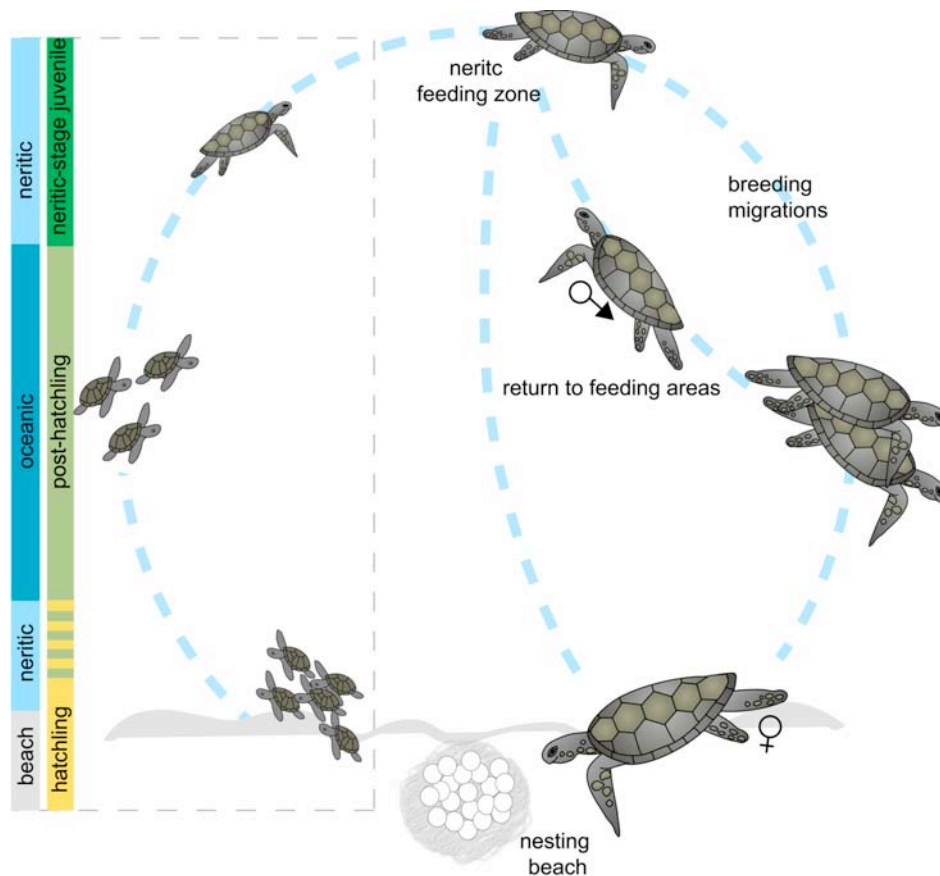


Post-hatchling sea turtle biology



This thesis is submitted for the degree of Doctor of Philosophy in the School of Marine and Tropical Biology at James Cook University, Townsville, Queensland, Australia by

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October 2006

Statement of sources

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references given.

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Statement of the contribution of others

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Glossary of terms

Many of these definitions are adapted from Lawrence, 1989 and Lincoln et al., 1998.

Anadromous	Referring to fishes that spend all or part of their adult life in salt water and return to freshwater streams and rivers to spawn.
Benthic	Organisms living in or on the bottom substrate of an aquatic environment.
Curved carapace length	A carapace length measurement for turtles. Measured from the anterior point at midline of nuchal scute to the posterior notch at midline between the supracaudal marginal scutes.
Clade	A monophyletic group of taxa sharing a closer common ancestry with one another than with members of any other group of taxa.
Colonisation	The invasion, and subsequent occupation of a new habitat by a species.
Colonisation bottleneck	A decrease in population density with resulting decrease in genetic variability through the process of colonisation.
Demersal	Organisms dwelling at or near the bottom of the sea or other body of water.
DNA	Deoxyribonucleic acid, the molecule of heredity that encodes genetic information.
DNA sequencing	The determination of the exact order of nucleotides in a segment of DNA.
Epipelagic	The upper part of the oceanic zone (normally photic) from the surface to about 200m depth.
Gene	A hereditary unit consisting of a sequence of DNA that occupies a specific location on a chromosome and determines a particular characteristic in an organism.
Genetic marker	A gene or segment of DNA with an identifiable physical location on a chromosome.
Haplotype	A unique DNA sequence usually referring to mtDNA.
Management Unit	Populations showing significant divergence in allele frequencies at nuclear or mitochondrial loci, regardless of phylogeny of alleles.
Microsatellite	A length of repetitive DNA composed of a variable number several to one hundred of more tandem repeats.
Monadrous	Referring to a mating system in which a female mates with only one male during a breeding season.
Natal origin	An individual's birth place.
Negative control	A PCR reagent to which no DNA has been added, used to indicate contamination of PCR reagents.
Neonate	With reference to marine turtles, a newly emerged hatchling, especially less than two days old.

Neritic		The inshore marine environment where bottom depths do not exceed 200m in depth.
Neuston		Minute organisms that float or swim on the surface of water.
Nucleotide		A subunit of DNA or RNA consisting of a nitrogenous base, a phosphate and a sugar molecule.
Oceanic		The open ocean environment where waters exceed 200m in depth.
Ontogenetic		The development history of an individual organism from its origin to its death.
Polymerase reaction (PCR)	chain	A technique for amplifying a region of DNA by separating the DNA into two strands and incubating it with flanking primers and DNA polymerase.
Pelagic		Organisms that occupy the water column in either the neritic or oceanic zone.
Philopatry		In animal behaviour, the tendency of a migrating animal to return to a specific location in order to breed or feed. Species that return to their birthplace in order to breed are said to exhibit natal philopatry.
Phylogeny		The evolutionary history of lineages or species.
Phylogenetic tree		A diagram showing the evolutionary relationships of a group of organisms that descended from a common ancestry. The distance of one group from the other groups indicates the degree of relationship.
Plankton		Tiny animals and plants floating in the sea or in lakes, usually near the surface.
Polyandrous		Referring to a mating system in which a female mates with several males during one breeding season.
Restriction length polymorphism (RFLP)	fragment polymorphism	Variations in the length of restriction fragments resulting from action by a specific endonuclease in a given genetic locus.
Stable Isotope		An isotope which does not spontaneously undergo radioactive decay.
Taq polymerase		A thermostable polymerase isolated from the thermophilic bacterium <i>Thermus aquaticus</i> . Often used in polymerase chain reaction.
Trophic shift		An organisms change of feeding habits.

Acronyms

BW	Beach washed (stranded)
CCL	Curved carapace length
CS	Coral Sea
DNA	Deoxyribonucleic acid
EAC	East Australian Current
MICRON	Micronesia
MR	Mon Repos, Queensland
MSA	Mixed stock analysis
mtDNA	Mitochondrial deoxyribonucleic acid
NC	New Caledonia
nDNA	Nuclear deoxyribonucleic acid
NEPNG	North-east Papua New Guinea
NGBR	Northern Great Barrier Reef
PF	Predatory fish
RFLP	Restriction fragment length polymorphism
SEC	Southern Equatorial Current
SGBR	Southern Great Barrier Reef
SIA	Stable isotope analysis
SR	Swains Reef, Queensland
WR	Wreck Rock, Queensland

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The post-hatchling stage of a sea turtle's life history has often been referred to as the 'lost years', reflecting the lack of understanding about this phase in their life. Obtaining information on where post-hatchlings go, or for how long, is significantly hindered by the elusiveness of a post-hatchling in its natural environment and the limitations of tagging technologies to track a hatchling as it leaves its nest. Consequently, much of what is understood of the post-hatchling life stage has been derived from indirect methods. As a result, our current understanding of post-hatchling biology is based on information gathered from stranded animals, opportunistic reports of sightings at sea, studies of hatchling behaviour, and more recently genetic based studies.

Although knowledge on the post-hatchling stage has progressed considerably in the last few years, studies have been limited primarily to loggerhead turtles in the northern Atlantic Ocean and northern Pacific Ocean. Thus there are substantial gaps in our knowledge of the life history of sea turtles for many regions of the world. The aim of this study is to increase the understanding of the ecology of loggerhead and green post-hatchling sea turtles in the southwest Pacific Ocean. The information acquired on the post-hatchling phase of sea turtle life history will help direct future regional management of these animals by providing region-specific information on the migratory routes and habitats occupied during the post-hatchling stage. This study also informs our global understanding of the sea turtle post-hatchling biology.

This study employed a multidisciplinary approach, incorporating ecological information from spatial and temporal distributions, diet and stable isotopes, and genetic methodologies. Post-hatchlings were sourced from strandings and from the stomachs of dolphin fish (*Coryphaena hippurus*). In addition, records were collated from the Queensland Environmental Protection Agency's database of marine wildlife strandings and deaths.

Data on the spatial and temporal distribution of post-hatchlings in relation to rookery location and oceanographic features compiled in this study provides evidence that loggerhead and green post-hatchlings from populations in the southwest Pacific region become entrained in oceanic currents and live a pelagic existence. Occupancy of an oceanic and pelagic habitat is supported by stable isotope signatures. In addition dietary investigations that show post-hatchlings in the southwest Pacific Ocean, from both of the investigated species, derive nutritional sustenance primarily from neustonic animal matter.

The spatial and temporal data on the two species of post-hatchlings however, indicates that the two species do not take the same migratory route after departing from the same coastal waters. The data provides strong evidence that loggerhead post-hatchling undergo trans-Pacific migrations within the southern Pacific sub-tropical gyre. This is suggested by; (i) incremental post-hatchling size increase in direction of this current away from nesting beaches, (ii) reports of loggerhead post-hatchlings are in New Zealand waters and on the eastern side of the southern Pacific, and (iii) loggerhead post-hatchlings larger than 13.7 cm CCL are not documented in the southwest Pacific Ocean. Although the current resolution of the genetic stocks in the southern Pacific does not allow differentiation between stocks on a regional scale, there is discrimination at the oceanic scale. Analysis of the haplotypes of the

loggerhead post-hatchlings shows that all specimens investigated in this study originated from southwest Pacific rookeries.

Whereas the data implies that loggerhead post-hatchlings embark on trans-Pacific migrations, it suggests that green post-hatchlings do not. Whilst this species also occupies offshore oceanic waters, it appears they remain in the southwest Pacific region. This is indicated by; (i) green post-hatchlings occupying waters around offshore seamounts (whereas loggerhead post-hatchlings appear absent), (ii) the absence of green post-hatchlings in New Zealand or southeast Pacific waters, and (iii) the occurrence of larger size classes of green post-hatchlings stranded on eastern Australian coast. Mixed stock analysis (using SPAM & TURTLE) performed with haplotypic information from post-hatchlings calculated that green post-hatchlings originate from the SGBR (60%), Coral Sea (27%) and New Caledonia (13%) rookeries.

This study is the first to describe the route that loggerhead and green post-hatchlings from the Australian region are taking. I demonstrate that these two species are undertaking significantly different migrations during this stage of their life. The principal findings of this study support the currently accepted view on the sea turtle's post-hatchling stage, for most species, is that of a pelagic oceanic existence.

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Chapter 1

General introduction: Post-hatchling sea turtle ecology

Extant sea turtles species

There are seven species of extant sea turtles, grouped into two families: Dermochelyidae and Cheloniidae. Dermochelyidae has a single species, the leatherback turtle (*Dermochelys coriacea*) and Cheloniidae contains six species in five genera; the green turtle (*Chelonia mydas*) (the black turtle (*C. agassizi*) is subjectively classed as a sub species of *C. mydas*), the flatback turtle (*Natator depressus*), the hawksbill turtle (*Eretmochelys imbricata*), the loggerhead turtle (*Caretta caretta*), the olive ridley turtle (*Lepidochelys olivacea*) and the Kemp's ridley turtle (*L. kempii*).

Global distribution

Sea turtles are primarily distributed throughout tropical, and to a lesser extent subtropical, waters of the Pacific, Indian and Atlantic Oceans. The exception to this is the leatherback turtle (*Dermochelys coriacea*), which is found world wide from the North Sea and the Gulf of Alaska in the Northern Hemisphere, to Chile and New Zealand in the Southern Hemisphere (Hamann *et al.*, 2006; James *et al.*, 2005; Luschi *et al.*, 2003b). Special adaptations allow this species to survive and function in very cold waters resulting in its broader geographic range (Friar *et al.*, 1972; Greer *et al.*, 1973).

The loggerhead and green turtle are both widely distributed in tropical and subtropical waters throughout the world, with the loggerhead turtle tending to be more common in temperate waters than the green turtle (Marquez, 1990). The loggerhead turtle has major breeding aggregations in Oman (Baldwin *et al.*, 2003) (Indian Ocean), the eastern USA (Ehrhart *et al.*, 2003) (Atlantic Ocean), southern Queensland (Limpus and Limpus, 2003b) and southern Japan (Kamazaki *et al.*, 2003) (Pacific Ocean), and Cyprus, Greece, and Turkey (Margaritoulis *et al.*, 2003) (Mediterranean Sea). The green turtle has major nesting colonies in north eastern Costa Rica, eastern Surinam, the barrier reef islands of Australia and on the remote oceanic islands of Ascension Island and Atol das Rocas (Pritchard, 1997).

The hawksbill turtle and the olive ridley turtle have the most tropical range of all the sea turtles species, occupying only the tropical waters of the Atlantic, Pacific and Indian Oceans. The hawksbill turtle is found in association with coral reefs throughout warm tropical waters of the central Atlantic and Indo-Pacific regions and rarely strays into temperate waters (Witzell, 1983). The Olive Ridley turtle is pan-tropical in distribution but is rarely found around oceanic islands. Major olive ridley breeding aggregations occur in Mexico, the west coast of Costa Rica, Surinam, and the east coast of India (Limpus, 1995a).

The black turtle, the flatback turtle and the Kemp's ridley turtle have the most restricted distributions of all the sea turtle species. The Kemp's ridley turtle is the rarest sea turtle in the world with only one major nesting site known from Mexico, while the black turtle is confined to the eastern Pacific. Australia's endemic flatback turtle has the most restricted range of all the sea turtle species, and is confined to the coastal waters throughout the northern regions of Australia, the Gulf of Papua New Guinea and southern Indonesia (Limpus *et al.*, 2000; Suarez, 2000).

The value of sea turtles

At the high population levels that are considered to once have existed in prehistoric times, extant sea turtles are presumed to have had a substantial influence on the marine systems they inhabited (Bjorndal and Jackson, 2003). Sea turtle species have diverse diets and occupy a wide range of habitats (Bjorndal and Jackson, 2003). Sea turtles thus fulfil a variety of ecological roles from causing broad scale modification of landscapes, consumers of primary, secondary and tertiary productivity and as habitat for other organisms that live on, in and with them (Bjorndal and Jackson, 2003).

Archaeological remains indicate that sea turtles have had nutritional, economic and spiritual significance for many civilisations dating back millennia (reviewed by Frazier, 2003). Artefacts show that turtles had a variety of uses, including; as a source of food, as ceremonial objects, as objects of trade or exchange, as ornamental objects, (e.g. tortoiseshell), cultural artefacts (e.g. hairpins, head ornaments), and for practical purposes (e.g. tortoiseshell for fishing hooks, carapaces for vessels). For many remote Indigenous and non-Indigenous communities sea turtles still play a significant part in day-to-day living, particularly as a subsistence food source using their meat, fat and eggs (reviewed by Campbell, 2003).

Sea turtle conservation status

Worldwide, many sea turtle populations have experienced significant declines, for which the primary causes can be categorised into three areas: habitat degradation (destruction and alteration), direct capture for meat, hides, eggs and carapace, and incidental capture in fisheries (reviewed by Lutcavage *et al.*, 1997). The most apparent result of these interactions have been changes in population numbers and their geographic distributions. Additionally however, these interactions have also affected behaviours such as timing, periodicity and location of nesting, mating and feeding activities and the alteration of a sea turtle's ecological function (Bjorndal and Jackson, 2003; Frazier, 2003).

Sea turtles are currently recognised internationally as species of conservation concern. All sea turtle species are included in the 2002 IUCN (World Conservation Union) Red List of Threatened Animals, and are protected from international trade to or from signatory countries, by the Convention on International Trade in Endangered Species of Flora and Fauna (CITES). In some countries, sea turtle species also receive protection from National and State legislation.

The recognition of declining sea turtle populations, and the resulting desire to protect their populations, has necessitated the need to increase our knowledge on their biology in order to

develop and prioritise conservation and management options. As a result of this requirement, in addition to the long held fascination that scientists have for sea turtles, a considerable amount of research has been conducted on sea turtles and subsequently a substantial body of literature exists.

Despite the voluminous research that has been conducted on sea turtles, there have been some areas of their life history that have been particularly challenging to elucidate. This is especially true for the post-hatchling stage, often referred to as ‘the lost years’, which accurately reflected the state of knowledge on this phase of a sea turtle’s life. Although knowledge has progressed considerably in the last few years, we are still limited by the fact that the majority of the information we have on the post-hatchling stage is based on loggerhead turtles in the northern Atlantic Ocean and northern Pacific Ocean. Consequently, the following summary of the post-hatchling stage focuses on the loggerhead turtles in these two geographical regions.

A sea turtle’s post-hatchling life history stage

Throughout the duration of the post-hatchling stage, a young turtle can occupy several habitats. This has resulted in the term ‘post-hatchling’ being somewhat ambiguous within the literature. Much of the ambiguity has been caused by researchers merging oceanographic terms with life history descriptive terms. Bolten (2003a) clarified the terminology applied to young juvenile stages in his review of the loggerhead post-hatchling stage in the Atlantic Ocean. In this review Bolten highlights the fact that life history descriptions are not synonymous with oceanographic descriptions. This thesis follows the definitions used by Bolten (2003a), except that distinctions will not be made between the post-hatchling transitional stages (neritic habitat) and the post-hatchling ‘proper’ stage (oceanic habitat) unless specified. The reader is referred to the glossary for oceanographic, and other definitions used in this thesis.

Life begins on the beach for a young hatchling (or neonate) emerging from its egg (Figure 1.1). After emergence from the nest, a hatchling actively orientates itself and moves towards the water using visual cues (reviewed by Lohmann and Lohmann, 2003). The hatchling stage continues in the near-shore waters as the young turtle orientates itself using wave direction and magnetic cues to swim away from beach and into offshore currents during an active swimming period known as the ‘swim frenzy’ (Wyneken and Salmon, 1992). The hatchling stage is short, and lasts only a few days, whilst the hatchling is nutritionally dependent on the remains of its egg yolk (Bolten, 2003a).

A hatchling becomes a post-hatchling when it begins to feed. Depending on the width of the continental shelf the hatchling has to swim across, this may or may not happen before the animal enters the oceanic zone (Bolten 2003a). Bolten (2003a) refers to a post-hatchling that is still in the neritic zone, as a turtle that is in the ‘post-hatchling transitional stage’. In the neritic zone, post-hatchlings live at or near the surface, and hence their movement into the oceanic zone is probably not denoted by any major behavioural shift, but just a change of location (e.g. neritic versus oceanic habitat). Bolten (2003a) indicates that the post-hatchling stage ‘proper’ begins when the post-hatchling enters the oceanic zone. Here, post-hatchlings are epipelagic, although they may become demersal when they are feeding in the vicinity of

seamounts, oceanic banks, and ridges that come close to the surface in the oceanic realm (Bolten, 2003a).

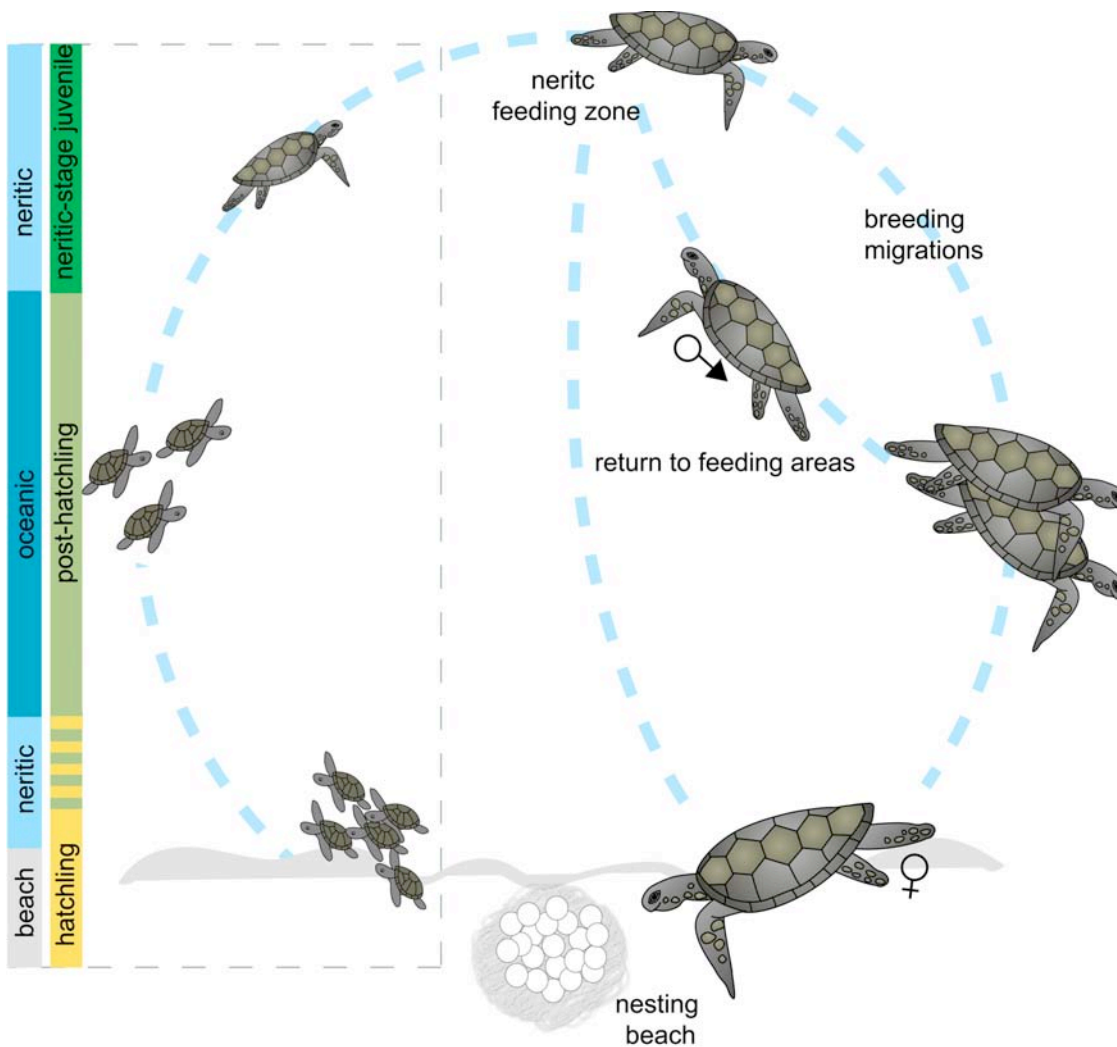


Figure 1.1. The generalised life cycle of sea turtles. The left hand side of the diagram depicts the habitat association of the hatchling, post-hatchling and early juvenile life stages. Diagram based on (Lanyon *et al.*, 1989).

After a period of time in the oceanic zone, a post-hatchling returns to coastal waters, and at this point the post-hatchling stage ends and the neritic juvenile stage begins (Figure 1.1). In the neritic zone, juveniles grow and develop into mature adults. Once turtles reach maturity they will again perform migrations, this time from feeding grounds to mating and breeding locations at intervals that vary depending on the individual and the species (Figure 1.1). Whilst some of these migrating mature animals do not leave the neritic zone in order to reach their breeding habitat, others will migrate back through oceanic zones (reviewed by Plotkin, 2003).

Ontogenetic habitat shifts are theorised to provide advantages either in terms of survival rate and/or growth rate (Werner and Gilliam, 1984). The function of an early developmental stage in the oceanic habitat is mostly likely the avoidance of higher predator pressure in the neritic habitat (Bolten, 2003b). Support for this function is provided by comparing the size of hatchling flatback turtles, who do not leave the coastal waters and are 1.8-3.0 times larger

than the hatchlings of other Cheloniidae species that all undertake habitat shifts to the oceanic environment (Walker and Parmenter, 1990). The larger size of the hatchling flatback turtles presumably helps reduce predator pressure in the neritic habitat by providing resistance against predation by crabs, gulls, egrets and smaller fish that feed on hatchlings of the other Cheloniidae species (Walker and Parmenter, 1990).

The sea turtle's early developmental stage in the oceanic habitat may also serve to reduce inter or intraspecific competition for food in the neritic habitat (Bolten, 2003b). Although the need for this may not be so evident in present times, historical data indicate that sea turtles once occurred in massive numbers (Jackson, 1997, 2001; Jackson *et al.*, 2001) and therefore resource partitioning would have been advantageous. A post-hatchling's return to neritic habitat could theoretically serve to maximise growth rates. This is supported by Bolten (2003a) who found growth rates of Atlantic oceanic loggerhead turtles are greater in neritic zone than in the oceanic zone after approximately 64 cm CCL, which is the size that most loggerhead turtles leave the oceanic zone.

Post-hatchling sea turtle ecology

Difficulty in tracking post-hatchling sea turtles

The tag and recapture studies that have been valuable for providing information on the periodic travel of nesting and feeding turtles are of limited use for determining post-hatchling movements. This is because high mortality of hatchlings through natural predation means large numbers of hatchlings would need to be tagged for there to be a probability that any significant numbers of individuals would survive until recapture. Additionally there is the problem that traditional body tags are unlikely to last throughout the length of the post-hatchling stage. A tag applied to a ~5 cm hatchling is unlikely to be retained as the turtle grows to a 30 cm or 70 cm juvenile, over a period that may extend beyond 10 years (Bjorndal *et al.*, 2000).

The technique of satellite tracking has been employed in sea turtle migratory research and is successfully beginning to reconstruct migratory pathways (Eckert, 1997; Renaud and Gitschlag, 1991). Satellite tracking has value over traditional tagging programs as they provide information on the behaviour and geographic patterns of migratory travel. However, present technology does not allow satellite tracking to be a feasible option for investigating post-hatchling migrations, as the transmitters that are currently applied to sea turtles are more than twice the size and weight of a hatchling. In addition, the high levels of post-hatchling predation combined with the expense of satellite transmitting units would render such research unjustifiably expensive. Consequently, much of what is understood of the post-hatchling life stage has been derived from indirect means, such as information gathered from stranded animals, opportunistic reports of sightings at sea and from studies of hatchling swimming behaviour and orientation.

The oceanic lifestyle of post-hatchling sea turtles

The notable absence of post-hatchling sea turtles from the neritic habitat, and an accumulation of observations of post-hatchlings associated with the Gulf Stream in the

Atlantic Ocean, signified to early researchers that this life history stage occupies offshore waters in association with oceanic currents (Carr, 1987). Beyond this, obtaining information on where the post-hatchlings went, or for how long, was significantly hindered by the elusiveness of a post-hatchling in its natural environment and the limitations of tagging technologies to track a hatchling as it leaves its nest.

The behaviour of a hatchling as it enters the water for the first time provides the first clue of a life history stage that has an offshore existence. When a hatchling enters the water, it swims unceasingly at the waters surface for 1-3 days in an offshore direction, using light and wave orientation cues (Lohmann and Lohmann, 1992; Wyneken *et al.*, 1990). This behaviour is referred to as the 'swim frenzy' stage and has been described for all species (Hamann, unpublished data; Wyneken, 1997). The distances that are covered during the swim frenzy removes a hatchling sea turtle further than what is necessary to escape immediate littoral and sub-littoral predators. Hence, it has been postulated that the purpose for a post-hatchling's swim-frenzy behaviour is to take the young turtle to offshore currents where they move into a semi-passive drifting phase (Bolten and Balazs, 1982; Wyneken and Salmon, 1992).

Numerous *in-situ* observations of post-hatchling sea turtles in the offshore currents provide convincing evidence of a life within border currents in the oceanic realm. The majority of reports are for loggerhead turtles in the northwest Atlantic Ocean in association with the Gulf Stream, although a few reports also exist for post-hatchling green and hawksbill turtles (Carr, 1987). Many of the *in-situ* sightings in the Atlantic Ocean have noted the association of post-hatchlings with *Sargassum* (reviewed by Carr, 1987), and it was this observation that gave rise to Carr's (1967) initial hypothesis on post-hatchling ecology, that "post-hatchling turtles inhabit *Sargassum* rafts". However, it is now recognised that, although *Sargassum* provides food and shelter for post-hatchling sea turtles, and numerous other organisms in the oceanic environment, it is not an indispensable habitat for post-hatchlings (Carr, 1987). Instead, it appears that sites of down-welling or convergence where buoyant material gathers (including *Sargassum* and other food resources), may generate essential habitat for post-hatchling sea turtles (Carr, 1987).

The extent of a post-hatchling green sea turtle's association with the *Sargassum* environment is poorly known. Although there have been at sea sightings of green turtles in *Sargassum* (see Carr, 1987), laboratory experiments found that whereas captive hatchling loggerhead and hawksbill turtles congregate in seaweed rafts, hatchling green turtles did not (Mellgren *et al.*, 2003). These results might be expected as the light plastron coloration of a hatchling green turtle, in comparison to the dark coloration of the hatchling loggerhead and hawksbill turtle's plastrons, would be more obvious to bottom-up predators in the seaweed.

The pelagic lifestyle of post-hatchling sea turtles

Investigations into the stomach contents of post-hatchling loggerhead turtles caught in the oceanic realm are in concordance with a pelagic lifestyle (reviewed by Bjorndal, 1997). Post-hatchling loggerhead turtles captured in the northern Pacific driftnet fisheries, had consumed a range of neustonic species, predominately the pelagic gastropod, *Janthina* spp. (Gastropoda), *Planes* spp. (Decapoda), *Carinaria cithara* (Heteropoda) and *Lepas* spp. (Cirripedia) (Parker *et al.*, 2005; Wetherall *et al.*, 1993b). Similarly dietary investigations

conducted in the Atlantic Ocean also found that post-hatchling loggerhead turtles in this region consume a wide range neustonic species. The taxonomic groupings were similar to those found in the Pacific, with the exception that *Sargassum*, and pelagic snails that associate with *Sargassum* (e.g. *Litiopa* sp. and *Diacria* sp.) featured regularly (reviewed by Bjorndal, 1997). The pelagic nature of the consumed items show that post-hatchling loggerhead turtles feed within the top few metres of the ocean's surface, and are likely to spend a reasonable portion of their time in this zone. Satellite telemetry and remote sensing studies conducted with post-hatchling loggerhead turtles in Azorean waters have confirmed the pelagic lifestyle of this life stage. Tracked turtles spent 75% of their time in the top 5 m, with the majority (80%) of their dives being to 2-5 m, where they were presumably foraging (Riewald *et al.*, 2000).

Trans-oceanic migration routes of post-hatchling loggerhead turtles

A hatchling loggerhead turtle's offshore migration to the Gulf Stream has been shown to be just the first step of a trans-oceanic journey. In the north western Atlantic there is a conspicuous gap in size class distribution of loggerhead turtles, where virtually no turtles between 20 and 40 cm curved carapace length (CCL) are found. However, in the eastern Atlantic, oceanic populations of feeding juveniles that range in size from 8.5 to 82.0 cm CCL, are known to occur around the Azores and Madeira (Bjorndal *et al.*, 2000; Bolten *et al.*, 1993). Carr (1987) hypothesised these small loggerhead turtles in the eastern Atlantic were derived from nesting populations in the western Atlantic. This relationship was first confirmed by tag and recapture studies (Bjorndal *et al.*, 1994; Bolten *et al.*, 1992), and was later supported with mitochondrial DNA sequence analyses (Bolten *et al.*, 1998).

A similar scenario exists in the Pacific Ocean, where loggerhead turtle nesting only occurs in Japan and Australia. Yet concentrations of juvenile loggerhead turtles are reported feeding off the Baja California coast (Peckham and Nichols, 2002; Pitman, 1990) and are captured in the North Pacific oceanic drift-net fishery (Wetherall *et al.*, 1993a). Mitochondrial DNA sequence analyses have linked these oceanic loggerhead aggregations back to the Japanese rookeries (Bowen *et al.*, 1995), thus confirming that post-hatchling loggerhead turtles in the northern Pacific Ocean undertake trans-oceanic migrations comparable to those undertaken by post-hatchlings of this species in the North Atlantic Ocean.

The size-class structure and geographical distribution of the oceanic aggregations of post-hatchling loggerhead turtles in relation to rookeries provided the initial evidence of trans-oceanic crossings being undertaken by post-hatchlings in the northern Atlantic and Pacific oceans. The lack of any equivalent aggregations for other loggerhead populations, or for any other species of sea turtle, may mean that the loggerhead population in the northern Atlantic and Pacific oceans are the only sea turtles making substantial oceanic crossings.

The role of currents in directing post-hatchling sea turtle migration routes

Hypotheses regarding trans-oceanic migrations of post-hatchling loggerhead turtles in the northern Atlantic and Pacific oceans assume they travel in the direction of the prevailing currents (Bolten *et al.*, 1998; Bowen *et al.*, 1995; Musick and Limpus, 1997). Adult or juvenile loggerhead turtles tracked using satellite transmitters indicate that the migratory routes of oceanic juvenile loggerhead turtles in the northern Pacific may be more complex than this, as

the majority of tracked turtles were observed swimming against prevailing currents (Polovina *et al.*, 2004; Polovina *et al.*, 2000). However, all turtles used in these tracking studies were greater than 41 cm in carapace length, and therefore their movements are possibly not representative of the migratory behaviour of smaller (and younger) post-hatchling turtles. In addition, these larger animals may represent turtles who have already undergone a trans-Pacific loop and did not exit the North Pacific gyre to take up residency in the neritic habitat when they returned to the western Pacific. Instead, they may have remained in the oceanic habitat, and as the tracking data show, continued to exploit the food resources provided by the Transition Zone Chlorophyll Front (TZCF) and the southern edge of Kuroshio current (Polovina *et al.*, 2004; Polovina *et al.*, 2006). The implication of these studies is that there remains considerable uncertainty of sea turtle post-hatchling migration behaviour. Nevertheless, the satellite telemetry data available indicate the importance of oceanic fronts and geostrophic currents as a foraging and migratory habitat for oceanic loggerhead turtles.

Duration of the sea turtle's post-hatchling stage

Based on growth models derived from length-frequency analysis and growth rates (Bjorndal *et al.*, 2000), it appears that the duration of the loggerhead turtle's pelagic stage in the South Pacific is longer than in the North Atlantic. The majority of the post-hatchling loggerhead turtles from northwest Atlantic rookeries recruit into neritic waters at between 46 to 64 cm CCL, which correlates to a range of 6 to 11 years of age (Bjorndal *et al.*, 2000). In contrast post-hatchling loggerhead turtles recruit to neritic waters in Queensland, Australia at a minimum size of 67 cm CCL (Limpus *et al.*, 1994b). Turtles of this carapace size are estimated to have a post-hatchling stage of approximately 15 years (Bjorndal *et al.*, 2000). These estimates are supported by direct evidence from an animal in the South Pacific region notched as a hatchling and recaptured at 75.6 cm CCL in Queensland waters 15.2 years after being notched (Limpus *et al.*, 1994b).

