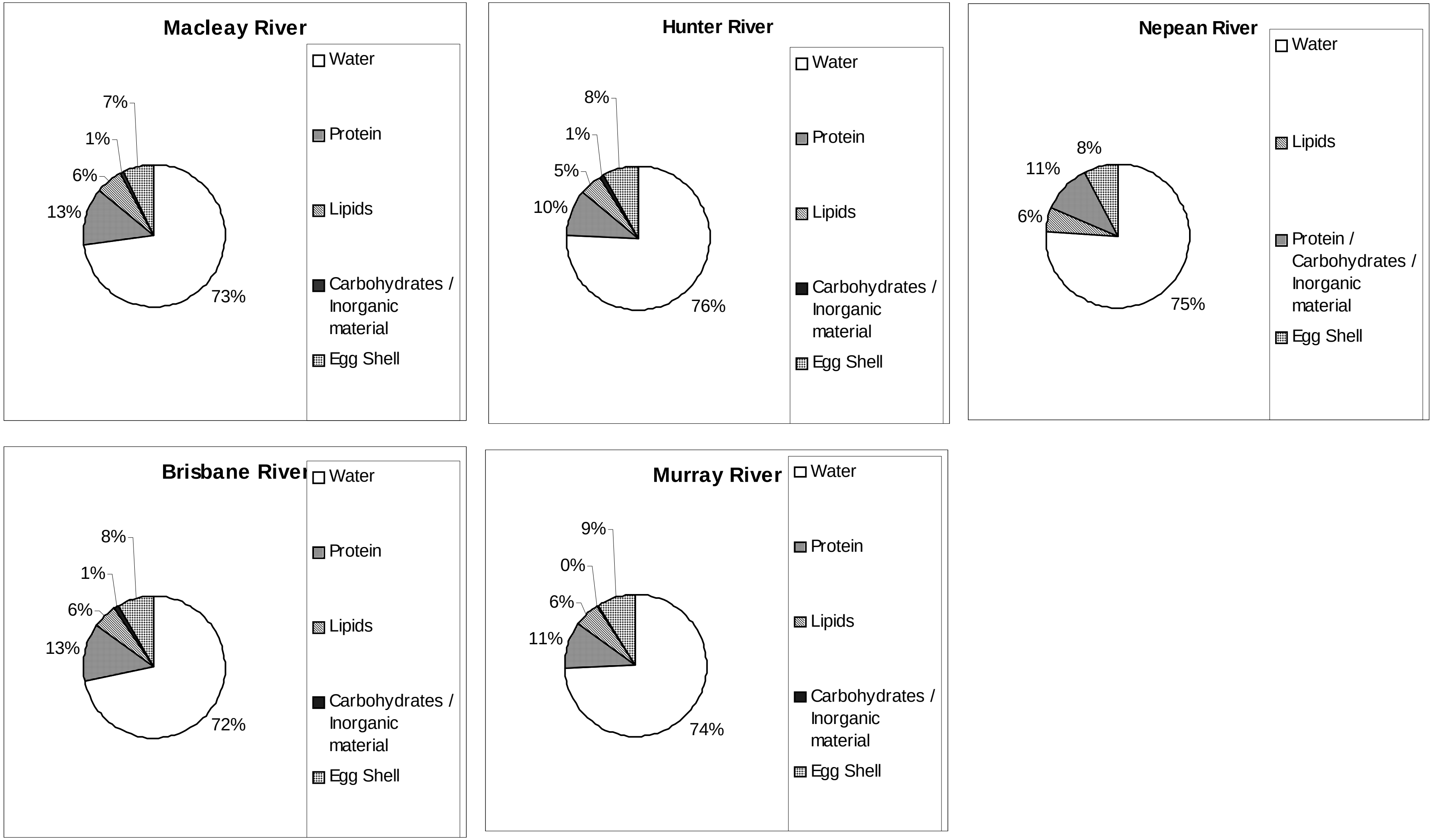


Figure 5.1. Comparison of egg components (wet mass) between the Macleay, Hunter, Nepean, Brisbane and Murray Rivers.

$p<0.05$; $r^2=0.15$) Rivers. Water content did not vary with egg size in the Hunter ($F=1.52$; d.f.=1,39; $p=0.23$) or the Brisbane ($F=2.81$; d.f.=1,31; $p=0.10$) Rivers.

There was a significant difference in total egg dry weight, after adjusting for differences in egg wet weight, among rivers ($F=10.19$; d.f.= 4,96; $p<0.0001$). Eggs from the Macleay and Brisbane Rivers had significantly greater total dry mass than eggs from the Murray, Hunter and Nepean Rivers. There was also a significant difference in yolk dry mass between rivers after adjusting for differences in egg wet weight ($F=39.96$; d.f.= 4,94; $p<0.0001$). Eggs from the Macleay and Brisbane Rivers had significantly the highest yolk dry mass, while the Nepean and Murray Rivers had the lowest (Table 5.8). Yolk dry mass in the Hunter River was significantly greater than the Nepean River, but not significantly different than the Murray River (Table 5.8).

Mean proportion egg lipid content ranged from 29.1 – 34.4 % (Table 5.9). There was a significant difference in the proportion of lipids in egg yolk among rivers ($F=14.20$; d.f.= 4,102; $p<0.0001$). Females from the Murray River produced eggs with the highest lipid content while females from the Brisbane River produced less lipid content. Eggs from the Nepean River had significantly higher proportion of lipids than the Brisbane River, but not significantly different from the Murray, Macleay or Hunter Rivers. The Macleay, Hunter and Brisbane Rivers were not significantly different from each other, and all had a significantly lower proportion of lipids than eggs from the Murray River. Protein makes up the greatest proportion of egg yolk, on average 65% (Table 5.9). The proportion of protein was inversely related to the proportion of lipids, being high in the Brisbane River but low in the Murray River (Table 5.9). The lipid component was negatively correlated to egg dry mass in the Nepean River ($F=8.00$; d.f.=1,12; $p<0.05$; $r^2=0.40$), while no relationship was found in the Murray ($F=2.93$; d.f.=1,22; $p=0.10$), Macleay ($F=1.51$; d.f.=1,20; $p=0.23$), Hunter ($F=0.003$; d.f.=0.96; $p=0.96$) or Brisbane ($F=0.05$; d.f.=1,23; $p=0.83$) Rivers.

Mean percent shell ash ranged from 73.2% in the Macleay River to 79.6% in the Murray River (Table 5.10). There was a significant difference in the shell ash

Table 5.8. Means ± standard errors for wet and dry weights of egg yolks and egg shells for the Macleay, Hunter, Nepean, Brisbane and Murray Rivers.

River	N	Yolk wet weight	Yolk dry weight	Yolk (% dry weight) (n)	Shell wet weight	Shell dry weight	% dry wt (n)
Macleay	25	6.8266 ± 0.1165	0.7822 ± 0.0256	22.02 ± 0.30 (20)	0.7822 ± 0.0256	0.5427 ± 0.0256	69.63 ± 1.08 (n=25)
Hunter	34	6.6301 ± 0.1198	0.8330 ± 0.0195	18.73 ± 0.20 (28)	0.8330 ± 0.0195	0.5980 ± 0.0133	72.03 ± 0.73 (n=34)
Nepean	8	4.4464 ± 0.4604	0.5737 ± 0.0381	18.46 ± 1.16 (8)	0.5737 ± 0.0381	0.4206 ± 0.0316	73.10 ± 1.47 (n=8)
Brisbane	23	7.3309 ± 0.1088	0.8833 ± 0.0279	21.99 ± 0.31 (20)	0.8833 ± 0.0279	0.7158 ± 0.0187	83.27 ± 2.44 (n=23)
Murray	31	8.0847 ± 0.2051	1.1130 ± 0.0362	19.30 ± 0.31 (26)	1.1130 ± 0.0362	0.8379 ± 0.0282	75.47 ± 0.90 (n=31)

Table 5.9. Proportion of lipids and protein in egg dry mass (g) from the Macleay, Hunter, Nepean, Brisbane and Murray Rivers.

River		Lipids	Protein
Brisbane	Mean ± SE	0.291 ± 0.007	0.658 ± 0.007
	Range	0.235 – 0.352	0.615 – 0.705
	n	25	15
Hunter	Mean ± SE	0.310 ± 0.005	0.636 ± 0.005
	Range	0.259 – 0.365	0.607 – 0.673
	n	23	15
Macleay	Mean ± SE	0.313 ± 0.004	0.655 ± 0.009
	Range	0.285 – 0.359	0.616 – 0.685
	n	22	7
Murray	Mean ± SE	0.344 ± 0.005	0.633 ± 0.009
	Range	0.309 – 0.380	0.579 – 0.673
	n	23	10
Nepean	Mean ± SE	0.331 ± 0.008	-
	Range	0.292 - 0.388	
	n	14	

Table 5.10. Means ± standard errors for eggshell dry mass, eggshell percentage ash and eggshell ash expressed as the percentage of total egg dry mass for *Emydura macquarii* from the Macleay, Hunter, Nepean, Brisbane and Murray Rivers.

River	Gravid Females (n)	Eggshell dry mass (g)	Eggshell % ash	Eggshell ash % of total egg dry mass
Macleay	24	0.5508 ± 0.0191	73.19 ± 0.64	19.93 ± 0.68 (19)
Hunter	31	0.6032 ± 0.0142	77.73 ± 0.38	25.61 ± 0.33 (26)
Nepean	5	0.3908 ± 0.0150	74.05 ± 2.06	24.37 ± 4.44 (5)
Brisbane	23	0.7260 ± 0.0189	79.20 ± 0.50	24.67 ± 0.66 (20)
Murray	27	0.8116 ± 0.0312	79.61 ± 0.59	27.72 ± 1.02
(21)				

content (relative to the egg shell dry weight) between rivers (F=8.07; d.f.=3,101; $p<0.0001$). Eggs from the Macleay River had the lowest shell ash content, although this was not significantly different from the Murray River. Eggs from the Murray, Brisbane and Hunter Rivers were not significantly different from one another. Sample size for the Nepean River was too small to warrant inclusion in the analysis. Shell ash expressed as a percentage of total egg dry mass, ranged from 19.9% in the Macleay River to 27.7% in the Murray River (Table 5.10). There was a significant difference in the shell ash content relative to the total dry weight of the egg (F=18.93; d.f.=3,81; $p<0.0001$). Eggs from the Murray and Hunters Rivers had the highest ash content, while eggs from the Macleay River had the lowest. Eggs from the Brisbane River had significantly lower ash content than eggs from the Murray River, although not significantly different from the Hunter River. Sample size for the Nepean River was to small for inclusion in the analysis.

Linear regressions of shell ash on yolk dry mass were significant in the Hunter (F=31.35; d.f.=1,24; $p<0.0001$; $r^2=0.57$), Macleay (F=5.91; d.f.=1,17; $p<0.05$; $r^2=0.26$) and Murray (F=5.44; d.f.=1,19; $p<0.05$; $r^2=0.22$) Rivers. That is, as egg dry weight increases the proportion of ash content increases. There was no significant relationship between shell ash and yolk dry mass in the Brisbane River (F=0.22; d.f.=1,18; p=0.65).

5.3.9 Hatchling results

Using the residuals from a linear regression on egg weight and hatchling weight, a significant difference in relative hatchling weight among rivers was found (F=48.67; d.f.= 4,537; $p<0.0001$). Relative to egg size, hatchlings from the Brisbane and the Macleay Rivers were the heaviest, followed by the Murray River, while hatchlings from the Nepean and Hunter Rivers were the lightest. There was also a significant difference in relative hatchling weight between temperatures (F=3.58; d.f.= 2,537; $p<0.05$). Hatchlings at the incubation temperature 26^{o}C were significantly heavier than hatchlings incubated at 30^{o}C, but not significantly different from those hatched at 28^{o}C. There was no significant difference in hatchling weights between the incubation temperatures 28^{o}C and 30^{o}C. No interaction between river and temperature was found (F=1.34; d.f.= 8,537; p=0.22).

There was a significant difference in hatchling weight (hatchlings incubated at 26^{o}C and 28^{o}C combined) among rivers (F=419.23, d.f.=4,391, p<0.0001). All rivers were significantly different from each other with the largest hatchlings from the Murray River, followed by the Brisbane River, the Macleay River, the Hunter River and lastly the Nepean River (Table 5.11).

Hatchling weight was significantly related to wet egg mass in all rivers (Table 5.12). The relationship was particularly strong in the Nepean and Hunter Rivers. Hatchling weight was also significantly related to egg width (Table 5.12). Again this relationship was strong for hatchlings from the Nepean and Hunter Rivers. Hatchling weight was significantly related to egg length in the Hunter, Macleay and Murray Rivers, but not significantly related in the Nepean and Brisbane Rivers. The relationship of both egg length and egg width to hatchling size is of similar magnitude in the Hunter, Macleay and Murray Rivers, while only egg width determines hatchling size in the Nepean and Brisbane Rivers. Hatchling weight was not related to female body weight in any river except the Hunter (Table 5.12).

5.3.10 Incubation regime

The hotter the temperature the faster the incubation time (Table 5.13). Eggs hatched on average from 39.7 days at 30^{o}C in the Hunter River to an average of 57.8 days at 26^{o}C in the Murray River (Table 5.13). Eggs from the Hunter River incubated faster than other rivers, while eggs from the Murray River incubated slower. Eggs from the Nepean, Brisbane and Macleay Rivers had similar incubation times.

With the river data pooled there was a significant difference in the incidence of scute deformities at different incubation temperatures (χ2=17.20; p<0.0001) (Table 5.14). Deformities were significantly more common at 30^{o}C and 32^{o}C than at 26^{o}C and 28^{o}C. There was also a significant difference in the proportion of eggs that failed to

Table 5.11. Size of *Emydura macquarii* hatchlings reared at 26°C and 28°C from the Macleay, Hunter, Nepean, Brisbane, and Murray Rivers.