Navigational mechanisms employed by post-hatchling sea turtles

Sea turtles have shown they have the capacity to navigate which is demonstrated by their strong site fidelity to both nesting and foraging habitats. Numerous examples exist of impressive breeding migrations undertaken by sea turtles (reviewed by Plotkin, 2003), and one exceptional example is the ability of breeding green turtles to migrate from their feeding grounds on the Brazilian coast to Ascension Island, a remote target in the Atlantic Ocean some 2300 km away (Luschi *et al.*, 1998). However, the sensory mechanisms employed by sea turtles to undertake these navigational feats, are still largely unknown. A variety of navigational mechanisms that sea turtles may employ have been described, such as navigation based on detecting magnetic fields, visual cues, water temperature gradients, waterborne chemical cues, windborne information, currents, and bathymetric features (reviewed by Lohmann *et al.*, 1997; Plotkin, 2003). Critical review of these mechanisms suggests that some are applicable in coastal waters such as visual cues and bathymetric features. Other mechanisms imply previous exposure and learned behaviour such as windborne information and waterborne chemical cues. Post-hatchlings undertaking migration in deep offshore waters must move through locations they have never traveled in and with no coastal guidance mechanisms. Navigation strategies such as biological compasses and water temperature gradients are the best suited to these environments, and should be evidenced in post-hatchling responses to these environmental cues.

Laboratory experiments have demonstrated that hatchling loggerhead turtles can detect magnetic inclination angle and magnetic field intensity (Lohmann and Lohmann, 1994, 1996). These hatchlings appear to use these magnetic fields as a guidance system (Lohmann *et al.*, 2001). For example, hatchlings taken from northwest Atlantic rookeries exposed to a magnetic field that replicated the one that exists offshore near northern Florida, swam east-southeast (Lohmann *et al.*, 2001). In this region, the Gulf Stream current divides, with one branch continuing eastwards into the North Atlantic gyre, and the other flowing northwards into the cold oceanic waters of Scandinavia. The response of a hatchling to move east-southeast in this region would lead the turtle away from the gyre's northern edge avoiding fatally cold water (Lohmann *et al.*, 2001). Exposure to magnetic fields that replicated the north eastern region of the gyre and the southern-most regions also resulted in hatchlings swimming in a direction that would be conducive of them remaining in the gyre (Lohmann *et al.*, 2001) (Figure 1.2). These directional responses ensure that post-hatchlings stay within the boundaries of a favourable habitat in the North Atlantic gyre. Based on the navigational abilities of hatching loggerhead turtles in the northern Atlantic, it seems reasonable to assume that all loggerhead hatchlings, and potentially hatchlings of other species, would have comparable navigational abilities.

Subsequent experiments however, have not provided supporting evidence for Lohmann *et al.*'s (2001) research (Akesson *et al.*, 2003; Luschi *et al.*, 2001; Luschi *et al.*, 2003a; Papi *et al.*, 2000). For example, Luschi *et al.*, (2001) and Akesson *et al.*, (2003) satellite tracked nesting female green turtles displaced from Ascension Island and found these turtles did not show any ability to compensate for displacement. Both studies concluded that the search trajectories of the turtles did not indicate the turtles were using true bi-coordinate geomagnetic navigation. Instead the winding search patterns shown by the displaced turtles were interpreted as the turtles searching for a sensory contact, presumably wind and/or water transported cues, to locate the island.

Another study attached powerful static magnets to green turtles making post-nesting migrations from Ascension Island to their Brazilian feeding grounds (Papi *et al.*, 2000). The magnets produced variable artificial fields that would make navigation based on geomagnetic maps impossible. As the turtles attached with magnets took similar courses to the turtles without magnets, the authors concluded that magnetic cues are not essential for these turtles to return to their feeding grounds (Papi *et al.*, 2000).

It should however, be noted that the experiments outlined above were conducted with mature turtles migrating between nesting and feeding locations. As these turtles had prior familiarity with the sites they were migrating to, they may have been relying on different mechanisms (e.g. windborne information) than those relied upon by turtles who have no prior familiarity with their migratory route, such as post-hatchlings or adults yet to breed. Evidently, it does appear that turtles employ different navigational mechanisms at different times throughout their life (reviewed by Lohmann *et al.*, 1997) and this may provide an explanation as to why adults making breeding migrations may not be solely relying upon, or even employing, geomagnetic cues for navigation.

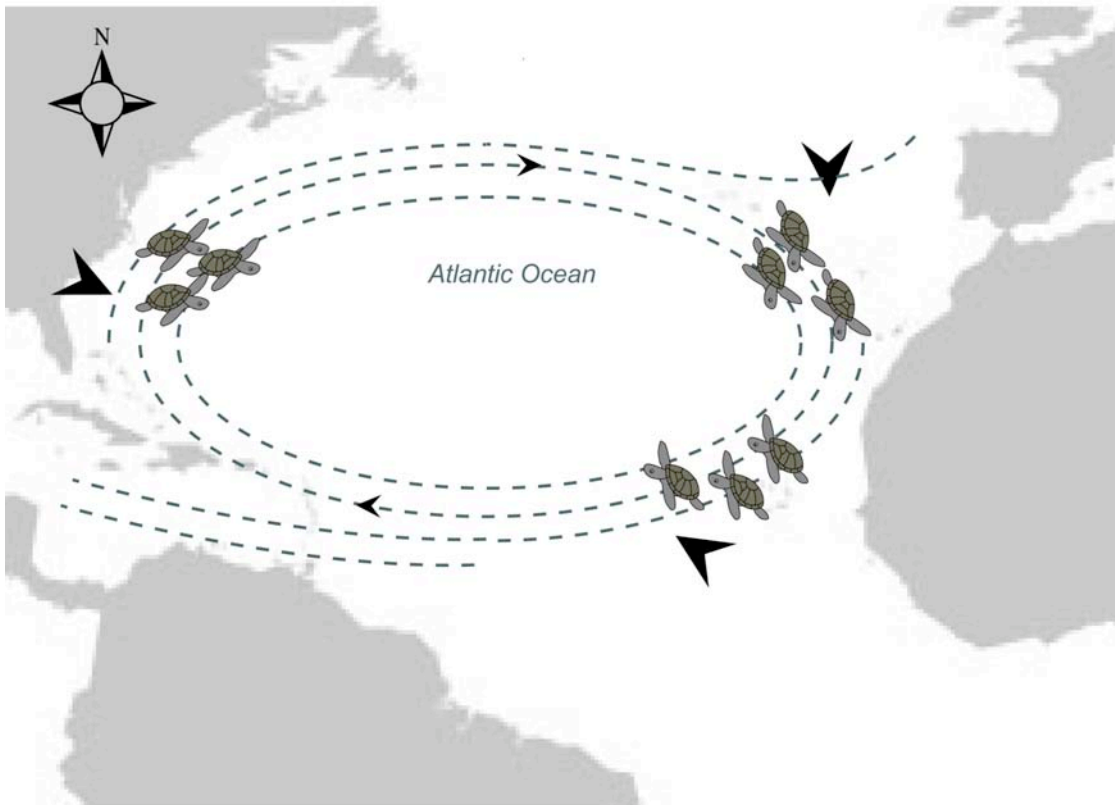


Figure 1.2. The orientation of hatchling loggerhead turtles (*Caretta caretta*) from eastern Florida, exposed to magnetic fields that represent three locations along their migratory route in the North Atlantic Ocean. Mean direction of orientation shown with large black arrow. Generalised currents of North Atlantic gyre are represented by dashed lines and small arrows. Derived from research conducted by Lohmann *et al.*, 2001.

Other post-hatchling loggerhead populations and other sea turtle species

Although knowledge on the sea turtle's post-hatchling stage has advanced considerably since Archie Carr coined the term 'the lost year', much of what we know is based on loggerhead post-hatchlings from northwest Atlantic rookeries, where post-hatchlings are pelagic and undertake trans-oceanic migrations in association with large oceanic gyres. Beyond these populations, our knowledge of post-hatchling ecology, and the developmental migrations they undertake, is still very limited and for the most part can only be speculated upon. For example, now that trans-oceanic migrations have been documented for northern Atlantic and northern Pacific populations, is this the general phenomenon for loggerhead turtle populations, and are trans-oceanic migrations also undertaken by post-hatchlings from other sea turtle species?

In light of our knowledge on loggerhead turtles, the absence of post-hatchlings of the remaining species in neritic waters, with the exception of the flatback turtle (discussed below), provides compelling evidence that post-hatchlings occupy an oceanic niche. In addition, the few observations of green, hawksbill and ridley turtles in the Gulf Stream (Carr, 1987), signifies a oceanic stage in association with offshore currents comparable to loggerhead post-hatchlings. Based on this speculation, several authors have used the

knowledge of local oceanographic conditions to provide insight on the likely dispersal paths for other sea turtle populations, including Kemp's ridley turtle populations from north western Gulf of Mexico (Collard and Ogren, 1990), green turtle populations from Tortuguero (Carr and Meylan, 1980) and loggerhead and green turtle populations in Australian waters (Walker, 1990).

Although the migrations of loggerhead populations provide a basis for speculation, it has been noted that morphological differences (e.g. countershading, flipper length, duration of pelagic stage, swimming behaviour and resource partitioning) observed between post-hatchlings of different species may be indicative of lifestyles variations in the oceanic-stage turtles (Bolten, 2003b). Indeed variations are known to exist for at least one species. In contrast to the generally accepted oceanic post-hatchling stage hypothesised for other sea turtle species, Australia's flatback turtle appears to remain in a coastal environment as post-hatchlings (Walker, 1990; Walker and Parmenter, 1990). This conclusion was developed from the presence of juvenile sized flatback turtles caught in coastal trawling operations and from skeletal remains observed at island feeding stations of white-bellied sea-eagles (*Haliaeetus leucogaster*) (Walker, 1990; Walker and Parmenter, 1990). Prey remnants of flatback turtles that ranged from 11–24 cm in carapace length (CL) have been found during extensive surveys of islands within the Great Barrier Reef (Limpus and Walker, 1990; Walker, 1990; Walker and Parmenter, 1990). The authors attributed the absence of the smaller sized carcasses (<11 cm CL) to the possibility that these sizes may be taken less frequently and/or may be consumed whole by the bird. In addition, because this species is not thought to disperse beyond the continental shelf, it is believed to have a life history without an oceanic post-hatchling phase.

Post-hatchlings in the southwest Pacific Ocean

Australia has some of the largest sea turtle nesting areas in the Indo-Pacific region (Limpus, 1990), and the only nesting populations of flatback turtles (Parmenter, 1991). There are five species of sea turtles that nest regularly within Australian waters; the green turtle, the loggerhead turtle, the flatback turtle, the hawksbill turtle and the olive ridley turtle. The leatherback turtle also occurs within Australian waters (Limpus, 1984; Limpus *et al.*, 1984), but there are only irregular reports (1-2 per year) of this species nesting on northern Australian beaches and no records of breeding in eastern Australia since 1996 (Hamann *et al.*, 2006).

All six sea turtle species that occur in Australian waters are protected by various State and Territory legislation and Federally by the Australian Government's Environment Protection and Biodiversity Conservation Act (EPBC Act 1999). Under this Act the loggerhead and olive ridley turtles are listed as Endangered, whilst the green, leatherback, hawksbill and flatback turtles are listed as Vulnerable. All of the species found in Australia are listed in the 2000 IUCN (World Conservation Union) Red List of Threatened Animals and under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). In addition, all sea turtles occurring in the Indo-Pacific region are a priority for conservation under the Convention on the Conservation of Migratory Species of Wild Animals (the Bonn Convention or CMS, Appendix I 2005, <http://www.cms.int/>).

Sea turtle research in Australia has for the most part paralleled global trends. Early research activities focussed intensely on the nesting beach (see Limpus, 1995b). As new technologies became available, research activities extended into coastal feeding grounds (Limpus *et al.*, 1994a; Limpus *et al.*, 1994b; Limpus and Limpus, 2003a; Limpus *et al.*, 2005). To date, research on post-hatchling ecology in the Indo-Pacific region has been limited to flatback turtles (Walker, 1990; Walker and Parmenter, 1990), reviews of post-hatchling specimens and observation records (Limpus and Walker, 1990; Reitemeyer, unpubl.; Walker, 1991), and one theoretical review of possible dispersal routes (Walker, 1990).

Based on the general acceptance that a sea turtle's life history pattern is characterised by an oceanic post-hatchling stage (Bolten, 2003b), this is the presumed pattern for post-hatchlings from Australian populations (except flatback turtles) (Walker, 1990). This assumption is supported by the following empirical evidence; (i) post-hatchling sized individuals are mostly absent from coastal Australian waters, and those that do occur in the Australian region are from stranded individuals at the smaller size end of the post-hatchling size range consistent with the size of recent hatchlings, (ii) the absence of post-hatchling turtles other than flatback turtles, found during the surveys of white-bellied sea eagle feeding stations within the Great Barrier Reef, despite larger populations of green and loggerhead turtles occurring in the same region, and sea eagles being common throughout the nesting areas of these species (Walker, 1991; Walker and Parmenter, 1990).

The evidence presented above indicates that post-hatchling loggerhead and green turtles originating from Australian nesting beaches possess an oceanic post-hatchling stage. In this thesis I will investigate the validity of this statement, and in doing so will improve knowledge of post-hatchling sea turtle biology in Australia and globally. For the purposes of this research a post-hatchling is an individual whose curved carapace length (CCL) is less than the smallest individual of this species that has been recorded residing in feeding grounds.

Research aims

The aim of this thesis is to establish the migration ecology of post-hatchling loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) sea turtles in the southwest Pacific Ocean.

My research approach has been to establish multiple sources of evidence to document the ecology of post-hatchling loggerhead and green turtles using different techniques. These will:

1. Assess the spatial and temporal distribution of post-hatchling loggerhead and green turtles in the southwest Pacific Ocean in relation to rookery location and dominant surface current features.
2. Analyse the genetic structure of post-hatchling sea turtles in the southwest Pacific Ocean region to provide insight into migratory routes.
3. Identify the habitats occupied by post-hatchling sea turtles in the southwest Pacific Ocean region.

The results from this research will have importance at both global and regional scales. On a global scale, this research will add to the overall information that has been acquired on post-hatchling ecology. At the regional level, this research will increase the understanding of the life history for two Australian sea turtle populations by providing region-specific information on the poorly understood post-hatchling stage. The information acquired on the post-hatchling stage in this thesis will help direct future regional management policy by providing information on the migratory routes and habitats occupied by these animals during their post-hatchling life history phase.

Thesis outline

This thesis is presented as seven chapters. This first chapter has provided an introduction to sea turtles and background information on their life history with particular reference to the post-hatchling stage. In Chapter 2 I investigate the sizes and the spatial and temporal distribution of post-hatchling loggerhead and green turtles in the southern Pacific Ocean in relation to rookery location and prevailing surface currents. The information presented in this chapter is critically analysed in respect to the theory that post-hatchling loggerhead and green turtles live in offshore waters where their migratory routes are significantly influenced by prevailing surface currents.

In Chapters 3 and 4 I employ molecular genetic tools to determine the population structure of post-hatchlings found stranded along Australia's eastern coast and of those that had been consumed by predatory fish. In Chapter 3 I investigate the global phylogeography of both study species compiling data available prior to this study along with newly collated data from the region. I use mitochondrial DNA sequences to assess the relationships amongst the currently recognised haplotypes of loggerhead and green sea turtles. Chapter 4 employs mixed stock analysis of post-hatchlings to determine their natal origin in order to gain insight into migratory routes. The investigation into employing mixed stock analysis techniques is also discussed in light of a need for reassessing the genetic stock structure of loggerhead and green turtle rookeries in the southwest Pacific, and even globally, due to a need for greater resolution of identification between rookeries.

Additional evidence for the migratory ecology of post-hatchling loggerhead and green turtles in the southwest Pacific is gained through dietary analysis (Chapter 5) and stable isotope (Chapter 6) investigations. In my final chapter (Chapter 7), I summarise the findings of the previous chapters and explore the outcomes in the context of what is observed in these species at a global scale, and offer some ecological explanations for the results I present. This final chapter also outlines the outcomes of this research in light of their implications for the management and conservation of loggerhead and green turtle populations in Australia.

Chapter 2

The spatial and temporal distribution of post-hatchling sea turtles in the southwest Pacific

Introduction

Sightings of post-hatchling turtles at sea and their absence in coastal waters provides evidence that this stage of a sea turtle's life history is spent in oceanic waters (Chapter 1). This is acknowledged to be the case for all sea turtle species with the exception of the flatback turtle (Walker, 1990; Walker and Parmenter, 1990). During this oceanic stage, the evidence available suggests that the direction and route taken by a post-hatchling are largely influenced by surface currents prevailing at the point and time that the hatchlings entered the ocean (Bolten, 2003b).

A post-hatchling turtle's small size, high mortality rate and cryptic nature in the oceanic environment has hindered researchers from directly tracking post-hatchlings to establish their migratory routes. However, an investigation into the surface currents that dominate the waters adjacent to sea turtle rookeries can provide insights into possible dispersal routes taken by the hatchlings leaving these rookeries. Such studies have enabled researchers to propose the migration routes taken by post-hatchlings for a number of sea turtle populations. These include Kemp's ridley (*Lepidochelys kempii*) hatchlings in the Gulf of Mexico (Collard and Ogren, 1990), loggerhead (*Caretta caretta*) hatchlings in the northwest Atlantic (Carr, 1987; Carr, 1986), and green (*Chelonia mydas*) and loggerhead hatchlings from major Australian rookeries (Bode *et al.*, 1995; Walker, 1990).

Post-hatchling-sized green and loggerhead sea turtles are largely absent from Australian coastal waters (Limpus, 2004a, 2004b). Thus it has been proposed that an oceanic juvenile stage is also characteristic of southwest Pacific sea turtle populations (Limpus and Walker, 1990; Walker, 1990). The consequence of this would be that hatchlings emerging from the Australian coast become entrained within the currents adjacent to their nesting beach, and their direction of travel away from the rookery and within the ocean will be to some extent dictated by the flow direction of these currents. In the absence of at sea sightings of green or loggerhead post-hatchlings in the southwest Pacific, flow characteristics of surface currents can be used as a conceptual basis for establishing a refined theory of possible dispersal paths for loggerhead and green post-hatchlings in this region.

In the southwest Pacific Ocean, the waters adjacent to the majority of green and loggerhead turtle rookeries are predominantly influenced by either the Southern Equatorial Current (SEC), the East Australian Current (EAC) or the North Queensland Current (NQC) (Figure 2.1). The Southern Equatorial Current (EAC) brings a broad westward flow of warm water across the tropical southern Pacific Ocean towards the Coral Sea. As the SEC flows into the Coral Sea, and encounters the islands of Fiji, New Caledonia and Vanuatu, it divides into strong and narrow jets (Ganachaud *et al.*, 2005). These jets are the South Caledonian Jet (SCJ),

the North Caledonian Jet (NCJ) and the North Vanuatu Jet (NVJ) (Figure 2.1). The Southern Equatorial Current bifurcates on the east coast of Australia at approximately 18°S, and becomes the source for the northward flowing North Queensland Current and the southward flowing East Australian Current (Ganachaud *et al.*, 2005).

The North Queensland Current is joined by the North Vanuatu Jet at about 15°S. This system continues to flow northwards and then eastwards following the northern Queensland and southern Papua New Guinea continental slopes (Burrage *et al.*, 1995; Ganachaud *et al.*, 2005). A portion of this current system exits into the Solomon Sea to feed the New Guinea Coastal Current (Ganachaud *et al.*, 2005), and the remaining portion recirculates clockwise around the northwest Coral Sea (Figure 2.1). Occasionally some of the water from this current may escape into the Torres Strait (Andrews and Clegg, 1989; Burrage, 1993; Church, 1987) (Figure 2.1).

The southward flowing branch of the Southern Equatorial Current is the source for the East Australian Current, which is the western boundary current of the South Pacific subtropical gyre. The East Australian Current flows along the continental slope of Australia's east coast until it turns eastward at about 35°S (Ganachaud *et al.*, 2005). After turning away from the Australian coast, the East Australian Current divides into two primary branches. One of these branches flows in a northwards direction where it generates numerous recirculating eddies in the Coral and Tasman seas and the other branch flows eastward across the Tasman Sea as the Tasman Front (Ganachaud *et al.*, 2005; Tilburg *et al.*, 2001; Tomczak and Godfrey, 1994). The residual of the EAC, also known as the EAC extension, continues to flow southward along the eastern Australian coast as far south as Tasmania, before turning westward into the Indian Ocean as the Tasman Outflow (Ganachaud *et al.*, 2005) (Figure 2.1). Superimposed on the simplified model described for the circulation of surface waters in the southwest Pacific, is a pattern of considerable spatial complexity that displays significant seasonal and inter-annual variability (Burrage *et al.*, 1995). However, using the general characteristics of these currents, hypotheses can be developed on the migratory routes taken by post-hatchling sea turtles in the southwest Pacific. Based on the knowledge of surface currents around the Australian coast, Walker (1990) speculated on the possible influence of currents on sea turtle hatchlings emerging from Australian beaches. Walker's (1990) speculations were based on post-hatchlings acting as passive drifters. He proposed that hatchlings from the southern Great Barrier Reef region will drift with EAC eddies in the Coral Sea and those from the northern Great Barrier Reef may disperse into the Gulf of Carpentaria. Since Walker's (1990) examination, improved satellite technologies and increased research into the currents off Australia's eastern coast, notably via the South Pacific Circulation and Climate Experiment (<http://www.ird.nc/UR65/SPICE>) and the Commonwealth Scientific and Industrial Research Organisation's (CSIRO) Blue Link Program (<http://www.marine.csiro.au/bluelink>), have resulted in greater understanding of surface water movement in this region. These improved data on currents, in conjunction with a significantly enhanced database on post-hatchling sightings, will allow greater insight into post-hatchling distribution.



Figure 2.1. The dominant surface currents in the southwest Pacific Ocean. As the Southern Equatorial Current (SEC) flows into the Coral Sea it divides into a number of jets: the North Vanuatu Jet (NVJ), the South Vanuatu Jet (SVJ), the North Caledonian Jet (NCJ), and the South Caledonian Jet (SCJ). These jets are the source of the current systems off eastern Australia, the East Australian Current (EAC), the North Queensland Current (NQC) and the New Guinea Coastal Current (NGCC). The seamounts located off the eastern Australian coast are shown as shaded regions.

If post-hatchling loggerhead and green turtles in the southwest Pacific region conform to the current understanding of an oceanic post-hatchling stage, their spatial and temporal distribution should reflect this and the following observations would be expected;

- observations of post-hatchling turtles will be missing from Australian coastal waters.
- post-hatchling turtles will be distributed away from rookeries in the direction of dominant currents.
- a positive relationship between the size of post-hatchling turtles and distance from closest rookery (i.e. potential source of natal origin) will exist. This is based on the assumption that the greater the distance a post-hatchling sea turtle is from its natal rookery, the older, and therefore larger, that animal will be.

- the majority of observations of post-hatchling turtles will occur during the months following the hatching season, with observations decreasing as time from hatching season increases. This is expected because sea turtle nesting in eastern Australia and the South Pacific is seasonal. Hence, each year, once all hatchlings have emerged from nests, fewer individuals are added to the post-hatchling distribution, while those produced earlier will have dispersed out of the region.

The aim of this chapter is to find evidence to support the above expectations. Evidence will be derived from the spatial distribution of the post-hatchling loggerhead and green turtles derived from by-catch reports, sightings at sea, stranded turtles and predator accumulation, and from size data. These biological attributes will then be discussed in light of current patterns in the South Pacific Ocean.

Methods

Collection of post-hatchling turtle data, specimens and samples

For the purpose of this research a post-hatchling turtle is an individual whose curved carapace length (CCL) is less than the smallest individual of this species that has been recorded residing in coastal feeding grounds. If any individual in the larger size class range for a post-hatchling had the physical appearance of a coastal inhabitant (e.g. epibiota type, colouration and plastron ridge abrasion) it was not considered a post-hatchling. Based on previous surveys in eastern Australia, the minimum size recorded for new loggerhead turtle recruits in coastal habitats is 66.7 cm CCL (mean 78.6 cm, range 66.7-93.9 cm) (Limpus and Limpus, 2003b), and the minimum size recorded for new green turtle recruits is 38.7 cm CCL (mean 43.6 cm, range 38.7-48.5 cm) (Limpus *et al.*, 2005).

Data collection on the distribution of post-hatchling turtles in Queensland has been ongoing for a number of years through *StrandNet*, the Queensland Parks and Wildlife Service's marine wildlife strandings and deaths database (Greenland *et al.*, 2002). *StrandNet* has been operating statewide (Queensland) since 1995, prior to this stranding data collection began in central Queensland in the early 1980's. *StrandNet* records information on injured, dying and dead sea turtles, cetaceans, pinnipeds, and dugongs throughout the Queensland State. Occasionally records of post-hatchling turtles observed in Australia's remaining states are also included in this database. Information kept by the database was made available for use in this study as the accumulation of information on post-hatchlings is slow and sporadic due to rare sightings and/or strandings. Had I relied solely on information I was able to collect during the three year time frame of my current study, my research would have been considerably limited.

In order to obtain stomach contents and samples for genetic analysis, requests for the collection of specimens and samples from post-hatchlings that stranded during this study was facilitated by the Queensland Sea Turtle Research Program. To extend sample collection beyond Queensland, other Australian agencies including the New South Wales Parks and Wildlife Service, Australian Sea Bird Rescue, Independent Seafood Producers and Ecofish were informed of the research and their help was enlisted. In addition, agencies from

neighbouring countries (New Caledonia and New Zealand) and islands (e.g. Lord Howe Island, New South Wales) were contacted to assist in the reporting and sampling of post-hatchlings in their region. Samples and information from stranded post-hatchlings were successfully obtained from New South Wales Parks and Wildlife Service and from New Zealand.

Post-hatchlings were also sourced from fishermen operating within the East Coast Tuna and Billfish Fishery (ECTBF), where occasionally post-hatchling turtles are found within the stomachs of *Coryphaena hippurus* (also known as dolphin fish or mahi mahi). These fisheries operate around the seamounts that occur offshore from the Sunshine Coast, Queensland and offshore from Coffs Harbour, New South Wales (refer to Figure 2.1). An attempt was also made to enlist the help of long-line fisheries operating out of Cairns (northern Queensland), however this was unsuccessful owing to the fishermen's concerns regarding the linking of their operations with sea turtles, no matter how indirect this link and despite assurances of anonymity.

Data recorded for each post-hatchling included species, date and location of capture and a series of morphometric measurements, based on those used in the Queensland Sea Turtle Research Program (Appendix B). A skin biopsy was removed from post-hatchling specimens for genetic analyses (Chapters 3 and 4) and stable isotope analyses (Chapter 6), and the crop and digestive tract were removed to enable the collection of stomach contents for dietary analyses (Chapter 5). The rationale and methodology of sample collection will be discussed further in their respective chapters.

Results

Spatial distribution of post-hatchling loggerhead and green turtles in the southwest Pacific

Documentation on the occurrence of post-hatchlings was scarce prior to the 1980s, with only 13 records existing, the first dating back to 1922. After 1980, recording of post-hatchling observations through *StrandNet* became more regular. Currently documentation for 123 loggerhead post-hatchlings and 172 green post-hatchlings in the southwest Pacific region exist (correct at date of this thesis submission, 10/09/06). These figures are based primarily on the QPWS stranding database in addition to the New Zealand records and those that have been reported in previous literature (Limpus and Walker, 1990), which include a number from New South Wales prior to 1991.

Post-hatchling loggerhead turtles

The majority of records that exist for post-hatchling loggerhead turtles in the southwest Pacific are for animals that have become stranded along Australia's eastern coast between Fraser Island in southern Queensland, southward, to the mid New South Wales coast (n>101). There is one stranding report for a 10 cm CCL loggerhead turtle north of the SEC's bifurcation point. Post-hatchling loggerhead turtles have also been found stranded on the northern New Zealand coastline (nine records), and there has been one stranding record

from Lord Howe Island. There are also 52 records for loggerhead post-hatchlings occurring in the waters off the coasts of southern Chile and northern Peru (Figure 2.2) (Alfaro-Shigueto *et al.*, 2004; Donoso *et al.*, 2000; Kelez *et al.*, 2003). These animals were documented as long-line fishery by-catch by these authors.

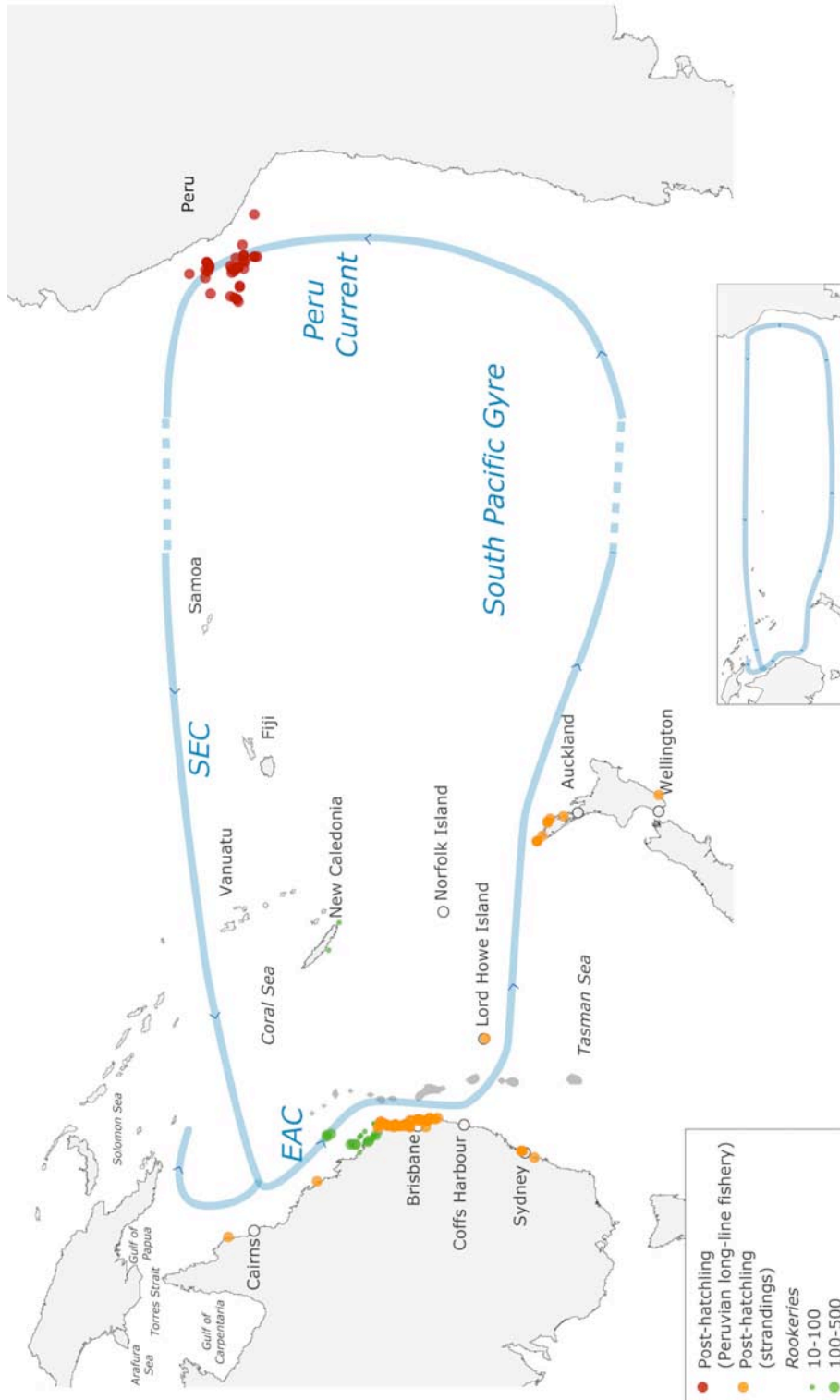


Figure 2.2. The distribution of post-hatchling loggerhead turtle (*Caretta caretta*) records for the southern Pacific Ocean, with rookeries and simplified primary currents shown.

Post-hatchling green turtles

Like loggerhead post-hatchlings, the majority of green turtle post-hatchling records are of individuals that have become stranded along Australia's eastern coast between Fraser Island (Queensland) and the mid New South Wales coast ($n > 128$). There is one record of a green post-hatchling stranded on Lord Howe Island and records of 18 animals that were found in the stomachs of *Coryphaena hippurus* that were caught off the southern Queensland and NSW coast (Figure 2.3). There are no records of green post-hatchlings northwards of the EAC's bifurcation. Nor are there any records of green post-hatchlings in New Zealand or in southeast Pacific waters.

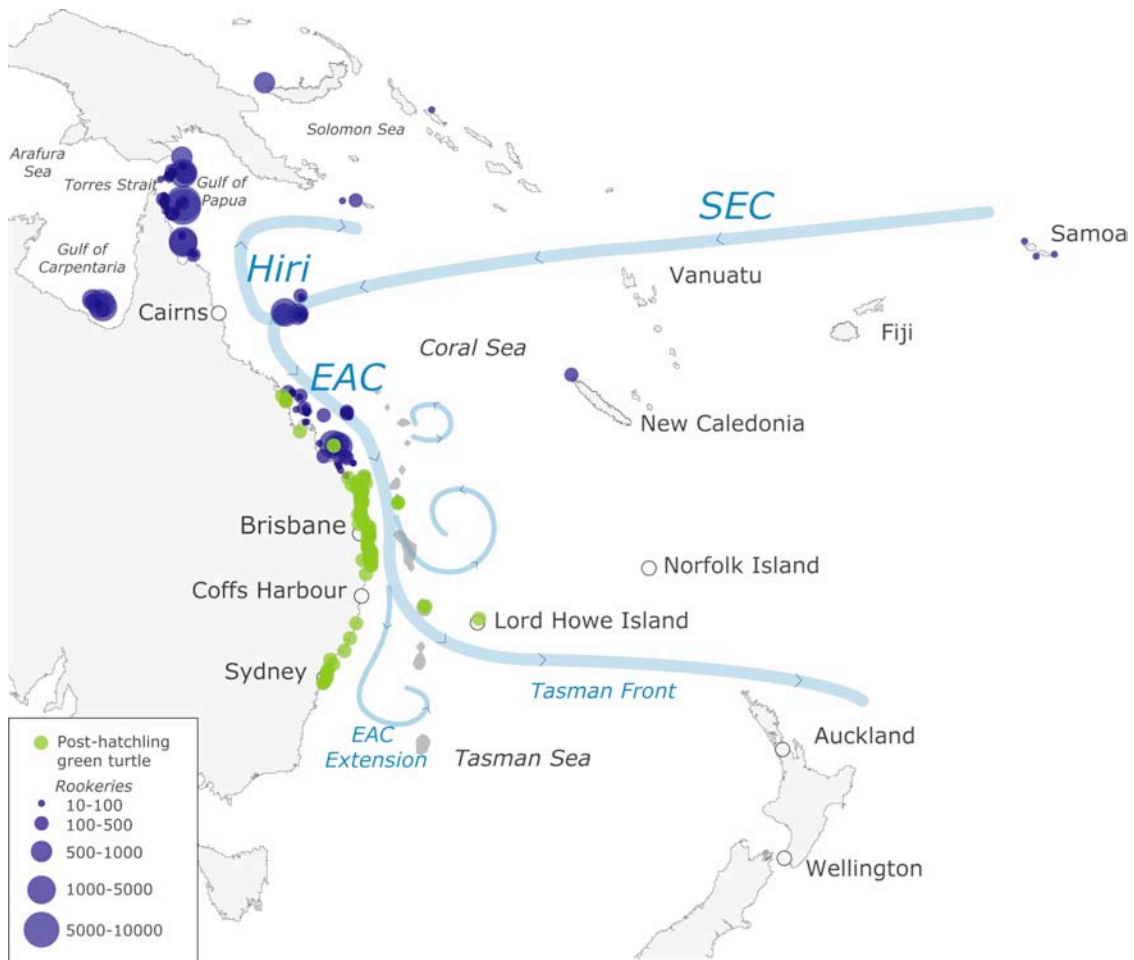


Figure 2.3. The distribution of post-hatchling green turtle (*Chelonia mydas*) records for the southwest Pacific region, with rookeries and simplified primary currents shown.

Size distribution of post-hatchling loggerhead and green turtles in the southwest Pacific

Post-hatchling loggerhead turtles

Post-hatchling loggerhead turtles recorded along Australia's eastern Pacific coast ranged in size from neonates (4.5 cm CCL) up to 13.7 cm CCL (Figure 2.4), with the majority (90%) of the individuals measuring less than 9.0 cm CCL.

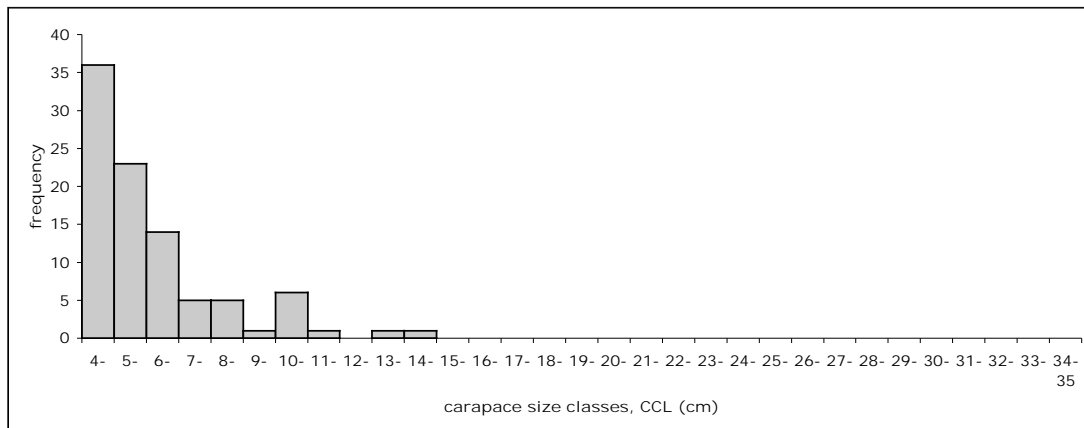


Figure 2.4. The size-frequency distribution of post-hatchling loggerhead turtles (*Caretta caretta*) stranded along Australia's Pacific coast.

The mean size of loggerhead post-hatchlings increases with distance from the primary rookery locations in the direction of the South Pacific sub-tropical gyre. The mean CCL measurements were 6.04 cm CCL along the Queensland coast, 8.05 cm CCL along the New South Wales coast, 13.82 cm CCL on New Zealand beaches and 53.3–71 cm CCL in the waters offshore from Peru and Chile (Table 2.1).

Post-hatchling green turtles

Post-hatchling green turtles recorded along the Australian Pacific coast ranged in size from neonates (4.9 cm) up to 34.7 cm CCL (Figure 2.5), with 81% being under 12 cm CCL. Green post-hatchlings found in the stomachs of *Coryphaena hippurus* ranged in size from 5.9 to 9.4 cm (mean = 7.3 cm, SD=1.18). The sole record for a green post-hatchling recorded at a locality offshore from Australia's east coast measured 10.2 cm CCL at Lord Howe Island.

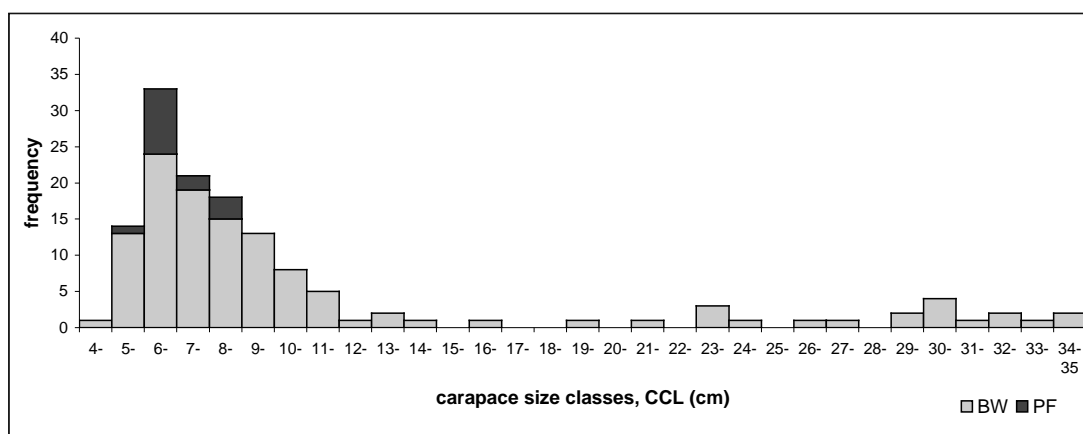


Figure 2.5. The size-frequency distribution of post-hatchling green turtles (*Chelonia mydas*) stranded (BW) and found in *Coryphaena hippurus* stomachs (PF) in the southwest Pacific.

The mean size of green turtle post-hatchlings does not vary with any pattern in regard to rookeries and currents. The smallest mean size was found in the post-hatchlings that had been consumed by dolphin fish (*Coryphaena hippurus*), although this is most likely determined by fish gape size and not location. The largest mean CCL measurements (15.1 cm CCL) were found amongst post-hatchlings that had become stranded north of Queensland's Sunshine Coast (Table 2.2).

Table 2.1. Size-range distribution of post-hatchling loggerhead turtles (*Caretta caretta*) recorded throughout the southern Pacific (adapted from Limpus *et al.*, 2005).

Geographical region	Curved carapace length (cm)				References
	mean	SD	Range	N	
Western South Pacific Ocean					
- SE Qld - Sunshine Coast to Gold coast (BW)	6.04	1.96	4.5-13.7	76	Limpus <i>et al.</i> , 1994b, EPA Stranding & Mortality Database
- NSW nth'n & central (BW)	8.05	2.51	5.1-13.0	11	
- New Zealand (BW)	13.82	9.86	8.6-33.0	9	
Eastern South Pacific Ocean					
- long-line fishery by-catch	57.0		48.5-62.5	7	Kelez <i>et al.</i> , 2003; Alfaro-Shigueto <i>et al.</i> , 2004, Donoso <i>et al.</i> , 2000
in sth'n Peru & nth'n Chile	56.6		41.0-70.0	29	
	53.3	10.8	26.0-65.5	15	
	71.0			1	
Recruitment size to coastal waters in Queensland					
-Capricornia reefs	79.05	4.34	68.0-93.9	53	Limpus & Limpus 2003a
-Hervey bay	78.63	1.86	76.0-80.0	3	
-Moreton bay	78.18	3.75	66.7-85.1	52	
combined	78.62	4.01	66.7-93.9	108	

Table 2.2. Size-range distribution of post-hatchling green turtles (*Chelonia mydas*) recorded throughout the southern Pacific (adapted from Limpus *et al.*, 2005) BW indicates stranded post-hatchlings, PF indicates turtles from predatory fish.

Region	Curved carapace length (cm)				References
	mean	SD	Range	N	
Western South Pacific Ocean					
- Qld - ntn Great Barrier Reef	15.1	14.73	5.1-34.5	6	EPA Stranding & Mortality Database
- SE Qld - Sunshine coast to Gold coast (BW)	12.2	8.18 6.32	5.4-34.7	82	
- NSW - northern & central (BW)	8.4		5.1-32.0	18	
- Lord Howe Island	10.2			1	
Fish stomachs (PF)	7.3	1.18	5.9-9.4	15	
Recruitment size to coastal waters in Queensland	43.6		38.7-48.5		Limpus & Limpus, 2005

Temporal distribution of post-hatchling loggerhead and green turtles in the southwest Pacific

Post-hatchling turtle strandings along Australia's eastern coast are observed throughout the year, however, most occur around the hatchling emergence season; March - May. Of all the loggerhead post-hatchling strandings, 89% (n=92) occurred during these three months, with one stranding occurring in each of July and November, two in each of June, August and September and three in October (Figure 2.6A). Of all the green post-hatchling strandings, 86% (n=98) occurred prior to June, with seven and six strandings occurring during August and September respectively and one per month during July, November and December (Figure 2.6B).

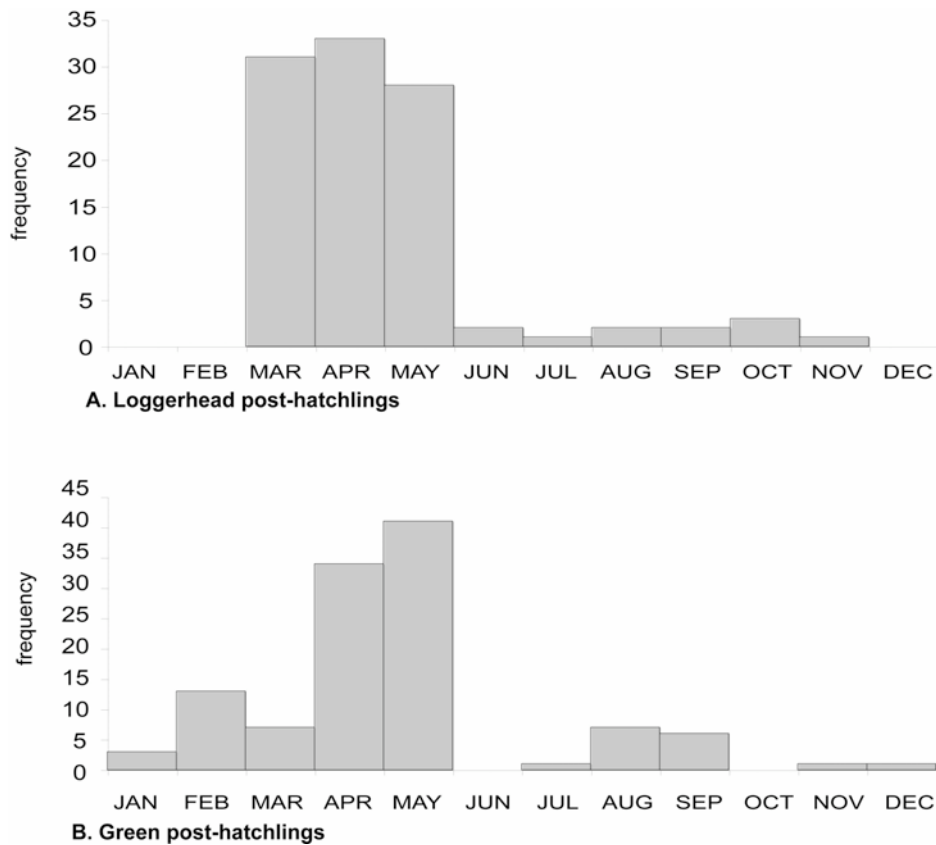


Figure 2.6. Seasonal distribution for the occurrence of post-hatchling loggerhead (*Caretta caretta*) and green turtles (*Chelonia mydas*) stranded along the east Australian coast.

Discussion

An oceanic existence

The lack of documentation of post-hatchling turtles in coastal waters, and the fact that the only records available for post-hatchlings in coastal waters are for stranded animals, provide supporting evidence that post-hatchling loggerhead and green turtles in the southwest Pacific Ocean lead an oceanic existence. Moreover, the majority of the stranding records are small post-hatchlings (i.e. <10 cm CCL) and neonates, and therefore relatively young animals. The young age of the turtles, combined with the fact that most strandings occur throughout, and immediately following the months when hatchlings emerge, provides further evidence that post-hatchlings are not lingering in coastal waters. If post-hatchlings were spending time within coastal waters it would be expected that the records would include a greater percentage of larger post-hatchlings with strandings occurring throughout the year, and the occasional *in-situ* observation. This conclusion is in keeping with all investigations to date, that post-hatchlings sea turtles occupy an oceanic habitat, with the exception of the flatback turtle.

The East Australian Current and post-hatchling dispersal patterns

As the Southern Equatorial Current brings water westwards into the Coral Sea it is likely to entrain loggerhead hatchlings emerging from Tokelau, Vanuatu and New Caledonian rookeries and green hatchlings emerging from New Caledonian and Coral Sea Platform rookeries. At the bifurcation, these entrained post-hatchlings will be directed either southwards within the EAC or northwards within the North Queensland Current in the Gulf of Papua.

Post-hatchlings emerging from rookeries in the southern Great Barrier Reef region, including Mon Repos, the Capricorn and Bunker Groups of islands and the Swain Reefs¹, will encounter the southward flow of the East Australian Current (EAC) (Figure 2.3). The concentration of post-hatchling strandings along the southern Queensland and northern New South Wales coasts strongly suggests that the EAC influences the initial migratory route taken by hatchlings emerging from rookeries in the southern Great Barrier Reef. The EAC may also contain a number of green and loggerhead post-hatchlings from offshore rookeries that have emerged into the flow of the Southern Equatorial Current (SEC).

The region (Figure 2.3) where the greatest number of records for stranded post-hatchlings occur, is also where the EAC crosses the continental shelf and moves closer inshore before turning eastward away from the Australian coast (Anderson, 1987; Tomczak and Godfrey, 1994). Post-hatchlings entrained within the EAC will be brought close to the shore in this region resulting in a greater chance of animals becoming washed ashore than in other regions, particularly during adverse weather conditions. This is supported by post-hatchling strandings occurring more commonly after strong winds and heavy seas (C. Limpus, pers. com, 2002).

Post-hatchlings stranded south of the SEC's bifurcation point would be predicted to be a mix of hatchlings from both the eastern Australian and offshore rookeries. It is likely that the greater proportion of these would originate from southeast Queensland rookeries, which are nearer. The spatial and temporal distribution of the post-hatchling turtles alone do not indicate whether this is the case. In order to ascertain the importance of the EAC in the distribution of post-hatchlings from offshore rookeries compared to rookeries within the southern Great Barrier Reef region, the genetics of these post-hatchlings needs to be investigated to determine their natal origin (see Chapters 3 and 4).

After the EAC swings away from the Australian coast, post-hatchlings using this current for transportation can embark on one of two likely migratory routes; (i) they can travel northwards and remain in the eddies offshore from the Australian coast or (ii) they can travel eastwards with the Tasman Front. If they remain with the Tasman Front, post-hatchlings would travel across the South Pacific Ocean, past Lord Howe Island and the north of New Zealand, across the southern Pacific Ocean, and past Peru and Chile via the Peru current (also known as the Humboldt Current), before returning to the east coast of Australia with the SEC (Figure 2.2) (Burrage, 1993).

¹ ¹ The latitudes and longitudes for the regional locations referred to in this thesis are provided in Table A1, Appendix A.

A number of factors indicate that the southwest Pacific loggerhead post-hatchlings are making trans-oceanic migrations similar to those undertaken by loggerhead post-hatchlings from south eastern USA and Japanese rookeries (Bolten *et al.*, 1998; Bowen *et al.*, 1995). The first line of evidence is the occurrence of oceanic stage loggerhead turtles stranded on northern New Zealand beaches and in fisheries operating in the waters off the Peruvian and Chilean coasts. As the only documented loggerhead rookeries in the Pacific Ocean occur in Japan in the northeast Pacific, and in eastern Australia and New Caledonia in the southwest Pacific Ocean, it would seem, based on distance and oceanographic features, that these post-hatchling loggerhead turtles have originated from southwest Pacific rookeries. Further evidence that this species undertakes trans-Pacific migrations is provided by the progressive increase in size of loggerhead post-hatchlings along the route of the anticyclonic subtropical gyre in the South Pacific Ocean. Additionally, the complete absence of records for loggerhead post-hatchlings larger than 13.7 cm CCL in Australian coastal waters suggests that post-hatchlings of this species inhabit waters far removed from the Australian coast.

Post-hatchlings that do not embark on migrations across the South Pacific Ocean could instead remain in the warm water eddies produced by the EAC offshore from the east Australian coast. Owing to its meandering nature, the EAC forms approximately three eddies each year that can persist as large-scale (ca. 1000 km across) anti-clockwise gyres in the deep water off the southern Queensland and northern New South Wales coasts, with four to eight eddies often co-existing at the same time (Burrage, 1993; Tomczak and Godfrey, 1994). Within these EAC eddies, post-hatchlings would embark on a counter-clockwise journey around the Coral and Tasman seas. The size distribution and spatial data available for green post-hatchlings suggest that this is what this species is doing. This is highlighted by the absence of records for post-hatchling sized green turtles in New Zealand or in waters beyond the southwest Pacific (compared with loggerhead records). Additionally, although the majority of stranding records for green post-hatchlings belong to small size classes, records do exist for all size classes up to the size observed that juveniles recruit back into coastal feeding grounds. That larger post-hatchlings do occasionally become stranded further supports the supposition that these animals occupy offshore oceanic waters and do not undergo trans-Pacific migrations that would remove them completely from the Coral and Tasman seas.

The difference in migration routes undertaken by the two species is further evidenced by the presence of only green post-hatchlings in the stomach contents of the dolphin fish, *Coryphaena hippurus*. These dolphin fish were caught in waters near offshore sea mounts. The lack of decomposition of the post-hatchlings, indicates that they were also inhabiting waters in the vicinity of these sea mounts. These seamounts lie beyond the typical path of the EAC but within the region that EAC eddies often exist. No loggerhead turtles were found in the stomachs of *C. hippurus*, which further attests that the post-hatchlings of this species, unlike post-hatchling green turtles, are not occupying waters near offshore sea mounts.

It is possible that the different plastron colouration of post-hatchling loggerhead and green turtles could account for the absence of loggerhead post-hatchlings in the stomachs of *Coryphaen hippurus*. However, as countershading (light underside) is a predator avoidance technique for marine pelagic organisms (Denton *et al.*, 1972; Johnsen, 2001), it would be expected that the dark plastrons of loggerhead post-hatchlings, would be more visible to fish predators than the light plastrons green post-hatchling turtles. Therefore, if post-hatchlings

of both species were in the same area, one would expect that loggerhead post-hatchlings are more likely to be preyed upon than green post-hatchlings. Loggerhead post-hatchlings have been found in the stomachs of predatory fish in other studies, including *C. hippurus* (Carr, 1987; Witham, 1974) and *Lutjanus* spp. (snapper) (Vose and Shank, 2003), and this suggests that if loggerhead post-hatchlings were available in these waters they would be preyed upon. The number of post-hatchlings collected from *C. hippurus* predation is reasonably low (n=18), further collection would enhance our understanding of the presence of each species in these environments to support the observation made here that loggerhead post-hatchlings do not frequent the same waters as green post-hatchlings.

The North Queensland Current and post-hatchling dispersal patterns

Records for post-hatchlings north of the SEC bifurcation point were considerably lower than records south of this point. Consequently, there was little evidence to indicate transportation by the North Queensland Current that were comparable to those available for the East Australian Current. Certainly, as hatchlings emerging from northern rookeries (e.g. Raine Island and Bramble Cay) will for some period be associated with surface waters in the Gulf of Papua, it would be anticipated that post-hatchlings occasionally strand along Australia's eastern coast northwards of Cairns and along the southern New Guinea coast. In southeast Queensland and northern New South Wales a high level of effort goes into collecting information on stranded animals, primarily in the form of public awareness generated by *StrandNet* and the response of EPA staff to stranding reports. This effort is not replicated in the coastal region north of Cairns primarily because of the relative remoteness of this area. Additionally the lower human occupancy of this region would considerably reduce the chances of a small post-hatchling turtle being found before it decomposes or is eaten by scavengers such as seabirds or crabs. It may also be that the low stranding records for the northern region are because fewer post-hatchlings actually do become stranded in this region. The northern coast is protected by the Great Barrier Reef from high-energy wave action, whereas the southern region is not, and consequently the northern coast is rarely subjected to rough seas that may weaken small post-hatchlings and contribute to them becoming stranded. Moreover, there is not an equivalent current that swings in as close to the coast as the EAC along the southeast coast.

The flow of surface waters in the northern Great Barrier Reef and Gulf of Papua region are complex and vary both spatially and temporally. The post-hatchlings that will most likely be caught in these complex drift schemes are green hatchlings emerging from rookeries in the far northern Great Barrier Reef and Torres Strait, and any hatchlings within the SEC that were directed northwards at the bifurcation point. Hatchlings that enter this system could follow one of several possible drift schemes. They may; (i) travel into the Torres Strait, (ii) be directed southwards into the EAC, (iii) remain within the gyre in the Gulf of Papua, or (iv) enter the Solomon Sea.

The net flow of water from the Gulf of Papua into the Torres Strait is low and surface drift is variable, generally flowing eastward from January to March and westward for the remainder of the year (Wolanski and Thomson, 1984). Therefore the route westwards from the Gulf of Papua New Guinea is likely to be a possibility only for hatchlings emerging after March, and even then because of low flow in this region it is unlikely that this route would be taken by

many post-hatchlings. If a post-hatchling was to embark on this journey, it would enter the waters of the Gulf of Carpentaria and eventually reach the Arafura Sea.

Under some conditions there is a southward flow of waters from the Gulf of Papua to southern Queensland. This is supported by observations of drift seeds from northern Queensland and Papua New Guinea germinating on cays in the southern Great Barrier Reef (Smith *et al.*, 1990) and from satellite-tracked buoys released in the northern Gulf of Papua being recovered in southern Queensland (MacFarlane, 1980). If post-hatchlings were to be directed southwards from the Gulf of Papua their ensuing route would be influenced by the EAC flow and the subsequent direction would be the same as for hatchlings emerging from southern rookeries.

The most likely routes for post-hatchlings in the Gulf of Papua are to remain within the circulation of the northern Coral Sea (the North Queensland Current), or to leave the Gulf of Papua via the Solomon Sea. The complex drift scheme in the Gulf of Papua may result in post-hatchlings travelling within numerous gyres until they take up residency in a local coastal feeding zone or are directed into another current system. The release of particles from Raine Island (Bode *et al.*, 1995) suggests that post-hatchling turtles associated with surface waters in the Gulf of Papua are most likely to be advected into the Solomon Sea and then along the northern coast of Papua New Guinea towards Indonesia. From Indonesia, currents flow south-eastwards towards Vanuatu, via the western coast of the Solomon Islands, and post-hatchlings associated with this flow may eventually be directed back towards the coast with the flow of the SEC (Bode *et al.*, 1995).

If post-hatchling loggerhead turtles emerging from offshore rookeries (e.g. New Caledonia) do become entrained within the SEC, and are directed northwards into the Gulf of Papua at the bifurcation point, it seems unlikely that they would undertake trans-Pacific migrations via the sub-tropical South Pacific gyre as the only way of reaching it would be through southwards advection into the EAC. Although this has been shown to be possible it is probably not common, for if loggerhead post-hatchlings were travelling southwards after first being advected in the northern current system, occasional strandings of larger loggerhead post-hatchlings along the path of the EAC would be expected.

Although the analysis of currents and models can provide insight into the probable migratory routes of post-hatchling turtles in the northern Coral Sea, the lack of stranded individuals available from this region does not allow substantiation of these hypotheses. To do this, greater information on stranded post-hatchlings along the far north eastern coastline of Cape York and the southern and northern coastline of Papua New Guinea would be required.

Conclusion

The spatial and temporal distribution of post-hatchling loggerhead and green turtles in relation to rookery locations and the local prevailing oceanic currents, support the hypothesis that post-hatchlings from populations in the southwest Pacific region become entrained in oceanic currents. However, the data also indicate that the two species do not

take the same migratory route after their departure from coastal waters. There is evidence that post-hatchling loggerhead turtles undergo trans-Pacific migrations within the southern sub-tropical gyre. This is shown by, (i) incremental size increase in direction of the current away from nesting beaches, (ii) loggerhead post-hatchlings occurring in New Zealand waters and on the eastern side of the South Pacific Ocean, and (iii) post-hatchlings larger than 13.7 cm CCL not being documented in the southwest Pacific.

In comparison the data suggest that although green post-hatchlings are not living in coastal waters, they remain in offshore oceanic waters in the southwest Pacific and do not undergo trans-Pacific migrations. This is indicated by, (i) green post-hatchlings occupying waters around offshore seamounts (whereas loggerhead post-hatchlings appear absent), (ii) the absence of green post-hatchlings in New Zealand or Peruvian and Chilean waters, and (iii) the occasional occurrence of larger size classes of green post-hatchlings stranded on the eastern Australian coast.

Chapter 3

A genetic approach to establishing post-hatchling turtle migration routes in the southwest Pacific Part I: mtDNA diversity in the southwest Pacific

Introduction

Molecular markers have provided tools for addressing a wide range of questions pertaining to sea turtle evolution, natural history, taxonomy and systematics (e.g. Dutton, 1996) and their global phylogeography (Bowen *et al.*, 1991, 1992, 1994, 1997; Dutton *et al.*, 1999a). Although a range of markers have been employed within sea turtle studies, such as allozymes, Restriction Fragment Length Polymorphisms (RFLPs), and microsatellites, the majority of contemporary studies employ mitochondrial DNA (mtDNA).

The first substantial mtDNA study of sea turtles (Bowen *et al.*, 1992) revealed a fundamental phylogenetic split in green turtles (*Chelonia mydas*) that distinguished those in the Atlantic-Mediterranean from those in the Indian-Pacific Oceans based on restriction-site analyses. A subsequent study investigating mtDNA haplotypes, again based on restriction-site analyses, for loggerhead (*Caretta caretta*) turtles also revealed a substantial phylogeographic split amongst major nesting populations investigated as well (Bowen *et al.*, 1994). Like the green sea turtles, two primary lineages were distinguished in the loggerhead populations, however unlike the green turtles, genotypes representing both lineages were found in all ocean basins.

The differences observed between these two species has been attributed to variations in their ecology and the geographic ranges they occupy (Bowen, *et al.* 1994). The green turtle is predominately a tropical species and populations from the Atlantic Ocean and Mediterranean Sea are isolated from populations in the Indian and Pacific Oceans by the cold temperate waters around the southern tips of Africa and South America, hence the formation of these two lineages through vicariance. These two lineages most likely shared a common ancestor prior to the formation of the Isthmus of Panama approximately three million years ago (Bowen *et al.*, 1992). In contrast, the loggerhead turtle is adapted to temperate waters and can utilise habitat around southern Africa and therefore populations would not have been isolated by the Isthmus of Panama (Bowen, *et al.* 1994).

In addition to delineating the major evolutionary lineages, assays of the maternally inherited mtDNA have also revealed geographic substructure within each ocean basin for green and loggerhead turtles (Bowen *et al.*, 1992; FitzSimmons *et al.*, 1996; Moritz *et al.*, 2002a; Norman *et al.*, 1994). Mitochondrial DNA studies of rookery structure have been extended to other sea turtle species, with significant matrilineal structure reported for hawksbills (Bass *et al.*, 1996; Broderick and Moritz, 1996; Broderick *et al.*, 1994), leatherbacks (Dutton *et al.*, 1999b), and ridley turtles (Bowen *et al.*, 1997). The observed differences in mtDNA lineages between geographic regions provides evidence that the high degree of fidelity showed by nesting

females to specific nesting beaches is a display of natal homing behaviour. This strong propensity for natal homing by nesting females has significance to conservation of these species, because any colony that is extirpated would be unlikely to be recolonised naturally (over ecological time scales), thus signifying that protection for sea turtle populations needs to be afforded at the rookery level (Awise, 2004).

Investigations into the mtDNA genetic structure of sea turtle populations beyond the nesting beaches (e.g. Bass *et al.*, 2004; Bolten *et al.*, 1998; Bowen *et al.*, 1995; Bowen *et al.*, 2004; Bowen *et al.*, 1996; Lahanas *et al.*, 1998) have shown that genetic structure is low at coastal feeding grounds and appears absent within some post-hatchling populations (Bowen *et al.*, 2005). This is typical of many migratory populations (see Bowen *et al.*, 2005), owing to the overlap of these populations at feeding habitats and during migrations.

Whereas mtDNA markers have been shown to reveal high levels of genetic structure between most rookeries², the levels of genetic structure revealed in assayed nuclear loci is lower. This pattern has been shown in sea turtle surveys (Karl *et al.*, 1992; Roberts *et al.*, 2004) including those on loggerhead and green turtles in Australian waters (FitzSimmons, In Press). The differences in the level of structure exposed by the two different markers comes about because of sex-biased behavioural differences. Such results have also been found in surveys of humpback whales (Baker *et al.*, 1998; Palumbi and Baker, 1994), harbour seals (Burg *et al.*, 1999), Dall's porpoise (Escorza-Trevino and Dizon, 2000) and the shortfin mako shark (Schrey and Heist, 2003). In these studies males are the primary mediators of gene-flow among populations or natal regions.

In addition to revealing the genetic structure of populations, studies using molecular markers have illuminated certain features of a sea turtle's life history that may have otherwise remained concealed. An example of this is the revelation of multiple paternity in sea turtles confirmed by allozyme and microsatellite assays (Bollmer *et al.*, 1999; Crim *et al.*, 2002; FitzSimmons, 1998; Harry and Briscoe, 1988; Hoekert *et al.*, 2002; Ireland *et al.*, 2003; Kichler *et al.*, 1999; Moore and Ball, 2002). These studies reveal that multiple paternity of clutches sired by multiple males is a relatively common (~10-60%) phenomenon amongst sea turtles. Where multiple paternity has been detected, the majority of studies have only been able to verify two fathers per clutch, with only two studies to date detecting more than two fathers (Lee and Hays, 2004; Moore and Ball, 2002). It is worth noting that these estimates of the number of fathers represented in a clutch will be conservative as fathers that share alleles will only be identified as the one father (Moore and Ball, 2002).

A mating system where females exhibit polyandrous behaviour can benefit the population or the female in numerous ways, including increased mean offspring fitness via sperm competition and fertility assurance if some males have poor-quality sperm (see Jennions and Petrie, 2000 for review, but also see Lee and Hays, 2004). In addition, as the life history of sea turtles means that colonisation of a new beach may typically be through one single gravid female, there would be significant benefits to establishment via polyandrous females due to

² A turtle rookery traditionally refers to a beach where the adult female lays her eggs, and in this sense the word 'rookery' can be interchangeable with the term 'nesting beach'. In this thesis the term 'rookery' refers to a nesting region, that may consist of more than one nesting beach that are in close proximity (typically adjacent) to one another.

the elevated levels of more genetic diversity they bring with them over and above a monoandrous female (Moore and Ball, 2002).

Mixed stock assessment

A practical application of molecular markers has been their use in assessing the genetic composition of populations that are comprised of individuals derived from multiple rookeries, known as 'Mixed Stock Analysis' (MSA). This application has been widely used in fisheries (e.g. (Beacham *et al.*, 2005c; Grant *et al.*, 1980; Koljonen *et al.*, 2005; Potvin and Bernatchez Giroq, 2001) and several sea turtle populations including green (Moritz *et al.*, 2002a), loggerhead (Bass *et al.*, 2004; Bowen *et al.*, 1995; Sears *et al.*, 1995) and hawksbill turtles (Bass, 1998; Bowen *et al.*, 1996). The results from such assessments can have some important management and conservation implications. For example, in species such as anadromous salmon, molecular markers have been used to determine the different river drainages (reproductive stocks) that are supplying the mixed oceanic fisheries and their proportionate contributions (e.g. Beacham *et al.*, 2005a; Beacham *et al.*, 2005b; Koljonen *et al.*, 2005). From this information harvesting strategies can be tailored to accommodate the management and conservation requirements of the respective reproductive stocks.

Before mixed stock analyses can be undertaken, several features of the populations in question need to be considered to determine whether such an analysis is suitable and to establish the level of confidence in the results. The primary factors that influence the effectiveness of MSA are the availability of genetic information to characterise all potentially contributing populations, the relative sample sizes of the populations being assessed and the degree of genetic differentiation between the contributing genetic stocks (Chapman, 1996). This thesis aims to employ MSA (Chapter 4) to determine the natal origin of the post-hatchlings investigated in this study to gain insight into their migration routes. Thus, what follows next is an overview of the current understanding of the loggerhead and green turtle genetic stocks in the southwest Pacific in view of their suitability for mixed stock analysis.

The potential for MSA with loggerhead and green turtles in the southwest Pacific region

To conduct mixed stock analyses genetic information is required for all potential source populations, and the sampling of these populations needs to be adequate enough to provide a true representation of the haplotype occurrence and frequency within these populations (Manel *et al.*, 2005). If this is violated the usefulness of MSA will be limited as results will not be based on true baseline information. Inadequate source population sampling may also result in the occurrence of haplotypes in the mixed stock that are not accounted for in the baseline populations, although this situation could also arise owing to rarer alleles being exposed and can be reduced by binning rare alleles into closely related common haplotypes (Bromaghin and Crane, 2005).

The source rookeries of post-hatchlings sampled along Australia's east coast, are most likely to be located in the southwest Pacific and to a lesser extent in South East Asia. Within these regions, the mtDNA genotypic frequencies have been investigated in conspecific nesting rookeries for green (Moritz *et al.*, 2002a) and loggerhead (Moritz *et al.*, 2002b) turtles. The genetic sampling that has occurred throughout the southwest Pacific region has been

geographically extensive and incorporates all known major breeding aggregations of both species, with the exception of the New Caledonian loggerhead rookery. It is accepted that nesting occurs in low numbers along the coast, and as genetic sampling has concentrated on those rookeries that are relatively centralised, the more remote areas with sporadic nesting are poorly represented in rookery sampling. This is more the case for green turtles who have more diffuse nesting than loggerhead turtles, whose nesting in Australian waters is concentrated in a few primary locations. The sample sizes vary across locations which can be reflective of rookery size but can also be due to the logistics of collecting samples at some localities (e.g. New Caledonia).

Previous studies of matrilineal genetic structure of loggerhead turtle rookeries in the southwest Pacific Ocean

Within the southwest Pacific Ocean, loggerhead turtle nesting occurs predominately at five rookeries (Figure 3.1). Four of these rookeries are located in eastern Australian waters at Wreck Rock, Mon Repos, Wreck Island and Swain Reefs, and one rookery is located in the southern province of New Caledonia. Genetic material has been collected from the four Australian rookeries and sequencing has revealed a lack of genetic variation between these rookeries (Moritz *et al.*, 2002b). Hence, this assemblage of rookeries is recognised as one homogenetic breeding group or genetic stock. This study investigated a ~380 nt sequence of the mtDNA region and found only two haplotypes which were distinguished by a single base-pair variation (Moritz *et al.*, 2002b). The most common haplotype (CCP1)³ was found in 98% (n=101) of samples, with the less common haplotype (CCP5) occurring in 2% (n=2) of samples (Moritz *et al.*, 2002b) (Figure 3.1).

The lack of heterogeneity amongst the east Australian loggerhead rookeries and the lack of genetic information on the loggerhead rookeries in New Caledonia currently precludes the use of MSA within the southwest Pacific region for loggerhead turtles.

Previous studies of matrilineal genetic structure of green turtle rookeries in the southwest Pacific Ocean

In contrast to loggerhead turtles, genetic heterogeneity does occur among regional sets of green turtle rookeries located in the southwest Pacific (Moritz *et al.*, 2002a). The mtDNA region sequenced for green turtles (Moritz *et al.*, 2002a) was the same as that used for loggerhead turtles, however for green turtles this region exposes 25 haplotypes that form 17 genetically discrete breeding groups in the South East Asian and western Pacific region (Figure 3.2). These breeding groups were determined by the presence of significant differences in the frequency of mtDNA variants (based on significant output from Exact tests). In the southwest Pacific region there are four genetically distinct green turtle breeding groups; Coral Sea Platform, New Caledonia, southern Great Barrier Reef and northern Great Barrier Reef. These regions consist of a number of geographically adjacent rookeries. The southern Great Barrier Reef assemblage consists of Heron, Lady Musgrave and North West

³ The names used to describe the haplotypes discussed in this thesis are those applied by the author that originally described them, and in keeping with the names used in GenBank.