River	Weight(g) Mean ± SE Range CV	Carapace Length (mm) Mean ± SE Range CV	Carapace Width (mm) Mean ± SE Range CV
Macleay (n=56)	4.17 ± 0.06 3.31 – 5.40 10.8	27.7 ± 0.2 22.8 – 29.7 5.1	26.2 ± 0.2 22.9 – 29.2 5.5
Hunter (n=149)	3.80 ± 0.03 2.80 – 4.75 10.6	25.9 ± 0.1 23.1 – 28.5 4.3	24.2 ± 0.1 21.2 – 25.8 4.0
Nepean (n=17)	2.43 ± 0.09 1.84 – 2.90 14.48	23.1 ± 0.3 20.9 – 25.3 5.2	22.3 ± 0.4 19.5 – 24.9 7.8
Brisbane (n=46)	4.86 ± 0.05 4.25 – 5.82 6.8	28.6 ± 0.1 25.4 – 30.3 3.4	26.2 ± 0.2 21.2 – 25.8 4.8
Murray (n=129)	5.39 ± 0.03 4.44 – 6.15 7.3	30.2 ± 0.1 27.6 – 32.1 2.8	28.0 ± 0.1 24.8 – 29.9 3.5

Table 5.12. Relationships of reproductive traits with hatchling weights for the Macleay, Hunter, Nepean, Brisbane and Murray Rivers.

River	Reproductive Trait	Intercept	Slope	r2	F	P	Df
Macleay	Egg Mass	0.96	0.4382	0.46	45.17	<0.0001	1,53
	Egg Width	-1.41	0.2924	0.21	13.68	<0.0010	1,53
	Egg Length	0.21	0.1172	0.22	15.34	<0.0005	1,53
	Female Mass	3.26	0.0019	0.11	4.06	=0.05	1,33
Hunter	Egg Mass	0.78	0.4121	0.73	386.42	<0.0001	1,146
	Egg Width	-4.61	0.4280	0.61	225.35	<0.0001	1,145
	Egg Length	-2.30	0.1909	0.58	198.03	<0.0001	1,146
	Female Mass	2.08	0.0015	0.26	12.16	<0.005	1,34
Nepean	Egg Mass	-1.02	0.7028	0.90	137.93	<0.0001	1,15
	Egg Width	-5.19	0.4411	0.88	111.99	<0.0001	1,15
	Egg Length	1.29	0.0419	0.05	0.84	=0.37	1,15
	Female Mass	-	-	-	-	-	-
Brisbane	Egg Mass	2.38	0.2919	0.39	28.07	<0.0001	1,44
	Egg Width	-0.74	0.2731	0.52	46.85	<0.0001	1,43
	Egg Length	4.80	0.0014	0.00	0.001	=0.97	1,43
	Female Mass	4.11	0.0004	0.13	2.35	=0.14	1,16
Murray	Egg Mass	1.35	0.4096	0.60	191.69	<0.0001	1,127
	Egg Width	-1.88	0.3399	0.31	57.20	<0.0001	1,127
	Egg Length	-0.47	0.1643	0.29	52.00	<0.0001	1,127
	Female Mass	4.92	0.0002	0.02	0.40	=0.54	1,16

Table 5.13. Comparison of incubation periods (days) of eggs incubated at 26^{o}C, 27^{o}C, 28^{o}C, 30^{o}C and 32^{o}C between *Emydura macquarii* from the Brisbane, Hunter, Macleay, Nepean and Murray rivers.

River		26^{o}C	27^{o}C	28^{o}C	30^{o}C	32^{o}C
Brisbane	Mean	56.9	49.8	47.7	43.7	42
	N	13	30	34	15	2
	Range	56 – 60	48 - 52	45 – 51	42 – 47	41 – 43
Hunter	Mean	47.6		44.8	39.7	40.1
	N	87		5	69	14
	Range	45 – 53		43 – 47	38 – 41	39 – 42
Macleay	Mean	55	49.6	47.0	44.2	41.3
	N	12	35	23	12	15
	Range	55	48 - 54	42 – 48	40 – 47	39 – 45
Nepean	Mean	51.6		48.0	43.1	
	N	8		9	8	
	Range	50 – 54		47 – 50	42 – 45	
Murray	Mean	57.8		50.8	45.1	43.3
	N	64		44	67	32
	Range	54 - 65		48 - 60	42 - 54	39 - 47

Table 5.14. Comparison of hatchling deformities and the number of eggs that failed to hatch between incubation temperatures. Data for each river was pooled.

	26°C	28°C	30°C	32°C
Hatchling Deformities	13	19	25	6
No. Hatchlings	163	232	113	19
Eggs that Failed to Hatch	31	30	26	22
No. Eggs	174	247	123	62

hatch at different incubation temperatures ($\chi 2=67.64$; $p<0.0001$) (Table 5.14). The highest proportion of eggs that failed to hatch was at 32°C, while the lowest was at 28°C. There was no significant difference between the two incubation temperatures 26°C and 30°C.

5.3.11 Summary

Females from the Murray River produced the largest clutch sizes, the largest eggs and the heaviest clutch mass of any of the populations. *Emdura macquarii macquarii* from the Macleay River produced the smallest clutch sizes and the lightest clutch mass. Clutch sizes were similar in the Brisbane, Hunter and Nepean Rivers, however egg size and clutch mass were significantly different. Eggs, and as a consequence clutch mass, were largest from the Brisbane River population, and smallest in the Nepean River. Egg weights of *Em. m. macquarii* in the Macleay River were similar to egg weights from the Hunter River, although egg shapes were longer and narrower in the Macleay River.

5.4 Discussion

5.4.1 Effect of body size on reproduction

Most of the variation in reproductive traits *among* populations was associated with differences in maternal body size. Maximum body size differed markedly among populations (see Chapter 4). Large turtles as found in the Murray and Brisbane Rivers were able to produce greater clutch sizes, larger eggs and a heavier clutch mass than small-bodied populations such as the Macleay River. Female chelonians retain an entire clutch before laying, hence reproductive output is limited by their internal volume. A larger body size provides greater body cavity space to produce more eggs and a greater clutch mass, while a narrow pelvic width in smaller turtles constrains egg width

(Tucker *et al.,* 1978; Congdon and Tinkle, 1982; Congdon *et al.,* 1983; Congdon and Gibbons, 1987).

Body size also accounted for the majority of reproductive variability *within* populations. Except for the Nepean River, clutch size, clutch mass, egg mass and egg width were all positively correlated with body size. The one reproductive trait that failed to correlate with body size was egg length. The only significant relationship between egg length and body size was found in the Macleay River, and even here the proportion of variability explained was small. A large number of studies have also failed to find a relationship between egg length and body size (Chessman, 1978; Tucker *et al.,* 1978; Kennett, 1987; 1994; Thompson, 1983; Congdon and Gibbons, 1983), although this is not always the case (Mitchell and Pague, 1990; Vogt, 1990; Iverson and Smith, 1993). Unlike egg width, egg length is not constrained by the pelvic width of the female. Therefore, the large variability in egg length was unrelated to body size. Egg length was more variable than egg width in every river, and in the Macleay River egg length differed between years but egg width did not.

The variability of egg size within a river was dependent on the maximum body size a population could reach. In larger-bodied populations such as the Murray and Brisbane Rivers, an increase in clutch mass with body size was mainly a result of increasing clutch size, especially in the Brisbane River where egg size appears to be relatively constant. For the smaller-bodied populations (Nepean, Macleay and Hunter Rivers), differences in egg size were also important in explaining variation in reproductive output. For the Nepean River, 70 % of the variation in clutch mass could be explained by differences in egg size. Apart from the Nepean River, most of the variation in egg size was due to egg width. Apparently, morphological constraints exist on egg size for the smaller populations, where an increase in body size also enables an increase in egg size.

Unlike the other populations, no reproductive variables were related to body size in the Nepean River. It was particularly unusual that even clutch mass failed to increase with body size. Although the sample size was small, only in the case of egg length may this have contributed to the non-significant result. Large individuals that were gravid were extremely rare, hence the range of body sizes was small relative to other populations. In regression, the smaller the range of the independent variable the less powerful the analysis. Most of the females caught were young in age and possibly only breeding for the first or second time. These small-bodied turtles produced eggs that were small and of poor quality and possibly responsible for the large proportion of clutches that failed to develop. Consequently, the data are probably not representative of a typical turtle population.

5.4.2 Other factors influencing reproductive variability

Differences in reproductive traits were not totally dependent on body size. When adjusted for body size, all reproductive traits were still significantly different among populations. Relative to their body size, *Em. m. macquarii* from the Brisbane and Macleay rivers tended to produce smaller clutch sizes of larger eggs. Populations from the Nepean and Hunter River populations produced relatively larger clutch sizes of smaller eggs. Females from the Murray River produced both relatively large eggs and large clutch sizes. Despite the relationship between most reproductive traits and body size a large proportion of variation was still unexplained. Thus, factors other than body size exert substantial influence on reproductive traits such as clutch size and egg size within rivers.

5.4.2.1 Temporal variation

A measure of reproductive variation can be explained by annual differences within a population. In this study, the Macleay River females produced significantly shorter and lighter eggs in 1995 than in 1996. In the Murray River, clutch size and clutch mass were smaller in 1996 than in 1995, although small sample sizes prevented a significant result. Annual variation in clutch mass, egg length, egg width and egg mass were found in *Chrysemys picta* (Schwarzkopf and Brooks, 1996). Other studies note that variability of clutch size within an individual is as high as variation among individuals (Gibbons *et al.*, 1982; Gibbons and Greene, 1990). Such high variability relating various reproductive traits and body size may reflect fluctuating environments where reproductive output is only be maximised under favourable conditions (Gibbons and Greene, 1990; Iverson, 1992). Ford and Seigel (1989, 1989a) found clutch mass and clutch size for the viviparous *snake Thamnophis marcianus,* was very plastic in response to different resource levels. Egg size in females from the Macleay River may be reduced in unfavourable conditions rather than clutch size due to the small number of eggs produced per clutch. That is, egg quality is sacrificed rather than egg number. Therefore, far from being fixed by natural selection, reproductive traits within an individual are relatively plastic in response to changing environmental conditions.

5.4.2.2 Egg size:clutch size trade-offs

Another source of reproductive variation is a trade-off between egg size and clutch size. Egg size:clutch size trade-offs have rarely been found in freshwater turtles (Congdon and Tinkle, 1982; Congdon *et al.*, 1983; Congdon and Gibbons, 1985; Thompson, 1983; Brooks, 1986; Vogt, 1990; Schwarzkopf and Brooks, 1986; Kennett, 1994; Roosenburg and Dunham, 1997), although this could be largely due to the failure to adjust for female body size before testing (Ford and Seigal, 1989). In this study where body size was adjusted for, a trade-off in egg size with clutch size was found both within and across populations. There was a negative correlation of egg size with clutch size in the Hunter, Macleay, Murray and Nepean Rivers, although a small sample size prevented a significant result in the Nepean. There was no such trade-off in the Brisbane River population where egg size is relatively constant. Egg length is traded off with clutch size in the Macleay, Hunter and Murray Rivers. Egg width is negatively correlated with clutch size in the Murray River, and especially the Nepean River. Egg length, but not egg width, has been found to be negatively correlated with clutch size in some species of turtles (Iverson *et al.*, 1991; Rowe, 1994) and snakes (Ford and Seigel, 1989). It is most likely that in small-bodied populations such as the Macleay and Hunter Rivers, pelvic width constraints result in egg length being traded off.

A trade-off between egg size and clutch size is an important component of optimal egg size theories (Smith and Fretwell, 1974; Brockelman, 1975). A trade-off was found in this study, however this was only apparent after adjustments in body size were made. The significant relationships between egg size and body size in *Em. m. macquarii* contradicts optimal egg size theories as it demonstrates that there is no single optimal egg size for the population, only for an individual (Congdon *et al.*, 1983). Given the fact that pelvic width constrains egg size in smaller turtles, it could be argued that the optimal egg size corresponds to the largest egg that a female can produce relative to its body size (Schwarzkopf and Brooks, 1986). However, this proposition is rejected by authors such as Congdon and Gibbons (1985) who view that any relationship between egg size and body size violates current optimal egg size theories. Even if egg size is not constant in a population, the theory predicts that changes in resources should affect the individuals clutch size rather than egg size (Smith and Fretwell, 1974; Brockelman, 1975). However, within individuals of *Em. m. macquarii* from the Macleay River, egg size was smaller in 1995 than in 1996. There was little or no growth in carapace length between years (see Appendix C), so an increase in body size cannot explain this variation. The difference in egg size between years clearly demonstrates that in the Macleay River at least, *Em. m. macquarii* contradicts current optimal egg size theories.

“It is becoming clear that the idea of an optimal egg size (Smith and Fretwell) is hard to defend” (Kaplan and Cooper 1984). Instead, Kaplan and Cooper (1984) presented a theory (“adaptive coin-flipping”) that the high interclutch variation in egg size may be a result of selection against an unpredictable environment. Females are unable to predict the environmental conditions that hatchlings may face. Therefore, females deliberately alter the egg size in a random order in the hope that the appropriate phenotype may by chance correlate with the right environmental conditions. Kaplan and Cooper (1984) believe that over the lifetime of an organism, this would provide a higher fitness than an optimal egg size.

5.4.2.3 The effect of latitude on reproduction

Egg size-clutch size trade-offs in turtles are more apparent on a larger geographical scale. A trade-off between egg size and clutch size in some North

American species of turtles is often related to latitude (Moll and Moll, 1990; Vogt, 1990). Across a tropical to temperate gradient, egg size gets smaller and clutch size gets larger. Legler (1985) suggested that a latitudinal gradient in reproductive traits does not exist in Australian chelids. However, geographic variation in reproductive traits of turtles may be obscured by the effects of food availability (Gibbons and Tinkle, 1969) and altitude (Christiansen and Moll, 1973). Legler (1985) looked only at absolute egg size when examining a latitudinal gradient in egg size. As egg size is related to body size, and body size is uncorrelated with latitude in *Em. macquarii* (see Chapter 4), it is unlikely that a trend with absolute egg size will be detected. That is, the relationship between reproductive traits and latitude is confounded by differences in body size.

This study revealed that relative clutch size was larger and relative egg size was smaller for the high latitude populations (Hunter, Nepean, and Murray Rivers) than was the case for low latitude populations (Brisbane and Macleay Rivers). This pattern continues towards the tropical north. *E. m. krefftii* from Fraser Island (North Queensland) produces very small clutch sizes of relatively large eggs (Georges, 1985). Other species of *Emydura* from Northern Australia produce even smaller clutch sizes of extremely large eggs (Georges & Thomson, unpubl. data). As more clutches per year can be produced in tropical zones (see below), selection appears to favour egg quality over egg number.

Similarly, clutch mass is noted to vary geographically. A strong positive gradient of relative clutch mass with latitude is generally found within and across turtle species (Iverson, 1992). That is, turtles from higher latitudes put greater effort into each clutch than populations from more tropical regions. *Em. m. macquarii* reflects this pattern as the relative clutch mass was smallest at lower latitude (Brisbane River) and greatest at higher latitude (Murray River).

Variation in reproductive output with latitude is likely due to differences in clutch frequencies. Annual reproductive output can be maximised by increasing clutch mass with body size and/or increasing clutch frequency. Here exists the trade-off between clutch frequency and individual clutch mass. An advantage of producing multiple, relatively lighter clutches is that “not all eggs are put in one basket”. That is, the chances increase that some eggs may escape predation (Christiansen and Moll, 1973) or environmental catastrophes (Georges, 1983). Multiple clutching would also enable reproductive output to increase without a need to increase body size, thereby enabling females to mature earlier (Georges, 1983). However, in temperate climates with a short nesting season, less time is available for clutch production and incubation. Hence, selection favours fewer relatively heavier clutches, possibly constrained by female body size (Moll, 1979; Wilbur and Morin, 1988; Iverson, 1992).

Clutch frequency in *Em. macquarii* ranges from one clutch in the Murray River (Thompson, 1983) to three clutches on Fraser Island (Georges, 1985) to as many as five clutches in North Queensland (Legler and Cann, 1980). However, it is known that fluctuating environmental conditions can have more of an effect on clutch frequency than clutch size (Gibbons, 1982; Gibbons *et al.,* 1982). Therefore, not all individuals produce the maximum number of clutches. In some cases females may skip breeding altogether (Gibbons and Greene, 1978; Tinkle *et al.,* 1981; Congdon *et al.,* 1983; Congdon *et al.,* 1987). Chessman (1978) reported that as many as three clutches could be produced by *Em. m. macquarii* from the Murray River, although Thompson (1983) found that less than 10% of individuals produced multiple clutches.

Overall, clutch frequency in *Em. macquarii* would appear to be greater at lower latitudes. Anecdotal evidence of clutch frequency in this study would not contradict this. *Em. m. macquarii* from the Albert River (Brisbane) can produce up to four clutches per year (Limpus, pers. comm), while in the more temperate climates, most populations are likely to lay between one and two clutches annually. The vast majority of females (79 – 88 %) in Murray River sites around Albury only produce one clutch per year (Spencer, 2000). The delayed start to the nesting season in the high altitude Hunter River site makes a second clutch unlikely. Two clutches are likely to be produced in the Macleay and Nepean River sites, although there is evidence to suggest that a small proportion of females may produce a third clutch.

Studies have shown that despite differences in the size and number of clutches per season, reproductive potential between populations of the same species is much the same (Christiansen and Moll, 1973; Iverson, 1992). That is, more clutches with fewer eggs against fewer clutches with more eggs. In *Em. m. macquarii* however, there is quite a large difference in annual reproductive potential between populations (Table 5.15). Populations from more tropical regions appear to have a distinct advantage in terms of reproductive output, due obviously to the large number of clutches that can be produced. For example, populations from North Queensland can produce on average more than six times as many eggs per year as populations from the Hunter and Macleay rivers. However it must be noted that the reported clutch frequencies represent the most common number of clutches per individual. It is known that a small proportion of females from populations such as the Macleay, Nepean and Murray Rivers may produce another clutch on top of what is reported. An increased reproductive output by tropical populations may be a response to higher predation rates.

5.4.3 Egg shape

The egg length-width ratio of *Em. m. macquarii* from the Brisbane, Nepean, Hunter and Murray Rivers was similar to that of most North American turtles (Congdon and Gibbons, 1990). However in the Macleay River, eggs were more ellipsoidal than is typically found. Iverson and Ewert (1991) predicted that rounder eggs would be favoured in larger clutch sizes as this favours more efficient packing and/or to reduce dessication of the egg during development. The narrow pelvic width in smaller turtles limits the width eggs can reach (Tucker *et al.,* 1978; Congdon and Tinkle, 1982; Congdon *et al.,* 1983; Congdon and Gibbons, 1987) resulting in more elongated eggs. The positive correlation of egg shape with body size in this study suggests that smaller turtles maximise hatchling size by producing more elongate eggs. Therefore, pelvic width restraints in *Em. m. macquarii* from the Macleay River

Table 5.15. Comparison of the average reproductive potential between populations of *Emydura macquarii macquarii* as well as two populations of *Emydura macquarii krefftii* from Fraser Island (Georges, 1985) and North Queensland (Legler and Cann, 1980).

River	Mean Clutch Size	Clutch Frequency	Reproductive Potential	Annual Clutch Mass
Macleay	6.8	2	13.6	99.2
Hunter	13.1	1	13.1	95.8
Nepean	14.4	2	28.8	146.0
Brisbane	13.2	3	39.6	340.5
Murray	20.1	1	20.1	190.4
Fraser Is	6.2	3	18.6	137.6
Nth QLD	16.4	4 – 5	65.6 – 82.0	639.6 – 799.5

constrain an increase in egg size, and hence larger and more viable hatchlings, to longer rather than wider eggs.

5.4.4 Egg components

5.4.4.1 Eggshell

Em. m. macquarii, like all chelid turtles, have rigid-shelled eggs. As is the case with rigid-shelled eggs from other turtles (Congdon and Gibbons, 1985), eggshell made up a high proportion of total egg dry weight in *Em. m. macquarii* (Table 5.16). However, the inorganic material in eggshells of *Em. m. macquarii* was much higher than rigid-shelled eggs from North American species of turtles (Table 5.16). Consequently, inorganic material in *Em. m. macquarii* contributed about 5 % more in terms of the relative proportion of inorganic material to total egg dry mass. Increased inorganic material in eggshells of *Em. m. macquarii* may be a mechanism to cope with stressful hydric conditions experienced during incubation. The hatching success and size of hatchlings are not influenced by the hydric conditions of the nest environment in *Em. m. macquarii* (Thompson, 1983). Therefore, increased inorganic material may reduce water loss enabling eggs of *Em. m. macquarii* to hatch successfully despite an often dry incubation environment.

Eggs from the Macleay River had relatively less shell, as well as lower levels of inorganic material in the shell, than other populations of *Em. m. macquarii* (Table 5.16). On average, only 26.7 % of an egg from the Macleay River were made up of shell, a figure that Congdon and Gibbons (1985) would classify as an intermediate shelled egg rather than rigid-shelled. Inorganic material was also positively related to egg size in all rivers (especially the Hunter) except that of the Brisbane River. Therefore, as egg size is positively related to body size, smaller younger turtles would be providing less inorganic material per egg. That is, calcium could be a limiting factor in terms of both growth and egg shell development. Younger turtles may trade-off in favour of growth over eggshell quality. This may be especially important in the Macleay River where an unproductive environment greatly reduces body size.

Table 5.16. Comparison of the proportion of lipids, water, eggshell, eggshell ash and energy content of eggs between *Emydura macquarii macquarii* and various other turtle species from North America.

	Lipid Fraction (%)	Total energy kJ/g	% Water	% Shell	% Eggshell Ash	Source
Intermediate-shelled eggs						
Chelydridae						
Chelydra serpentina	33.8[a]	26.34	72.6		-	1
	23.5[b]	-	68.3	23.5	40.0	2,7
Flexible-shelled eggs						
Emydidae						
Chrysemys picta	33.0[b]	-	66.5	20.0	-	2
Deirochelys reticularia	31.5[b]	-	-	19.2	-	3
	32.4[b]	-	70.7		35.9	2,7
Malaclemys terrapin	26.4[b]	-	68.9		-	4
Pseudemys concinna	27.7[b]	-	72.8	15.8	38.3	2,7
Pseudemys floridana	29.0[b]	-	72.0	18.1	38.5	2,7
Terrapene carolina	25.8[b]	-	67.9	20.7	41.5	2,7
Trachemys scripta	30.4[b]	-	72.2	18.6	39.3	2,7
	40.7[a]	30.11	-		-	8
Rigid-shelled eggs						
Chelidae						

Table 5.16 (Continued)

	Lipid Fraction (%)	Total energy kJ/g	% Water	% Shell	% Eggshell Ash	Source
Emydura macquarii macquarii						
Brisbane River	29.1[a]	27.06	72.1	30.2	79.2	6
Macleay River	31.3[a]	27.86	72.7	26.7	73.2	6
Hunter River	31.0[a]	27.29	75.4	32.9	77.7	6
Nepean River	33.1[a]	27.96	75.8	31.4	74.1	6
Murray River	34.4[a]	28.57	74.4	34.4	79.6	6
Kinosternidae						
Kinosternon subrubrum	31.6[b]	-	61.2	43.9	52.8	2,7
Sternotherus *odoratus*	25.8[b]	-	64.5	40.5	52.2	2,7
Testudines						
Gopherus polyphemus	25.6[b]	-	65.3	36.4	52.1	2,7
	33.6[a]	28.91	66.1	33.1	76.8	5
Trionychidae						
Trionyx ferox	28.0[b]	-	70.7	31.4	50.4	2,7

1, Wilhoft, 1986 2, Congdon and Gibbons, 1985 3, Congdon et al., 1983 4, Ricklefs and Burger, 1977 5, Linley and Mushinsky, 1994 6, This study 7, Lamb and Congdon, 1985 8, Caudle, 1984

[a] includes both polar and nonpolar lipids

[b] includes only nonpolar lipids

5.4.4.2 Egg yolk

Lipids in this study averaged 31.8 % (of yolk dry weight) across populations (Table 5.16). Total lipid levels in the North American species *Trachemys scripta* were much higher, while *Chelydra serpentina* and *Gopherus polyphemus* were slightly greater than the average found here, although within the range (Table 5.16). Comparison of lipid levels with other studies listed in Table 5.16 is difficult as these figures only relate to nonpolar lipids (as opposed to polar and nonpolar in this study). Results from Table 5.16 suggest that polar lipids make up around 24 to 30 % of total lipids. If this were the case for *Em. m. macquarii*, then nonpolar lipids would average between 22.2 and 24.2 %. This would be well below reported levels in many North American species (Table 5.16). Delayed emergence of hatchlings from the nest is believed to be an important factor in increased lipids levels in some North American species of turtle (Congdon *et al.*, 1983). Delayed emergence is unknown in *Em. macquarii* and hence would explain the lower lipid levels relative to some species.

The previous report of lipid levels (total lipids) in the eggs of *Em. m. macquarii* (Murray River) gave much lower concentrations (18.8 %) than was the case in this study (Thompson, 1983). That value seems quite low when compared with overseas studies and therefore must be questioned. However, lipid levels were quite variable both within and among populations in this study. Lipid composition of egg yolk can be influenced by maternal diet (Noble, 1987b; Naber and Biggert, 1989), therefore extreme variation may exist in wild species (Romanoff and Romanoff, 1949). This may explain why turtles from the two most productive rivers, the Murray and Nepean, produced eggs with the highest proportion of lipids.

Compared to fowl, crocodilians and snakes, the proportion of yolk lipid used by embryos for development is very low in turtles (Noble, 1992). Only 38 % of lipids were depleted during the development of *Malaclemys terrapin*, *Chrysemys picta* and *Emydoidea blandingi* (Congdon and Tinkle, 1982; Congdon *et al.*, 1983). Most egg lipids are stored by hatchlings for use in maintenance or growth rather than for embryonic development (Congdon *et al.*, 1983; Congdon and Gibbons, 1985; Wilhoft, 1986). Residual yolk, a form of preovulatory parental investment in care, can fuel a turtle for a considerable time after hatching. For *Trachemys scripta,* stored lipids can support a standard metabolic rate at 28°C for approximately 55 days (Fischer, unpubl. data). Lower levels of lipids in the Brisbane River may not effect the size or quality of the hatchling, but its ability to survive once it is hatched. This may explain why despite low lipid levels, hatchlings from the Brisbane River were very large relative to egg size. The fact that *Em. m. macquarii* from the Brisbane River can lay up to four clutches per season may result in a trade-off in favour of more hatchlings over better quality ones. That is, the supply of high energy lipids is limited because of the large reproductive potential. In the Murray River, where one clutch is normally produced per season, lipids are not so limited, and more investment can be provided per egg.

Water content was negatively related with egg size in the Nepean, Murray and Macleay Rivers. Higher water content is positively correlated with a larger hatchling mass (Finkler, 1997). The smaller eggs may be compensated by increased water content enabling relatively larger hatchlings. This relationship was particularly strong in the Nepean River where egg size was significantly smaller than all other populations. Lipid levels were not related to egg size in any population apart from the Nepean River. Therefore, lipid levels are added proportionally as egg size increases.

The total energy levels of eggs in this study (Table 5.16) fell within the range of most reptilian species (25.1-29.3 kJ/g - Linley and Mushinsky, 1994). As would be expected, eggs with high percentages of lipids have high total energy values (Table 5.16).

5.4.5 Hatchlings

Eggs incubated at warmer temperatures tended to produce lighter hatchlings. Incubation temperature has a notable effect on the extent of yolk lipid uptake (Deeming and Ferguson ,1991). An incubation temperature of 34°C reduced yolk lipid uptake by 23% compared to eggs incubated at 28°C in *Crocodylus johnstoni* (Manolis *et al.*, 1987). The differences in the nest environment (both within and among clutches) can effect the size of the hatchling and the amount of nutrient reserves (Packard *et al.*, 1993). Warmer temperatures also increased the proportion of eggs that failed to hatch as well as the proportion of hatchlings with deformities. Failures and abnormalities were particularly magnified at temperatures above 30°C, which appears to be above an optimum incubation environment for *Em. m. macquarii*.