Islands as they comprise a homogenous genetic group. Similarly, the northern Great Barrier Reef assemblage consists of Bramble Cay, Raine Island and No.8 Sandbank.



Figure 3.1. The genetic structure of loggerhead turtle (*Caretta caretta*) rookeries in the southwest Pacific Ocean as determined by Moritz *et al.*, (2002b; based on ~384 nt). There are five rookeries in the region, and based on frequencies of mtDNA haplotypes the east Australian rookeries form one genetically distinct breeding group. The eastern Australian rookeries are; Swains Reef (SR), Wreck Island (WI), Wreck Rock (WR), and Mon Repos (MR). The combined genetic structure of these rookeries is represented by a pie-graph, with the coloured portions symbolising different haplotypes. At the time of Moritz *et al.*'s (2002b) study the genetic structure of the primary rookery in New Caledonia, Lá Roche Percee (RP), was unknown.

A number of the haplotypes that occur in the southwest Pacific region occur across multiple rookeries at high frequencies (e.g. haplotypes A2, C1 and C3), while others are found only among several adjacent rookeries (e.g. haplotypes A1, B1, B3, B5, C4, C5, C8, C9, D2, B5) and a few are rookery specific (e.g. haplotypes B4, C2, C7, C12, C1, J1,J2) (Moritz *et al.*, 2002a). Haplotypes that have broad geographic distributions and variable frequencies pose limitations to MSA by reducing the resolution between populations. To minimise this problem, fisheries based research has inclined towards using microsatellites, which have a greater number of potential expressions than other marker types, in multilocus assignment tests (Beacham *et al.*, 2005c; Potvin and Bernatchez Giroq, 2001; Withler *et al.*, 2004). However

the employment of microsatellites for population resolution is not so effective in situations where gene flow is male-mediated, and where levels of genotypic diversity are low such as in sea turtle populations (Awise *et al.*, 1992). In such populations, microsatellite data do not reveal the level of population structure reflected in parallel mtDNA surveys as is observed in sea turtles. Therefore to detect population structure in sea turtle populations, mtDNA markers are the marker of choice.

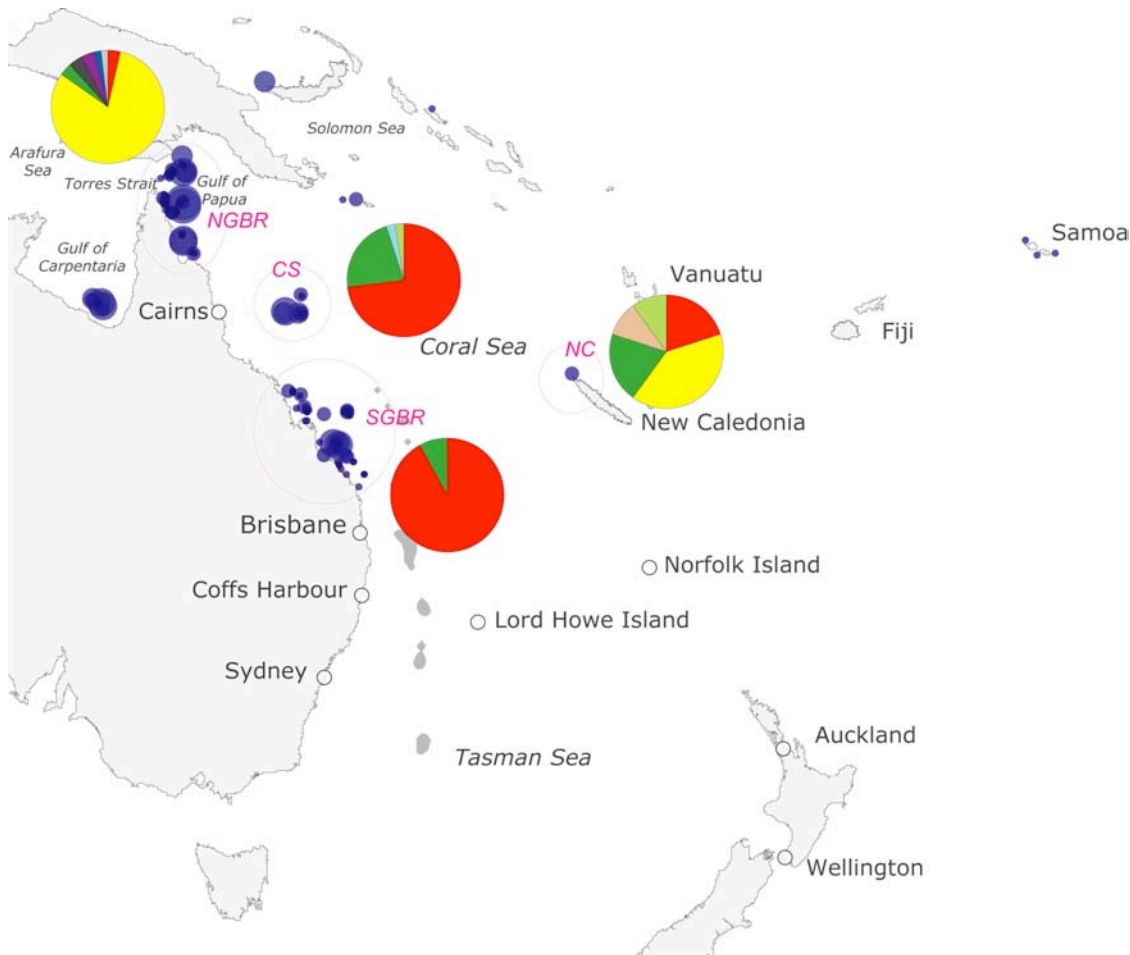


Figure 3.2. The genetic structure of green turtle (*Chelonia mydas*) rookeries in the southwest Pacific Ocean as determined by Moritz *et al.*, (2002a; based on ~384 nt). Based on frequencies of mtDNA haplotypes, these authors suggest there are four genetically distinct breeding groups; the northern Great Barrier Reef (NGBR), the Coral Sea (CS), the southern Great Barrier Reef (SGBR) and New Caledonia (NC). The genetic structure of the breeding groups is represented by a pie-graph, with the coloured portions symbolising different haplotypes.

The mtDNA variation observed between the green turtle rookeries provides a potential basis for employing MSA to determine the stock structure at a broad scale and also at a regional scale. Moritz *et al.* (2002a) used the stock structure of rookeries in the southwest Pacific and South East Asia to determine the stocks contributing to feeding aggregations in northern and Western Australia and eastern Indonesia, and for harvests from Indonesia, northern Australia and the Torres Strait. The output of the MSA that they employed provided a guide to which stocks are making contributions to these feeding grounds and harvests. MSA was

most effective for mixed stocks that contained alleles that were not widely distributed and less effective for stocks that contained alleles with a broader geographic distribution with variable frequencies (e.g. C1 and C3 at Fog Bay and Ashmore Reef feeding grounds). The current limitations to mixed stock analysis in the southwest Pacific region for green turtles is the sharing of haplotypes between regions that provides greater error margins in MSA, and the low sample sizes collected from some populations (e.g. New Caledonia).

Improving MSA for southwest Pacific loggerhead and green turtles

Increased resolution of green and loggerhead rookeries in the southwest Pacific would enhance the capabilities of mixed stock analyses within this region. Currently, the information on the genetic structure of loggerhead and green turtle rookeries in the southwest Pacific and South East Asia is based on a ~384 nt region within the mtDNA control region (Moritz *et al.*, 2002a, 2002b). It may be possible to yield information that will provide finer resolution to the stocks in this region through using multilocus techniques or investigating a longer region of the mtDNA control region. Multilocus techniques have been investigated by FitzSimmons (in prep.) for both green and loggerhead turtles in the southwest Pacific region and it was found that these populations register lower genetic structure in nDNA assays relative to mtDNA, as has been the case for all published data to date (FitzSimmons *et al.*, 1996; Schroth *et al.*, 1996). To date, sequencing a longer region of the mtDNA has not been investigated and could provide a way of finding greater resolution between turtle rookeries in the southwest Pacific Ocean region.

Aim of this chapter

This chapter addresses the limitations of the current baseline rookery information for green and loggerhead turtles in the southwest Pacific for the employment of mixed stock analysis in the following chapter (Chapter 4). This will be achieved in two ways; (i) by improving the mtDNA resolution of potential source rookeries for the post-hatchling loggerhead and green turtles investigated in this study, and (ii) by addressing the need for baseline reference mtDNA information for the New Caledonian loggerhead rookery. To increase the resolution of these rookeries I will investigate a longer region of the mtDNA control region than has been examined in previous investigations (~1100 nt this study versus ~384 nt used by Moritz *et al.*, 2002a, 2002b). Additionally, the mtDNA data for this study will be used to establish the relationship of the haplotypes found in the southwest Pacific rookeries with current phylogeographic data of both study species globally. Previously phylogenies based on mtDNA have been derived from haplotypes determined from restriction analysis, making this study the first to examine the relationships based on nucleotide sequence information from the mtDNA d-loop.

Methods

Sample collection and DNA extraction

In order to increase the genetic resolution of green and loggerhead turtle stocks in the southwest Pacific region, stored tissue from previous investigations into the genetic structure of southwest Pacific rookeries (Moritz, *et al.* 2002a, 2002b) was accessed for re-sequencing (Dr Nancy FitzSimmons; Applied Ecology Research Group, University of Canberra). In total 95 samples from loggerhead (n=40) and green (n=55) east Australian rookeries were re-sequenced. The samples for loggerhead turtles were from Wreck Island (WI) (n=15), Swains Reef (SR) (n=15) and Mon Repos (MR) (n=10) (Figure 3.1). The samples from green turtle rookeries were spread across the northern Great Barrier Reef (NGBR) (n=5 each from Bramble Cay and Raine Island), southern Great Barrier Reef (SGBR) (n=20), Coral Sea (CS) (n=15) and New Caledonia (NC) (n=10) (Figure 3.2). During the laboratory work a genetic sample from a juvenile green turtle that had recently recruited into coastal Queensland waters was supplied. This sample was subsequently found to represent haplotype C3 (Moritz, *et al.* 2002b) and was incorporated into this present study for the long sequence information that it could provide on this haplotype.

To obtain information on the genetic structure of the New Caledonian loggerhead rookery, skin samples were taken from 29 adult female turtles during a nesting survey conducted at La Roche Percee (S21°37.989; E165°27.807) in January, 2005 (Figure 3.1). La Roche Percee is the primary loggerhead turtle rookery in New Caledonia, beyond this beach loggerhead nesting is reported to occur sporadically and only at low densities (J. Pierre pers. comm., 2005). Skin samples were removed with a sterile scalpel blade from the upper shoulder region of the turtle when she had finished depositing eggs. All turtles were tagged with standard turtle flipper tags to avoid repeated sampling.

Tissue samples were stored in 20% dimethyl sulfoxide (DMSO) saturated with 5M NaCl (no EDTA), routinely used to preserve Chelonid tissue (Dutton, 1996). Tissue samples were removed from the DMSO, rinsed in distilled water, and minced up with a sterile scalpel blade to optimise DNA extraction. Genomic DNA was then isolated from approximately 0.1 mg of tissue by proteinase K digestion in 250 μ l of Digsol extraction buffer containing, 50 mM Tris, 20 mM EDTA, 120 mM NaCl and 1% sodium dodecyl sulphate (SDS). The DNA was then recovered from the solution using ethanol precipitation in the presence of 4 M ammonium acetate and resuspended in TE buffer (10 mM Tris, 0.1 mM EDTA, pH 7.5).

PCR amplification and sequencing

A 1120 nt length fragment anchored in the Cytochrome B and control region of the mitochondrial genome was amplified with polymerase chain reaction (PCR) methodology, using the primers TCR6 (5'-GTA CGT ACA AGT AAA ACT ACC GTA TGC C-3') and TCR1 (5'- GGA TCA AAC AAC CCA ACA GG -3') designed for sea turtles (Norman *et al.*, 1994).

TCR1 is positioned in the Cyt B region and TCR6 is positioned in the control region (Figure 3.3).

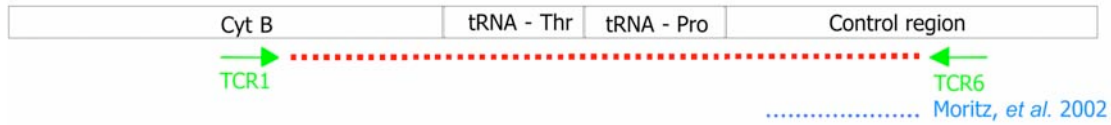


Figure 3.3. The region sequenced for loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) turtles within the mtDNA genome. The red line represents the region analysed in the present study and the blue line represents the region analysed by Moritz *et al.* (2002a, 2002b).

PCR conditions were optimised using methods described by Cobb & Clarkson (1994). PCR amplifications were performed in a 25 μ l reaction volume containing, 3 ng of DNA template, 1x QIAGEN PCR Buffer (containing Tris-Cl, KCL, $(\text{NH}_4)_2\text{SO}_4$, 15 nM MgCL₂; pH 8.7 @ 20°C), 0.32 mM of each dNTPs, 4 mM MgCL₂, 0.40 mM of each primer (TCR1 + TCR6), 0.04 μ g/ μ l BSA and 0.5 units of Taq DNA polymerase (QIAGEN). PCR amplifications included a negative (DNA free) control reaction to test for contamination. The amplification conditions were as follows: 95°C for 2 mins followed by 34 cycles at 94°C for 25 sec, 48°C for 15 sec, 72°C for 45 sec with a final extension at 72°C for 5 sec.

The amplified products were electrophoresed in agarose gels and excised from the 1.5% agarose gels (containing 40 mM Tris-acetate, 1 mM EDTA) and purified using a gel extraction kit (QIAGEN). Quantification of DNA concentrations were performed with ImageJ 1.33 software (<http://rsb.info.nih.gov/ij>). Cycle sequencing reactions were conducted in both directions with the amplification primers (TCR1 and TCR6) in ET terminator (Amersham Biosciences™) half reactions according to manufacturers instructions and analysed in the Genetic Analysis Facility in James Cook University's Advanced Analytical Centre on a MegaBase 1000 (Amersham Biosciences™), and at Macrogen Inc.

Genetic data analysis

Sequence alignments

Resulting sequences were edited with Sequencher 4.2.2 (GeneCodes, 1991), and manually aligned using Se-AL v 2.0a11 (Rambaut, 2002). Variability at sites was confirmed with the associated chromatograms for sequences in both directions. Sequences of loggerhead and green turtles were downloaded from the GenBank database (National Centre for Biotechnology Information, <http://www.ncbi.nlm.nih.gov>), the Archie Carr Centre for Sea Turtle Research (<http://accstr.ufl.edu/genetics.html>) and obtained from Moritz *et al.* (2002a, 2002b) for phylogenetic comparisons. The long sequences obtained in this study were compared to previously described haplotypes and assigned letter codes following the conventions established in the GenBank database and Moritz *et al.* (2002a, 2002b). Sequences that resolved into previously undescribed haplotypes were considered to be 'new' haplotypes and were assigned unique codes and registered with GenBank.

Datasets used within genetic analyses

Genetic analyses are enhanced by the use of large data sets and for comparative purposes data are often required across a large spatial scale. Owing to the logistics and finances involved in such large scale studies, research into establishing genetic information for sea turtles is typically a collaborative effort involving many researchers. For this reason, a number of the analyses that I used in this study incorporated data collected during previous studies. The three datasets, from which analyses were conducted, are outline below and in Table 3.1.

The first dataset (A) consists solely of long sequence (1120 nt) information derived from this immediate study. This dataset is comprised of newly obtained samples (e.g. New Caledonian loggerhead turtles), and from a subset of samples used by Moritz *et al.* (2002a, 2002b) during their examination of genetic structure in southwest Pacific rookeries, that I re-sequenced for the present study (Table 3.1).

The second dataset (B) combined the information obtained from A (see above) plus all the information obtained during Moritz *et al.*'s (2002a, 2002b) study. This currently equates to the most comprehensive data set available for green and loggerhead turtles in the southwest Pacific region (Table 3.1). The combining of these data from A and additional data from Moritz *et al.*'s (2002a, 2002b) results in a dataset that has a combination of long sequences (1120 nt) and short (~384 nt) sequences. However, as the majority of samples are represented by short sequences, all analyses bar one, conducted with this dataset were based on haplotypes that were only evident in the short sequence region. The analysis that used haplotypic information derived from both the long and short sequences was a mixed stock analysis, and is explained further in the following chapter (Chapter 4).

The third dataset (C) is a collation of haplotype information for loggerhead and green turtles based on a global scale, including the southwest Pacific region (Table 3.1). This dataset is limited to what has been placed on the internet and published, and hence does not represent a complete set.

Table 3.1. The three datasets derived from nesting loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) turtles that were used in the genetic analyses employed in this study.

Dataset	Description	Sample size	Source
A	New samples obtained during this study for both loggerhead and green turtles. Long sequences: 1120 nt	loggerheads n=65; greens n = 43	Present study
B	Samples from dataset A and information on loggerhead and green nesting regions in the southwest Pacific. Long and short sequences: 1120 nt and 384 nt	loggerheads n=132; greens n = 210	Present study, (Moritz, <i>et al.</i> 2002a, 2002b)
C	Haplotype information contained within datasets A and B, and information on loggerhead and green turtle mtDNA haplotypes available at a global scale. Short sequences: 384 nt	loggerheads n=38; greens n = 120	Present study, (Moritz, <i>et al.</i> 2002a, 2002b), online sources

Diversity indices

Sequences were analysed within ModelTest 3.7 (Posada and Crandall, 1998) to determine the best-fit nucleotide substitution model for implementation in Arlequin 3.0 (Excoffier *et al.*, 2005). Estimates of nucleotide diversity for each species amongst the adult sequences were then calculated using Arlequin 3.0 based on Kimura 2P measures, as determined from ModelTest.

Haplotype diversity was calculated using the following equation (Nei, 1987),

$$h = \frac{n(1 - \sum x_i^2)}{n - 1}$$

where n is the number of individuals in the population and x_i represents the frequency of the i th haplotype within the population. The diversity indices for both nucleotides and haplotypes, were estimated with dataset B for loggerhead turtles, and with dataset A for green turtles.

Minimum spanning analyses

To visualise the relationships between the mtDNA haplotypes described in the results, minimum spanning networks were constructed based on the pairwise differences calculated in Arlequin 3.0 (Excoffier *et al.*, 2005). The analyses were conducted with different datasets for loggerhead and green turtles owing to the varying sequence analyses results between the two species. Because of the lack of variation amongst the loggerhead turtles between the long versus short sequences, minimum spanning networks were constructed with collated haplotype information for this species for the southwest Pacific (dataset B). Dataset A was used to provide a comparison between information provided by long (~1100 nt) and short (~380 nt) sequences of green turtles, as variation that was found in the long sequences was lost when the sequences are investigated solely at the short region.

Phylogenetic relationships

To explore the relationship that exists between the haplotypes found in the southwest Pacific Ocean and those that exist in other oceanic regions, information was collated to develop a global dataset for green and loggerhead turtles based on mtDNA sequence data. This dataset is restricted to what has been posted on the internet and published, and as a result does not represent a complete global data set. As available haplotype information is currently typically based on a shorter sequence region (~380 nt) than that used in this study, the analyses performed with this dataset are limited to only including haplotypes that are defined within this shorter region. Consequently, any haplotypes that emerge only as a result of the long sequence information of this current study will not be represented.

Bayesian inference and Markov Chain Monte Carlo (MCMC) were used to estimate the phylogenetic relationships amongst the green and loggerhead turtles using MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003). Unlike other programs (e.g. PAUP and Phylip) that use methods of parsimony and maximum likelihood to estimate phylogeny, Bayesian inference is based upon estimating the posterior probability of the parameter. Prior to implementation

of MrBayes, the best-fit model of nucleotide evolution needs to be specified. The models for nucleotide evolution were calculated separately for loggerhead and green turtle sequences using MrModeltest 2.2 (Nylander, 2004). Models were selected under the Akaike information criterion (AIC; (Akaike, 1973), which is based on maximum likelihood estimates. The models of best-fit were the HKY+G (Hasegawa *et al.*, 1985) model for the loggerhead turtle sequences, and GTR+I+G (Tavare, 1986) for green turtle sequences. Prior distributions for the model parameters were set to the program's defaults.

The same parameters were used for both the loggerhead and green turtle models. Three runs, each consisting of four Markov chains were run simultaneously, initiating each chain from a random tree and branch lengths. Each chain was run for 9,000,000 generations, sampling from the chain every 100th generation. To confirm convergence of the independent runs the parameter means and variances between runs and the output (plot of each parameter of the model of the independent runs and potential scale reduction factor) values were examined. The first 30,000 of the sampled generations were discarded, and hence inferences were based upon a sample of 180,000 (3 runs x 60,000 samples). For the green turtles, a prior distribution for the nucleotide substitution rate ratios of the GTR model was specified as a Dirichlet prior, Dir (1,1,1,1,1).

Results from the independent analyses (n=3 for loggerhead turtles, n=3 for green turtles) were summarised and a 50% majority rule consensus tree with nodal posterior probability support and branch lengths was generated. The loggerhead turtle phylogenetic tree was rooted using a representative from the green turtles and the green turtle phylogenetic tree was rooted using mtDNA sequence information from a leatherback turtle. These out-groups were selected based on groups that were basal to the species of interest (Pritchard, 1997).

Results

Mitochondrial DNA analysis

After editing and alignment, long sequences of 1120 nt in length were obtained for the majority of specimens. A number of samples did not amplify successfully, due to DNA degradation, and hence genetic information was not obtained for these animals. For a number of samples amplification by only one primer (i.e. in one direction) was successful. Samples for which only the forward sequence was obtained were omitted from following analyses as the region sequenced did not span the region that has been used in previous analyses (Moritz *et al.*, 2002a, 2002b) and therefore could not be used for comparative purposes. Because those samples with a successful reverse sequence spanned the region that has been previously analysed, they were included in subsequent analyses.

The obtained sequences provided two primary datasets; (i) loggerhead turtle rookeries, and (ii) green turtle rookeries. Each primary dataset could be looked at as (a) long sequences (1120 nt) or (b) short sequences (384 nt). The short sequences replicate the region sequenced in previous mtDNA studies (Moritz, *et al.*, 2002a, 2002b) in the western Pacific and South East Asian region.

Loggerhead turtle rookeries in the southwest Pacific Ocean

Haplotypes and diversity indices

Genetic material representing east Australian and New Caledonian loggerhead turtle rookeries was successfully obtained from 65 individuals (Table 3.2). All 36 adult females from the east Australian rookeries were found to be haplotype CCP1, while 27 of the 29 females sampled at the New Caledonian rookery were haplotype CCP1 and two were haplotype CCP5. The codes applied to these sequences are consistent with those used in GenBank, where CCP1 relates to haplotype 'A' and CCP5 relates to haplotype 'B' in Moritz, *et al.* (2002a).

Table 3.2. Haplotype frequencies found at loggerhead turtle (*Caretta caretta*) rookeries in the southwest Pacific Ocean.

Location	Sample size (N)	CCP1	CCP5
Swains Reef, East Australia	14	1.0	0
Wreck Island, East Australia	12	1.0	0
Mon Repos, East Australia	10	1.0	0
New Caledonia	29	0.93	0.07

The extended sequences (1120 nt) did not show any finer resolution between the two haplotypes (CCP1 and CCP5) from that already reported in Moritz *et al.* (2002b). However, there was a consistent variation in the sequences obtained in this study from those reported by Moritz *et al.* (2002b) at two positions (714 and 726). These positions are near the beginning of Moritz *et al.*'s (2002a) sequences where the sequence may have been unclear and consequently incorrectly interpreted using older methodologies. Despite this variation in the nucleotide changes reported at the these two positions, it remains that only one polymorphic site exists between the two loggerhead haplotypes found in the southwest Pacific (CCP1 and CCP5) (Table 3.3). Full sequence alignment is shown in Appendix C and have been submitted to GenBank (Acc. No EF033112 & EF033113).

Table 3.3. Aligned mtDNA haplotypes of southwest Pacific loggerhead turtles (*Caretta caretta*). The haplotype names are consistent with those used in GenBank.

Haplotypes	Nucleotide base position		
	714	726	845
CCP1 – this study, 2006	A	G	G
CCP1 – Moritz <i>et al.</i> , 2002a	T	A	G
CCP5 – this study, 2006	A	G	A
CCP5 – Moritz <i>et al.</i> , 2002a	T	A	A

As the extended length sequences did not reveal any new haplotypes amongst southwest Pacific loggerhead turtles, or any further resolution between the two haplotypes that do exist, the sequence information obtained from the New Caledonian natal region was combined with the sequence information available for the Australian rookeries (Moritz, *et al.* 2002b) for the diversity indices calculations.

With only one polymorphic site and two haplotypes observed in loggerhead turtles, the resulting nucleotide and haplotype diversity amongst the loggerhead turtles in the southwest Pacific is very low. The highest nucleotide diversity was observed at the New Caledonian rookery (0.0007), although this is still a very low value (Table 3.4). The low diversity values were enhanced in the longer sequences, revealing just how genetically similar this species is in the southwest Pacific. The haplotype diversity values are accordingly low with a value of only 0.061 for the combined New Caledonian and Australian rookeries (Table 3.4).

Table 3.4. The nucleotide and mtDNA haplotype diversity of loggerhead (*Caretta caretta*) turtles in the east Queensland region, the New Caledonian region and within these two regions combined (southwest Pacific).

Region	Sequence length	N	N haplotypes	Haplotypic diversity	Nucleotide diversity π (\pm SD)
East Australia	short	103	2	0.0384	0.0002 (\pm 0.00)
	long	36	2	0.0384	0.0001 (\pm 0.00)
New Caledonia	short	29	2	0.1330	0.0007 (\pm 0.00)
	long	29	2	0.1330	0.0002 (\pm 0.00)
Southwest Pacific (=E. Aust + N. Cal.)	short	132	2	0.0592	0.0003 (\pm 0.00)
	long	65	2	0.0592	0.0001 (\pm 0.00)

Updated haplotype information for loggerhead turtle rookeries in the Southwest Pacific Ocean

The present study did not find any information that changed the current understanding (derived from Moritz *et al.*, 2002b) on haplotype frequency in the Australian rookeries. The investigation into the genetic stock structure of the New Caledonian loggerhead rookery did however, enhance our knowledge of loggerhead turtle stocks in the southwest Pacific region. Both the Australian and New Caledonian rookeries are comprised of only two haplotypes. Haplotype CCP1 is the dominant haplotype occurring at 98% in the Australian rookeries and 93% in the New Caledonian rookeries (Figure 3.4).



Figure 3.4. The genetic structure of loggerhead turtle (*Caretta caretta*) rookeries in the southwest Pacific Ocean as determined in this study. Each cluster of geographically adjacent rookeries is represented by a pie-graph, with the coloured portions symbolising different mtDNA haplotypes. The recognised genetic structure of these rookeries has not changed for the eastern Australian region from Moritz *et al.*, (2002b; based on ~384 nt), to the present study (based on ~1200 nt). Prior to the present study there was no information on the New Caledonian rookeries.

Minimum spanning analyses

The minimum spanning network (MSN) illustrates the low divergence between the two loggerhead haplotypes that are found in the southwest Pacific (Figure 3.5). They do however hint that CCP5 may be more prevalent in New Caledonia, although this could be owing to the low sample size for the New Caledonian rookery compared to the east Australian rookeries. The single base-pair difference between the two haplotypes strongly suggests that these haplotypes are derived from the same lineage. The relationship depicted in the MSN is the same for both the short and long sequences.

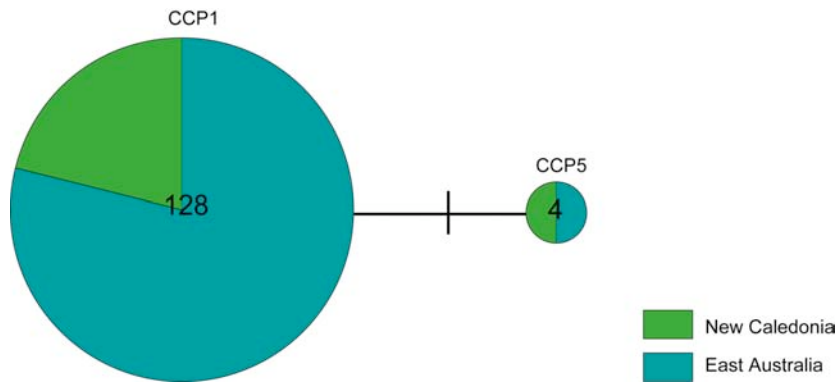


Figure 3.5. Minimum spanning network showing the relationship between the mtDNA haplotypes (based on long sequences, 1120 nt) of loggerhead turtles (*Caretta caretta*) from east Australian (n=103) and New Caledonian (n=29) rookeries. The colours indicate the location of the rookery containing that haplotype, and the size of each circle and the number within it indicates the number of individuals with that haplotype. The single dash represents one base pair difference between the haplotypes.

Loggerhead phylogeography

The phylogeny tree derived from loggerhead mtDNA nucleotide sequences (dataset C) shows three primary clades. Two of these clades are comprised of haplotypes that have only been found in the Atlantic Ocean (blue text), and one consists of haplotypes found in the both the Pacific Ocean (red text) and Atlantic Ocean (Figure 3.6). All three clades contain haplotypes that have been recorded at both feeding grounds and rookeries, however owing to the migratory nature of feeding turtles, haplotypes that are found in rookeries are more indicative of the haplotype origin. One of the Atlantic lineages is constructed of haplotypes that predominately occur in rookeries from the southwest Atlantic. This appears to be the basal clade from which the other clades are derived, a slightly more derived but weakly supported Atlantic clade ($p \geq 0.50$), the strongly supported Pacific Clade ($p \geq 0.95$) and a more derived and strongly supported Atlantic clade ($p \geq 0.95$). The more derived Atlantic clade contains haplotypes that are found in rookeries located predominately in the northern Atlantic. The Pacific clade contains haplotypes found at rookeries in the northern and southern Pacific Ocean but also shows the occurrence of Pacific rookery haplotypes in the Atlantic Ocean. The phylogram clearly shows the low haplotype diversity in the Pacific (with only four haplotypes having been reported at rookeries) in comparison to those reported in the Atlantic Ocean. Haplotypes that represent those found in Indian rookeries are notably absent from this analysis, owing to the lack of published information from this region.

Green turtle rookeries in the southwest Pacific Ocean

Haplotypes and diversity indices

Genetic material was successfully obtained from 43 samples representing east Australian green turtle rookeries: SGBR n=16, NGBR n=9, CS n=12, NC n=6. Alignment of the sequences obtained from rookery samples revealed 46 polymorphic sites that resolve into

eight haplotypes within the long (1120 nt) sequenced region (Table 3.5). Four of these haplotypes correspond to previously described sequences (A2, B1, B3, C13 in Moritz *et al.* 2002a), whilst four are previously undescribed. I retained the previous haplotype designations and assigned the undescribed sequences codes on the basis of homology to previously defined haplotype, followed by an unique letter determined by the order of observation. Two of the new haplotypes group closely with A2 and have been labelled A2b (1 nt additional substitution compared with A2) and A2c (3 nt additional substitutions compared with A2) accordingly. One of the new haplotypes aligned closely with B1 (1 nt additional substitution) and has been assigned the code B1b and one new haplotype was assigned the code name B3b (1 nt additional substitution compared with B3) to signify its close relationship with haplotype B3. Full sequence alignment have been submitted to GenBank (Acc. No. EF029117 - EF029126) and are shown in Appendix D.

The variable sites that occur prior to position 726 in the long sequence are outside of the region previously sequenced to define haplotypes (Table 3.5). In this way these nucleotide variations further define; A2 from B1, B3, C3 and C13 (position # 375, 429, 512, 640, 641, 642, 643), C3 from A2, B1, B3, C13 (position # 70, 366, 592) and C13 from A2, B1, B3, C3 (position # 240, 643, 719).

In the long sequence dataset, A2b was represented by two individuals (1xCS, 1xSGBR), A2c was represented by one individual (1xCS), B1b represented by one individual (1xSGBR) and B3b was represented by two individuals (2xNC) (Table 3.6). The A2 haplotype was the most common haplotype occurring at 49% across all rookeries, with the majority of A2 coming from the SGBR rookeries (Table 3.6). B1 was the next most commonly occurring haplotype at a frequency of 27.5%. The remaining haplotypes were represented by two individuals with the exception of A2c that was represented by only one individual (Table 3.6).

Table 3.5. Aligned mtDNA haplotypes of southwest Pacific green turtles (*Chelonia mydas*). Haplotype A2 is the reference sequence, where a period (.) indicates a common sequence, a (?) represents missing data, with variable sites indicated by the base concerned, and (*) indicates previously undescribed haplotypes. The haplotype name is consistent with those assigned by Moritz *et al.*, 2002a.

	Nucleotide base position					
					11111	111111
	2223344	5566666667	7777777788	8888899999	9999900000	000011
	4570456724	1911144441	7888899900	7888901467	7889922234	444690
haplotype	9108026591	2278901239	1156756945	9369492286	8245612362	349098
A2	TATCCGCTAC	TTAAT----T	GGTTGCATAA	ATCATAGAAC	AGGTCTCCTA	CGTTGC
A2b*T.
A2c*C..	G...C.....
B1	CT.T.A.CTA	C....TAAC.	.A...TG...	..TG..AG.T	GAA.TCTTCG	TA..A.
B1b*	CT.T.A.CTA	C....TAAC.	.A...TG...	..TG..AG.T	GAA.TCTTCG	T...??
B3	CT.T.A.CTA	C....TAAC.	.A...TG...	...G..AG.T	GAA.TCTTCG	TA..A.
B3b*	CT.T.A.C.A	C....TAAC.	.A...TG...	...G..AG.T	GAA.TCTTCG	TA..AA
C3	..C...TCT.	CC---TAAC.	..CCAT....	.C...G..GT	GAAC.CTTCG	TAC.??
C13T..CT.	C.---TAATC	A..CAT..GGG.	G...T.TTCG	T..CA.