Selection would favour larger hatchlings as they have a greater chance of survival (Gutze and Packard, 1984; Janzen, 1990; 1993; Bobyn and Brooks, 1994), possibly due to differences in survival from nest to water (Janzen, 1993). However, hatchling size varied both within and among populations. Differences in hatchling size among rivers were largely a result of differences in the size of eggs. That is, the largest hatchlings come from the Murray River, where *Em. m. macquarii* produces the largest eggs, and the smallest hatchlings come from the Nepean River where eggs are extremely small. Hatchling size was also significantly related to egg size within each population. Yet not all variation in hatchling size can be explained by differences in egg size. Relative to egg size, hatchlings from the Brisbane River and Macleay River were significantly larger than other populations. Despite similar egg sizes between the Macleay and Hunter Rivers, hatchlings from the Macleay River were significantly heavier.

Variation in hatchling weights may partly relate to the length of the incubation periods which differed greatly between rivers. For example at 26°C, eggs from the Murray River took on average 12 days longer to hatch than eggs from the Hunter River. Legler (1985) found that incubation time in *Emydura* was inversely related to latitude. In contrast, this study documented that the most southerly located population (Murray River) had the longest incubation period. No pattern of incubation time with latitude, egg size or any other variable is apparent. Eggs from the Hunter River were the fastest to incubate, although the outcome was traded off with smaller hatchlings relative to egg size. Was this a response to the late breeding season and short growing period in the Hunter River? That is, selection favouring a shorter incubation period providing hatchlings with an extended growth period before winter.

5.4.6 Summary

Reproductive traits in *Em. m. macquarii* are highly variable, both within and across populations. Body size has a major significant effect on the reproductive traits of *Em. m. macquarii*. Large turtles produce more eggs, bigger eggs and a greater reproductive output than smaller turtles. However, reproductive traits vary greatly as a function of factors other than body size. Within female variation may be an important source of variation in reproductive traits of *Em. m. macquarii*. In turtles from the Macleay River, egg size differed within individuals from one year to another. Latitudinal effects are also an important factor in relation to the breeding biology of *Em. m. macquarii*. *Em. m. macquarii* at higher latitudes tend to lay fewer clutches, have a larger clutch mass, produce larger clutch sizes of smaller eggs and have a lower reproductive potential. Finally, some variation may be explained by the trade-off between egg size and clutch size. The negative correlation between clutch size and egg size suggests that a trade-off in favour of more eggs or larger eggs occurs to some degree. However, despite this trade-off, it was considered that *Em. m. macquarii* did not fit optimal egg size models.

Variation in egg components such as lipids, eggshell and water content between populations of *Em. m. macquarii* was also great. Some of this variation may be explained by differences in egg size, resource availability and selection pressures. Overall, *Em. m. macquarii* produces eggs with a high proportion of eggshell and a low proportion of lipids relative to turtles from North America. Hatchling size is not totally dependent on egg size in *Em. m. macquarii*. Incubation temperature, incubation periods, and egg quality may also be important in determining hatchling size.

Chapter 6

Synopsis

This is the first comprehensive study of variation in life history attributes for an Australian turtle. It contributes several significant advances to the understanding of turtle biology in general. This thesis provides data on fundamental biological parameters such as body size, growth, reproduction, and population structure of five populations of the widely distributed turtle *Emydura macquarii macquarii*. Detailed biological data are reported for the first time on populations of *Em. m. macquarii* from the coastal rivers of southeast Australia. Comparisons of the populations revealed new insights into patterns and causes of regional variation in life history traits. Furthermore, a detailed population study was conducted for the recently discovered Sydney Short-necked Turtle to assess the status of the population, as well as any key threats to its conservation.

Life history traits of *Emydura macquarii macquarii* were highly variable, both within and across turtle populations. This finding agrees with studies on emydid turtles where variability in life history traits is extensive at every level from the individual to the species. Even so, *Em. macquarii* displays greater plasticity in life history traits than other Australian turtles. It is a hardy generalist adapted to nearly any aquatic habitat over a wide distribution. Its inability to migrate overland has resulted in isolated populations in habitats of varying quality. Hence, habitat productivity is likely a major influence on the life history attributes. Turtles from productive lowland habitats such as the Murray River and Coopers Creek attain much larger maximum sizes than populations from the coastal flowing rivers along the Great Dividing Range. Unlike the case in the Northern Hemisphere, temperature is likely to play a minor role in determining life history characteristics relative to that of habitat productivity.

The most influential trait in life history variation within *Em. m. macquarii* was body size, largely through its effect on reproductive output, both within and among populations. Large turtles produced more eggs, larger eggs and a greater reproductive output per clutch than smaller turtles. A larger body size confers higher fitness to females as a fecundity advantage, but to males as an advantage in sexual selection. However, large differences in body size were found among populations. For example, maximum carapace length ranged from 185.4 mm in the Macleay River to 303.5 mm in the Murray River. Minimum carapace length at maturity ranged from 136.8 mm in the Macleay River to 222.5 in the Murray River. Differences of maximum body size among populations were shaped by juvenile growth rates, which in turn determined the size at maturity. For example, rapid juvenile growth rates result in a delayed size at maturity and a larger maximum size in the Murray River.

6.1 Models to explain variability

The critical question is whether the high variability in life history traits among populations is genetically fixed or a result of high phenotypic plasticity. If hatchlings from the small-bodied population in the Macleay River were released into the Murray River, would they develop a size similar to that of the resident population? Or would small body size be maintained by natural selection in their novel environment? I discuss four models that may explain the variation in life history traits.

- Founder effect

Founder effect is a random drift in the gene pool from the few individuals that colonise a novel population. Genetic drift combined with strong selection pressure can result in a rapid shift of haplotype frequency to a new coadapted combination (Barton and Charlesworth, 1984). The fewer the colonisers, the lower the probability that the gene pool will represent the parent population. A famous example of founder effects is the evolution of fauna indigenous to the Galapagos Islands. Mechanisms that operate in founder effect speciation are the loss of genetic variability, the relaxation of selection during population expansion or the deviations of genotypic frequencies from Hardy-Weinberg expectations (Barton and Charleworth, 1984).

Models of founder effects infer that variation in life history traits are the result of a few colonisers that reach each previously uninhabited drainage. The interaction of genetic drift and a unique environment results in turtles with phenotypes unlike that of the donor population. An ongoing process of colonisations as *Em. macquarii* expands its distribution results in a series of populations with greatly different body sizes, reproductive traits and other phenotypes. Although the theory predicts that phenotypic variation among rivers is a random draw from the gene pool of initial colonists, there is little empirical evidence that bottlenecks accelerate genetic change (see reviews by Barton and Charleworth, 1984; Carson and Templeton, 1984) or promote marked changes in phenotype (Barton and Charlesworth, 1984; Bryant and Meffert, 1990; 1996). Bottlenecks are usually too short to affect substantial genetic change through drift. Even a few invaders return sufficient genetic variation from the parent population and its erosion through small sample sizes takes time. Therefore, founder effects are unlikely to explain the high variability in life history traits among populations of *Em. macquarii*.

- Selection

In a natural selection, evolutionary processes shape the direction of variation in life history attributes. Selection favours the phenotypes that work best in their environment. Variation in maximum body size among island populations of tiger snakes *Notechis ater niger* were explained by differences in available prey size (Schwaner, 1985). For example on an island where the sole food source is large mutton-bird chicks, very large tiger snakes have evolved. Phenotypic variation was an expression of selection for island specific prey types.

Evolutionary processes are also responsible for latitudinal shifts in body size and reproductive traits. For a shorter breeding season, selection has favoured temperate populations to mature later and larger and reach larger maximum sizes than tropical populations (Sibly and Atkinson, 1994). The disadvantages of a lower clutch frequency in temperate environments become offset by the capacity of a larger body size to produce a greater reproductive output per clutch. In this sense, a phenotypic response to temperature is adaptive (Nunney and Cheung, 1997).

Therefore, this model predicts that when a new drainage was colonised, the novel environmental conditions selected for a phenotypic shift in traits from that of the original population. The problem with a selective model to explain body sizes (eg., Georges, 1985)) is that after intense selective pressure, variation in body size should have been dramatically reduced. There was no evidence of this factor in the Fraser Island populations (Georges, 1984) nor in populations in this study. Because growth rates and sizes at maturity were so variable within a population, other factors apart from selection must be influencing body size in *Em. macquarii*.

- Direct phenotypic response

When the environment varies over the range of a species, it is unlikely that the fitness of any single phenotype will be high under all conditions. Therefore, a species should evolve a genotype encompassing phenotypic variability to adjust to different environments in order to increase fitness in uncertain conditions (Kaplan, 1980; Cooper and Kaplan, 1982; Caswell, 1983; Kaplan and Cooper, 1984; Zhivotovsky *et al.,* 1996). This ability to change phenotypes may still be favoured even when the population has achieved a higher level of phenotype-environment matching (Moran, 1992).

Phenotypic plasticity is regarded as a character in its own right and under the control of selection (Perkins and Jinks, 1973; Weber and Scheiner, 1992; Scheiner 1993; Gotthard and Nylin, 1995; Blanckenhorn, 1998). Adaptive phenotypic plasticity is viewed in terms of a genotype-environment interaction (Moran, 1992). First, changes in developmental environments result in high plasticity with divergent phenotypes expressed under each unique set of environmental conditions. Second, selection favours those phenotypic alternatives that have the highest fitness.

The evolution of phenotypic plasticity shapes the evolution of generalists and specialists (van Tienderen, 1997). Fluctuating environmental conditions are thought to favour generalists by forcing all genotypes to perform a range of activities (Taper and Case, 1985; Lynch and Gabriel, 1987; Seger and Brockman, 1987). In the case of specialists, a high fitness in one environment would necessarily entail a low fitness in another environment due to differences in the technique and behaviour required in exploiting resources (Wilson and Yoshimura, 1994). Therefore, phenotypic plasticity is predicted to coincide with generalism whenever plasticity is advantageous (van Tienderen, 1997).

Australia turtles have evolved within an environment that is at the very least, as variable and as unpredictable as on other continents. *Emydura macquarii* has a generalist strategy in a wide range of environmental conditions. Consequently, it may have evolved a highly plastic phenotype in response to changing habitat quality. Put simply, as *Em. macquarii* colonises a novel area, it matches its phenotype to the demands of the new environment. The response would be unique for every individual, hence a range of phenotypes would result. Natural selection then favours phenotypes that provide the greatest fitness. Georges (1985) offered this explanation for the small body size of *Em. m. krefftii* from Fraser Island relative to that of mainland populations.

Several studies demonstrate that turtles alter the phenotype under different environmental conditions. Growth rates and age at maturity for male *Chrysemys picta* in a Michigan population were faster and younger in the late 1980’s than earlier in the decade due to warmer temperatures (Frazer *et al.,* 1993). Increased productivity and water temperature from the thermal effluent of a nuclear reactor resulted in greater growth rates and increased body size in a population of *Pseudemys scripta* (Gibbons, 1970; Gibbons *et al.,* 1979). Kennett (1994) recorded that an experimental reduction in the turtle density resulted in a direct change for many life history traits of *Chelodina rugosa*. However, captive rearing experiments suggest that variation in body size is not simply phenotypic. Hatchlings from Fraser Island raised in an ad-libidum pond grow to become small adults (Legler in pers. comm. with Georges) while pygmy crocodiles from Arnhemland when fed well in captivity become overweight rather than larger in body size (Banks, pers. comm.).

- Maternal Effects

Maternal effects are a “direct effect of a parent’s phenotype on the phenotype of its offspring” (Bernado, 1996, p 87). The maternal effect is a part of an offspring’s phenotype that does not result from the action of its own genes and the interaction of those genes with its environment (Bernado, 1996). For example, body size in some organisms may reflect the environmental conditions experienced by the mother. Egg size and composition mirror the female’s physiological state and size, which are determined by her pre-ovulatory lipid state. Egg size and composition can in turn effect progeny growth (Ebert, 1993; Bernado, 1996a) and in some cases may effect progeny body size at maturity (Fox and Savalli, 1998). Therefore maternal effects can influence offspring body size (Fox and Savalli, 1998). In fact, many demographic parameters are susceptible to maternal effects including age structure, sex ratio and recruitment rates (Bernado, 1996 and references within).

Maternal effects have recently been recognised as important in the evolution of natural populations (Kirkpatrick and Lande, 1989; Mousseau and Dingle, 1991). Maternal effects can persist in offspring phenotypes for a considerable time, even into subsequent generations (Ebert, 1993; Fox and Savalli, 1998). For example, egg size and quality can effect offspring fitness (Bernado, 1996a), which in turn can affect the subsequent propagule size. As a result, maternal variation in body size and egg size can be transmitted across multiple generations. Maternal effects may be particularly important in progeny phenotype early in ontogeny (Rossiter, 1996), yet the persistence of environmental variation across generations is apparently uncommon in animals (Roach and Wulff, 1987; Mousseau and Dingle, 1991; Fox, 1993; 1994; 1997; Bernado, 1996a), though not unknown (Fox, 1997).

The demonstration of inter-generational effects on phenotype opens up an intriguing possibility to explain geographic variation in body size. Consider a feedback loop whereby (a) smaller females invest less in eggs either by way of size or contents, (b) eggs with a lesser investment produce smaller hatchlings and (c) smaller hatchlings grow into smaller adults. The process of inter-generational reduction in body size in response to low per capita production does not invoke genetic change. The mechanism allows a rapid (relatively) response from population to population, and also explains why captive hatchlings from small-bodied populations grow fat rather than bigger when well fed.

6.1.1 Conclusions

Moran (1992) concluded that alternative phenotypes result more often from plasticity than genetic variability. However, it is difficult to distinguish between nonadaptive plasticity caused by physical and chemical constraints and adaptive plasticity (Gotthard and Nylin, 1995). To determine whether a particular trait is a result of adaptive or nonadaptive plasticity, reciprocal transplantations can reveal great insight (Gotthard and Nylin, 1995). Logistical constraints have prevented such work in turtles. Niewiarowski and Roosenburg (1993) employed reciprocal transplantations to determine the environmental dependence of growth rates in two widely separated populations of fence lizards, *Sceloporus undulatus*. The authors found that differences in growth rates were genetic based due to differences in the daily potential activity. However, Madsen and Shine (1993) concluded from a captive-reared study, that differences in body size of two localised populations of grass snakes (*Natrix natrix*) were due to phenotypic plasticity rather than adaptive selection.

The extreme variability in life history traits across populations of *Em. macquarii* reflects an interaction of adaptive selection, phenotypic plasticity and maternal effects. Adaptive selection would be an important factor in terms of life history variation over larger scale distributions where climatic differences (especially temperature) result in adaptive responses to a unique environment. However, differences between relatively localised populations would be more a result of phenotypic plasticity, where differences in food availability and food quality result in different phenotypic responses. Maternal effects may be responsible for some of the variation within populations, however it is unlikely to contribute greatly to the large differences between rivers. Even so, the ability of maternal effects to affect selection may confound our interpretation of within and among population variation in morphological and life history traits (Sinervo, 1990).

6.2 Nepean River population

An intensive population study on *Em. m. macquarii* was carried out at Nortons Basin in the Nepean River. This population was considered to be of conservation concern, as it was believed to be rare and on the decline. Life history traits of turtles from the Nepean River differed markedly with that of other populations of *Em. macquarii* in both this (chapters 4 and 5) and other studies (Chessman, 1978; Georges, 1983, Thomson, 1983). Growth rates were rapid, even after maturity. Maturity was hastened, rather than delayed so that body size could be enlarged. The Nepean River population is the only study of *Em. macquarii* with a male bias. The population was dominated by juveniles with a lack of old individuals. Annual reproductive output was high, while egg size from females in the Nepean River were the smallest ever reported for an *Emydura* species.

A significant difference between the Nepean River and all other rivers was the population density. While other populations were at, or close to the carrying capacity, the Nepean River population appeared to be well below that which could be supported. Therefore, the life history traits of turtles from the Nepean River are not currently influenced by density dependency factors. Kennett (1990) looked at the effect of population density on life history traits of turtles by removing 55 % of a population of *Chelodina rugosa*. Compared to before the harvest as well as two other control populations, adult and juvenile growth rates were rapid, the age at maturity was greatly reduced, clutch size was possibly increased and there was an increase in the number of juveniles. Therefore, these outcomes are similar to a contrast of the Nepean River population against other populations of *Em. macquarii*. Kennett (1994) hypothesised that this plasticity in life-history traits evolved in response to stochastic mortality during overland migration or as a result of traditional hunting activities by Aboriginal people. These traits enabled *C. rugosa* to recover quickly from such mortality events.

The life-history traits displayed by *Em. m. macquarii* in the Nepean River mainly reflect the low population. As the current population density is well below the carrying capacity, rapid growth, early maturation, and high reproductive output would enable the population to increase faster. This contradicts conventional wisdom that turtles as long-lived organisms have evolved life-history traits such as delayed maturity, low fecundity, and high adult survivorship, which combined, limit their ability to respond to increased mortality and hence low population densities (Brooks *et al.*, 1990; Congdon *et al.*, 1993; 1994).

While *Em. m. macquarii* in the Nepean River has both a spotty distribution and a low population density, the life history data suggests that the population is thriving and in an expansion phase. The question remains as to why the population is at such a low density to start with? Chapter Three mentioned that the Nepean population was at the latitudinal limit of its coastal distribution. Therefore, sublethal environmental conditions may be responsible for the small and fluctuating population sizes. However, an absence of *Em. m. macquarii* from coastal catchments south of the Nepean River does not necessarily reflect unfavourable environmental conditions, as populations in the Murray River catchment extend much further south into Victoria. Breeding populations also exist below Burrinjuck Dam near Canberra, a region that has very cold winters. Conditions for these populations are far more extreme than the coastal rivers south of Sydney. A more likely explanation is that *Em. m. macquarii* have never reached these catchments because of their inability to migrate between isolated waterbodies.

Therefore, it is improbable that the low density of turtles in the Nepean River is a result of unfavourable environmental conditions. An alternative hypothesis is that *Em. m. macquarii* are not native in the Nepean River but became established from turtles introduced by humans from source populations elsewhere in the range of *Em. macquarii*. There are several anecdotes to support this case:

- *Em. m. macquarii* was only discovered in the Nepean River in the late 1970’s. This appears to be quite remarkable considering its location within Australia’s largest city. Turtles are extremely easy to sight when basking on the sandstone rocks along the river.

- The distribution of *Em. m. macquarii* in the Nepean River was concentrated primarily near the more populous areas that are also potential sites of introduction. None were seen or caught in the remote areas above Warragamba Dam.

- The marginal location of population distribution gives credibility to the introduction hypothesis.

- The lack of old turtles in the population supports the premise that a population was established from a handful of individuals.

- Several turtles caught during this study were clearly individuals from other populations of *Em. macquarii*. These were most likely dumped pets.

- Non-native turtles have been introduced in other waterbodies around Sydney with seven species of turtles recorded in Centennial Park lakes (Stepherson 1986). The American turtle (*Trachemys scripta elegans*), which was a popular pet in the 1970's, is now established in the Sydney region, including the Nepean River.

There is little work published comparing the biology of introduced turtles with that of natural populations. Lindeman (1996) compared life history parameters of painted turtles *Chrysemys picta* between a natural lake and three recently created waste water lagoons (1969). The results were very similar to those found in this study. That is, the introduced population matured earlier, had rapid growth rates, a high proportion of juveniles, higher clutch sizes, a male bias, laid multiple clutches and was dominated by young cohorts.

All evidence for the introduction hypothesis is anecdotal however. The only way to establish whether the Nepean population is natural or introduced is through genetic studies. This work is currently being done by Dr. Brad Schaefer (UC-Davis) and preliminary results indicate that at least one individual in the Nepean population had the identical genetic sequence to that of individuals from the Hastings and Macleay Rivers. This is despite the population (Hunter River) between the Nepean and Macleay/Hastings Rivers having a genetic sequence that is distinctively different from all other populations of *Em. macquarii*. The most sensible interpretation is that this individual was introduced or is a descendent of turtles released from the Macleay/Hastings Rivers. The fact that this individual was a female larger (carapace - 192mm) than any recorded the putative donor individuals suggests that it is a descendent of turtles from the Macleay/Hastings Rivers, as opposed to being directly released.

However, the DNA analysis is still preliminary so no firm conclusions can be drawn. There is no doubt that some turtles in the population were released. The difficulty may be in determining whether there was an initial baseline population. At present, the most feasible explanation is that the Nepean River population of *Em. m. macquarii* was established by individuals released from other populations. Although the population is currently at a very low density, the situation is likely to change rapidly over the next 10-15 years as the population expands. In particular, the present sparseness of adult females will shortly be augmented by an abundance of recruiting females.

6.2.1 Management considerations

Low densities of *Em. m. macquarii* in the Nepean River could result from two possibilities. Either it is a marginal population with a patchy spatial distribution and the sparse fluctuating population is a natural feature or the population is an introduction from other populations of *Em. m. macquarii*. Each scenario calls for a different management strategy. For the first scenario, no major management actions would be required. There is no eminent threat to the population although at low density they are always at risk from a sudden catastrophe. The major threat to *Em. m. macquarii* at Nortons Basin is incidental mortality from recreational fishermen. Fishing is very popular in this region and short-neck turtles regularly get caught on fishing lines. One individual fisherman caught eight turtles in one day. Some turtles get released unharmed, but many die from the swallowed hook (stainless steel hooks) or drown when the fishing line gets entangled around logs.

If the introduction scenario is true, then more urgent management actions may be required. *Em. macquarii* is a robust and adaptable turtle that is generally found at very high densities and has a significant influence on the riverine ecosystem. If indeed *Em. m. macquarii* is introduced, the Nepean River population may increase to much higher densities in the future and possibly alter the riverine environment. In such circumstances it would be preferable that *Em. m. macquarii* be removed from the Nepean River.

6.3 Further research

Is Emydura macquarii macquarii native to the Nepean River?

It is unknown whether *Em. m. macquarii* in the Nepean River is a rare population on the limits of it distribution or whether the population was established from dumped individuals from other drainages. Until genetic studies are completed, uncertainty prevents any recommendations regarding the conservation of the population, and precludes the assessments the assessment of the unusual life history traits the population exhibits. Dr. Brad Schaffer (UC-Davis) is hopelessly finalising this research within the next few years.

What would be the effects of an increase in turtle density on the life history traits of the Sydney Short-necked Turtle?

The unusual life history traits of *Em. m. macquarii* from the Nepean River make it an ideal population for long-term study. Of particular interest would be the effect of increasing population density on the life history strategies. Would the life history attributes eventually converge with those of other populations of *Em. macquarii* as the Nepean River population increases? A long term study may also provide insights into the respective roles of adaptive selection, phenotypic plasticity and maternal affects as mechanisms that influence a population's life history attributes, especially if the population were found to be introduced.

What is the role of clutch frequency in relation to the life history attributes of Emydura macquarii macquarii?

This study was limited by a lack of hard data on clutch frequency for each population. Clutch frequency is often highly variable within both an individual and a population. Individuals may only produce one clutch while others will produce multiple clutches (Tinkle *et al.,* 1981; Gibbons, 1982; Schwarzkopf and Brooks, 1986; Wilbur and Morin, 1988; Mitchell and Pague, 1990). For example, in Lake Bonney in South Australia, less than 10% of *Em. m. macquarii* produced more than one clutch per season (Thompson, 1983). In some cases, independent of the previous reproductive season, females may skip reproduction in a year altogether (Tinkle *et al.,* 1981; Schwarzkopf and Brooks, 1986; Wilbur and Morin, 1988). Thus, clutch frequency may be a more important source of variation in annual reproductive output than clutch size (Gibbons, 1982). Accurate determination of the proportion of females that may produce multiple clutches or skip reproduction in a season is difficult (Schwarzkopf and Brooks, 1986) and far beyond the scope of this thesis. The focus of this thesis was primarily on trade-offs of clutch size-egg size and reproductive variation within a single clutch. Nevertheless, variation within a clutch maybe heavily influenced by the total annual reproductive output. Clutch frequency is an important component of the life history patterns of turtles (Iverson, 1992), and needs to be considered when determining geographic variation in egg size and clutch size.

What are the life history attributes of populations from other parts of the species range?

This study only looked at the variability in life history attributes of populations of *Em. macquarii* from south-east Australia (subspecies *macquarii*). Studies from other regions of Australia would give a more complete picture of variation across the species range. Of specific interest would be the population at Coopers Creek. This population exists in a true desert environment which would likely have an extreme effect on its biology. Investigations of populations from tropical environments in North Queensland would also raise new insights.

What is the variability in life history traits within a drainage?

The high degree of phenotypic plasticity in life history traits obscures whether the trait value represents a true value for that region. Gibbons (1990a) stated that a central problem with geographic variation studies in turtles is that variation of life history traits within a region is not determined before comparisons are made among regions. That is, a sample from one region is collected from a single population, then compared with another from a different region to show there is a significant difference. Hence, local variation will not be accounted for despite the plethora of studies that claim variation within a region can be high (Gibbons, 1967; Gibbons and Tinkle 1969; Gibbons *et al.*, 1982; Webb, 1984). It is critical that local variation be documented before differences among regions are attributed to geographic variation (Gibbons, 1990a).

References

Andrews, R.M. (1982). Patterns of growth in reptiles. In Vol. 13 of Biology of the Reptilia, edited by C. Gans and F.H. Pough, 273-320. Academic Press, London.

Atkinson, D. and R.M. Silby (1997). Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. Trends in Ecology and Evolution (TREE) 12 (6): 235-239.

Banks, C. B. (1987). Nesting, incubation and hatchling growth in captive Krefft's Tortoise, *Emydura krefftii*. Aust. Wildl. Res., 14: 551-558.

Barron, J.N. (1997). Condition-adjusted estimator of reproductive output in snakes. Copeia, 1997 (2): 306-318.

Barton, N.H. and B. Charlesworth (1984). Genetic revolutions, founder-events, and speciation. Ann. Rev. Ecol. Syst. 15: 133-164.

Bernado, J. (1996). Maternal effects in animal ecology. Amer. Zool., 36: 83-105.

Bernado, J. (1996a). The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. Amer. Zool., 36: 216-236.

Berry, J.F. and R. Shine (1980). Sexual size dimorphism and sexual selection in turtles (Order Testudines). Oecologia (Berlin) 44: 185-191.

Bjorndal, A.B. and A.B. Bolten (1988). Growth rates of immature green turtles, *Chelonia mydas*, on feeding grounds in the southern Bahamas. Copeia 1988: 555-564.

Blanckenhorn, W.U. (1998). Adaptive phenotypic plasticity in growth, development, and body size in the yellow dung fly. Evolution, 52 (5): 1394-1407.

Bobyn, M.L. and R.J. Brooks (1994). Interclutch and interpopulation variation in the effects of incubation conditions on sex, survival and growth of hatchling turtles (*Chelydra serpentina*). J. Zool., London, 233: 233-257.

Brockelman, W.Y. (1975). Competition, fitness of offspring, and optimal clutch size. Am. Nat. 109: 677-699.

Broin F. de (1987). The late Cretaceous fauna of Los Alamitos, Patagonia, Argentina. Part IV. Chelonia. Rev. Mus. Argent. Cienc. Nat., Bernardino Rivadavia, Paleontologia, 3 (3): 131-139.

Brooks, R.J., G.P. Brown, and D.A. Galbraith (1990). Effects of a sudden increase in natural mortality of adults on a population of the common snapping turtle (*Chelydra serpentina*). Can. J. Zool. 69: 1314-1320.

Brooks, R.J., C.M. Shilton, G.P. Brown, and N.W.S. Quinn (1992). Body size, age distribution, and reproduction in a northern population of wood turtles (*Clemmys insculpta*). Can. J. Zool. 70: 462-469.