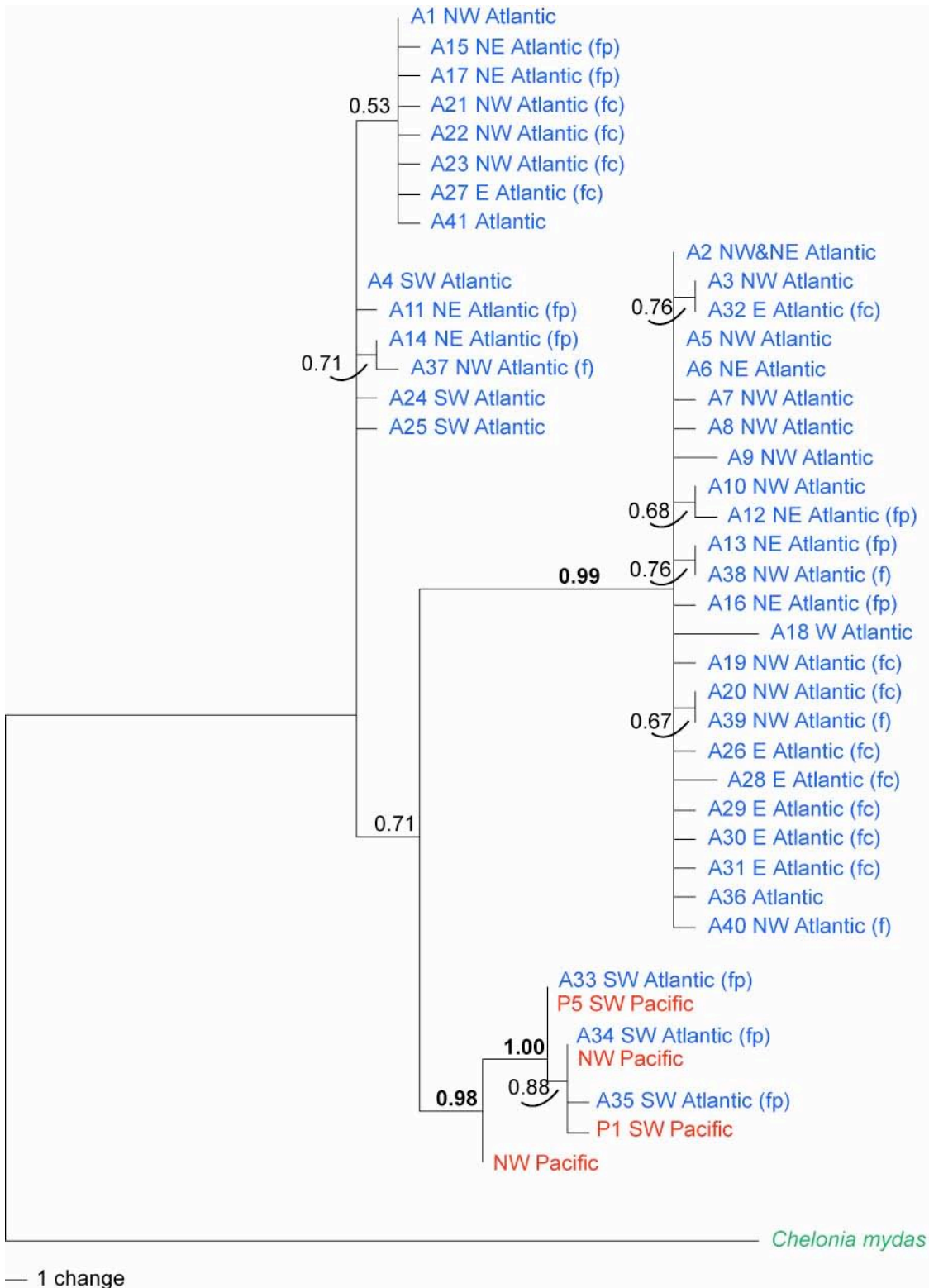


Figure 3.6. Majority rule consensus tree derived from Bayesian MCMC phylogenetic analyses of the loggerhead turtle (*Caretta caretta*) mtDNA haplotypes under the HKY+G model of nucleotide evolutions. Nodal posterior probabilities are indicated with those greater than 0.95 shown in bold. The different geographical regions are colour coded. Those haplotypes that are annotated with either (fp) or (fc) refer to those haplotypes occurring at coastal (fc) or pelagic (fp) feeding locations. Unannotated haplotypes were collected from nesting beaches.

Table 3.6. Haplotype frequencies as defined in long sequences, found at green turtle (*Chelonia mydas*) rookeries in the southwest Pacific Ocean. (n=43). (*) denotes haplotypes previously undescribed. SGBR = southern Great Barrier Reef, NGBR = northern Great Barrier Reef, CS = Coral Sea, NC = New Caledonia.

Haplotypes	Haplotype frequency at locations				Total haplotype frequency
	SGBR n = 16	NGBR n = 9	CS n = 12	NC n = 12	
A2	0.82 (13)	0.11 (1)	0.59 (7)		0.49 (21)
A2b*	0.06 (1)		0.08 (1)		0.05 (2)
A2c*			0.08 (1)		0.02 (1)
B1	0.06 (1)	0.89 (8)		0.50 (3)	0.28 (12)
B1b*	0.06 (1)				0.02 (1)
B3			0.17 (2)		0.05 (2)
B3b*				0.33 (2)	0.05 (2)
C13			0.08 (1)	0.17 (1)	0.05 (2)

To compare the information that is obtainable from the longer sequenced region (1120 nt), to that from the shorter sequenced region (~384 nt) that was used in previous studies (Moritz *et al.*, 2002a), the same adult sequences were analysed again, but this time based on the shorter sequence region. This resulted in a reduction in the number of polymorphic sites and haplotypes detected amongst the sequences (Table 3.7). Haplotypic diversity (*h*) decreased at the SGBR and CS rookeries, where haplotypes A2b and B3b collapsed into A2 and B3 respectively, which resulted in an overall decrease from eight to six haplotypes (Table 3.8). Nucleotide diversity was higher across all regions in the short sequence region. Although the actual number of haplotypes remained the same at New Caledonia, the two samples that were a B3 within the short sequence become new haplotypes (B3b) in the long sequence.

Table 3.7. Summary of long and short sequence information obtained for green turtles (*Chelonia mydas*). Long sequence information based on 1087 nucleotides and short sequence information based on 362 nucleotides.

	Adults long sequence	Adults short sequence
# haplotypes	8	6
# polymorphic sites	46	29
# of observed sites with transitions	44	29
# of observed sites with transversions	4	0
# of observed sites with substitutions	49	29
# of observed sites with indels	7	0
Nucleotide composition	C : 25.25% T : 28.61% A : 34.23% G : 11.91%	C : 16.08% T : 33.20% A : 35.40% G : 15.32%
Sample size	43	43

Table 3.8. The nucleotide and mtDNA haplotype diversity of green turtles (*Chelonia mydas*) as calculated with both long and short sequences within regions and with the regions combined to represent all the rookeries in the southwest Pacific Ocean. SGBR = southern Great Barrier Reef, NGBR = northern Great Barrier Reef, CS = Coral Sea, NC = New Caledonia, SWP = Southwest Pacific..

Region	Sequence length	N	Number of haplotypes	Haplotypic diversity	Nucleotide diversity π (\pm SD)
SGBR	short	16	3	0.242 (\pm 0.135)	0.012 (\pm 0.007)
	long	16	4	0.350 (\pm 0.148)	0.006 (\pm 0.013)
NGBR	short	9	2	0.222 (\pm 0.166)	0.017 (\pm 0.007)
	long	9	2	0.222 (\pm 0.166)	0.006 (\pm 0.013)
CS	short	2	4	0.561 (\pm 0.154)	0.022 (\pm 0.012)
	long	12	5	0.667 (\pm 0.141)	0.011 (\pm 0.006)
NC	short	6	3	0.733 (\pm 0.155)	0.017 (\pm 0.011)
	long	6	3	0.733 (\pm 0.155)	0.009 (\pm 0.006)
SWP	short	43	6	0.639	0.030 (\pm 0.015)
	long	43	8	0.690	0.013 (\pm 0.007)

Updated haplotype information for green turtle rookeries in the southwest Pacific Ocean

The additional haplotype information obtained in the present study has further defined our current understanding of the genetic structure of green turtle rookeries in the southwest Pacific region (Figure 3.7). Four haplotypes are now recognised to occur amongst the southern Great Barrier Reef rookeries as opposed to the previous two. An additional two haplotypes were found amongst the Coral Sea rookeries, taking the haplotypes from four to six in this region. This study has also revealed that both of the B3 haplotypes that had been found at New Caledonian rookeries resolve into new haplotypes in the longer sequences, which at this present time appears to be unique to this rookery. There were no changes to the haplotypes recognised amongst the northern Great Barrier Reef rookeries.

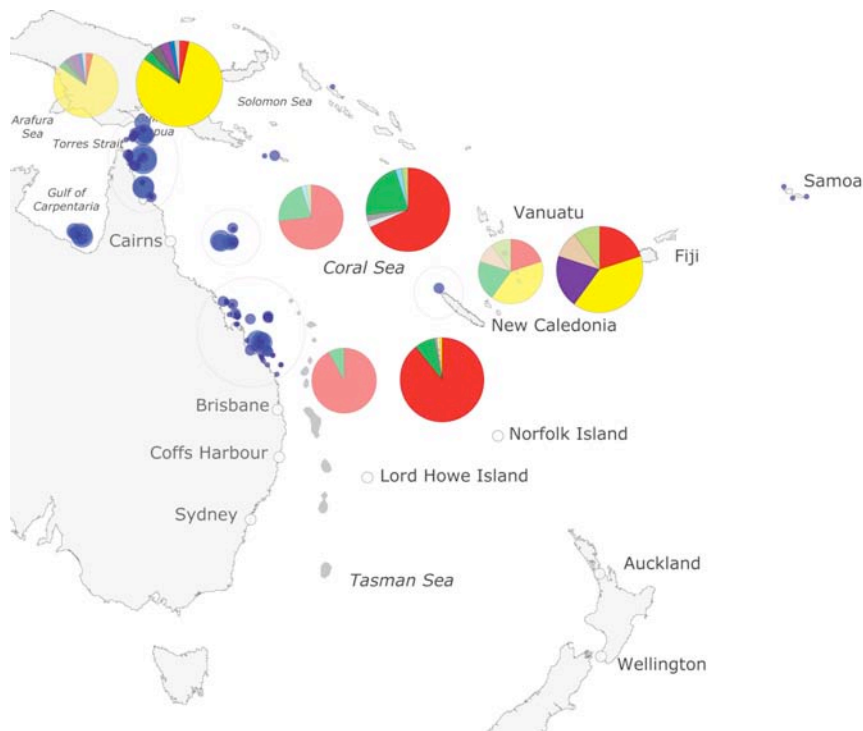


Figure 3.7. The genetic structure of green turtle (*Chelonia mydas*) rookeries in the southwest Pacific Ocean as determined in this study. Each genetically distinct region is represented by a pair of pie-graphs, with the coloured portions symbolising different haplotypes. The smaller, faded pie-graph represents the recognised structure prior to this study (based on ~384 nt by Moritz *et al.*, 2002a), and the larger, brighter pie-graph represents the updated structure derived from the present study (based on ~1200 nt).

Minimum spanning analyses

The minimum spanning networks (MSN) show that three lineages exist amongst the green turtle rookeries in the southwest Pacific. The distance between the lineages is increased in the MSN produced with the longer sequences (Figure 3.8.B) compared to those produced with the shorter sequences (Figure 3.8.A). The MSN also illustrate the collapse of A2b into A2 and B3b into B3 when only the short sequences are analysed.

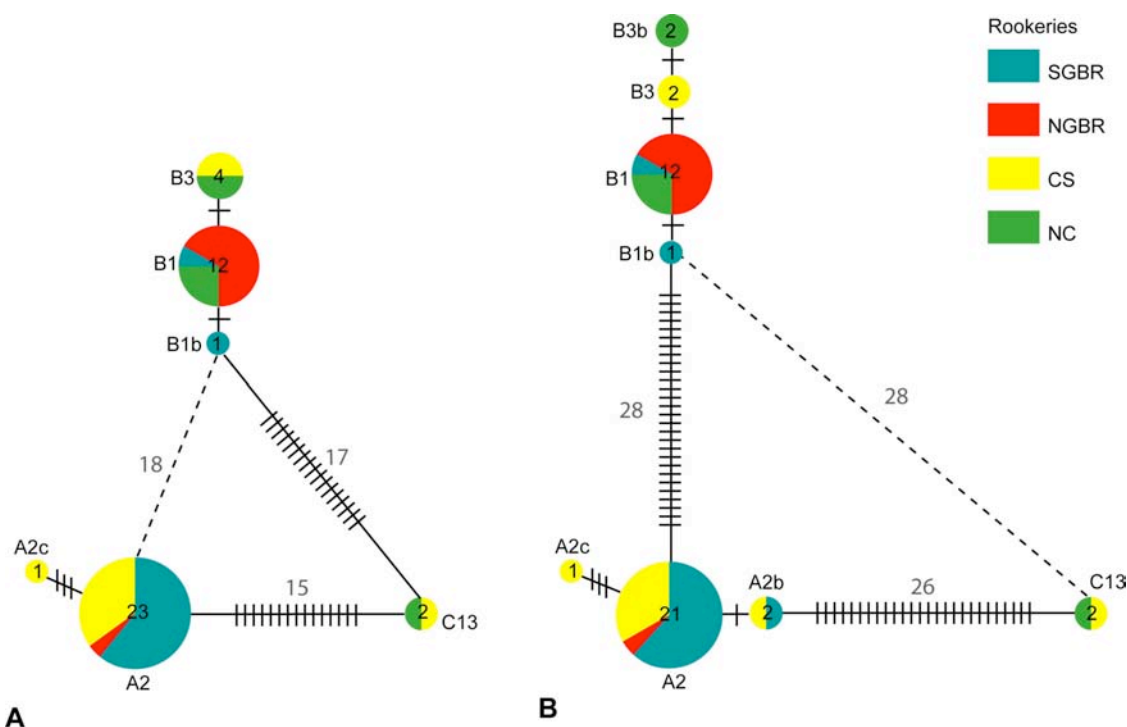


Figure 3.8. Minimum spanning networks showing the relationship between the mtDNA haplotypes of green turtles (*Chelonia mydas*), **A**; based on short, 384 nt and **B**; based on long, 1120 nt, sequences. Sampling regions are southern Great Barrier Reef (SGBR, n=16), northern Great Barrier Reef (NGBR, n=9), Coral Sea (CS, n=12) and New Caledonian (NC, n=6) rookeries. The colours indicate the location of the rookery containing that haplotype. The size of each circle and the number within it indicates the number of individuals with that haplotype. Each dash represents a single base pair difference between the haplotypes.

Green turtle phylogeny

The phylogenetic tree derived from green turtle mtDNA nucleotide sequences (384 nt) shows two major lineages, both of which have strong support ($p \geq 0.99$). One of these lineages is comprised of exclusively Atlantic Ocean haplotypes, and the other is comprised of exclusively Pacific and Indian Ocean haplotypes (Figure 3.9). The phylogenetic tree suggests that the Pacific/Indian lineage is basal to the derived Atlantic lineage. Both lineages exhibited similar haplotype diversity. Some internal structure within the Pacific lineage exists, with a clustering of haplotypes from the central eastern Pacific rookeries. In addition to the two primary lineages, there are some smaller lineages comprising of Pacific and Indian Ocean haplotypes that are derived from the basal Pacific-Indian clade. These smaller groups consist of Pacific-Indian haplotypes with one grouping consisting only of Indian Ocean haplotypes that have been identified in the Oman rookery. These smaller groups illustrate that clear geographic partitioning of the haplotypes in the Pacific and Indian Oceans is possible and further exploration of these relationships may reveal this.



Figure 3.9. Majority rule consensus tree resulting from Bayesian MCMC phylogenetic analyses of the green turtle (*Chelonia mydas*) mtDNA haplotypes under the GTR+I+G model of nucleotide evolutions. Nodal posterior probabilities are indicated with those greater than 0.95 shown in bold. The different geographical regions are colour coded.

Discussion

Loggerhead turtle diversity

An investigation into 1120 nt of the mtDNA genome of loggerhead turtles did not result in the detection of rookery heterogeneity in the southwest Pacific region, nor did it reveal any further variation between the two previously described haplotypes. Therefore, it remains that loggerhead nesting populations in the southwest Pacific region (rookeries MR, WR, WI, SR, NC) consist of only two haplotypes; CCP1 at 97%, and CCP5 at 3%.

The low level of variability in mtDNA amongst loggerhead turtle rookeries in the southwest Pacific is reflective of the overall pattern in the entire Pacific ocean. Three haplotypes have been detected in Japanese rookeries (Hatase *et al.*, 2002a), one of which is CCP1, making a total of four haplotypes in the Pacific (Bowen, *et al.*, 1995). Low levels of genetic diversity are also reported for the Indian Ocean, with only two haplotypes being described based on RFLPs (Bowen *et al.*, 1994). In comparison to the low levels of genetic diversity of loggerhead rookeries in the Pacific and Indian oceans, the Atlantic Ocean populations are considerably more diverse. In a companion study, Bolten *et al.* (1998) and Encalada *et al.* (1998) detected 17 haplotypes among 380 loggerhead samples from the Atlantic (including a small number of Greek samples), based on the analysis of 380 nt in the mtDNA control region. Also, Laurent *et al.* (1998) reported 11 haplotypes among 259 samples from the Mediterranean (including a small number of American samples), based on the analysis of 452–458 nt in the same region. Although it is not clear of the overlap of haplotypes that may have occurred between these two sets of studies, it is apparent that haplotype diversity for loggerhead turtles in the Atlantic Ocean is considerably greater than in the Pacific or Indian oceans.

The low heterogeneity of loggerhead turtles in the Pacific Ocean could be attributed to a number of factors. It may be that sampling has not been expansive enough, or that the markers used are not targeting variable DNA regions, or that diversity simply does not exist for loggerhead turtles in this region. Given that sampling has been extensive in both geographic range and sample size (Moritz *et al.*, 2002b, this study), it is doubtful that under-sampling is the problem. The markers used in this study are the same as those used in the Atlantic Ocean, where they have been able to find sufficient heterogeneity to distinguish rookeries on a regional scale (Encalada *et al.*, 1998). The same markers have also elucidated adequate resolution between natal regions for other species, which suggests that the markers used in the present study are typically adequate for detecting some amount of variability. It is almost certain then, that the very low genetic diversity of loggerhead turtles in the Pacific and Indian Oceans is not an artefact of the markers used or the extensiveness of the sampling. The phylogenetic tree that was constructed in the present study indicates that the Pacific loggerhead lineage is derived from a basal Atlantic group, possibly from the southwest populations. Therefore, the observed low diversity in the Pacific loggerhead turtle lineage is consistent with a historical colonisation bottleneck.

Regardless of the contributing factors, the low heterogeneity, and consequent lack of resolution between loggerhead populations within the southwest Pacific prevents the use of Mixed Stock Analysis for providing insight into the origin, and hence the migration routes of the loggerhead post-hatchlings investigated in this study. However, the heterogeneity that

occurs between loggerhead rookeries in the southern and northern Pacific Ocean will provide insight into migratory routes at an oceanic scale.

Green turtle diversity

The investigation into an extended sequence length (1120 nt vs 384 nt) of the mtDNA for a sample of green turtles from southwest Pacific rookeries found that the longer sequences revealed greater nucleotide diversity. The increased nucleotide diversity provides further resolution between known haplotypes. Haplotype diversity was also found to increase for the southwest Pacific region, whereas only six haplotypes were found in the short sequences, two additional haplotypes were revealed when the extended length of the mitochondrial DNA was investigated. The increase in haplotype diversity presents the potential to provide greater resolution between natal regions. One of the new haplotypes found is a promising candidate for defining the New Caledonian rookery further from other rookeries in the southwest Pacific region. Haplotype B3 is shared across a range of rookeries when the short sequence is investigated, however when the long sequence is investigated both the B3s that have been described at the New Caledonian rookery resolved into a new haplotype that so far has not been found elsewhere. The other new haplotype found in the long sequenced region occurred twice, once each at the southern Great Barrier Reef and Coral Sea rookeries, and is therefore not unique to either natal region.

Interestingly, two new haplotypes were also detected among the short sequences. This discovery hints that the current description of natal regions for green turtles in the southwest Pacific may be inadequate. Alternatively, the new haplotypes discovered in this study may simply represent rare alleles that occur within the southwest Pacific rookeries. As both of these new haplotypes only occurred once each it is likely that they are rare alleles. Rare alleles can be a feature of any population and attempting to account for all alleles that occur at low frequencies may be a futile task for animals that exist in such large numbers (more than 100,000 breeding animals are estimated in Queensland waters alone).

The low success rate for re-sequencing Moritz *et al.*'s (2002a, 2002b) rookery samples, limits the ability of the present study to make specific assessments on the occurrence of the new haplotypes found in this study. To gain a greater understanding of the frequency at which the newly described haplotypes occur a broader and more intensive genetic survey of rookeries in the southwest Pacific needs to be undertaken. Such a survey may resolve whether the haplotypes that appeared to be rookery-unique in this study maintain this status. The study would need to investigate the longer sequence lengths that were used in the present study and ensure adequate sample sizes were attained for all major rookeries.

The phylogenetic tree based on mtDNA haplotypes, supports the finding that fine scale resolution within the sampled populations may not exist. The lack of clear partitioning of haplotypes between geographic regions in the Pacific Ocean lineage, suggests that natal philopatry may not be as absolute in the Pacific Ocean lineage as in the Atlantic Ocean lineage. Alternatively, the Atlantic Ocean green turtle lineage may be more restricted due to the colonisation bottleneck implied by the global phylogeny. In which case, the resolution of natal philopatry may be coarser than currently assumed. Another perspective on this phenomenon may be that regional differences in nesting beach habitat availability on a

geological time scale has resulted in differing degrees of natal homing in search of nesting beaches. For example, the east coast of Australia, has had dramatic changes in coastal habitat availability during the past 18,000 years, when sea level was lower and the entire Great Barrier Reef region was reduced to the edge of the coastal shelf. At this time, the location of nesting beaches would have been dramatically different. In this scenario, females who do not have such highly refined homing for a natal beach, are more likely to find suitable nesting habitat. Thus, green turtles (at least in the southwest Pacific) may be adapted to take advantage of returning to known locations once found, but are not restricted to a single nesting location. It would be interesting to generate a method to track sufficient numbers of nesting females to model this behaviour.

Summary

The intent of this chapter was to address the limitations of the current baseline rookery information for green and loggerhead turtles in the southwest Pacific in order for mixed stock analysis to be employed in the following chapter. The lack of information on the loggerhead rookery in New Caledonia was addressed through the sampling of females during the nesting season. However no new haplotypes were found that distinguished this region from the east Australian rookeries. The phylogenetic tree supports a finding that the Pacific loggerhead lineage was derived from the Atlantic lineage with limited diversity in this region. This explains the lack of diversity among sequences and the overall low haplotype diversity in the whole Pacific Ocean.

In contrast to the loggerhead turtles, green turtles in the southwest Pacific Ocean exhibit significant haplotype diversity. However, the Pacific and Indian Ocean lineages do not exhibit clear partitioning of haplotypes into geographic regions. This is due to many haplotypes being shared across different natal regions. These findings indicate a potential for mixed stock analysis techniques for green turtles in this region, but large variances may be associated with the output estimates. Examining an extended sequence length to that currently used may provide some further resolution between rookeries in the southwest Pacific in the future. Phylogenetic hypotheses generated suggest that green turtles in the southwest Pacific do not have highly precise natal philopatry and therefore sharing of haplotypes between rookeries is an inherent feature of this species in this region. Therefore, whilst greater resolution in terms of greater numbers of haplotypes, appears to be possible through sequencing a longer region of mtDNA the utility of such an exercise remains to be evaluated.

Chapter 4

A genetic approach to establishing post-hatchling turtle migration routes in the southwest Pacific

Part II: mixed stock analysis

Introduction

The application of mixed stock analyses in sea turtle populations can have important conservation and management implications. During the non-nesting phases of a sea turtle's life history, populations comprise mixed stocks for which the reproductive origin of the individuals is obscure (Bowen *et al.*, 1996; Encalada *et al.*, 1998; Lahanas *et al.*, 1998). Mortalities resulting from direct harvesting and incidental capture in coastal and offshore fisheries can be high during the non-nesting phases. For example, an estimated 20,000 juvenile loggerhead turtles (*Caretta caretta*) are incidentally caught in the Mediterranean long-line swordfish fishery annually, of which at least 20% perish (Bowen and Avise, 1996). Through employing mixed stock analysis researchers have been able to estimate which reproductive stocks (rookeries, or clusters of rookeries) are being adversely affected through mortalities in pelagic long-line fisheries (Bowen *et al.*, 1995; Laurent *et al.*, 1998), benthic trawl fisheries (Laurent *et al.*, 1998) and direct harvesting (Dethmers and Broderick, 2002; Encalada *et al.*, 1994; Moritz *et al.*, 2002a).

Molecular markers and their application within mixed stock analysis programs have also been used to infer migratory routes of sea turtles. This application has been particularly useful for providing insight into the movements of post-hatchlings from rookeries. Based on mtDNA assays and mixed stock analyses, it has been determined that post-hatchling loggerhead turtles in the Azores and Madeira have undergone trans-Atlantic migrations from rookeries in the southern United States and Mexico (Bolten *et al.*, 1998), and post-hatchling loggerhead turtles occupying waters offshore from Baja California have migrated across the Pacific from Japanese rookeries (Bowen *et al.*, 1995).

In the same manner that previous researchers have employed genetic techniques to determine the stock structure of non-nesting populations of sea turtles, this thesis will investigate the stock structure of the post-hatchlings investigated during this study and employ mixed stock analyses to determine the contributing rookeries. The results from this analysis will then allow inferences to be made on the migration routes taken by post-hatchling turtles in the Australian-Pacific region.

The low heterogeneity and consequent lack of resolution between the loggerhead turtle populations in the southwest Pacific Ocean prevents the use of mixed stock analysis as a technique to provide insight into the migration routes of the loggerhead post-hatchlings investigated in this study. However, as heterogeneity does exist between loggerhead rookeries in the southern and northern Pacific, haplotype information on these post-

hatchlings can be used as an indication of migration routes at an oceanic scale in the Pacific (see Bowen *et al.*, 1995).

In contrast to the loggerhead turtles, green turtles (*Chelonia mydas*) in the southwest Pacific Ocean are considerably more variable in terms of their haplotype diversity. Although there is higher diversity of haplotypes, there is weak partitioning of haplotypes into geographic regions, with many haplotypes being shared across different natal regions. The sharing of haplotypes without fixed allele frequencies across the geographic regions reduces the statistical power of a mixed stock analysis and can result in large variances around the estimates. In spite of this, sufficient resolution amongst southwest Pacific populations exists to provide at least a modest insight into post-hatchling green turtle migrations in this region. This was indicated by Moritz *et al.*'s (2002a) investigation into the natal source of green turtles harvested in Indonesia and the Torres Strait and northern Australia.

The objective of this chapter is to investigate the stock structure of the loggerhead and green post-hatchling turtles obtained in this study and to use this information to gain insight into their possible natal origin. The stock structure of the post-hatchlings will be resolved through sequencing the same region of mitochondrial DNA as sequenced in Chapter 3 so that the haplotypes of the post-hatchlings can be determined. For post-hatchling green turtles this information will be analysed with rookery baseline data in two MSA programs (SPAM & TURTLE) so that insight can be gained into their natal origin. Although the lack of genetic distinction between the loggerhead rookeries in the southwest Pacific negates the use of MSA to obtain natal information for post-hatchlings of this species on a regional scale, the determination of the post-hatchling loggerhead turtles haplotypes will provide origin information at the oceanic scale.

Methods

Sample collection and DNA extraction

Tissue samples for genetic analysis were collected from the post-hatchling loggerhead and green turtles obtained via the methods described in Chapter 2. All of the post-hatchlings genetically sampled were sourced from the central and southern Queensland and New South Wales regions, with the exception of three post-hatchling loggerhead turtles that stranded on New Zealand beaches. From dead post-hatchlings, a small piece of skin was removed from the underside of the pelvic region with a sterile scalpel blade. From live post-hatchlings, a small notch from the outer edge of the 10th or 11th marginal scute was removed with a sterile leather punch.

Tissue storage, DNA extraction, PCR amplification and sequencing followed the procedures described in the previous chapter (Chapter 3).

Genetic data analysis

As described in the previous chapter (Chapter 3), sequences were edited with Sequencher 4.2.2 (GeneCodes, 1991), and manually aligned using Se-AL v 2.0a11 (Rambaut, 2002), with variable sites being confirmed with the associated chromatograms. The sequences obtained from post-hatchling individuals were compared to previously identified haplotypes and assigned letter codes following the GenBank database and Moritz *et al.*, (2002a, 2002b). New haplotypes were assigned new codes and registered with GenBank.

Diversity indices

Basepair variations and nucleotide diversity amongst the post-hatchling sequences were determined using MEGA and Arlequin 2000 (Schneider *et al.*, 2000) as described for adult sequences in Chapter 3.

Phylogenetic relationships

The relationships between the haplotypes found in the green and loggerhead post-hatchlings were calculated in Arlequin 2000 (Schneider *et al.*, 2000) and visualised as a minimum spanning network following the methodology described in Chapter 3.

Estimation of stock composition

Post-hatchling mixed stock

To determine whether the post-hatchling green turtles represented a mixed stock derived from multiple rookeries or a single stock derived from one rookery, a Chi-square goodness of fit test was conducted using the haplotype frequencies of the post-hatchlings and of the rookeries. After establishing that these post-hatchlings do characterise a mixed stock, the relative contributions from the nesting populations to the sample of post-hatchlings were estimated using two Mixed Stock Analysis packages; Statistical Package for Analysing Mixtures (SPAM), version 3.7 (ADF&G, 2001) and TURTLE (Bolker and Okuyama, 2002). The lack of genetic resolution amongst loggerhead rookeries in the southwest Pacific negated further analysis (e.g. MSA) for loggerhead post-hatchlings.

Baseline and mixture files for MSA

MSA requires the construction of baseline files and mixture files. A baseline file contains information on the haplotypic frequencies of all potential contributing stocks, in this case green turtle rookeries. A mixture file contains information on the haplotypic frequencies of the mixed stock for which the origin stocks are to be estimated, in this case the post-hatchling green turtles. Two baseline files were constructed for this study, each with 273 samples per file. The first baseline file was constructed from the mtDNA data in Moritz *et al.* (2002a), and included the 15 haplotypes that were observed within a 384 nt sequence length. The second included the same information as the first but with the additional haplotype information (19 haplotypes) that was determined in this study (see Chapter 3) for 53 of the samples through investigating the extended sequence length (1120 nt).

The data for the two baseline files are presented in Table 4.1. In the short sequence dataset, haplotypes discovered in this present study (denoted with *) are binned with their adjacent haplotype (based on minimum spanning network analysis, Chapter 3). Thus A2b and A2c become A2, B1b becomes B1 and B3b becomes B3. Both baseline files contained haplotype information for six nesting regions that, based on geographical location, could be the potential origin of the post-hatchlings sampled in this study. These regions were the southern Great Barrier Reef (SGBR, n=107), northern Great Barrier Reef (NGBR, n=52), Coral Sea (CS, n=41), New Caledonia (NC, n=10), north-eastern Papua New Guinea (NEPNG, n=18) and Micronesia (Micron, n= 50) (Figure 3.2). Although populations beyond these regions could theoretically supply the post-hatchlings in this study, the distances involved in relation to the sizes of the post-hatchlings (see Chapter 2) would make them unrealistic contributors.

Table 4.1. Frequencies of mtDNA alleles used for baseline file construction for implementation in the SPAM & TURTLE mixed stock analyses. mtDNA allele data is derived from Moritz *et al.* (2002a) and the current study. (*) denotes haplotypes that were included in the 19 haplotype baseline file only. Rookery size estimates are derived from *Limpus, in press and ^the Queensland Sea Turtle Database.

Haplotype	SGBR	NGBR	CS	NC	NEPNG	MICRON
A1						7
A2	96	2	30	2		2
A2b*	1		1			
A2c*			1			
A3					16	26
A4						2
B1	1	42		4		
B1b*	1					
B3	8	2	9	2		
B3b*				2		
C1			1			
C3			1	1	1	
C4		1				
C7					1	
C12		2				
C13				1		
E1/E2						13
J1		2				
J2		1				
Total	107	52	41	10	18	50
Estimated rookery size	~8000* females per average breeding season	~41,000* females per average breeding season	~2000^	~600^	~600^	Many hundreds annually^

The mixture file was derived from the haplotype frequencies of the sampled green post-hatchlings. Haplotypes that occurred amongst the post-hatchlings that have not been recorded at any rookery were binned with their closest haplotype as they provide no useful information on origin of the post-hatchling. Binning consisted of combining the haplotype

that occurred in the post-hatchlings (but are unassigned to a rookery) with an adjacent haplotype that did occur in the baseline file.

MSA using the program SPAM

Maximum likelihood estimates for the contribution of rookeries to the mixture sample (the post-hatchlings) were obtained by the Statistical Package for Analysing Mixtures (SPAM), version 3.7 (ADF&G, 2001). SPAM employs maximum likelihood methods using Bayesian modelling of baseline haplotype frequency distributions to estimate relative contributions of discrete populations in a mixture of several populations. The Pella-Masuda baseline posteriors (Pella and Masuda, 2001) correction to baseline haplotype frequency option was implemented so that if a rookery has a sampled frequency of zero for a haplotype, it will be not be ruled out as an impossible source for a mixture individual with that haplotype. This is important in studies for which some stocks have small sample sizes (e.g. New Caledonia in this study), as a smaller sample size increases the chance of haplotypes going undetected.

Prior to using empirical data, the accuracy and precision of stock composition estimates from the baseline file was evaluated using simulation studies. The simulation studies were conducted with SPAM's simulation procedure, where simulated mixture samples of 95 turtles were generated by randomly re-sampling with replacement the baseline populations. Within the simulation procedure, SPAM calculates the average maximum likelihood estimates of stock composition from the known mixture file and compares the result to the known true contribution. The estimated contributions of the rookeries for the known mixture populations are reported as the mean of 1000 bootstrap replicates, with 90% confidence limits and standard deviations.

After simulations, maximum likelihood estimates of stock compositions from the baseline file to the unknown post-hatchling mixture population were derived. Estimations were calculated for each of the six nesting regions as separate entities and also with the six regions divided into two groups. The groups were chosen based on the theoretical distribution of the post-hatchlings by surface currents (see Chapter 2), with the southern Great Barrier Reef, New Caledonian and Coral Sea rookeries as one group and the northern Great Barrier Reef, north-eastern Papua New Guinean and Micronesian rookeries as the second group. The estimated contributions of rookeries for the post-hatchling mixture population are reported as the mean of 10000 bootstrap replicates, with 90% confidence limits and standard errors, where both the baseline and mixture files were re-sampled .

MSA using the program TURTLE

A second MSA program, TURTLE (Bolker and Okuyama, 2002), was employed to test the results for congruence of the two methodological approaches. TURTLE is designed specifically to conduct stock analysis of sea turtle populations using data on mitochondrial haplotypes from rookeries and a mixed stock (Bolker *et al.*, 2003). The same baseline files and mixture files that were used in SPAM were used in TURTLE. Stock estimations using unconditional maximum likelihood (UML) and Markov Chain Monte Carlo (MCMC) functions were calculated within TURTLE. Estimates are provided as mean estimations with bootstrap 95% confidence intervals determined by re-sampling the data 1000 times with replacement.

Results

Mitochondrial DNA analysis

After editing and alignment, sequences of 1120 nt in length were obtained for the majority of post-hatchlings. A number of samples did not amplify successfully, due to DNA degradation, and hence genetic information was not obtained for these animals. These failed amplifications were most prevalent amongst post-hatchling samples that had been in storage for an extended period of time (> 5 years).

The obtained sequences provided two primary datasets for; (i) loggerhead post-hatchlings, and (ii) green post-hatchlings. Each dataset was analysed as long sequences (1120 nt) or short sequences (384 nt) for comparative purposes with previous studies.

Post-hatchling loggerhead turtles: haplotypes and diversity indices

Mitochondrial DNA sequences were successfully obtained from 19 loggerhead turtle post-hatchling samples. There were no polymorphic sites between the sequences obtained and all sequences conformed to the haplotype CCP1 that is described in the previous chapter. Consequently the haplotype diversity was zero for the loggerhead post-hatchlings for both sequence lengths.

Post-hatchling green turtles: haplotypes and diversity indices

Mitochondrial DNA sequences were successfully obtained from 56 green turtle post-hatchling samples for short sequences (384 nt) and 53 green post-hatchling samples for long sequences (1120 nt). Within the post-hatchling long sequenced region 46 polymorphic sites were detected which resolved into six haplotypes (Table 4.2). Four of these haplotypes have been previously described (A2, B1, B3, C13: Moritz *et al.*, 2002a), whilst two were previously undescribed. Haplotypes codes were assigned to novel sequences that correspond to the closest previously defined haplotype. One of the new haplotypes aligned closely with A2 and I labelled this A2d (1 nt difference compared with A2), and the other aligned closely to B3 and was labelled B3c (3 nt differences compared with B3). A2d and B3c were represented by only one individual each. The minimum spanning network shows the relationship between the new post-hatchling haplotypes found in this study with those described in the previous chapter for the haplotypes found amongst the adult green turtles (Figure 4.1).