Brown, G.P., C.A. Bishop, and R.J. Brooks (1994). Growth rate, reproductive output, and temperature selection of snapping turtles in habitats of different productivities. J. Herpetology, 28 (4):405-410.

Bryant, E. H., and L.M. Meffert (1990). Multivariate phenotypic differentiation among bottleneck lines of the housefly. Evolution 44: 660-668.

Bryant, E. H., and L.M. Meffert (1996). Morphometric differentiation in serially bottlenecked populations of the housefly. Evolution 50:935-940.

Bull, J.J., J.M. Legler, and R.C. Vogt (1985). Non-temperature dependent sex determination in two suborders of turtles. Copeia, 1985 (3): 784-786.

Bury, R.B. (1979). Population ecology of freshwater turtles, pp 571-602. In: Turtles: Perspectives and Research. M. Harless and H. Morlock (eds.). Wiley Interscience, New York.

Cann, J. (1969). Preliminary notes on some short-necked tortoises from eastern New South Wales. Vict. Nat. 86: 191193.

Cann, J. (1998). Australian Freshwater Turtles. Beumont Publishing, Singapore. 292 pp.

Cann, J., and J. Legler (1994). The Mary River Tortoise: a new genus and species of short-necked chelid from Queensland, Australia (Testudines: Pleurodira). Chelonian Conservation and Biology 1: 81-96.

Carson, H.L. and A.R. Templeton (1984). Genetic revolutions in relation to speciation phenomena: the founding of new populations. Ann. Rev. Ecol. Syst., 15:97-131.

Caswell, H. (1983). Phenotypic plasticity in life-history traits: demographic effects and evolutionary consequences. Am. Zool. 23:35-46.

Caudle, A.L. (1984). Effects of nest humidity and egg size on hatching success and growth of embryonic freshwater chelonians. Master's thesis. University of Georgia.

Chessman, B. (1978). Ecological studies of freshwater turtles in southeastern Australia. PhD Thesis, Monash University, Melbourne.

Chessman, B. (1984). Evaporative water loss from three South-eastern Australian species of freshwater turtle. Aust. J. Zool., 32: 649-655.

Chessman, B. (1988). Habitat preferences of freshwater turtles in the Murray Valley, Victoria and New South Wales. Aust. Wildl. Res., 15: 485-491

Christiansen, J.L. and E.O. Moll (1973). Latitudinal reproductive variation within a single subspecies of painted turtle, *Chrsemys picta bellii*. Herpetologica 29: 152-163.

Cogger, H.G., E.E. Cameron, and H.M. Cogger (1983). Zoological Catalogue of Australia Vol 1: Amphibia and Reptilia. Australian Government Publishing Service, Canberra.

Cogger, H.G. (1992). Reptiles and Amphibians of Australia. 5th Ed. (Reed:Sydney).

Congdon, J.D. (1989). Proximate and evolutionary constraints on energy relations of reptiles. Physiol. Zool. 62: 356-373.

Congdon, J.D. and D.W. Tinkle (1982). Reproductive energetics of the painted turtle (*Chrysemys picta*). Herpetologica 38: 228-237.

Congdon, J.D., A.E. Dunham, and R.C. van Loben Sels (1993). Delayed sexual maturity and demographics of Blanding’s Turtles (*Emydoidea blandingi*): Implications for conservation and management of long-lived organisms. Conserv. Biol. 7: 826-833.

Congdon, J.D., and R.C. van Loben Sels (1993). Relationships of reproductive traits and body size with attainment of sexual maturity and age in Blanding's turtles (*Emydoidea blandingi*). J. Evol. Biol. 6: 547-557.

Congdon, J.D., A.E. Dunham, and R.C. van Loben Sels (1994). Demographics of common snapping turtles (*Chelydra serpentina*): Implications for conservation and management of long-lived organisms. Amer. Zool., 34: 397-408.

Congdon, J.D., and R.C. van Loben Sels (1991). Growth and body size in Blanding's turtles (*Emydoidea blandingi*): relationships to reproduction. Can. J. Zool. 69: 239-245.

Congdon, J.D., J. Whitfield Gibbons, and J.L. Greene (1983). Parental investment in the chicken turtle (*Deirochelys reticularia*). Ecology, 64: 419:425.

Congdon, J.D., S.W. Gotte and R.W. McDiarmid (1992). Ontogenetic changes in habitat use by juvenile turtles, *Chelydra serpentina* and *Chrysems picta*. Canadian Field-Naturalist, 106 (2): 241-248.

Congdon, J.D., G.L. Breitenbach, R.C. van Loben Sels, and D.W. Tinkle (1987). Reproduction and nesting ecology of snapping turtles (*Chelydra serpentina*) in southeastern Michigan. Herpetologica 43: 39-54.

Congdon, J.D. and J. Whitfield Gibbons (1983). Relationships of reproductive characteristics to body size in *Pseudemys scripta*. Herpetologica 39 (2): 147-151.

Congdon, J.D., and J. Whitfield Gibbons (1985). Egg components and reproductive characteristics of turtles: relationships to body size. Herpetologica, 41:194-205.

Congdon, J.D. and J. Whitfield Gibbons (1987). Morphological constraint on egg size: a challenge to optimal egg size theory? Proc. Natl. Acad. Sci. USA. 84: 4145-4147.

Congdon, J.D. and J.Whitfield Gibbons (1990). Turtle eggs: Their ecology and evolution, p109-123. In: Life History and Ecology of the Slider Turtle. J.W. Gibbons (ed.). Smithsonian Institution Press, Washington D.C.

Congdon, J.D. and J.Whitfield Gibbons (1990). The evolution of turtle life histories, p45-54. In: Life History and Ecology of the Slider Turtle. J.W. Gibbons (ed.). Smithsonian Institution Press, Washington D.C.

Congdon, J.D., D.W. Tinkle and P.C. Rosen (1983). Egg components and utilization during development in aquatic turtles. Copeia 1983 (1): 264-268.

Cooper, W.S. and R.H. Kaplan (1982). Adaptive “coin-flipping”: a decision-theoretic examination of natural selection for random individual variation. J. Theor. Biol. 94:135-151.

Cox, W.A., J.B. Hazelrig, M.E. Turner, R.A. Angus, and K.R. Marion (1991). A model for growth in the musk turtle, *Sternotherus minor*, in a north Florida spring. Copeia 1991: 954-968.

Doddridge, L.K. (1992). The autecology of the Cooper Creek Tortoise *Emydura sp.* (aff. *kreffti*). Graduate Diploma Thesis: University of New England, Armidale N.S.W.

Dunham, A.E. (1978). Food availability as a proximate factor influencing individual growth rates in the iguanid lizard S*celoporus merriami*. Ecology 59: 770-778.

Dunham, A.E., and D.B. Miles (1985). Patterns of covariation in life-history traits of squamate reptiles: The effects of size and phylogeny reconsidered. Am. Nat. 126: 231-257.

Dunham, A.E. and J.W. Gibbons (1990). Growth of the slider turtle, p135-145. In: Life History and Ecology of the Slider Turtle. J.W. Gibbons (ed.). Smithsonian Institution Press, Washington D.C.

Ebert, D. (1993). The trade-off between offspring size and number in *Daphnia magna*: The influence of genetic, environmental, and maternal effects. Arch. Hydrobiol. Suppl. 90: 1-21.

Edmonds, J.H. and R.J. Brooks (1996). Demography, sex ratio, and sexual size dimorphism in a northern population of common musk turtles (*Sternotherus odoratus*).

Ehmann, H. (1992). Encyclopedia of Australian Animals-Reptiles. Harper Collins Publishers Pty Ltd Pymble Australia.

Ernst, C.H. (1990). Systematics, taxonomy, variation, and geographic distribution of the slider turtle, p57-67. In: Life History and Ecology of the Slider Turtle. J.W. Gibbons (ed.). Smithsonian Institution Press, Washington D.C.

Fabens, A.J. (1965). Properties and fitting of the von Bertalanffy growth curve. Growth 29: 265-289.

Finkler, M.S. (1997). Impact of egg content on post-hatching size, body composition, and performance in the common snapping turtle (*Chelydra serpentina*). Chelonian Conservation & Biology: 2 (3): 452:455.

Fitch, H.S. (1981). Sexual size differences in reptiles. Misc. Publ. Mus. Nat. Hist., Univ. Kansas 70: 1-72.

Ford, N.B. and R.A. Seigel (1989). Relationships among body size, clutch size, and egg size in three species of oviparous snakes. Herpetologica, 45 (1): 75-83.

Ford, N.B. and R.A. Seigel (1989a). Phenotypic plasticity in reproductive traits: evidence from a viviparous snake. Ecology, 70(6): 1768-1774.

Ford, N.B. and R.A. Seigel (1994). An experimental study of the trade-offs between age and size at maturity: effects of energy availability. Functional Ecology 8: 91-96.

Forsman, A. and R. Shine (1995). Sexual size dimorphism in relation to frequency of reproduction in turtles (Testudines: Emydidae). Copeia 1995 (3): 727-729.

Fox, C.W. (1993). Maternal and genetic influences on egg size and larval performances in a seed beetle (*Callosobruchus maculatus*): multigenerational transmission of a maternal effect? Heredity 73: 509-517.

Fox, C. W. (1994). The influence of egg size on offspring performance in the seed beetle, *Callosobruchus maculatus*. Oikos 71: 321-325.

Fox, C. W. (1997). The ecology of body size in a seed beetle, *Stator limbatus*: persistence of environmental variation across generations? Evolution, 51 (3): 1005-1010.

Fox, C.W. and U.M. Savalli (1998). Inheritance of environmental variation in body size: superparatism of seeds affects progeny and grandprogeny body size via a nongenetic maternal effect. Evolution, 52 (1): 172-182.

Frazer, N.B. and L.M. Ehrhart (1985). Preliminary growth models for green, *Chelonia mydas*, and loggerhead, *Caretta caretta*, turtles in the wild. Copeia 1985: 73-79.

Frazer, N.B. and R.C. Ladner (1986). A growth curve for green sea turtles, *Chelonia mydas*, in the U.S.Virgin Islands, 1913-1914. Copeia 1986: 798-802.

Frazer, N.B., J.W. Gibbons, and J.L. Greene (1990). Exploring Fabens' growth interval model with data on a long-lived vertebrate, *Trachemys scripta* (Reptilia: Testudinata). Copeia 1990: 112-118.

Frazer, N.B., J.W. Gibbons, and J.L. Greene (1991). Growth, survivorship and longevity of painted turtles *Chrysemys picta* in a southwestern Michigan marsh. Am. Midl. Nat. 125: 245-258.

Frazer, N.B., J.L. Greene, and J.W. Gibbons (1993). Temporal variation in growth rate and age at maturity of male painted turtles, *Chrysemys picta*. Am. Midl. Nat. 130: 314-324.

Freund, R. J. and R. C. Littel (1991). SAS procedures guide, Release 6.04 Edition, SAS Institute Inc., Cary, NC, USA.

Gaffney, E.S. (1977). The side-necked turtle family Chelidae: a theory of relationships using shared derived characters. American Museum Novitates 2620: 1-28.

Galbraith, D.A., and R.J. Brooks (1987). Survivorship of adult females in a northern population of common snapping turtles, *Chelydra serpentina*. Can. J. Zool., 65: 1581-1586.

Galbraith, D.A., C.A. Bishop, R.J. Brooks, W. Len Simser, and K.P. Lampman (1988). Factors affecting the density of populations of common snapping turtles (*Chelydra serpentina serpentina*). Can. J. Zool. 66: 1233-1240.

Galbraith, D.A., R.J. Brooks, and M.E. Obbard (1989). The influence of growth rate on age and body size at maturity in female snapping turtles (*Chelydra serpentina*). Copeia 1989: 896-904.

Georges, A. (1982a). Ecological studies of Krefft's River Tortoise, *Emydura krefftii* (Gray), from Fraser Island, Queensland. Ph.D. Thesis: University of Queensland.

Georges, A. (1983). Reproduction of the freshwater turtle *Emydura krefftii* (Chelonia: Chelidae). J. Zool., Lond. 201, 331-350.

Georges, A. (1985). Reproduction and reduced body size of reptiles in unproductive insular environments. Pages 311-318 in Biology of Australasian frogs and reptiles ed. by Grigg, G., R. Shine, and H. Ehmann, Royal Zoological Society of New South Wales.

Georges, A. (1994). Setting conservation priorities for Australian freshwater turtles. Herpetology in Australia. p49-58.

Georges, A. and J. M. Legler (1994). *Emydura sp.* (Fraser Island). Fraser Island short-necked turtle. In The Consevation Biology of Freshwater Turtles ed by P. C. H. Pritchard and A. G. J. Rhodin. IUCN Publications: Gland: Switzerland.

Georges, A. (1982). Diet of the Australian freshwater turtle *Emydura krefftii* (Chelonia: Chelidae), in an unproductive environment. Copeia, 1982 (2): 331-336.

Georges, A. and M. Adams (1992). A phylogeny for Australian chelid turtles based on allozyme electrophoresis. Australian Journal of Zoology, 40: 453-476.

Georges, A. and M. Adams (1996). Electrophoretic delineation of species boundaries within the short-necked freshwater turtles of Australia (Testudines: Chelidae). Zoological Journal of the Linnean Society, 118: 241-260.

Georges, A., C.J. Limpus, and C.J. Parmenter (1993). Natural history of the Chelonia. In Glasby, C.J., G.J.B. Ross, and P.L. Beesley (eds.), Fauna of Australia. Vol. 2A Amphibia and Reptilia, pp. 120-128. Australian Government Publishing Service. Canberra.

Gibbons, J.W. (1967). Variation in growth rates in three populations of the painted turtle, *Chrysemys picta.* Herpetologica Vol. 23, No.4: 296-303.

Gibbons, J.W. (1970). Reproductive dynamics of a turtle (*Pseudemys scripta*) population in a reservoir receiving heated effluent from a nuclear reactor. Can. J. Zool. 48: 881-885.

Gibbons, J.W. (1982). Reproductive patterns in freshwater turtles. Herpetologica 38: 222-227.

Gibbons, J.W. and J.L. Greene (1979). X-ray photography: a technique to determine reproductive patterns of freshwater turtles. Herpetologica: 35 (1) 86-89.

Gibbons, J.W. and D.W. Tinkle (1969). Reproductive variation between turtle populations in a single geographic area. Ecology 50:340-341.

Gibbons, J.W., and R.D. Semlitsch (1982). Survivorship and longevity of a long-lived vertebrate species: How long do turtles live? J. Animal. Ecology, 51: 523-527.

Gibbons, J.W., J.L. Greene, and K.K Patterson (1982). Variation in reproductive characteristics in aquatic turtles. Copeia 1982: 776-784.

Gibbons, J.W., R.D. Semlitsch, J.L. Greene, and J.P. Schubauer (1981). Variation in age and size at maturity of the slider turtle (*Pseudemys scripta*). Am. Nat. 117 :841-845.

Gibbons, J.W. (1970). Sex ratios in turtles. Res. Population Ecol. 12: 252-254.

Gibbons, J.W. (1990). Sex ratios and their significance among turtle populations. In: Gibbons, J.W. (Ed.). Life History and Ecology of the Slider Turtle. Washington, DC: Smithsonian Inst. Press, pp. 171-182.

Gibbons, J.W. (1990a). Recommendations for future research on freshwater turtles: What are the questions? In: Gibbons, J.W. (Ed.). Life History and Ecology of the Slider Turtle. Washington, DC: Smithsonian Inst. Press, pp. 311-317.

Gibbons, J.W. and J.L. Greene (1990). Reproduction in the slider and other species of turtles. In: Gibbons, J.W. (Ed.). Life History and Ecology of the Slider Turtle. Washington, DC: Smithsonian Inst. Press, pp. 124-134.

Gibbons, J.W., G.H. Keaton, J.B. Schubauer, J.L. Greene, D.H. Bennett, J.R. McAuliffe, and R.R. Sharitz (1979). Unusual population structure in freshwater turtles on barrier islands. Georgia J. Sci. 37: 155-159.

Gibbons, J.W. and J.E. Lovich (1990). Sexual dimorphism in turtles with emphasis on the slider turtle (*Trachemys scripta*). Herpetol. Monogr. 4: 1-29.

Gotthard, K. and S. Nylin (1995). Adaptive plasticity and plasticity as an adaptation: a selective review of plasticity in animal morphology and life history. Oikos, 74: 3-17.

Griffiths, K. (1987). Reptiles of the Sydney Region. Three Sisters Productions, Winmalee.

Griffiths, K. (1997). Frogs and Reptiles of the Sydney Region. University of New South Wales Press, Sydney.

Gutzke, W.H.N. and G.C. Packard (1984). Hatching success in relation to egg size in painted turtles (*Chrysemys picta*). Canadian J. Zool., 63:67-70.

Hinton, T.G., P.D. Fledderman, J.E. Lovich, J.D. Congdon, and J.W. Gibbons (1997). Radiographic determination of fecundity: is the technique safe for developing turtle embryos? Chelonian Conservation and Biology, 2 (3): 409-414.

Iverson, J.B. (1982). Biomass in turtle populations: a neglected subject. Oecologia, 55: 69-76.

Iverson, J.B. (1991). Patterns of survivorship in turtles (Testudines). Can. J. Zool., 69: 385-391.

Iverson, J.B. (1992). Correlates of reproductive output in turtles (order Testudines). Herpetological Monographs, 6: 25 – 42.

Iverson, J.B. and P.E. Moler (1997). The female reproductive cycle of the Florida softshell turtle (*Apalone ferox*). J. Herpetology, 31(3): 399-409.

Iverson, J.B., E.L. Barthelmess, G.R. Smith and C.E. DeRivera (1991). Growth and reproduction in the mud turtle *Kinosternon hirtipes* in Chihuahua, Mexico. J. Herpetology 25 (1): 64-72.

Iverson, J.B. and M.A. Ewert (1991). Physical characteristics of reptilian eggs and a comparison with avian eggs, p. 87-100. In: Egg incubation: its effect on embryonic development in birds and reptiles. D. Deeming and M.W.J. Ferguson (eds.). Cambridge Univ. Press, New York, New York.

Iverson, J.B., C.P. Balgooyen, K.K. Byrd, and K.K. Lyddan (1993). Latitudinal variation in egg and clutch size in turtles. Can. J. Zool. 71: 2448-2461.

Janzen, J.J. (1993). An experimental analysis of natural selection on body size of hatchling turtles. Ecology, 74 (2): 332-341.

Jolly, G.M. (1982). Mark-recapture models with parameters constant in time. Biometrics 38: 301-321.

Jones, R.L. and P.D. Hartfield (1995). Population size and growth in the turtle *Graptemys oculifera*. Journal of Herpetology, 29 (3): 426-436.

Kaplan, R.H. (1980). The implications of ovum size variability for offspring fitness and clutch size within several populations of salamanders (Ambystoma). Evolution 34:51-64.

Kaplan, R.H. and W.S. Cooper (1984). The evolution of developmental plasticity in reproductive characteristics: an application of the “adaptive coin-flipping” principle. Am. Nat, 123 (3): 393-410.

Kennett, R.M. (1987). Reproduction, growth and dispersal of the eastern long-necked turtle *Chelodina longicollis* Shaw (Testudinata: Chelidae) in the Jervis Bay Territory. Honours Thesis: Australian National University, Canberra.

Kennett, R.M. (1994). Ecology of two species of freshwater turtle, *Chelodina rugosa* and *Elseya dentata* from the wet-dry tropics of Northern Australia. Ph.D. Thesis: Northern Territory University, Darwin.

Kennett, R.M. and A. Georges (1990). Habitat utilization and its relationship to growth and reproduction of the eastern long-necked turtle, *Chelodina longicollis* (Testudinata: Chelidae), from Australia. Herpetologica, 46 (1): 22-33.

Kirkpatrick, M. and R. Lande (1989). The evolution of maternal effects. Evolution 43: 485-503.

Lake, P.S. (1994). Understanding the ecology of Australian running waters: an assessment of current endeavours and future research priorities. LWDRRDC occasional paper series no. 13/94. LWRRDC; EPA; Dept. of the Environment, Sport and Territories.

Lamb, T. and J.D. Congdon (1985). Ash content: Relationships to flexible and rigid eggshell types of turtles. J. Herpetol. 19: 527-530.

Legler, J.M. (1960). A simple and inexpensive device for trapping aquatic turtles. Proc. Utah Acad. Sci. 37: 63-66.

Legler, J.M. (1977). Stomach flushing: A technique for chelonian dietary studies. Herpetologica 33: 281-284.

Legler, J.M. (1985). Australian chelid turtles: reproductive patterns in wide-ranging taxa. p117-123 in Biology of Australasian Frogs and Reptiles ed. by G. Grigg, R. Shine and H. Ehmann, Royal Zoological Society of New South Wales, 1985.

Legler, J.M. and A. Georges (1993). Family Chelidae. In Glasby, C.J., G.J.B. Ross, and P.L. Beesley (eds.), Fauna of Australia. Vol. 2A Amphibia and Reptilia, pp. 142-152. Australian Government Publishing service. Canberra.

Limpus, C.J. (1984). Identification of ovarian follicles and oviducal eggs by cloacal examination of live Australian freshwater crocodiles, *Crocodylus johnstoni*. Aust. Wildl. Res. 11: 203-204.

Linley, T.A. and H.R. Mushinsky (1994). Organic composition and energy content of eggs and hatchlings of the gopher tortoise. Fish and Wildlife Research 13: 113-128.

Lindeman, P.V. (1993). Aerial basking by hatchling freshwater turtles. Herpetological Review 24 (3): 84-87.

Lindeman, P.V. (1996). Comparative life history of painted turtles (*Chrysemys picta*) in two habitats in the inland pacific northwest. Copeia, 1996 (1): 114-130.

Lovich, J.E. (1996). Possible demographic and ecologic consequences of sex ratio manipulation in turtles. Chelonian Conservation and Biology, 2 (1): 114-117.

Lovich, J.E. and J.W. Gibbons (1990). Age at maturity influences adult sex ratio in the turtle *Malaclemys terrapin*. Oikos, 59: 126-134.

Lovich, J.E. and J.W. Gibbons (1992). A review of techniques for quantifying sexual size dimorphism. Growth, Development, & Aging, 56: 269-281.

Lowry, O., N.J. Rosebrough, A.L. Farr, and R.J. Randall (1951). Protein measurement with the folin phenol reagent. Journal of Biological Chemistry 193:265-275.

Lynch, M. and W. Gabriel (1987). Environmental tolerance. Am. Nat. 129: 283-303.

McNicol, K. and A. Georges (1980). Observations on the eggs and hatchlings of *Emydura krefftii* from Fraser Island. Herptofauna 12 (1): 10-12.

MacCulloch, R.D. and D.M. Secoy (1983). Demography, growth and food of western painted turtles, *Chrysemys picta bellii* (Gray), from southern Saskatchewan. Can. J. Zool. 61: 1499-1509.

Madsen, T. and R. Shine (1993). Phenotypic plasticity in body size and sexual size dimorphism in European grass snakes. Evolution, 47:321-325.

Manning, B., and C.P. Kofron (1996). Evolution and zoogeography of Australian freshwater turtles. Memoirs of the Queensland Museum 39 (2): 319-331.

Manolis, S.C., G.J.W. Webb and K.E. Dempsey (1987). Crocodile egg chemistry. In Wildlife Management: crocodiles and alligators, ed. G.J.W. Webb, S.C. Manolis and P.J. Whitehead, pp. 445-472. Sydney: Surrey Beatty Pty Ltd.

Mayeaux, M.H., D.D. Culley. JR., and R.C. Reigh (1996). Effects of dietery energy: protein ratio and stocking density on growth and survival of the common snapping turtle *Chelydra serpentina*. J. World Aquaculture Society, 27 (1): 64-73.

Merila, J. and M. Bjorklund (1995). Fluctuating asymmetry and measurement error. Syst. Biol. 44(1): 97-101.

Mitchell, J.C. (1985). Female reproductive cycle and life history attributes in a Virginia population of painted turtles, *Chrysemys picta*. J. Herpetology, 19: 218-226.

Mitchell, J.C. (1988). Population ecology and life histories of the freshwater turtles *Chrysemys picta* and *Sternotherus odoratus* in an urban lake. Herpetological Monographs, 2: 40-61.

Mitchell, J.C. and C.A. Pague (1990). Body size, reproductive variation, and growth in the slider turtle at the northeastern edge of its range. In Life History and Ecology of the Slider Turtle ed. J. W. Gibbons. Smithsonian Institution Press, Washington D.C. p146-151.

Moll, E.O. (1973). Latitudinal and intersubspecific variation in reproduction of the painted turtle, *Chrysemys picta*. Herpetologica 29: 307-318.

Moll, E.O., and J.M. Legler (1971). The life history of a neotropical slider turtle, *Trachemys scripta*, in Panama. Nat. Hist. (Sci.) 11:1-102.

Moll, D. and E.O. Moll (1990). The slider turtle in the neotropics: adaptation of a temperate species to tropical environment. In Life History and Ecology of the Slider Turtle ed. J. W. Gibbons. Smithsonian Institution Press, Washington D.C. p 152 - 161.

Moran, N. (1992). The evolutionary maintenance of alternative phenotypes. Am. Nat., 139: 971-989.

Mousseau, T.A. and H. Dingle (1991). Maternal effects in insect life histories. Annu. Rev. Entomol. 36: 511-534.

Naber, E.C. and M.D. Biggert (1989). Patterns of lipogenesis in laying hens fed a high fat diet containing safflower oil. Journal of Nutrition, 119: 690-695.

Neter, J., M.H. Kutner, C.J. Nachtsheim, and W. Wasserman (1996). Applied linear statistical models. 4th Edition. Irwin, Burr Ridge, Illinois.

Niewiarowski, P.H. and W. Roosenburg (1993). Reciprocal transplant reveals sources of variation in growth rates of the lizard *Sceloporus undulatus*. Ecology, 74: 1992-2002.

Nunney, L. and W. Cheung (1997). The effect of temperature on body size and fecundity in female *Drosophila melanogaster*: evidence for adaptive plasticity. Evolution, 51 (5): 1529-1535.

Noble, R.C. (1991). Comparative composition and utilisation of yolk lipid by embryonic birds and reptiles. In Egg Incubation: its effects on embryonic development in birds and reptiles, ed. D.C. Deeming and M.W.J. Ferguson, pp. 17-28. Cambridge University Press.

Noble, R.C. (1987). Egg lipids. In Egg Quality: Current Patterns and Recent Advances, ed. R.G. Wells & C.G. Belyavin, pp. 159-177. London: Butterworths.

Packard, G.C., K. Miller, and M.J. Packard (1993). Environmentally induced variation in body size of turtles hatching in natural nests. Oecologia, 93: 445-448.

Palmer, A.R. and C. Strobeck (1986). Fluctuating asymmetry: measurement, analysis, patterns. Ann. Rev. Ecol. Syst. 17:391-421.

Palmer, A.R. and C. Strobeck (1992). Fluctuating asymmetry as a measure of developmental stability: implications of non-normal distributions and power of statistical tests. Acta Zool. Fennica 191:57-72.

Pappas, M.J. and B.J. Brecke (1992). Habitat selection of juvenile Blanding's Turtles, *Emydoidea blandingii*. J. Herpetology, 26 (2): 233-234.

Parker, W.S. (1984). Immigration and dispersal of slider turtles *Pseudemys scripta* in Mississippi farm ponds. Am. Midl. Nat. 112: 280-293.

Parmenter, C.J. (1976). The natural history of the Australian freshwater turtle *Chelodina longicollis* Shaw (Testudinata: Chelidae). Ph.D. Thesis, University of New England, Armidale.

Parmenter, R.R. (1980). Effects of food availability and water temperature on the feeding ecology of pond sliders (*Chrysemys s. scripta*). Copeia 1980: 503-514.