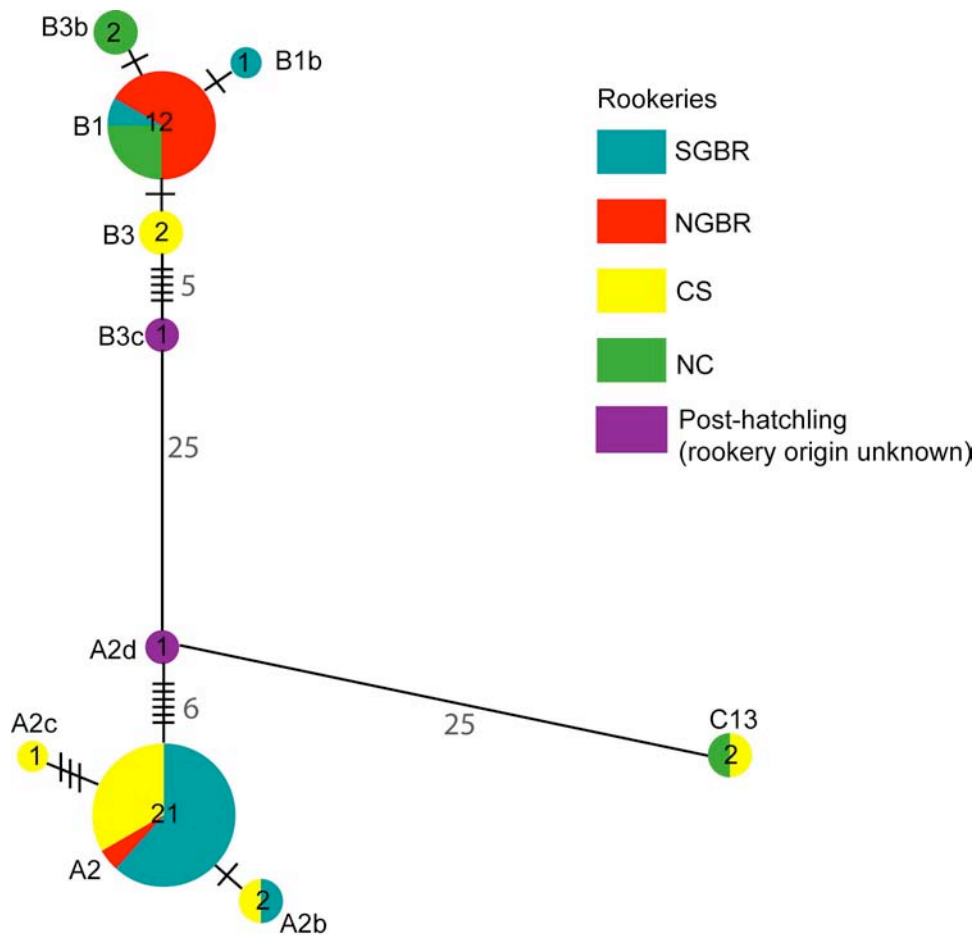


Figure 4.1. Minimum spanning network showing the relationship between the haplotypes of post-hatchling green turtles (*Chelonia mydas*) found in this study and haplotypes described for green turtles in Chapter 3 (based on 1120 nt). The size of each circle and the number within it indicates the number of individuals with that haplotype. Each dash represents a single base pair difference between the haplotypes.

Analysis of the shorter sequences revealed just 27 polymorphic sites and five haplotypes, as haplotype B3c collapsed into B3 when only the short sequence was investigated. Full sequence alignments have been submitted to GenBank (Acc. No. EF029120 & EF029125) and are shown in Appendix D. For the purpose of mixed stock analysis, the two previously undescribed haplotypes that were found in the post-hatchling population A2d and B3c were binned with the closest common haplotypes, A2 and B3 respectively.

The majority of the post-hatchlings were haplotype A2 which accounted for 75% of the samples, followed by B3 at 14%, B1 at 5% and C13, A2d and B3c at 2% each (Table 4.3). Haplotypic diversity in the post-hatchling samples was $h=0.456$ ($n=53$) in the long (1120 nt) sequences and $h=0.450$ ($n=56$) in the short (384 nt) sequences (Table 4.4). Nucleotide diversity was low amongst both sequence lengths, and was lowest in the long sequence length (Table 4.4). Chi-square analyses revealed that the haplotype frequencies of the post-hatchlings were significantly different from those encountered at any one nesting rookery, and therefore it can be assumed that the post-hatchlings investigated came from a mixture of rookeries.

Table 4.2. Aligned post-hatchling green turtle (*Chelonia mydas*) mtDNA haplotypes. Haplotype A2 is the reference sequence. A period (.) indicates common sequence, a (?) represents missing data, with variable sites indicated by the base concerned and (*) denotes haplotypes previously undescribed. The haplotype name is consistent with those assigned by Moritz *et al.* (2002a).

Haplotype	Nucleotide base position				
	22234456	6666667777	7777888899	9999990000	1111 11111
	4504572411	1144441678	8899008814	6778892223	44469
	9180259127	8901239011	6756456922	8682461236	23409
A2	TACCGTACTA	AT----TGGG	TGCAAACAGA	ACAGGCTCCT	ACGTG
A2d*A	AT----.A..?
B1	CTT.ACTACA	ATTAAC...A	..TG..TGAG	.TGAATCTTC	GTA.A
B3	CTT.ACTACA	ATTAAC...A	..TG...GAG	.TGAATCTTC	GTA.A
B3c*	..T.A.TACA	ATTAAC...A	..TG...GAG	.TGAATCTTC	GTA.A
C13	...T.CT.C-	--TAATC.A.	CAT.GG....	G.G..T.TTC	GT.CA

Table 4.3. The frequencies (and number) of mtDNA haplotypes in the studied post-hatchling green turtles (*Chelonia mydas*). (*) denotes haplotypes previously undescribed.

Post-hatchling haplotypes	Haplotype frequency (numbers)
A2	0.75 (42)
A2d*	0.017 (1)
B1	0.056 (3)
B3	0.143 (8)
B3c*	0.017 (1)
C13	0.017 (1)

Table 4.4. Summary of sequence information for long (1120 nt) compared to short (384 nt) for post-hatchling green turtles (*Chelonia mydas*).

	Post-hatchling 1120 nt	Post-hatchling 384 nt
# haplotypes	6	5
# polymorphic sites	46	27
# of observed sites with transitions	35	26
# of observed sites with transversions	4	1
# of observed sites with substitutions	39	27
# of observed sites with indels	7	0
Nucleotide composition	C : 25.30% T : 28.70% A : 34.15% G : 11.85%	C : 16.41% T : 33.27% A : 35.29% G : 15.04%
Sample size	53	56
Nucleotide diversity	0.0101 +/-0.0052	0.0232 +/-0.0122
Haplotype diversity	0.456	0.450

Mixed stock analyses

Mixed stock analyses outputs – SPAM

Both of the simulation studies had mean maximum likelihood estimates similar to the true contributions, although the estimates that were derived from the baseline file that comprised of 19 haplotypes were closer to the actual contribution for the majority of populations (SGBR, NGBR, CS, NC) (Table 4.5). In keeping with this outcome, the standard deviation was smaller and the confidence intervals narrower in the SGBR and CS populations for the simulation that was run with the 19 haplotype baseline file in comparison to the 15 haplotype baseline file (Table 4.5).

Table 4.5. Efficiency of mixed stock analysis to estimate the contributions of southwest Pacific green turtle (*Chelonia mydas*) rookeries for a simulated mixture composed of the baseline populations. SGBR = southern Great Barrier Reef, NGBR = northern Great Barrier Reef, CS = Coral Sea, NC = New Caledonia, NEPNG = north-eastern Papua New Guinea, MICRON = Micronesia.

Data set	Regions	Actual contribution	Mean Estimate	Std. Dev.	Lower 90% CI*	Upper 90% CI*
Simulated 15 haplotypes	SGBR	0.4500	0.4315	0.1956	0.0137	0.6941
	NGBR	0.1000	0.1096	0.0624	0.0000	0.2156
	CS	0.2000	0.2285	0.2053	0.0000	0.6562
	NC	0.1500	0.1188	0.0921	0.0000	0.2849
	NEPNG	0.0500	0.0509	0.0355	0.0000	0.1141
	MICRON	0.0500	0.0490	0.0336	0.0000	0.1086
Simulated 19 haplotypes	SGBR	0.4500	0.4442	0.1562	0.1519	0.6707
	NGBR	0.1000	0.1079	0.0632	0.0000	0.2092
	CS	0.2000	0.2114	0.1640	0.0000	0.5315
	NC	0.1500	0.1204	0.0947	0.0000	0.2977
	NEPNG	0.0500	0.0524	0.0354	0.0000	0.1139
	MICRON	0.0500	0.0480	0.0340	0.0000	0.1111

*based on Symmetrical Percentile Bootstrap Confidence Intervals

Mixed stock analysis within the post-hatchling mixture file indicates that the majority of the post-hatchling green turtles investigated in this study are being supplied by the southern Great Barrier Reef rookeries (60%), followed by the Coral Sea rookeries (28%) and the New Caledonian rookery (12%) (Table 4.6). Large standard errors exist for all contribution estimates when rookeries are investigated as separate units, however when they are divided into two groups, the output strongly suggests that the post-hatchling mixed stock population constitutes animals primarily (99%) from these three rookeries (SGBR, NC, CS) (Table 4.6).

Table 4.6. Estimates for the contribution of southwest Pacific rookeries to post-hatchling green turtles (*Chelonia mydas*) collected from eastern Australia derived from mixed stock analysis from the program SPAM. SGBR = southern Great Barrier Reef, NGBR = northern Great Barrier Reef, CS = Coral Sea, NC = New Caledonia, NEPNG = north-eastern Papua New Guinea, MICRON = Micronesia.

Data set	Regions	Mean Estimation	Standard Error
Estimated from 15 haplotypes	SGBR	0.6005	0.2681
	NGBR	0.0001	0.0006
	CS	0.2771	0.2824
	NC	0.1222	0.0640
	NEPNG	0.0000	0.0000
	MICRON	0.0000	0.0000
	SGBR+NC+CS	0.9999	0.0006
	NGBR+NEPNG+MICRON	0.0000	0.0000

Mixed stock analyses outputs – TURTLE

Mixed stock analysis within the program TURTLE indicates that the majority of the post-hatchlings investigated in this study are supplied by the southern Great Barrier Reef rookeries, with UML estimates of 60% and MCMC 68% (Table 4.7). The second largest contributing rookery was the Coral Sea rookeries with estimates of 26% (UML) and 18% (MCMC) followed by the New Caledonian rookery with estimates of 14% (UML) and 12% (MCMC) (Table 4.7). Estimates for northern Great Barrier Reef, north-eastern Papua New Guinean and Micronesian breeding groups suggest that these rookeries only have negligible contribution, if any, to the post-hatchlings found along Australia’s eastern coast (Table 4.7). Large standard errors exist for mean estimations of the three rookeries (SGBR, NC, CS) that are indicated as contributing to the mixed stock, however the low contribution of the remaining rookeries are strongly supported by the narrow 95% confidence intervals (Table 4.7).

Table 4.7. Estimates for the contributions of southwest Pacific rookeries to post-hatchling green turtles (*Chelonia mydas*) collected from the southwest Pacific derived from mixed stock analysis from the program TURTLE, using UML (unconditional maximum likelihood) and MCMC (Markov Chain Monte Carlo) methods. The regions investigated were SGBR = southern Great Barrier Reef, NGBR = northern Great Barrier Reef, NC = New Caledonia, CS = Coral Sea, NEPNG = north-eastern Papua New Guinea, MICRON = Micronesia.

Data set	Region	Mean estimation	Lower 95% CI	Upper 95% CI
UML	SGBR	0.6059	0.0000	0.9792
	NGBR	0.0000	0.0000	0.1132
	CS	0.2586	0.0000	0.9613
	NC	0.1355	0.0000	0.3178
	NEPNG	0.0000	0.0000	0.0000
	MICRON	0.0000	0.0000	0.0000
MCMC	SGBR	0.6784	0.0000	0.9454
	NGBR	0.0145	0.0000	0.0890
	CS	0.1800	0.0000	0.9270
	NC	0.1211	0.0000	0.3088
	NEPNG	0.0029	0.0000	0.0240
	MICRON	0.0031	0.0000	0.0258

Discussion

Post-hatchling loggerhead turtles

All the loggerhead post-hatchlings genotyped in this study were found to be haplotype CCP1. This haplotype occurs almost exclusively within the southwest Pacific region, with only one record of a nesting female at a Japanese rookery having this haplotype (Bowen *et al.*, 1995), and 15 juveniles from an oceanic habitat in the southwest Atlantic (L. Soares pers. com., 2005). The majority of post-hatchlings found in this study were very small (i.e. less than 10 cm CCL, see Chapter 2) and based on growth rates of wild post-hatchling loggerhead turtles, most of them would be no more than one to two months old (Bjorndal *et al.*, 2000, 2003). This suggests that the majority of the post-hatchlings investigated here had not travelled any significant distance, and would therefore be from nearby rookeries. Hence, the small size of the post-hatchlings, in addition to the relative exclusivity of CCP1 to the southwest Pacific region, provides strong evidence that all the loggerhead post-hatchlings genetically assessed in this study originated from rookeries located in the southwest Pacific.

Genetic information was unobtainable from the three post-hatchlings that had stranded on New Zealand beaches, owing to DNA degradation, and therefore there is no genetic evidence that these post-hatchlings originated from southwest Pacific rookeries. A concurrent investigation has genotyped a number of juvenile pelagic loggerhead turtles captured in long-line fisheries operating off the coasts of northern Chile and southern Peru,

found that these turtles are CCP1, the southwest Pacific haplotype (X. Velez pers. com., 2006). The finding of this haplotype amongst pelagic juveniles in the southeast Pacific provides evidence for trans-Pacific migrations of post-hatchling Australian loggerhead turtles.

Mixed stock analysis could not be used for post-hatchling loggerhead turtles due to low resolution between rookeries in the southwest Pacific. The global phylogeny constructed in previous chapter (Chapter 3), confirms that haplotype diversity is very low in the Pacific Ocean, probably owing to a colonisation bottleneck effect, and consequently resolution between the eastern Australian and New Caledonian rookeries is unlikely to be found.

Post-hatchling green turtles

There were six haplotypes detected among the post-hatchling green turtles examined in this study. Four of these haplotypes have been previously described and accounted for in rookeries, whilst two are previously undescribed. Three of the four previously recorded haplotypes occur across multiple rookeries and therefore do not provide any distinctive rookery origins from which the turtle may have migrated. Only one post-hatchling emerged as a haplotype that is unique to a region. In this case the post-hatchling was haplotype C13, and as this haplotype has thus far only been reported from the New Caledonian rookery, this provides support that this turtle originated from New Caledonia.

The simulation studies conducted within SPAM indicate that the baseline files derived from currently available rookery data can provide modestly accurate estimates for relative contributions to the post-hatchling population by potential source rookeries. The results from both of the MSA packages (SPAM and TURTLE) concurred in that they suggest the largest contributor to the post-hatchling population was the southern Great Barrier Reef rookeries, followed by Coral Sea rookeries and New Caledonian rookeries. The two different analyses agreed that the north-eastern Papua New Guinean and Micronesian rookeries did not contribute whilst the northern Great Barrier Reef rookery had minimal, if any, contribution. There were large standard errors and confidence intervals associated with the mean estimates derived from both the simulation studies and from the empirical data for the three primary contributing rookeries. These large confidence intervals are brought about from the sharing of haplotypes across regions which reduces the resolution between the rookeries. For example, haplotypes A2 and B3 occur at all three of these rookeries whilst any haplotypes that are unique to these rookeries, only occur at low frequencies (e.g. C1 at 2% at the Coral Sea rookeries).

An investigation into a sample of green turtles from southwest Pacific populations (Chapter 3) demonstrated that increasing the region of the mtDNA examined has the potential to increase the resolution between these populations. Despite the limited number of samples for which long sequence information was obtained (see Chapter 3), the additional information yielded in this dataset resulted in estimations that were closer to the true contributions with narrower confidence intervals. This finding suggests that if a broad scale study that investigated the long sequence region, as described in the previous chapter was to be undertaken, further resolution would be found that would enhance MSA results for future studies. The degree to how much resolution between the rookeries can be improved is

questionable, as the global phylogenies shown in the previous chapter (Chapter 3), indicate that natal philopatry is not absolute, and therefore significant levels of resolution between rookeries may not exist for this species.

Regardless of the potentially large errors associated with the estimated contribution values, the congruence between SPAM and TURTLE for the actual order of contribution (SGBR > CS > NC) suggest that the estimated values do provide a good indication. Additionally, the low standard errors and narrow confidence intervals that were calculated for the three rookeries that are estimated to have little or no contribution support that the southern Great Barrier Reef, Coral Sea and New Caledonian rookeries are the most likely contributors. Overall the results suggest that mixed stock analyses provide a useful guide to the stocks that are making contributions and those that are not.

When the mixed stock analysis results are considered in light of biological probability, additional support is gained for the contribution estimates provided by the programs. As with the loggerhead post-hatchlings, the majority of green post-hatchlings in this study were relatively small (i.e. less than 10 cm CCL, see Chapter 2). Limited information is available for growth rates of green post-hatchlings, however based on this species having a similar diet to loggerhead post-hatchlings (see Chapter 5), growth rates between the two species are likely to be comparable. Therefore, based on growth rates of post-hatchling loggerhead turtles, it can be estimated that the majority of the post-hatchling green turtles studied would be no more than one or two months old. This provides further support to the MSA outputs that suggest that the majority of the genotyped post-hatchlings were from nearby rookeries (e.g. SGBR).

The low contribution estimates for the northern Great Barrier Reef, north-eastern Papua New Guinea and South East Asian rookeries are also in accordance with theories based on oceanic currents (see Chapter Two). Dominant currents in the waters adjacent to these three rookeries would not carry post-hatchlings directly to the central and southern east Australian coast. Although it is not irrefutable that hatchlings from these rookeries may eventually end up in this region through an indirect route, it would take longer and it would therefore be expected that the animals arriving from these rookeries would be somewhat larger than those that were investigated.

Conclusion

The low haplotype diversity and a lack of resolution among loggerhead turtle rookeries in the southwest Pacific means that mixed stock analysis was not a viable technique for determining migratory routes for post-hatchling loggerhead turtles at a regional level in this region. However beyond this regional scale, mtDNA markers provide compelling evidence that loggerhead post-hatchlings from southwest Pacific rookeries undertake trans-Pacific migrations. These migrations are comparable to the trans-oceanic migrations that post-hatchling loggerhead turtles perform in the northern Pacific and northern Atlantic oceans as evidenced by mtDNA markers.

The accurate results obtained in the simulation study run in SPAM suggest that, despite the moderate levels of resolution between the green turtle rookeries in the southwest Pacific, MSA techniques can provide a reasonable indication of contribution rookeries to a mixed stock for this species in this region. This is supported by the fact that the results obtained from the two mixed stock analysis programs were in congruence with one another. Based on mtDNA haplotypes, the MSA results indicates that the post-hatchlings found along the central and southern Queensland and New South Wales coasts originate from three rookeries (southern Great Barrier Reef, New Caledonia and Coral Sea), with the southern Great Barrier Reef rookery being the main contributor to the mixed stock. These results support the dispersal hypotheses derived from the size of the turtles and the oceanic features adjacent to rookeries (Chapter 2).

Chapter 5

The diet of post-hatchling loggerhead and green turtles: an insight into habitat association

Introduction

Dietary information from the stomach contents of post-hatchling turtles has contributed to our knowledge of their ecology in a variety of ways such as: habitat use (Tomas *et al.*, 2001; Witherington, 1998), foraging strategies (Tomas *et al.*, 2001), energetics (Mann *et al.*, 1999), diet contaminants (McCauley and Bjorndal, 1999), and the relative health of individual turtles (McCauley and Bjorndal, 1999). Most information on the diet of pelagic post-hatchling turtles has been obtained from loggerhead turtles (*Caretta caretta*) in the northern Atlantic and northern Pacific oceans (reviewed by Bjorndal, 1997). These post-hatchlings were caught in high-sea driftnets in the central northern Pacific Ocean (Parker *et al.*, 2000), in the waters around the Azores (Bolten and Balazs, 1982; van Nierop and den Hartog, 1984) and in the Atlantic Ocean's Gulf Stream (Richardson and McGillivray, 2001). Several dietary studies have also examined post-hatchling loggerhead turtles captured in the neritic habitat, including waters near nesting beaches in the south-eastern USA and South Africa (Hughes, 1974) and from stranded individuals found along the coast of Texas (Plotkin, 1999), and Florida (Carr and Meylan, 1980).

In comparison with loggerhead post-hatchlings, only scarce documentation is available for green (Frick, 1976; Hughes, 1974), hawksbill (Meylan, 1984), Kemp's ridley (Shaver, 1991), leatherback (Brongersma, 1970) and flatback (Zangerl *et al.*, 1988) post-hatchlings, whilst there appears to be no documentation on the diet of olive ridley post-hatchlings. Of the extant sea turtle species the flatback turtle is the only one believed to have a post-hatchling stage restricted to coastal waters (Walker and Parmenter, 1990). The dietary items found in the stomachs of flatback post-hatchlings reflect this and include benthic organisms, such as sea pens and sea horses in addition to planktonic organisms (Zangerl *et al.*, 1988; pers. obs.). It is thought that all the remaining sea turtle species have a pelagic post-hatchling stage. The stomachs of post-hatchlings of these species contain neustonic species, dominated by pelagic molluscs and crustaceans (e.g. Cirripedia & Amphipoda), hydrozoans, *Sargassum* spp. and fish eggs, and lack benthic organisms. In addition to organic items, dietary investigations into post-hatchling turtles often report the consumption of anthropogenic debris such as plastics, styrofoam and tar (Parker *et al.*, 2005; Richardson and McGillivray, 2001; Witherington, 1994a). These stomach contents suggest oceanic dwelling post-hatchling turtles feed in a relatively non-selective manner. The true extent of their lack of selectivity is unclear. For example, Witherington (1998) compared material ingested by post-hatchlings captured off Florida with material in the surrounding environment and found that there was some discrimination of items taken, with animal material, in particular hydroids and copepods, selected over plant material.

Because post-hatchlings feed opportunistically on potential food items, their stomach contents can provide information on the habitat they occupy (Tomas *et al.*, 2001; Witherington, 1998). For example, items such as the seaweed *Sargassum* floats and leaf parts, and snails (*Litiopa melanostoma*) associated with the *Sargassum*, are found within the stomachs of loggerhead post-hatchlings stranded along the Florida coastline. This has been used to associate these post-hatchlings to the *Sargassum* rafts that occur along the convergence zones within the Atlantic Ocean (Carr and Meylan, 1980; Plotkin, 1999). Similarly, pelagic post-hatchling loggerhead turtles inhabiting regions of up-welling off the coast of Baja California have a diet that is dominated by pelagic red crabs (*Pleuroncodes planipes*) that are characteristic of this region (Nichols *et al.*, 1999).

A considerable portion of the dietary information for post-hatchlings has come from stranded individuals, so it is possible that this information is not entirely representative of a healthy turtle's diet. However, the similarity of items retrieved from live turtles found *in-situ* and from stranded dead and moribund turtles in the south-western north Atlantic Ocean (Carr and Meylan, 1980; Plotkin, 1999 vs Richardson and McGillivray, 2001; Witherington, 1994a), suggests that stranded post-hatchlings provide a good indication of the feeding ecology of this life stage and are a valuable source of information on the diet of post-hatchlings in the absence of accessible *in-situ* samples.

The information available for post-hatchling loggerhead turtles in the northern Atlantic and northern Pacific oceans, suggests that the stomach contents of pelagic post-hatchlings in the southwest Pacific region will contain organisms that are epipelagic and oceanic, and no benthic organisms. I anticipate that in general the organisms that comprise the diet of post-hatchlings in this region will belong to similar taxonomic groups as those found in previous studies (e.g. Bolten and Balazs, 1982; Hughes, 1974; Parker *et al.*, 2000; Plotkin, 1999), although species level distinctions are expected owing to the different geographical location.

The aim of this chapter is to qualitatively investigate the diet of post-hatchling loggerhead and green turtles from the southwest Pacific Ocean region in order to obtain information regarding the habitat occupied by post-hatchlings of these two species. Additionally, it will provide the first documentation of the diet of post-hatchling turtles in the southwest Pacific region and contribute to the currently sparse records on the diet of post-hatchling green turtles globally.

Methods

Stomach content retrieval and identification

Stomach contents were recovered for qualitative dietary analysis from the green (n=35) and loggerhead (n=7) stranded or beach-washed (BW) post-hatchlings and from green post-hatchlings retrieved from the stomachs of predatory fish (PF) (n=13). BW comes from the term 'beach-washed'. I have elected to use this abbreviation to remain consistent with the coding used by the Queensland Sea Turtle Research Program. Necropsies were performed to remove the stomach and intestinal tract from deceased post-hatchlings, and the mouth cavity and oesophagus were examined to check for any food items that had been ingested prior to

death. The contents were stored at -4°C to prevent degradation of the material before it was examined.

The digestive tract was divided into anterior (stomach) and posterior (large and small intestine) portions and gross observations of the contents were made under a dissecting microscope. The contents were then sorted and a reference collection was made of the discernible items. These items were identified to the lowest possible taxon and kept to aid following identifications. A selection of items that were difficult to identify were sent to the Marine Invertebrate Group at the Australian Museum, Sydney. Alcian Blue-Safranin and Youngs Eosin stains were applied to the remaining indistinguishable material to differentiate plant from animal matter.

Volumetric and biomass estimates

Previous dietary analysis studies on sea turtles have attempted to estimate the volume or biomass of various prey items (Plotkin *et al.*, 1993). However, as different types of items are digested at different rates, this provides biased information on the actual biomasses ingested. For example, non-digestible, hard-bodied items will persist in the digestive tract longer than soft-bodied items, which are rapidly digested; resulting in hard-bodied items being over-represented. Hence, volume and biomass were not estimated in this study.

Statistical analyses

The frequency of occurrence of identified dietary items among individual post-hatchlings was calculated for three groupings, (1) green post-hatchlings that had been stranded, (2) green post-hatchlings that had been found in fish stomachs, and (3) loggerhead post-hatchlings that had been stranded. The percentage frequency of occurrence of components was calculated by:

$$\frac{\text{total number of stomachs in which prey items found}}{\text{total number of stomachs examined}} \times \frac{100}{1}$$

A multi-response permutation procedure (MRPP) using Blossom version 2005.08.26 (Slauson *et al.*, 1991) was conducted to establish whether there were any significant differences in the proportions of stomachs containing dietary items in each class, between (1) stranded green and loggerhead turtles, and (2) green turtles that were stranded and those that were found within the stomachs of fish. As only green post-hatchlings were found as prey items within fish, the later analysis was restricted to this species.

MRPP is a nonparametric procedure used to determine whether groups differ in one or more measured attributes; in this case the presence or absence of dietary items, and the groups are defined by species or method of capture; BW, PF (Zimmerman *et al.*, 1985). Unlike parametric tests, the application of MRPP does not depend on restrictive assumptions, such as the data being from normal populations and having homogeneous variances (Zimmerman *et al.*, 1985). The probability value of an MRPP statistic is derived through permutation, and remains sensitive even when sample sizes are small (Zimmerman *et al.*, 1985).

To perform MRPP, prey items were categorised into 11 groupings based on taxonomic classes (Table 5.1). For each post-hatchling, the presence or absence of items belonging to each prey group was recorded. These presence/absence values for the individual post-hatchling were the observations. The MRPP null hypothesis for this analysis was that the group (e.g. species or method of capture) to which the observations (dietary information) were assigned, was random. A small probability value indicates meaningful grouping, whilst a high probability value indicates lack of grouping.

Table 5.1. The 11 groups (A-K) of prey items used in the multi-response permutation procedure (MRPP) statistical analysis.

Group	Grouping	Group items included:
A	Synthetic debris	nylon, plastics (hard & sheet),
B	Natural debris	feathers, wood, pumice
C	Class Hydrozoa	all Hydrozoans
D	Class Insecta	Orders Coleoptera, Diptera, Hemiptera
E	Class Malacostraca	Orders Amphipoda, Euphausiacea, Isopoda
F	Class Maxillopod	Order Calanoida
G	Class Cirripedia	Order Thoracica
H	Class Gastropoda pelagic	Order Thecosomata
I	Class Gastropoda non-pelagic	Order Pterioda
J	Sand	
K	Plant material	all items of plant origin

Results

Stomach contents were obtained from 55 post-hatchling sea turtles: 35 green turtles that had been stranded (BW), 13 green turtles that had been preyed upon by fish (PF) and seven stranded (BW) loggerhead turtles. Although a greater number of loggerhead post-hatchlings were obtained for dietary analysis the majority of these turtles were very small and still hatchlings with large egg yolk supplies, and were thus not useful for dietary studies.

The mean curved carapace length (CCL) of the green post-hatchlings was 7.5 cm (SD=1.04, range=5.5-11.3) and of the loggerhead post-hatchlings was 6.4 cm (SD=1.4, range=4.6-10.6) (see Figure 2.1). All of these animals were under the minimum size for their respective species that have been observed foraging in neritic habitats (i.e. <66 cm CCL loggerhead turtles and <38 cm CCL green turtles, refer to Chapter 2), and were therefore considered to be post-hatchlings.

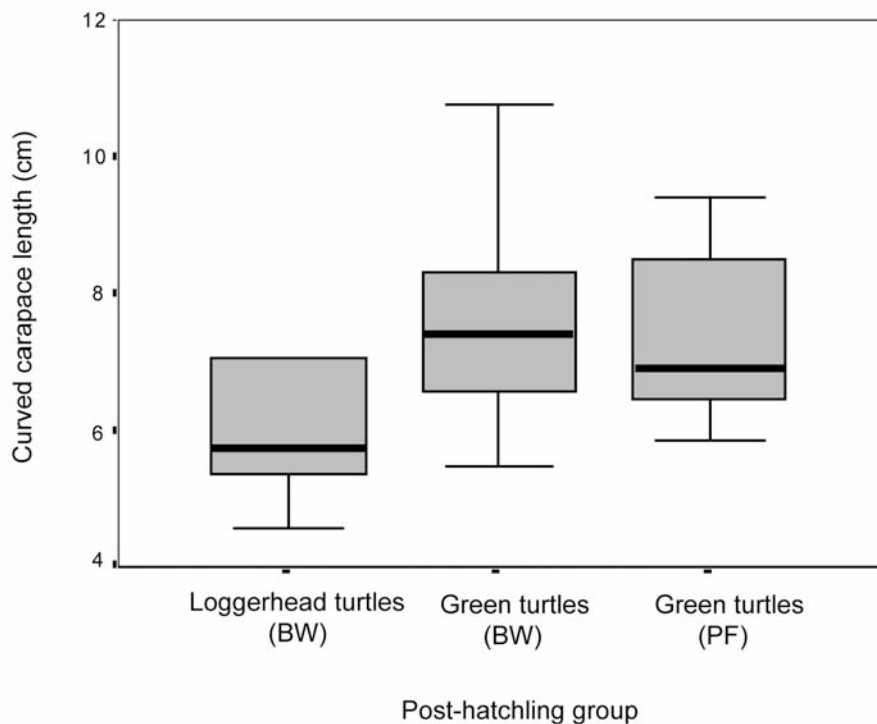


Figure 5.1. A box plot of the carapace lengths, showing median, range and SD, of the three groups of post-hatchlings, loggerhead turtles (BW), green turtles (BW), and green turtles (PF), that had their stomach contents analysed. BW = stranded (i.e. beach washed), PF = fish prey.

Frequency of occurrence of prey items

The majority of material within the digestive tract was highly digested and for the most part was unidentifiable. It was not possible to categorise this digested matter into plant or animal material by conventional staining techniques. Items that could be identified were primarily found in the anterior portion of the stomach, with the exception of synthetic material, pumice and shell fragments, which occurred throughout the digestive tract.

The identifiable components of the stomach contents were categorised into 12 broad taxonomic groups (Table 5.2). These groupings were the same as the 11 used for MRPP, with the exception that pelagic gastropods were divided into two groups.

Table 5.2. Prey species encountered in the stomachs of post-hatchling green (*Chelonia mydas*) and loggerhead (*Caretta caretta*) turtles, and the percentage frequency (%F) of occurrence, with the number of stomachs the item occurred within (n). OW = oceanic waters, CW = coastal waters, BW = stranded, PF = fish prey.

Diet component	Habitat	Loggerhead-BW (total n=7) % F (n)	Green-BW (total n=34) % F (n)	Green-PF (total n=13) % F (n)
Synthetic flotsam				
Plastics styrofoam nylon cord/string	Pelagic, OW, CW	57 (4)	74 (25)	46 (6)
Natural flotsam				
feather wood pumice	Pelagic, OW, CW	14 (1)	26 (9)	31 (4)
Phylum Cnidaria				
unidentified <i>Porpita</i> sp.	epipelagic, OW, CW	43 (3) 0 (0)	50 (16) 3 (1)	62 (7) 3 (1)
Phylum Arthropoda				
Cl. Insecta	Terrestrial, airborne	0 (0)	3 (1)	15 (2)
Phylum Arthropoda				
Sub Ph. Crustacea				
Cl. Malacostraca	Various,	29 (2)	56 (19)	69 (9)
Cl. Maxillopod	Marine	0 (0)	3 (1)	0 (0)
Cl. Cirripedia		0 (0)	12 (4)	38 (5)
Phylum Mollusca				
Cl. Gastropoda	Pelagic, OW, CW			
Tonnidae		14 (1)	26 (9)	0 (0)
Cavoliniidae		0 (0)	24 (8)	0 (0)
Pterioda*		0 (0)	3 (1)	0 (0)
Plant	Various, OW, CW	14 (1)	12 (4)	15 (2)
Sand	Benthic, OW, CW	29 (2)	12 (4)	0 (0)

*adult stage Pterioda occupies a benthic habitat.

Synthetic flotsam occurred most frequently in the stomachs of the stranded post-hatchlings (both species) and was the third most frequently occurring item in the stomachs of green post-hatchlings that had been preyed upon by *Coryphaena hippurus* (dolphin fish) (Table 5.2). This synthetic flotsam was dominated by hard plastic pieces but also included plastic film, styrofoam and nylon cord (Figure 5.2 O,P). The occurrence of synthetic items varied between animals from single pieces to volumes that nearly filled the stomach.

Natural flotsam (e.g. pumice, wood and feathers) occurred relatively frequently in the stomach contents of the three groups of post-hatchlings, ranging from 14% in stranded loggerhead post-hatchlings to 31% in the green post-hatchlings predated by fish (Table 5.2). Organisms from the subphylum Crustacea and the phylum Cnidaria (class Hydrozoa) also occurred frequently in the stomachs examined (Table 5.2). Cnidarians, were recognised by the remains of *Porpita* sp. disks and various tentacles (Figure 5.2 I), and Crustaceans were identified through the remains of exoskeletons, which were often in pieces with no soft parts remaining. Malacostraca was the most dominant class of Crustacea and consisted primarily of amphipods, *Hyperia* sp. (Hyperiididae), Isopods, *Idotea metallica* (Idoteidae) and krill

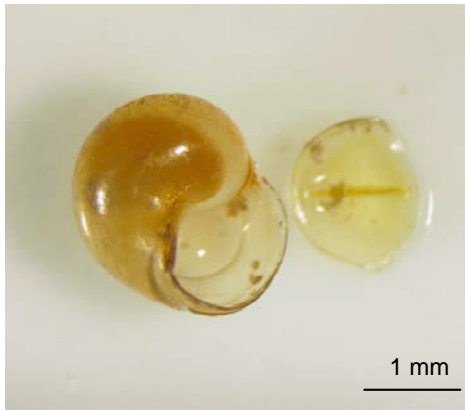
(Euphausiacea) (Figure 5.2 G,J,K,L). Of the identifiable prey items, organisms from the class Malacostraca were the most common item within green post-hatchlings that had been preyed upon by fish (69%) and the second most common item found in stranded green post-hatchlings (56%) (Table 5.2). Other Crustacea found included copepods (Calanoida) from the class Maxillipoda, and barnacles and cyprid larva belonging to the family Lepadidae (*Lepas* sp.) from the class Cirripedia (Figure 5.2 C).