Perkins, J.M., and J.L. Jinks (1973). The assessment and specificity of environmental and genotype-environmental components of variability. Heredity 30:111-126.

Plummer, M.V. (1977). Reproduction and growth in the turtle *Trionyx muticus*. Copeia 1977: 440-447.

Pollock, K.H., J.D. Nichols, C. Brownie, and J.E. Hines (1990). Statistical inference from capture-recapture experiments. Wildlife Monographs 107, 1-97.

Preston, F.W. (1969). Shapes of birds' eggs: extant North American families. Auk 86: 246-264.

Pritchard, P.C.H. (1979). Encyclopedia of Turtles. T.F.H. Publications: Hong Kong.

Quinn, A.J., and J.L. Christiansen (1972). The relationship between pond bottom type of western painted turtles *Chrysemys picta belli* in Iowa, a preliminary report. Proc. Iowa Acad. Sci. 73: 67-69.

Ream, C., and R. Ream (1966). The influence of sampling methods on the estimation of population structures in painted turtles. Am. Midl. Nat. 75: 325-338.

Ricklefs, R.E., and J. Burger (1977). Composition of eggs of the diamondback terrapin. Am. Midl. Nat. 97: 232-235.

Roach, D.A. and R.D. Wulff (1987). Maternal effects in plants. Annu. Rev. Ecol. Syst. 18:209-235.

Romanoff, A.L. and A.J. Romanoff (1949). The Avian Egg. New York: John Wiley and Sons.

Roosenburg, W.M. and A.E. Dunham (1997). Allocation of reproductive output: egg and clutch size variation in the Diamondback Terrapin. Copeia 1977 (2): 290-297.

Rosen, S. (1995). Losing ground: an environmental history of the Hawkesbury-Nepean catchment. Hale & Iremonger Pty Ltd Sydney.

Rossiter, M. (1996). Incidence and consequences of inherited environmental effects. Annu. Rev. Ecol. Syst., 27: 451-476.

Rowe, J.W. (1992). Observations of body size, growth, and reproduction in Blanding's turtle (*Emydoidea blandingii*) from western Nebraska. Can. J. Zool. 70: 1690-1695.

Rowe, J.W. (1994). Egg size and shape variation within and among Nebraskan painted turtles (*Chrysemys picta bellii*) populations: relationships to clutch and maternal body size. Copeia 1994 (4): 1034-1040.

Scheiner, S.M. (1993). Genetics and evolution of phenotypic plasticity. Annu. Rev. Ecol. Syst., 24:35-68.

Schoener, T.W. and A. Schoener (1978). Estimating and interpreting body-size growth in some *Anolis* lizards. Copeia 1978: 390-405.

Schwaner, T., (1985). Population size and structure of black tiger snakes (*Notechis ater niger*) on offshore islands of South Australia. Pp. 35-46 in “Biology of Australasian Frogs and Reptiles” ed. by G. Grigg, R. Shine, and H. Ehmann. Roy. Zool. Soc. NSW, Sydney.

Schwarzkopf, L. and R.J. Brooks (1986). Annual variations in reproductive characteristics of painted turtles (*Chrysemys picta*). Can. J. Zool. 64: 1148:1151.

Seber, G.A.F. (1982). The estimation of animal abundance and related parameters. Second ed. MacMillan, New York, N.Y. 654 pp.

Seger and Brockman (1987). What is bet-hedging? Oxford surveys in evolutionary biology, 4: 181-211.

Shine, R. and J.B. Iverson (1995). Patterns of survival, growth and maturation in turtles. Oikos, 72: 343-348.

Sibly, R. M. and D. Atkinson (1994). How rearing temperature affects optimal adult size in ectotherms. Funct. Ecol. 8: 486-493.

Sinervo, B. (1990). The evolution of maternal investment in lizards: an experimental and comparative analysis of egg size and its effects on offspring performance. Evolution 44: 279-294.

Smith, C.C. and S.D. Fretwell (1974). The optimal balance between size and number of offspring. Am. Nat. 108: 499-506.

Smith, J. and P. Smith (1990). Fauna of the Blue Mountains. Kangaroo Press, Sydney.

Snedecor, G.W. and W.G. Cochran (1980). Statistical Methods. 7th ed. Iowa State University Press, Ames, Iowa. 507 pp.

Spencer, R. (2000). Population dynamics, nesting ecology and impact of the introduced red fox, *Vulpes vulpes*. PhD thesis, University of Sydney. <www.geocities.com/emydura1/rspencer.pdf>

St. Clair, R., P.T. Gregory, and J.M. Macartney (1994). How do sexual differences in growth and maturation interact to determine size in northern and southern painted turtles? Can. J. Zool. 72: 1436-1443.

Stearns, S.C. (1976). Life-history tactics: A review of the ideas. Quart. Rev. Biol. 51: 3-47.

Stearns, S.C. and J.C. Koella (1986). The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at sexual maturity. Evolution 40: 893-913.

Stepherson, G. (1986). A checklist of turtles living in Centennial Park, Sydney. Reptile Keepers Association, Issue 23.

Sydney Water (2000). Annual Environment and Public Health Report 2000, Sydney Water. (Report can be found at http://www.sydneywater.com.au/html/aer2000/welcome.htm)

Taper, M.L. and T.J. Case (1985). Quantitative genetic models for the coevolution of character displacement. Ecology, 66:355-371.

Thompson, M.B. (1983). The physiology and ecology of the eggs of the pleurodiran tortoise *Emydura macquarii* (Gray), 1831. Unpubl. PhD Thesis, Univ. Adelaide, Australia.

Thompson, M.B. (1983a). Populations of the Murray River tortoise, *Emydura* (*Chelodina*): the effect of egg predation by the red fox, *Vulpes vulpes*. Aust. Wildl. Res., 10: 363-371.

Thompson, M.B. (1985). Functional significance of the opaque white patch in eggs of *Emydura macquarii*. In ‘Biology of Australian Frogs and Reptiles’. (Eds. G. Grigg, R. Shine and H. Ehmann) pp. 387-395. (Royal Zoological Society of New South Wales, Sydney).

Thompson, M.B. (1988). Influence of incubation temperature and water potential on sex determination in *Emydura macquarii* (Testudines: Pleurodira). Herpetologica 44, 86-90.

Timms, B.V. (1973). A limnological survey of the freshwater coastal lakes of east Gippsland, Victoria. Aust. J. Mar. Freshw. Res. 24: 1-20.

Tinkle, D.W., J.D. Congdon, and P.C. Rosen (1981). Nesting frequency and success: implications for the demography of painted turtles. Ecology, 62: 1426-1432.

Tucker, J.K., R.S. Funk, and G.L. Paukstis (1978). The adaptive significance of egg morphology in two turtles (*Chrysemys picta* and *Terrapene carolina*). Bulletin Maryland Herp. Soc., 14 (1): 10-22.

Van Loben Sels, R.C., J.D. Congdon and J.T. Austin (1997). Life history and ecology of the Sonoran Mud Turtle (*Kinosternon sonoriense*) in southeastern Arizona: a preliminary report. Chelonian Conservation and Biology 2(3): 338-344.

Van Tienderen, P. H. (1997). Generalists, specialists, and the evolution of phenotypic plasticity in sympatric populations of distinct species. Evolution, 51 (5):1372-1380.

Vogt, C. (1990). Reproductive parameters of *Trachemys scripta venusta* in Southern Mexico. In Life History and Ecology of the Slider Turtle ed. J.W. Gibbons. Smithsonian Institution Press, Washington D.C. p162-168.

Warren, J.W. (1969). Chelid turtles from the mid-Tertiary of Tasmania. Journal of Paleontology 43:179-182.

Webb, G.J.W. (1984). A look at the freshwater crocodile. Pp. 395-399 in “Vertebrate Zoogeography and Evolution in Australasia” ed. By M. Archer and G. Clayton. Hesperian Press: Western Australia.

Weber, S. L. and S.M. Scheiner (1992). The genetics of phenotypic plasticity. IV. Chromosomal localization. J. Evol. Biol. 5: 109-120.

Wells, R.W. and C.R. Wellington (1985). A classification of the Amphibia and Reptilia of Australia. Australian J. Herpetology, Supplementary Series Number 1: 1-61.

Whitfield Gibbons, J., J.L. Greene, and J.P. Schubauer (1978). Variability in clutch size in aquatic chelonians. British Journal of Herpetology, Vol. 6: 13-14.

Wilbur, H.M. and P.J. Morin (1988). Life history evolution in turtles. In Vol. 16 of Biology of the Reptilia, edited by C. Gans and R. Huey, 387-439. A. R. Liss., New York.

Wilhoft, D. (1986). Eggs and hatchling components of the snapping turtle *Chelydra serpentina*. Comp. Biochem. Physiol. 84A: 483-486.

Wilson, D.S. and J. Yoshimura (1994). On the coexistance of specialists and generalists. Am. Nat. 144: 692-707.

Witzell, W.N. (1980). Growth of captive hawksbill turtles, *Eretmochelys imbricata*, in Western Samoa. Bull. Mar. Sci. 30: 909-912.

Wood, J.R., F.E. Wood, K.H. Critchley, D.E. Wilde, and M. Bush (1983). Laparoscopy of the green sea turtle *Chelonia mydas*. Brit. J. Herpetol. 6: 323-327.

Zar, J.H. (1984). Biostatistical analysis. 2nd ed. Prentice-Hall, Englewood Cliffs, N.J. 718 pp.

Zhivotovsky, L.A., M.W. Feldman, and A. Bergman (1996). On the evolution of phenotypic plasticity in a spatially heterogeneous environment. Evolution, 50: 547-558.

Appendix A

Construction of the formula used in determining growth rates from scute annuli is as follows:

$CL = aD + b$ Relationship between scute size and carapace length now

$CLo = aDo + b$ Relationship between scute size and carapace length at birth

$b = CLo - aDo$

$CL = aD + CLo - aDo$

$CLt = a(-Dt + Dnow) + CLnow$

$CLo = a(-Do + Dnow) + CLnow$

$a = (CLo - CLnow)/(-Do + Dnow)$

$CLt = (((CLo - CLnow)/ (-Do + Dnow)) * (Dt - Dnow)) + CLnow$

Appendix B

Results from Jolly-Seber (Model B) analysis for the Murray River.

Parameters	Mean ± SE	95% CI
PHI	0.93 ± 0.18	0.57 - 1.28
p	0.14 ± 0.04	0.06 - 0.22
M	178.78 ± 73.80	34.13 - 323.43
B	31.08 ± 81.44	-128.54 - 190.69
N (2)	455.67 ± 106.54	246.86 - 664.49
N (3)	715.98 ± 197.02	329.83 - 1102.14
N (4)	472.08 ± 134.25	208.96 - 735.20
Mean	547.91 ± 230.73	95.69 - 1000.13

The Goodness of Fit test for the Jolly-Seber Model B (constant survival rate per unit time and time specific capture probabilities) demonstrated the data adequately fitted the model ($\chi 2=2.56$; D.F.=4, p=0.63), The mean population size for Mungabareena Reserve in the Murray River was 547.9. However the estimate of 715.98 for N(3) would appear to be inflated, and a more reliable estimate would be the average of N(2) and n (4) - 464. The survival rate was high at 0.94. However, caution must be applied for these results as the capture probability (0.14) was low.

Appendix C

Comparison of reproductive data between years for individual females in the Murray and Macleay Rivers.

Murray River

ID	Year	Female Carapace Length	Clutch Mass	Egg Weight	Egg Length	Egg Width	Clutch Size
113	1995	267.2	158.72	9.92	36.68	21.08	16
	1996	267.5	173.33	10.2	37.42	21.2	17
121	1995	291.5	189.06	9.95	37.42	20.91	19
	1996	289.8	191.85	9.59	37.23	20.71	20
	1997		187.12	9.85	37.76	20.8	19
31	1995	287.1	223.17	10.14	35.89	21.63	22
	1996	285.7	195.33	9.77	34.76	21.68	20
32	1995	242.8	143.97	8.47	34.98	19.88	17
	1996	242.8	128.83	8.05	35.6	19.49	16
33	1995	253.5	179.39	8.97	33.96	20.85	20
	1996	251.9	161.45	9.5	34.36	21.12	17
94	1995	269.5	180.41	10.02	36.89	21.04	18
	1996	268.7	152.55	10.17	37.03	21.30	15
200	1996	284.7	213.93	8.91	35.58	20.29	24
	1997	285.3	204.53	8.18	34.67	19.74	25

Macleay River

ID	Year	Female Carapace Length	Clutch Mass	Egg Weight	Egg Length	Egg Width	Clutch Size
501	1995	167.0	49.57	7.08	33.51	18.73	7
	1996	168.7	49.47	8.25	34.80	19.75	6
504	1995	170.5	57.37	7.17	31.36	19.69	8
	1996	172.3	61.81	7.73	33.29	19.71	8
505	1995	166.8	47.32	6.76	31.91	18.59	7
	1996	166.3	38.62	7.72	36.16	18.5	5
506	1995	160.3	50.89	7.27	31.87	19.61	7
	1996	160.3	46.52	7.75	33.97	19.42	6
529	1995	166.2	48.38	8.06	34.53	19.53	6
	1996	166.0	55.90	7.99	34.61	19.43	7
530	1995	152.3	44.78	5.60	30.70	17.48	8

	1996	152.9	47.39	6.77	33.87	18.30	7
536	1995	170.5	36.64	7.33	35.96	18.44	5
	1996	170.2	44.55	6.36	32.63	18.07	7
543	1995	150.7	40.83	5.83	30.53	17.89	7
	1996	151.2	47.02	6.72	32.96	18.26	7
545	1995	154.5	51.54	7.36	34.19	18.73	7
	1996	155.2	47.39	7.90	36.12	18.78	6
553	1995	161.8	51.79	7.40	33.81	19.03	7
	1996	164.5	48.46	8.08	35.90	19.37	6
561	1995	155.0	46.25	7.71	35.52	18.87	6
	1996	153.7	47.77	7.96	37.80	18.53	6
563	1995	159.7	45.46	6.49	33.39	18.06	7
	1996	160.4	40.90	6.82	33.97	18.25	6