Organisms identified from the class Gastropoda (phylum Mollusca), consisted of tonnoid veliger larvae, and pteropods; *Cavolinia* sp. and *Creseis* sp. (Figure 5.2 A,B,E,F,H) These items were relatively common in stranded green post-hatchlings (26%) and to a lesser extent in the stranded post-hatchling loggerhead turtles (14%), but were absent in post-hatchling green turtles that had been sourced from *Coryphaena hippurus* (PF) (Table 5.2). In one stranded post-hatchling green turtle, a single specimen of *Pinctada* sp. (pearl oyster) was present (Figure 5.2 D).

Other items present in the post-hatchling green and loggerhead turtle stomachs included floating plant matter (seed pods and spores etc), insects (Figure 5.2 M,N) and sand grains (Table 5.2). The insects, one each from the orders Diptera, Hemiptera and Coleoptera, occurred in three green post-hatchlings (1xBW, 2xPF). Sand grains were present in the stomachs of six post-hatchlings that had been stranded: two from loggerhead and four from green turtles. The sand occurred throughout the digestive tract, but there was no evidence of sand in the mouth or oesophagus.

Dietary differences between species

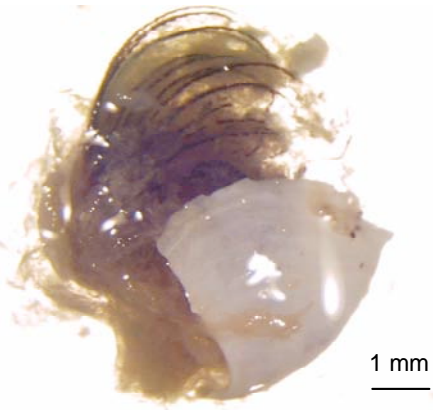
A multi-response permutation procedure (MRPP) indicated that the frequency of occurrence of the consumed items did not differ significantly between the stranded loggerhead and the stranded green post-hatchlings ($p=0.59$). Similarly, the MRPP statistical test did not detect a significant difference in dietary items occurring between green post-hatchlings that had been found in the stomachs of predatory fish and green post-hatchling turtles that had been stranded ($p = 0.24$).



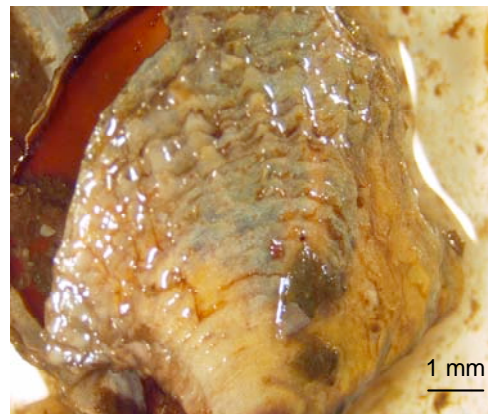
A Tonnoid larvae



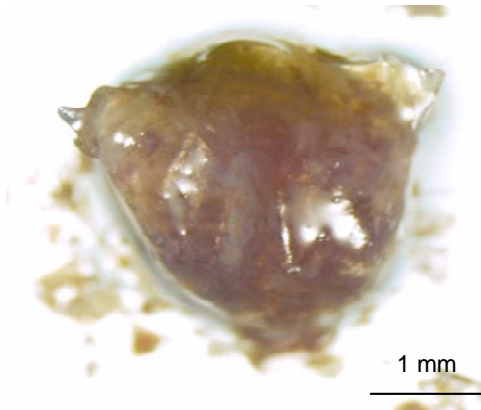
B Tonnoid larvae



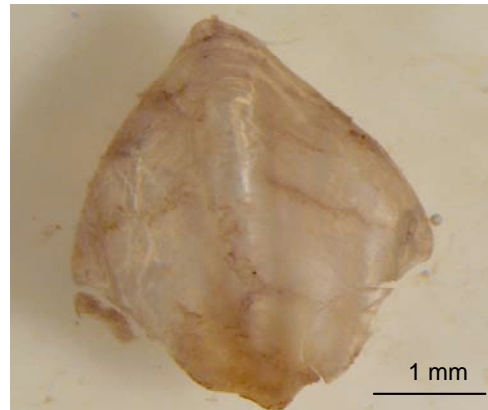
C Cirripedia: *Lepas* sp.



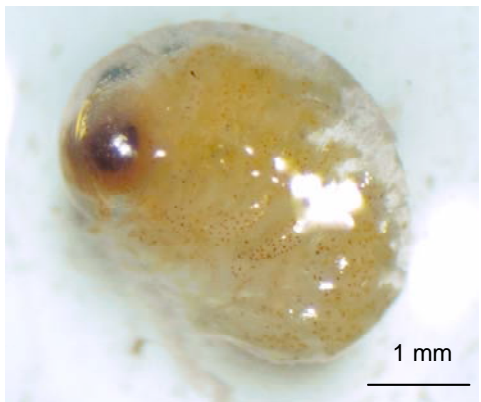
D Pteriidae: *Pinctada* sp.



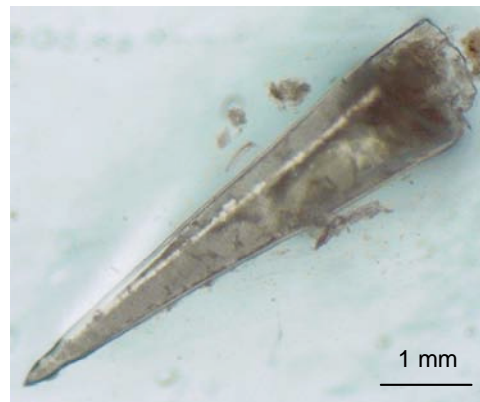
E Cavoliniidae: *Cavolinia* sp.



F Cavoliniidae

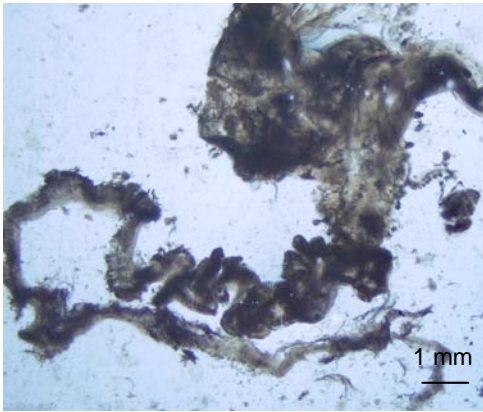


G Crustacean larvae



H Cavoliniidae: *Creseis* sp.

Figure 5.2. Items found in the stomach contents of post-hatchling loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) turtles in the southwest Pacific Ocean.



I Hydrozoa tentacles



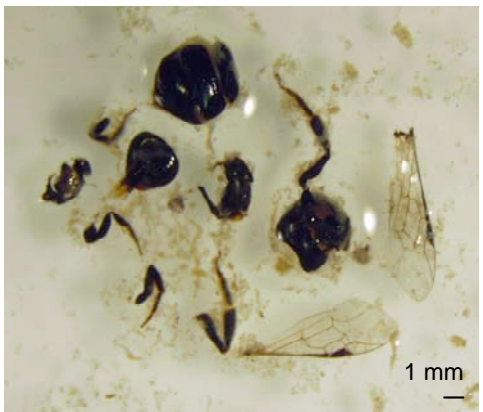
J Idoteidae: *Idotea metallica* Bosc, 1802



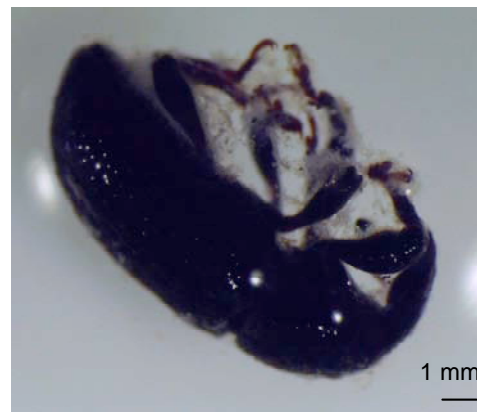
K Euphausiacea (krill)



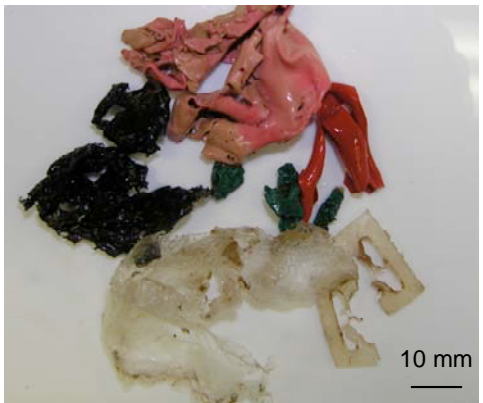
L Hyperiidae: *Hyperia* sp



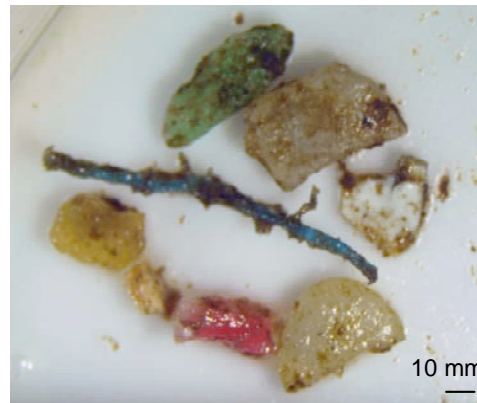
M Insecta: Hemiptera



N Insecta: Coleoptera Curculionidae



O Example of plastics found in one turtle



P Example of plastics found in one turtle

Figure 5.2 (cont.). Items found in the stomach contents of post-hatchling loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) turtles in the southwest Pacific Ocean.

Discussion

Post-hatchling loggerhead and green turtles in the southwest Pacific Ocean appear to feed opportunistically. This is indicated by the presence of a wide range of items, including non-nutritional items, in their stomach contents. Opportunistic feeding behaviour is observed in many oceanic pelagic fish (e.g. wahoo, billfish, tuna and mackerel) and particularly in the juvenile life stages (Bernard *et al.*, 1985; Bertrand *et al.*, 2002). In the oceanic environment food resources converge at current boundaries and occur at regions of high productivity, such as areas of up-welling. At such locations a diverse array of potential prey items will co-occur. An opportunistic approach to feeding allows an animal to take advantage of whatever resources are available.

As an opportunistic feeder, a post-hatchling turtle's diet is largely determined by the relative abundance of potential food objects in the environment. Studies conducted in the Atlantic Ocean (e.g. Bolten and Balazs, 1982; Plotkin, 1999; Richardson and McGillivray, 2001; van Nierop and den Hartog, 1984; Witherington, 1994a) frequently report *Sargassum* and other plant material in the stomach contents of loggerhead post-hatchlings. For example, 26% of loggerhead post-hatchlings caught off the coast of Florida, were found with *Sargassum* in their stomachs (Witherington, 1994a), whilst the stomachs of the two loggerhead post-hatchlings caught 93 km east off Florida's coast contained *Sargassum* fragments (Richardson and McGillivray, 2001). Studies conducted in the Pacific Ocean (this study, Parker *et al.*, 2005; Peckham and Nichols, 2002; Nichols *et al.*, 1999) have not reported the same consistency of plant matter as the aforementioned studies. Both Nichols *et al.*, (1999) and Peckham (2002) found that the stomach contents of post-hatchling loggerhead turtles caught off the Baja coast, consisted entirely of pelagic red crabs (*Pleuroncodes planipes*). Similarly, Parker *et al.*'s (2005) study on post-hatchling loggerhead turtles caught in the central northern Pacific Ocean, reported stomach contents that were animal matter, primarily *Carinaria cithara*, *Janthinia* sp., *Lepas* sp., *Planes cyaneus* and *Pyrosomas*, with a small mention of *Cystoera* sp., a brown algae.

A comparison between studies conducted in the two oceanic regions implies that loggerhead post-hatchlings have a stronger tendency towards carnivory in the Pacific Ocean (Table 5.3). This would be expected because the pelagic *Sargassum* habitat in the Pacific Ocean is considerably smaller than in the Atlantic Ocean. Therefore, a post-hatchling feeding opportunistically will be more likely to consume plant matter in the Atlantic Ocean than one feeding in the Pacific Ocean because chances of exposure to plants are less.

Many of the organisms found in stomach contents (e.g. synthetic and natural flotsam, cnidarians) occur in both coastal and oceanic waters, and therefore specific feeding habitats cannot be discerned from these items. However, a few of the organisms within the stomach contents suggest that the investigated post-hatchlings were feeding and hence inhabiting oceanic environments, and hence that this is the region that the investigated post-hatchlings were inhabiting. These items were; *Porpita* sp., a floating hydroid which typically inhabits pelagic oceanic and continental shelf waters (Wrobel and Mills, 1998), the organisms belonging to the family Cavoliniidae (*Creseis* sp. and *Cavolinia* sp.) which are open-ocean pelagic gastropods (Lalli and Gilmer, 1989), and the planktonic larval stage of the Tonnoid gastropod, which are widely dispersed in ocean currents (Beu, 2001), the neustonic isopod *Idotea metallica* (Smith, 1977), *Hyperia* sp. a planktonic amphipod (Smith, 1977), and the

calanoid copepod which are usually planktonic (Smith, 1977). Based on the occurrence of oceanic organisms in the post-hatchling stomach contents, two of the seven loggerhead, and 23 of the 34 green turtle post-hatchlings could be confirmed as oceanic foragers.

Table 5.3. Stomach contents of post-hatchling loggerhead (*Caretta caretta*) turtles in the Atlantic and Pacific oceans showing that post-hatchlings feed on pelagic organisms. % refer to the percentage of post-hatchlings with the specified dietary item in their stomach contents.

Atlantic Ocean			Pacific Ocean			
Witherington, 1994	Richardson & McGillivray, 2001	Witherington, 1998	Carr & Meylan, 1980	Parker, 2005	Peckham & Nichols, 2002* Villanueva, 1991^	This study
off Florida, at sea (n=50)	off Florida, at sea (n=2)	off Florida, at sea (n= 66)	Florida, stranded (n=5)	North Pacific (n=52)	Off California (*n=7; ^n=19)	E. Australia, stranded (n=7)
Cnidaria (40%)	<i>Sargassum</i> (100%)	<i>Sargassum</i>	<i>Sargassum</i>	Gastropods	<i>Pleuroncodes planipes</i> – pelagic crab (100%)	Synthetics (57%)
Tar (34%)	Other plant material (100%)	Other plant material	Gastropods	Cephalopods		Cnidaria (43%)
Synthetics (32%)	Insects (100%)	Cnidaria	Crustaceans	Crustaceans		Crustacea (29%)
<i>Sargassum</i> (26%)	Crustaceans (100%)	Copepods		Cnidaria		Gastropods (33%)
Crustaceans (18%)	Cnidaria (100%)	Insects		Urochordata		Plant material (14%)
Hydrozoans (16%)	Tar (100%)	Plastics & tar (5.1%)		Fish		
Insects (4%)	Fish eggs (50%)	Polycheates		Annelids		
Gastropods (4%)	Plastics/synthetics (50%)	Bryozoan		Algae		
Other plant material (8%)						

The almost complete absence of benthic organisms provide additional support to the hypothesis that the investigated post-hatchlings were inhabiting oceanic waters. Because diving studies have shown that loggerhead post-hatchlings can dive to depths of 200 m (Riewald & Bolten, unpubl.) and post-hatchling loggerhead turtles held in aquariums browse upon benthic food (Davenport and Clough, 1986), it would be expected that benthic organisms would form part of the diet of post-hatchlings living in the coastal zone. The only benthic organisms found within the stomach contents were a single juvenile *Pinctada* specimen and sand grains. *Pinctada* spp. have previously been recorded on the ocean's surface, although this is not the typical habitat of the post-larval stages of these species.

Pinctada spp. have a pelagic larval dispersal stage (Southgate and Lucas, 2003), with settlement occurring on hard substrata, typically in coastal waters. However, spatfall (settled larva) have been observed in the open sea (Alagarwami, 1977), and therefore it is possible that the juvenile *Pinctada* specimen was consumed by a post-hatchling in the pelagic environment.

The presence of sand grains in the post-hatchling's stomach contents could indicate that they were foraging on benthic food, as sand grains can be introduced into a turtle's stomach indirectly from material ingested as the turtle forages on benthic foods (McCauley and Bjorndal, 1999; Seminoff *et al.*, 2002). However, if this was the case it would be expected that, in addition to the sand grains, benthic organisms would have been found in the stomach contents. The overall lack of benthic organisms in the stomach contents investigated suggests that this is not the case. Alternatively, sand grains could also have been ingested as the hatchlings made their initial swim offshore through the surf zone. It has been shown that as hatchlings enter the surf they take in water (Bennett *et al.*, 1986; Marshall and Cooper, 1988); if this water is ingested where turbulent wave action causes sand particles to become suspended, sand could be ingested along with the water. That sand only occurred in stranded individuals suggests that either the sampled post-hatchling had only recently left the beach, and sand ingested at that time had not yet passed through their stomachs, or the sand was ingested whilst the turtle became stranded. It would be expected that if sand grains were picked up during the stranding process, sand would occur in the mouth. Sand was not obvious in the mouth of any of the individuals, and occurred throughout the digestive tract, suggesting that these individuals had only recently left the coastal environment.

Terrestrial insects are not usually associated with the ocean waters. However their occurrence in the oceanic environment has previously been documented (Peck, 1994), as insects can be transported large distances by moving air masses (Johnson, 1969) and in this way can end up floating on the ocean's surface. They could thus become potential prey items for a post-hatchling that is feeding at the ocean's surface and in this way insects have been found in the stomachs of post-hatchlings that were caught *in-situ* in a pelagic oceanic habitat (Richardson and McGillivray, 2001; Witherington, 1994a).

The presence of pelagic and oceanic organisms, and the lack of benthic organisms, in the stomach contents of the investigated post-hatchling turtles, provides evidence that they occupied pelagic oceanic waters. However, the majority of prey organisms do not explicitly identify whether the post-hatchlings were inhabiting surface waters, or waters below the surface. A few items, e.g. *Porpita* sp., plastics, pumice, and aerial insects, all occur floating on the ocean's surface, and therefore provide evidence that the post-hatchlings were feeding, at least for some period of time, at the surface. Other planktonic organisms that were found in the stomach contents, such as *Idotea metallica*, *Hyperia* sp. a calanoid copepod, could have been ingested at the surface or below the surface.

Previous dietary studies (Peckham and Nichols, 2002; Richardson and McGillivray, 2001; Witherington, 1994a, 1998), and *in-situ* observations (Carr and Meylan, 1980; Witherington, 1994a) have concluded that post-hatchling turtles spend the majority of their time feeding on the surface. This conclusion is supported by data obtained from two remote sensing and satellite telemetry studies of loggerhead post-hatchling turtles in the northern Atlantic Ocean. One of these studies concluded that post-hatchling loggerhead turtles in the waters

around the Azores, were spending 75% of their time in the top 5m of water (Bolten & Reiwald, unpubl.), whilst the other found that loggerhead post-hatchlings spent most of their time just below the surface, between 0-1m deep (Dellinger and Freitas, 1999). Because of the energetics involved for a small post-hatchling to remain below the surface (they buoy to the surface when they cease swimming; Davenport and Clough, 1986) and respiratory requirements, it would be energetically advantageous for this life history stage to be spent floating at or near the surface.

Although plastics are most likely over-represented owing to their non-degradable nature, their high occurrence in the stomachs of post-hatchlings does raise health concerns. Even though the effects cannot be directly assessed, it has been shown that post-hatchlings do not compensate for the consumption of non-nutritional items, which results in reduced energy and nitrogen uptake (McCauley and Bjorndal, 1999). The post-hatchlings that were consumed *in-situ* by *Coryphaena hippurus* were presumably healthier animals than those that had become stranded, which may not have been. It is therefore interesting to note that fewer '*in-situ*' post-hatchlings (46%) had consumed plastic compared with those that had become stranded (69%). To further explore the relationship between stranded post-hatchlings and the consumption of plastics, the quantity of plastics in the stomach needs to be compared between stranded and animals those caught *in-situ*.

Summary

Analysis of stomach contents of post-hatchling sea turtles showed that in the southwest Pacific Ocean post-hatchling loggerhead and green turtles derive nutritional sustenance primarily from animal matter, and they feed on a variety of neustonic species in a non-selective manner. The dietary information obtained for post-hatchling loggerhead turtles in this study are in keeping with previous studies conducted at locations in the northern Atlantic and northern Pacific Oceans. Prior to this study, very little has been documented on the diet of post-hatchling green turtles. The dietary items found in post-hatchling green turtles in the southwest Pacific Ocean suggests that this species exhibits pelagic feeding behaviour, similar to loggerhead turtles during this stage of their life history.

Although the organisms that comprised the diet of post-hatchling loggerhead and green turtles in the southwest Pacific Ocean region belong to similar taxonomic groups as those found in previous studies on loggerhead post-hatchlings, some distinctions occurred owing to the different geographical location. In particular the absence of *Sargassum* and the organisms that specifically associate with the *Sargassum* rafts were absent in the stomachs of post-hatchlings feeding in the southwest Pacific Ocean.

Chapter 6

Using stable isotopes ($\delta^{13}\text{C}$ & $\delta^{15}\text{N}$) to identify diet and habitat shift of post-hatchling green turtles

Preamble:

This chapter, based on the assessment of stable isotope ratios, represents a collaboration between Drs Karen Arthur (University of Queensland, Brisbane) and Col Limpus (Queensland Environmental Protection Agency, Brisbane) and myself. The collaborators have each had an integral role in providing samples from different life history stages. In this chapter I will present data collated from samples collected by all three authors in order to provide an overview of the collaborative study, however, the main interpretation presented here focuses on the post-hatchling data as this represents my contribution. The additional data presented is done so with permission from my colleagues as this brings the post-hatchling results into context. Interpretations of the data and the written work presented here are my own interpretation of the results.

Introduction

The previous chapters of this thesis provide evidence that post-hatchling loggerhead and green turtles in the southwest Pacific lead a pelagic existence in oceanic waters. In both species this lifestyle represents a contrast to the habitat occupied by the remaining life history stages, which occur in coastal waters (Limpus, 2004a, 2004b). The shift in habitat between the post-hatchling and the latter life stages is accompanied by a change in feeding behaviour. Whereas post-hatchlings of both species appear to feed primarily on neustonic animals in the oceanic environment (Chapter 4), in the neritic habitat juveniles and adults feed primarily on benthic associated organisms. In coastal water habitats loggerhead turtles are primarily carnivorous, using their enlarged jaws to feed on hard-bodied crustaceans and molluscs (Bjorndal, 1997; Limpus and Limpus, 2001), whilst green turtles take up a herbivorous diet, feeding predominately on macro-algae and seagrass (Bjorndal, 1997). Therefore, for green turtles, the shift in habitat from the oceanic environment to coastal waters entails a marked ontogenetic dietary shift, from one of predominately carnivory, to one of predominately herbivory.

Dietary shifts from carnivory to herbivory have been documented in several turtle families, including Emydidae (Hart, 1983; Parmenter and Avery, 1990), Chelidae (Chen and Lue, 1999) and Cheloniidae (Bjorndal, 1997). These diet shifts are often accompanied by habitat shifts which suggests that differences in prey availability may be the underlying reason for a dietary shift. An animal's ability to physically manipulate or digest the food will also play a critical role in determining food preferences. For example, herbivorous turtles require microbial symbionts in their hindgut to aid in the digestion of plant material and the

carnivorous loggerhead turtle has enlarged jaws that allow them to dine upon hard shelled molluscs and crustaceans (Bouchard and Bjorndal, 2006).

Shifts in diet from one food source to another throughout a species' life history have been successfully identified using stable isotope analysis (SIA) in an array of organisms including cichlid fish (Genner *et al.*, 2003), coral reef fish (de la Moriniere *et al.*, 2003), largemouth bass (Post, 2003), Brook charr (Power *et al.*, 2002), Arctic charr (McCarthy *et al.*, 2004) and cephalopods (Cherel and Hobson, 2005). Stable isotope analysis has also been used in loggerhead turtles (Hatase *et al.*, 2002b), northern fur seals (Burton *et al.*, 2001) and sea birds (Hobson, 1987) to detect animals feeding in benthic versus pelagic, and near-shore versus offshore environments.

Two elements commonly used in stable isotope analysis for dietary investigations are carbon and nitrogen. As animals ingest organic matter, metabolic processes cause isotopic fractionation of ^{13}C : ^{12}C and ^{15}N : ^{14}N stable isotope pairs (Jacob *et al.*, 2005). Consequently, progressive enrichment of the heavier isotope (^{13}C and ^{15}N) occurs as consumers of higher-trophic-level organisms develop their tissues (Biasatti, 2004). The level of enrichment varies between the two isotopes and enrichment is typically lower for carbon ($\sim 1\%$) than it is for nitrogen (~ 3 to 5%).

The modest transfer of carbon isotopic compositions between an animal and its diet means that the animal's $\delta^{13}\text{C}$ signature can be used in tracing its food sources where large $\delta^{13}\text{C}$ values exist between food sources (Michener and Schell, 1994). For example, within the marine system, $\delta^{13}\text{C}$ variations exist between inshore or benthic food webs and offshore or pelagic food webs, with the former having higher $\delta^{13}\text{C}$ values than the latter (e.g. Dunton *et al.*, 1989; Hobson *et al.*, 1994). This pattern has been confirmed in Godley *et al.*'s (1998) study that found the $\delta^{13}\text{C}$ values of loggerhead and green turtles were elevated compared to those of leatherback turtles. The lower values of the leatherback turtle are consistent with the pelagic nature of this species, while the comparatively enriched values of loggerhead and green turtles are consistent with their neritic feeding preferences (Godley *et al.*, 1998).

The enrichment of ^{15}N that occurs within an organism results from the preferential excretion of ^{15}N -depleted nitrogen, in the form of urea and ammonia (Michener and Schell, 1994). This progressive ^{15}N enrichment between a consumer and its diet, make $\delta^{15}\text{N}$ measurements useful indicators of an individual's trophic position. Nitrogen isotope signatures have been used to successfully delineate trophic relationships in marine ecosystems for sea turtles (Godley *et al.*, 1998), seabirds (Forero and Hobson, 2003; Hobson *et al.*, 1994), fish (Davenport and Bax, 2002; McCarthy *et al.*, 2004) and cephalopods (Cherel and Hobson, 2005).

The variation in trophic shift observed amongst individual consumers can be substantial and is dependent on diet type and analytical methods employed (McCutchan *et al.*, 2003). For example, the trophic shift for C ($\Delta\delta^{13}\text{C}$) and N ($\Delta\delta^{15}\text{N}$) is higher for unacidified samples than for acidified samples, and $\Delta\delta^{13}\text{C}$ is higher for consumers analysed using muscle tissue versus the whole organism (McCutchan *et al.*, 2003). Moreover, $\Delta\delta^{15}\text{N}$ is higher in individuals consuming an invertebrate diet ($+1.4\%$), compared to those on a plant and algae diet ($+2.2\%$) and to those consuming a high-protein diet ($+3.3\%$) (McCutchan *et al.*, 2003). McCutchan *et al.* (2003) found that individuals that switched from depleted to more enriched

diets had a lower $\Delta\delta^{13}\text{C}$ than those that changed from an initially enriched diet to a depleted one.

The isotopic signature of a dietary source does not immediately manifest in the consumer's tissues, but is integrated gradually over a period that is dependent upon the elemental turnover rate of the tissue investigated (Hobson, 1999). Tissue with a high metabolic rate will turn over more quickly, and therefore will provide more recent information than tissue with a slower turnover rate that will provide dietary information on a longer-term basis. The turnover rate of tissue is correlated with body mass and growth rate of the organism (Post, 2002), and can range from a few days, in tissue such as liver and blood plasma, to weeks or months, in tissue such as muscle and skin (Hobson, 1999). Metabolically inert tissues such as hair, feathers and nails retain an isotope record that reflects where the tissue was synthesised (Cherel *et al.*, 2000; Schell *et al.*, 1988). Laboratory studies are required to determine the turnover rates of sea turtle tissues.

The more conventional methods used to elucidate foraging choices rely primarily upon direct observation and stomach content analysis. Both of these techniques have inherent drawbacks (see Chapter 5), including that they focus on taxonomic relationships and are restricted to providing information on what the animal has eaten recently. Stable isotope analysis techniques can complement these studies by providing information on trophic relationship positions and feeding locations, and by providing a non-lethal tool for the measurement of assimilated, not just ingested, diet over a longer period of time (Davenport and Bax, 2002).

To my knowledge, only one previous study has investigated the trophic shift of green turtles using stable isotope techniques. This study was conducted by Godley *et al.* (1998) who examined the stable isotope signature in the bone collagen and keratin of green turtles that ranged from 21 to 85 cm CCL, from the northeast Atlantic Ocean. The smaller turtles in this study were post-hatchlings, presumably with an omnivorous feeding behaviour in the pelagic environment, whereas the larger animals were largely herbivorous in coastal waters (Bolten, 2003b). Therefore it would be anticipated that a size-related relationship existed in the isotopic signatures across the size range examined. However, Godley *et al.* (1998) did not find any such relationship. Most studies that investigate trophic shifts choose a relatively metabolically active tissue (Genner *et al.*, 2003; Hobson *et al.*, 1994), with a relatively short retention time. As bone collagen and keratin retain long-term information, at least for other species, this may have reduced the ability of Godley and his colleagues to detect any isotopic size-related relationships.

Aim

The previous chapter (Chapter 5) represents the first work that has been conducted on the diet of post-hatchling green turtles in the southwest Pacific region. The results from this work, combined with the results presented in Chapter 2, favour the conclusion that in the southwest Pacific post-hatchling green turtles have a primarily carnivorous diet composed of neustonic species consumed in offshore waters. When these post-hatchlings leave the oceanic environment to take up residency in coastal feeding grounds, their diet shifts to one that consists primarily of algae and seagrass gathered from the benthic environment (Brand-

Gardner *et al.*, 1999; Read and Limpus, 2002). This change of habitat, and the associated shift in diet, represents a substantial trophic shift within the green turtle's life history.

The overall focus of the broader study was initiated to (i) investigate the stable isotope signature of ^{13}C and ^{15}N across the life history stages of green turtles in southeast Queensland, and (ii) determine whether we could identify the shift in habitat and food source that occurs within this species' life history, through stable isotope analysis. To this end, we chose to analyse the ^{13}C and ^{15}N isotopic composition of skin as it has a shorter retention time than bone collagen and keratin, used by Godley *et al.* (1998) in a comparable study.

More specifically for this thesis, I was particularly interested in examining whether the isotopic signature of post-hatchling green turtles supported my dietary findings presented in the previous chapter (Chapter 5); that post-hatchling green turtles have a primarily carnivorous diet obtained in the offshore environment they inhabit.

Methods

Sample collection

Epidermal tissue was collected for stable isotope analysis (SIA) from green turtles across a range of life-history stages (Table 5.1). Post-hatchlings were obtained using the methods described in Chapter 2, with tissue for SIA being removed during the autopsy procedure. For hatchlings, eight eggs were retrieved from four clutches (four separate mothers) from the Wreck Rock Island rookery in central Queensland and incubated at 29.0-30.5°C, with hatchlings being sampled upon emergence. The remaining samples were collected during ongoing population demographic studies of green turtles in Queensland inshore feeding habitats. Live turtles were captured in Moreton Bay using the turtle rodeo technique (Limpus and Reed, 1985), and were tagged, measured and sexed in accordance with the Queensland Turtle Conservation Project protocol (Limpus *et al.*, 1994b).

Immature turtles were divided into two categories: small (<65 cm CCL) and large (>65 cm CCL). Within the former category, twelve individuals that had recently recruited into the coastal habit from an offshore habitat were identified. These animals are referred to as 'new recruits'. New recruits were recognised by a clean white plastron with obvious ridges that had not been worn down by abrasion that is consistent with living in an inshore area (Limpus and Limpus, 2003a) (Table 6.2). Additionally, the presence of gooseneck barnacles (*Lepas* spp.) and burrowing barnacles, which are indicative of animals living in the pelagic environment, and the absence of coastal barnacles, provided evidence that these animals had recently recruited into the coastal habitat (Limpus and Limpus, 2003a). As the Moreton Bay sea turtle populations have been extensively studied for a number of years, the years since recruitment from the pelagic to the coastal environment was known for nine of the individuals that were sampled for this study (Table 6.2). These animals are referred to as NR⁺n, where n is the time in years since recruitment into the coastal habitat.

Table 6.1. Samples collected from green turtles (*Chelonia mydas*) from southeast Queensland, Australia, for stable isotope analysis. Samples were collected by ⁺Limpus, ^{*}Boyle & [^]Arthur.

Life history stage	Sample size (n)	mean CCL (cm)	CCL range (cm)	Collection method
Hatchling ⁺	8	-	-	nests
post-hatchlings [*]	9	7.2	(6.6 – 22.6)	strandings
immature <65 cm [^]	25	48.3	(41.1-61.1)	rodeo, coastal waters
immature >65 cm [^]	6	87.1	(67.5-106.2)	rodeo, coastal waters
mature adult ⁺	7	110	(105.1-115.0)	rodeo, coastal waters

Table 6.2. Individual green turtles (*Chelonia mydas*) sampled during the study for which the number of years since recruitment into the coastal habitat is known. NR = new recruit, NR⁺1yr is a new recruit from the previous year and NR⁺4yrs is a new recruit from four years previously.

Life history stage	Sample size (n)	Mean CCL, cm	CCL range (cm)
NR ⁺ 0yr	12	43.9	41.1 - 49.0
NR ⁺ 1yr	1	43.4	
NR ⁺ 2yrs	2	45.7	45.7 - 45.8
NR ⁺ 3yrs	3	52.9	47.5 - 56.8
NR ⁺ 4yrs	1	54.6	
NR ⁺ 7yrs	1	54.3	
NR ⁺ 8yrs	1	60.8	

Samples obtained for SIA consisted of a small piece of epidermal tissue (~1 cm²), removed from the inguinal region of the turtle, except for hatchlings. Prior to the removal of the biopsy, the skin was scraped with a sterile scalpel blade to remove any ectoparasitic material. As hatchlings were too small for this type of sampling, a small piece of skin (~0.5 cm²) was removed from the hind flippers.

In addition to tissue samples, the stomach contents of a dead immature turtle retrieved from Moreton Bay were collected by Dr Arthur. Samples of the seagrasses *Syringodium isoetifolium*, *Halophila ovalis* and *Zostera marina* and of unidentified jellyfish were retrieved from the crop and stomach (n = 1 for each).

Stable isotope analysis

The removed tissue and dietary samples were stored frozen until they were required, at which time they were oven dried at 60°C for approximately 48 hrs. The stable isotope analysis of δ¹³C and δ¹⁵N was performed by staff at the Faculty of Environmental Sciences at Griffith University (Queensland), using the Dumas Combustion Technique (REF) with GV Instruments Isoprime coupled to a EuroVector EA 3000 elemental analyser.

Isotopic ratios are expressed as δ values in parts per thousand (‰), by the following equation:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = (R_{\text{sample}} / R_{\text{standard}} - 1) \times 1000$$

where R is the corresponding ratio, $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. PeeDee Belemnite (PDB) was used as the carbon isotope standard and atmospheric nitrogen was used as the nitrogen isotope standard.

Statistical analysis

Differences between average stable isotope composition of green turtle life history classes were tested with a Student's *t*-test for independent samples (Zar, 1998).

For analytical purposes a number of groupings were made. These groupings were based on carapace lengths, stable isotope data and biological knowledge. The groupings were as follows:

(1) The single NR⁺¹ yr specimen was combined with the NR⁺⁰ yr specimens. This grouping was justified by (i) the stable isotopic data for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for the NR⁺¹ yr individual were well within the range of values obtained from the 12 NR⁺⁰ yr, (ii) combining these data did not alter the mean $\delta^{15}\text{N}$, and varied the $\delta^{13}\text{C}$ mean by only 0.2, and (iii) depending on when these animals were caught and registered as new recruits, they may have only recruited a few months apart. From hereafter in my thesis this grouping (NR⁺⁰ yr and NR⁺¹ yr) is referred to as new recruits (NR), unless explicitly stated otherwise. (2)

(2) Individuals collected from coastal waters were grouped as <65 cm CCL immatures, >65 cm CCL immatures, and mature adults. The <65 cm CCL immature grouping did not include NR (NR⁺⁰ yr and NR⁺¹ yr) animals. The >65 cm CCL immatures included all the individuals with a curved carapace length (CCL) greater than 65 cm except the sexually mature animals (determined by laparoscopy) which were grouped as mature adults.

(3) Post-hatchlings were grouped into two categories; those <10 cm and those >10 cm CCL. Because of the lag period from time of food assimilation until it is expressed in their tissue, it is possible that smaller post-hatchlings retain a larger portion of their mother's isotopic signature than larger post-hatchlings who would have acquired their isotopic signature from food they have ingested.

Results

Variations of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ across life history stages of green turtles

The carbon and nitrogen isotopic signature of epidermal tissue (skin) from green turtles that were sourced from eastern Australia, varied across the different life history classes (Table 6.3/ Figure 6.1). The results show a general trend of decreasing $\delta^{15}\text{N}$ values and increasing

$\delta^{13}\text{C}$ values from life history classes that have spent the most time in the pelagic environment (new recruits and >10 cm post-hatchlings) towards the life history classes who had lived longer in the coastal habitat (immature individuals >65 cm and mature adults) (Table 6.3/ Figure 6.1). The exceptions to this trend were hatchlings and post-hatchlings <10 cm.

As expected, dietary items that are procured by turtles in the offshore, pelagic environment (jellyfish and zooplankton) have lower ^{13}C values compared to dietary items from the inshore, benthic environment (seagrass). The large post-hatchlings and new recruits had ^{13}C and ^{15}N values similar to jellyfish, whilst the animals feeding in the coastal habitat (with the exception of PH<10 cm and hatchlings) had ^{13}C and ^{15}N values similar to seagrass (Figure 6.1).

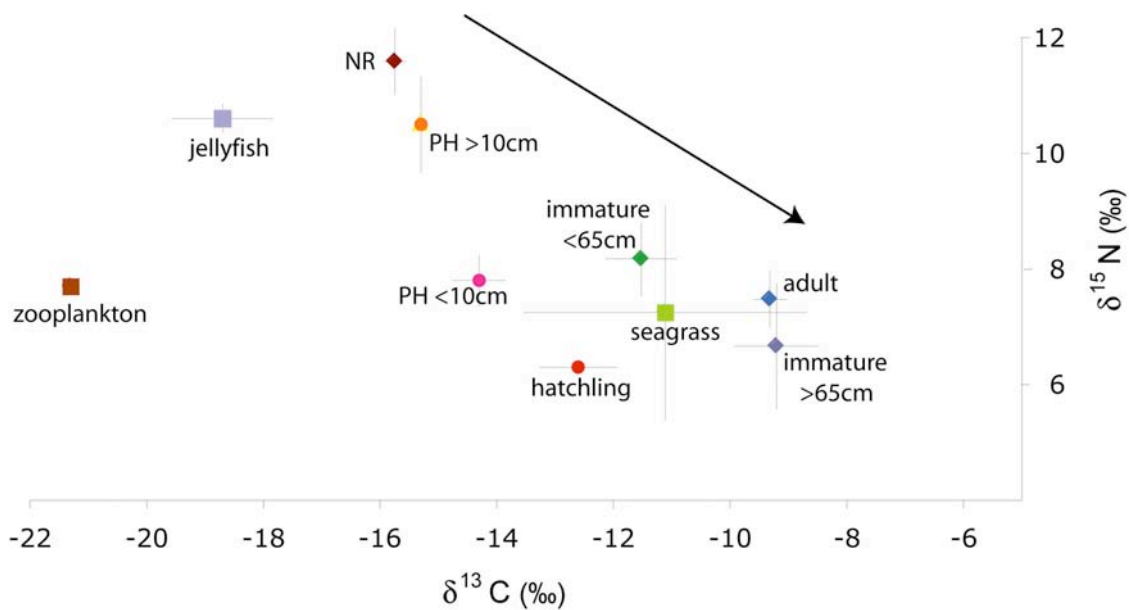


Figure 6.1. Stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) analysis of epidermal tissue from green turtles (*Chelonia mydas*) sampled in eastern Queensland, Australia. Data are provided as mean \pm SE. Seagrass values are from foraging turtles combined with data from (Udy and Dennison, 1997). Jellyfish values are from one sample in this study and 8 jellyfish (2 spp.) (Hatase *et al.*, 2002c). Zooplankton data are from (Davenport and Bax, 2002). PH = post-hatchlings, NR = new recruits. All sizes are in cm CCL. Detailed data available in Appendix E.

Large $\delta^{13}\text{C}$ ranges existed for all groups, with the exception of post-hatchlings >10 cm CCL, with most groups having ranges that overlap those of the other life history classes (Figure 6.2A). This is also the case for the observed ranges of $\delta^{15}\text{N}$, where most groups had ranges that overlapped those of the other life history classes (Figure 6.2B). The low ranges for post-hatchlings >10 cm CCL are possibly the result of the small sample size.

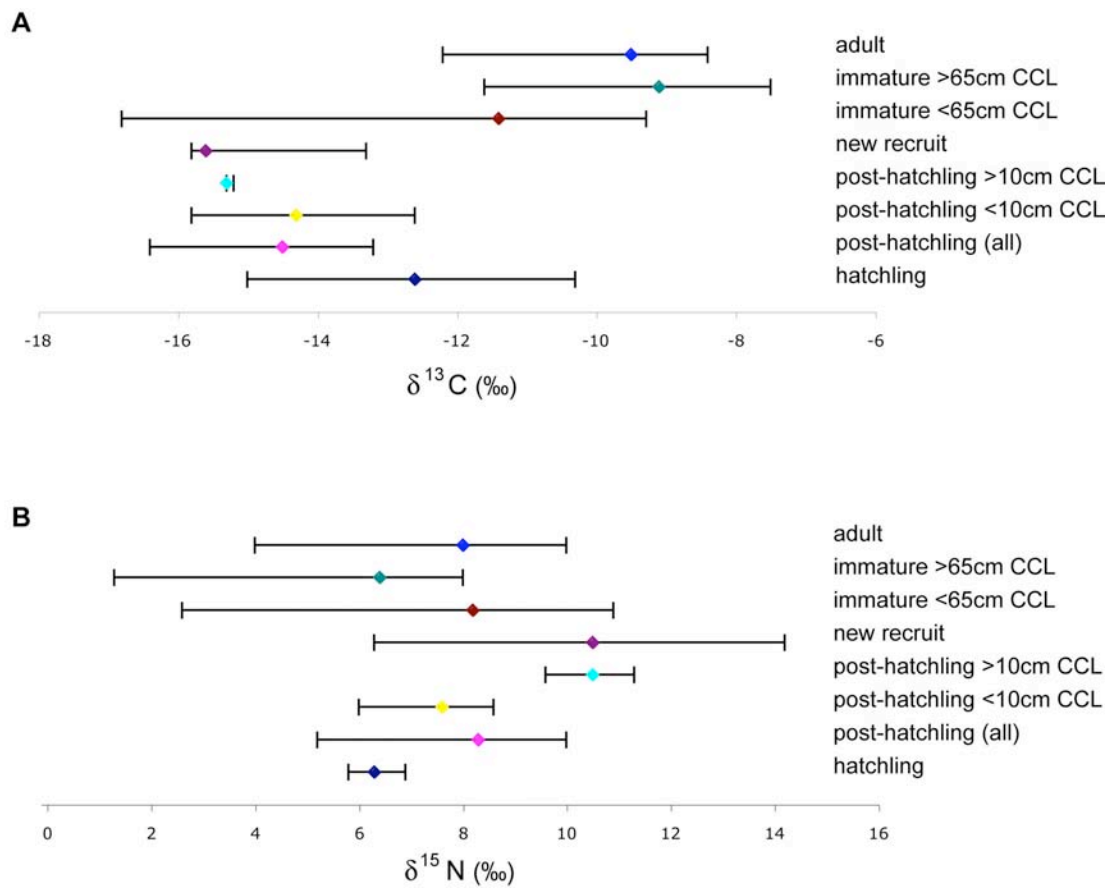


Figure 6.2. The mean $\delta^{13}\text{C}$ (A) and $\delta^{15}\text{N}$ (B) values and the ranges found within life history classes of green turtles (*Chelonia mydas*).

Table 6.3. Comparison of the stable isotopic signature of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ throughout the life history classes of green turtles (*Chelonia mydas*). ns = non-significant, s* = significant to one decimal places. s** = significant to two decimal places. Based on students *t*-test for independent samples.

	hatchling	Post-hatchling <10 cm CCL	Post-hatchling >10 cm CCL	New recruit	Immature <65 cm CCL	Immature >65 cm CCL
Post-hatchling <10 cm CCL	$\delta^{13}\text{C}$: ns $\delta^{15}\text{N}$: s*					
Post-hatchling >10 cm CCL	$\delta^{13}\text{C}$: ns $\delta^{15}\text{N}$: s**	$\delta^{13}\text{C}$: ns $\delta^{15}\text{N}$: s*				
New recruit	$\delta^{13}\text{C}$: s** $\delta^{15}\text{N}$: s**	$\delta^{13}\text{C}$: s* $\delta^{15}\text{N}$: s*	$\delta^{13}\text{C}$: ns $\delta^{15}\text{N}$: ns			
Immature <65 cm CCL	$\delta^{13}\text{C}$: ns $\delta^{15}\text{N}$: s*	$\delta^{13}\text{C}$: s* $\delta^{15}\text{N}$: ns	$\delta^{13}\text{C}$: s* $\delta^{15}\text{N}$: ns	$\delta^{13}\text{C}$: s** $\delta^{15}\text{N}$: s*		
Immature >65 cm CCL	$\delta^{13}\text{C}$: s** $\delta^{15}\text{N}$: ns	$\delta^{13}\text{C}$: s** $\delta^{15}\text{N}$: ns	$\delta^{13}\text{C}$: s** $\delta^{15}\text{N}$: s*	$\delta^{13}\text{C}$: s** $\delta^{15}\text{N}$: s**	$\delta^{13}\text{C}$: s* $\delta^{15}\text{N}$: s*	
Mature adult	$\delta^{13}\text{C}$: s** $\delta^{15}\text{N}$: s*	$\delta^{13}\text{C}$: s** $\delta^{15}\text{N}$: ns	$\delta^{13}\text{C}$: s** $\delta^{15}\text{N}$: ns	$\delta^{13}\text{C}$: s** $\delta^{15}\text{N}$: s*	$\delta^{13}\text{C}$: s* $\delta^{15}\text{N}$: ns	$\delta^{13}\text{C}$: ns $\delta^{15}\text{N}$: ns

Despite the large overlap across ranges, significant differences exist between the means of many life history classes (Table 6.3). However, differences in means for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for post-hatchlings >10 cm CCL and new recruits, and for immature individuals >65 cm CCL and mature adults, were not significant.

Variations of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ across post-hatchling green turtles of varying size

A relationship existed between post-hatchling size, and the $\delta^{15}\text{N}$ value of their epidermal tissue ($R^2 = 0.69$), with larger post-hatchlings (>10 cm CCL) having more N^{15} enriched tissue in comparison to the smaller post-hatchlings (<10 cm CCL). There was less variation of $\delta^{13}\text{C}$ values across the different sized post-hatchlings, with a weak relationship existing of decreasing $\delta^{13}\text{C}$ values with increasing post-hatchling size ($R^2 = 0.24$) (Figure 6.3).

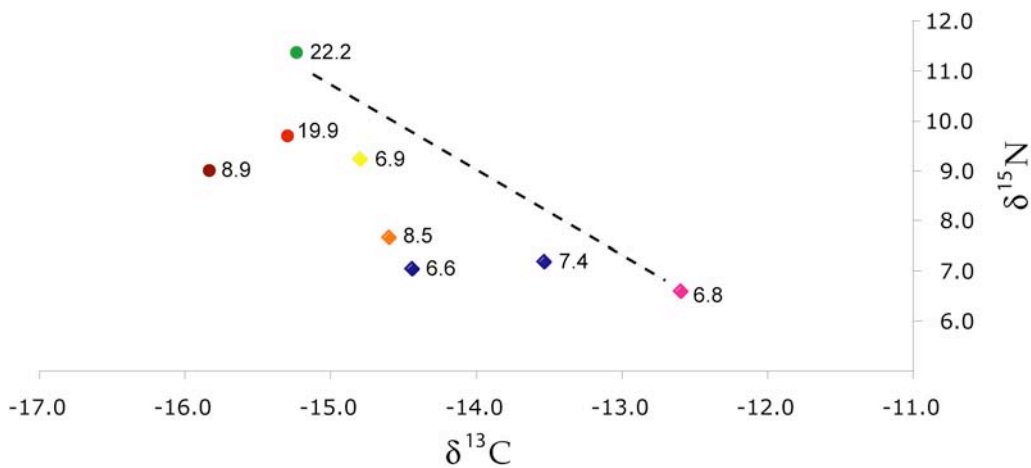


Figure 6.3. Isotopic signature of post-hatchling green turtles (*Chelonia mydas*), showing the change in signature with increasing carapace size. Carapace lengths are given beside the data point in cm CCL.

Variations of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ across green turtles of known years since recruitment into a coastal habitat

New recruits had the highest $\delta^{15}\text{N}$ values and the lowest $\delta^{13}\text{C}$ values of the individuals for which time since recruitment is known. The remaining groups had similar $\delta^{15}\text{N}$ values, ranging between 7‰ to 8.6‰, although they were spread across a wider range of $\delta^{13}\text{C}$ values, -12.8‰ to -9.7‰ (Figure 6.4). Across all groups there is a general trend from high $\delta^{15}\text{N}$ and low $\delta^{13}\text{C}$ towards decreasing $\delta^{15}\text{N}$ values and increasing $\delta^{13}\text{C}$ values as individuals spend more time in coastal habitats (Figure 6.4). With the exclusion of NR⁺⁸ and adults from these data, this relationship has an R^2 value of 0.69. The data from NR⁺⁸ are based on one individual. With more individuals representing this class it is likely the data point would be closer to the adults and N⁺⁷ classes.

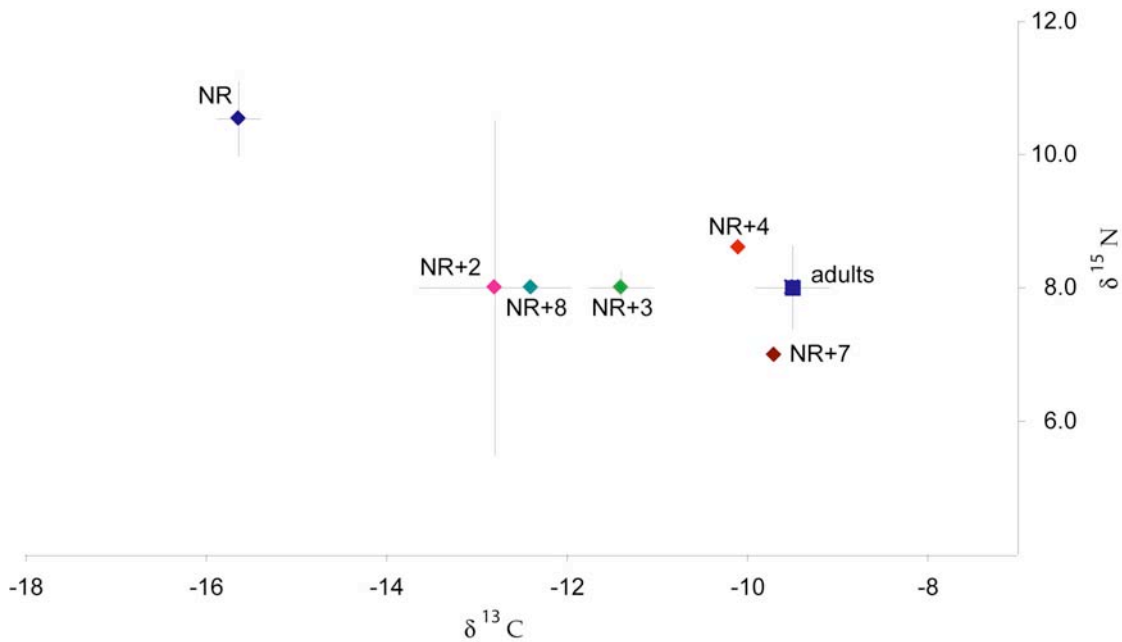


Figure 6.4. Isotopic signature of juvenile green turtles (*Chelonia mydas*) of known years since recruitment into the coastal habitat, showing the change in signature with years post recruitment. Mature adults are included for comparative purposes. NR = new recruit and NR +4yrs is a new recruit from four years previously.

Discussion

This is the first study to our knowledge that has measured the stable isotope signature of green turtles across all of their life history stages. The stable isotope data generally conformed to anticipated trends given our knowledge about the habitat and diet of green turtles in southeast Queensland and the nature of stable isotopes (e.g. lag time).

Post-hatchlings, and animals that had newly recruited into the coastal habitat, had higher $\delta^{15}\text{N}$ and lower $\delta^{13}\text{C}$ values than the remaining life history classes. This is consistent with expectations based on the findings of previous chapters (see Chapters 2 and 5) that found post-hatchlings procure their nutrition from the oceanic environment and have a diet that is dominated by animal matter. There was a trend for $\delta^{13}\text{C}$ values to increase and for $\delta^{15}\text{N}$ values to decline across the life history classes that had spent greater time feeding in coastal waters where green turtles have a diet that is dominated by plant matter.

New recruits had more negative $\delta^{13}\text{C}$ values and higher $\delta^{15}\text{N}$ values than the larger post-hatchlings, which suggests that even around the 20 cm CCL size, post-hatchlings still retain some of their mother's isotopic signature. As the carapace length cannot be used to determine the age of a sea turtle, the size of the post-hatchling cannot give an indication of the lag time for the absorption of ^{13}C and ^{15}N into the epidermal tissue of sea turtles. However the data available for the juveniles for which time since coastal recruitment was known, suggests that a green turtle's skin retains some of the $\delta^{15}\text{N}$ signature from its oceanic stage for two years, and perhaps longer for $\delta^{13}\text{C}$. New recruits (NR⁺⁰) and new recruits that

had been in coastal feeding grounds for one year (NR+1) had similar values, but by the time a new recruit had been in coastal feeding grounds for two years (NR+2) their $\delta^{15}\text{N}$ values had become similar to remaining life history classes living in coastal habitats. The low sample sizes limit data interpretation but with the single new recruit of eight years (NR+8) removed from the dataset, there is a trend for $\delta^{13}\text{C}$ values to continue to increase through to seven years following recruitment, at which point they become comparable to adult values.

Although it is most likely that the lag period is the reason new recruits have isotopic signatures similar to post-hatchlings, other factors may also contribute to this observation. For example, as a post-hatchling moves into the neritic feeding habitat, its foraging habits may not make a defined switch from one of pelagic animal matter to one of benthic plant matter. Instead they may have a transitional stage during which they feed on both types of items. Additionally, the microflora they require for the digestion of cellulose in algae and seagrass is most likely acquired as they begin feeding in the neritic habitat. Therefore, the assimilation of this food source would initially be low and would improve with time spent foraging in coastal waters as higher concentrations of microflora are obtained. More research into the stomach contents of newly recruited animals and into the lag time of isotopes within the epidermal tissue of sea turtles is needed to determine what combination of these factors causes the long lag phase.

Hatchlings' versus adults' isotopic signature

As the tissue from a hatchling that has not yet begun feeding is derived solely from the mother's protein, we would have predicted that the hatchlings would have had isotopic values similar to the adults. However there was a significant difference in the mean values of $\delta^{13}\text{C}$ between hatchlings and adults in this study. Nannarelli *et al.*, (2001), suggested that chemical and physical processes occurring in the developing eggs may lead to ^{13}C enrichment of the incubating hatchling. This could explain the findings of Godley *et al.* (1998), where hatchlings were more enriched in ^{13}C (-10.8‰) than adults (carapace scutes 14.8‰, bone collagen -15.4‰). However in our study, ^{13}C enrichment occurred in the opposite direction to that predicted if enrichment of the egg occurs, with adults having a mean $\delta^{13}\text{C}$ of -9.5‰ that reduced to $\delta^{13}\text{C}$ -12.6‰ in hatchlings. The variation in findings may be due to the different fractionation of isotopes that occurs between different tissue types (Gannes *et al.*, 1997). If our study had used tissue from carapace scutes or bone collagen as used by Godley *et al.* (1998) hatchlings may have been more enriched in comparison to adults.

This study also found a difference in the mean values of $\delta^{15}\text{N}$ between hatchlings and adults, with adults having higher $\delta^{15}\text{N}$ values (8.0‰) than hatchlings (6.3‰). This ^{15}N enrichment of adults in comparison to hatchlings was also reported by Godley *et al.* (1998) who found adults' bone collagen (9.4‰) and carapace scute (5.2‰) values were more ^{15}N enriched than hatchlings (4.4‰). Godley *et al.* (1998) also investigated the stable isotope signature of loggerhead turtles and found a relationship similar to that found for green turtles in our study, where adult loggerhead turtles were more enriched in both $\delta^{15}\text{N}$ (20.0‰) and $\delta^{13}\text{C}$ (-14.6‰) than hatchlings ($\delta^{15}\text{N}$, 7.8‰ and $\delta^{13}\text{C}$, -17.2‰).

The adults sampled in our study were feeding in Moreton Bay, however it is not known where the mothers of the sampled hatchlings were feeding. It is documented that regional variance exists for stable isotope signatures in aquatic ecosystems (Boon and Bunn, 1994; Hatase *et al.*, 2002c; Jennings *et al.*, 1997; Schell *et al.*, 1988) and therefore it is possible that regional differences can account for some of the variation observed between hatchlings and adults. The study by Godley *et al.* (1998), did not sample mothers and their associated hatchlings and therefore regional differences could also account for the variations found within their study. To explore these differences further, and to assess the level of enrichment that occurs between hatchling and mother for both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, if any, it would be valuable to conduct a study that samples mother-hatchling pairs.

What does the stable isotopic data tell us about the diet of post-hatchling turtles?

Owing to the delay in time before the isotopic signature of a consumer's diet is represented in its tissues, post-hatchlings >10 cm CCL and new recruits provide the best representatives of post-hatchling diet. The $\delta^{13}\text{C}$ values of the post-hatchlings and new recruits were lower than the remaining life stages that occupy coastal feeding grounds and that share $\delta^{13}\text{C}$ values closer to seagrass. All the life history classes that were sampled in coastal feeding grounds clustered within the range of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic values of seagrass and algae. In comparison, larger post-hatchlings and new recruits had more negative $\delta^{13}\text{C}$ and had higher $\delta^{15}\text{N}$ values, indicating that these stages are influenced by a oceanic diet of a trophic level that is higher than the remaining life history stages. The stable isotope data therefore support the findings of the dietary investigation outlined in Chapter 5, that at least a reasonable proportion of a post-hatchling's diet is comprised of animal matter, with plant matter unlikely to be a major component.

Summary

Our study has shown stable carbon and nitrogen isotope ratios in the tissue of green turtles reflect the different dietary habits of the life history stages and the ontogenetic dietary shift that occurs from the pelagic post-hatchling stage to the neritic habitat stages. Pelagic post-hatchling's had more negative $\delta^{13}\text{C}$ values and higher $\delta^{15}\text{N}$ values than the remaining life stages that occupy coastal feeding grounds. These isotopic differences reflect that post-hatchlings feed at a higher trophic level than the life history classes consuming predominately seagrass and algae, and therefore support the dietary investigation outline in Chapter 4, that found post-hatchlings have a diet dominated by animal matter obtained from offshore pelagic waters. These results provide details on how stable isotopes can be used in conjunction with dietary information to provide information on the ecology of post-hatchlings in a specific region.

Chapter 7

General discussion: advances in the biology of post-hatchling sea turtles in the South Pacific

The research presented in this thesis is the first study to undertake a detailed investigation into post-hatchling loggerhead turtles in the South Pacific region and the first to undertake an investigation of post-hatchling green turtles in any region. The results obtained from; spatial and temporal distribution records in relation to dominant surface currents (Chapter 2), genetic analyses (Chapters 3 and 4), and dietary (Chapter 5) and stable isotope investigations (Chapter 6), confirm the thesis' initial hypothesis, that 'post-hatchling loggerhead and green turtles originating from southwest Pacific rookeries lead an oceanic existence'. Moreover, the data also provide substantial evidence that after the post-hatchlings of these two species leave coastal waters, the migration route they take, and hence the habitats they occupy, diverge.

In this chapter, I begin by summarising the findings of the previous chapters. Following this summary, I explore the outcomes derived from the data presented in this thesis in relation to what is observed in the study species on a global scale. As this study has found that considerable differences exist in regards to the spatial scale at which the study species undertake their post-hatchling life stage, I explore the results in an ecological context and offer some possible explanations for the varying migration routes of the two species. Finally, this chapter outlines potential future research in this field and highlights the outcomes of the presented research in light of the implications for management and conservation of loggerhead and green turtle populations in Australia.

Evidence of an oceanic, pelagic post-hatchling lifestyle

The improved knowledge of the spatial and temporal distribution of post-hatchling loggerhead and green turtles in the southwest Pacific region supports their having an oceanic existence. For these two species post-hatchlings are not observed *in-situ* in coastal waters, nor have their skeletal remains been found at sea eagle nests like those of post-hatchling flatback turtles. The only post-hatchling records that do exist for these species are of turtles not significantly larger than neonates that have become stranded along the coast. That the majority of these strandings occur during the months following the hatching season, with observations decreasing after this, is consistent with a life history stage that does not reside in coastal waters. Moreover, given that strandings are concentrated along the southern Queensland and northern New South Wales coasts, south of the main rookeries, provides evidence of the East Australian Current's role in determining the initial migratory routes of emerging hatchlings. This provides firm evidence that the migrations of post-hatchlings, at least for the early part of this life stage, are influenced by prevailing surface currents.

Qualitative dietary analyses provided supporting evidence for an oceanic and pelagic post-hatchling stage for loggerhead and green turtles in the southwest Pacific Ocean. The diets of these two species were not significantly different and were dominated by neustonic animal

matter. Although the neustonic nature of the diet indicates a pelagic lifestyle, many of the items consumed by the post-hatchlings of both species occur in both coastal and offshore waters. However, the absence of benthic organisms, in addition to the presence of a few items that only occur offshore (e.g. *Porpita* sp. and cavolinids), provides strong support that these post-hatchlings lead an oceanic existence. The observation of a similar diet between post-hatchling loggerhead and green turtles contrasts with these species' feeding behaviour in the neritic life stages, when loggerhead turtles are primarily carnivorous and green turtles are primarily herbivorous.

The results presented in the dietary analysis (Chapter 5) concluded that post-hatchling loggerhead and green turtles have a diet dominated by zooplankton, in particular crustaceans, with minimal plant material. However, one caveat to the examination of stomach contents for the purpose of determining dietary preferences is the potential for over estimating organisms that persist in the gut, such as those with an endo- or exoskeleton. Thus, the conclusions reached from the dietary analysis could be due to the persistence of skeletal over plant tissue in the stomach of a sea turtle. However, the stable isotope analysis supported the findings of the dietary investigation for post-hatchling green turtles, i.e. they have diet relatively high in zooplankton. Analysis of the carbon isotopes also supported the conclusion of the dietary investigation, that post-hatchling green turtles procure their diet from offshore waters. Furthermore, the isotopic signatures of green turtles newly recruited to the coastal habitat provides evidence that the post-hatchlings of this species retain similar dietary habits throughout this life stage.

Migratory routes of post-hatchling loggerhead turtles in the southwest Pacific

The complete absence of records for post-hatchling loggerhead turtles larger than 13.7 cm CCL in Australian coastal waters, coupled with the progressive size increase of post-hatchlings, starting at southern Queensland nesting beaches, along the flow path of the subtropical gyre in the South Pacific Ocean, indicates that Australian post-hatchling loggerhead turtles make trans-Pacific migrations (Figure 7.1). Genetic studies have since confirmed the occurrence of southwest Pacific haplotypes among oceanic loggerhead turtles captured in the eastern Pacific (X. Velez pers. com., 2006), thus confirming trans-Pacific migration are undertaken by post-hatchling loggerhead turtles emerging from southwest Pacific rookeries. This migration route is comparable to the trans-oceanic migrations undertaken by loggerhead post-hatchlings from south eastern USA and Mexican rookeries across the northern Atlantic Ocean and from Japanese rookeries across the northern Pacific Ocean (Bolten *et al.*, 1998; Bowen *et al.*, 1995). Loggerhead hatchlings emerging from New Caledonia would be likely to take the same migratory route, with the South Equatorial and South East Australian Current systems first taking them via Australia's eastern coast. It would therefore follow that a portion of post-hatchling loggerhead turtles stranded along Australia's eastern coast would be of New Caledonian origin. The lack of genetic resolution between loggerhead turtle rookeries in the southwest Pacific Ocean means that this can not be confirmed by contemporary genetic techniques. However, the larger sized post-hatchling loggerhead turtles that become stranded are likely candidates for New Caledonian stock origin. It is also possible a number of the post-hatchling loggerhead turtles that originated from the offshore rookeries (New Caledonian and Vanuatu) are directed northwards at the SEC bifurcation or with the North Vanuatu Jet. If this happens these post-hatchlings would

not be in a current system that facilitates trans-Pacific migrations. The finding of one nesting loggerhead turtle with an otherwise exclusive Australian haplotype at a Japanese rookery may have resulted from such a situation.

Migratory routes of green post-hatchlings in the southwest Pacific

The spatial and size distribution of post-hatchling green turtles suggests an alternative route of migration to that taken by loggerhead post-hatchlings (Figure 7.2). The absence of records for post-hatchling sized green turtles throughout the southern Pacific (e.g. New Zealand and eastern Pacific waters, as found for loggerheads) and the occasional record of larger size-class post-hatchlings stranding along the eastern Australian coast suggests that, instead of undertaking trans-Pacific migrations, post-hatchling green turtles remain in oceanic waters in the southwest Pacific Ocean. Their occurrence in the stomachs of dolphin fish, and association with the East Australian Current suggests that they most likely remain in the warm water gyres that are formed by the EAC in the offshore waters off the eastern Australian shelf.

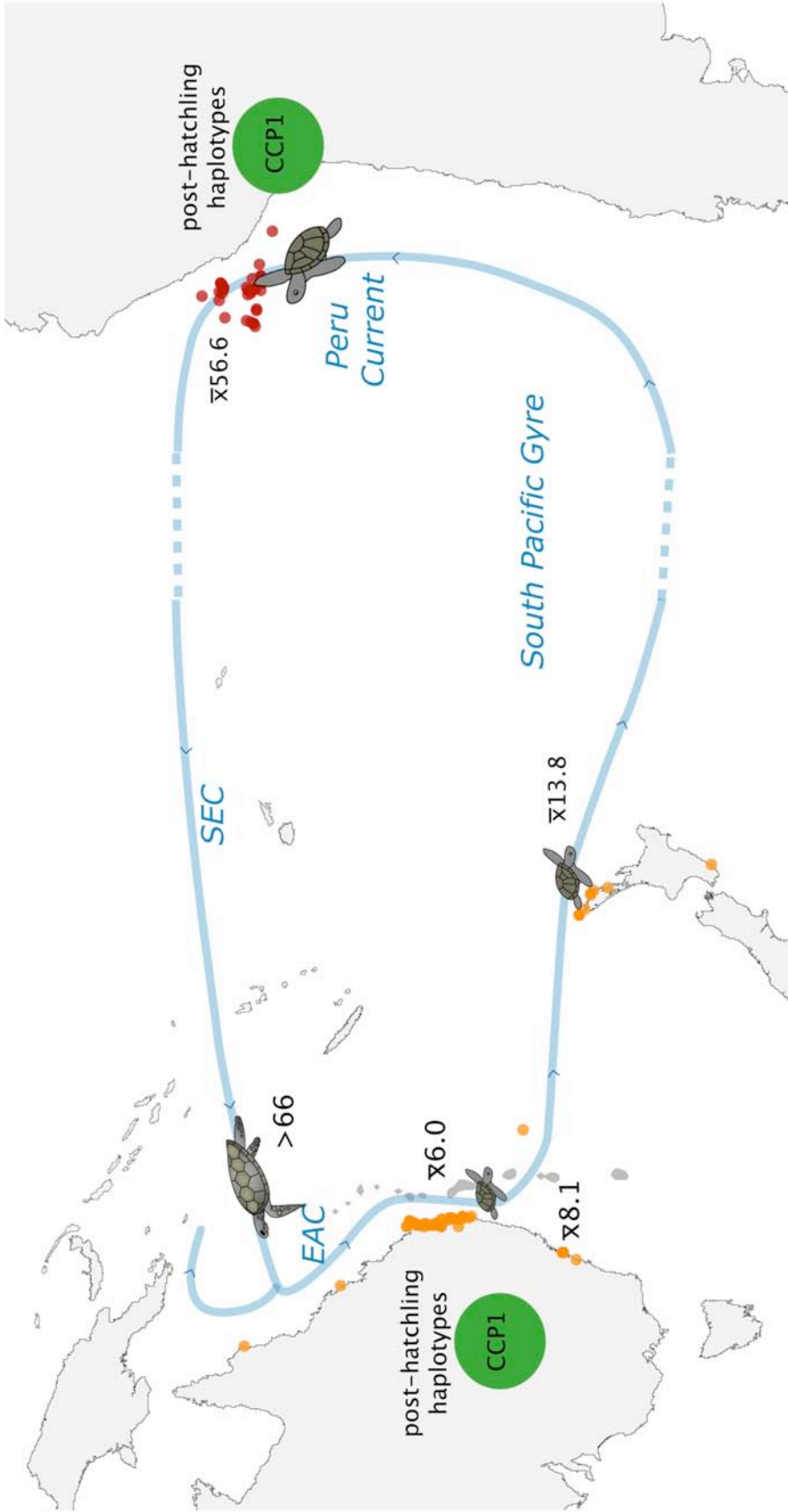


Figure 7.1. Model of the trans-Pacific migration route undertaken by post-hatchling loggerhead turtles (*Caretta caretta*) from southwest Pacific Ocean rookeries established in this study. Post-hatchling mean carapace sizes (shown in cm CCL) are indicated along the route of the South Pacific subtropical gyre. Haplotype type codes are explained in chapters 3 & 4.

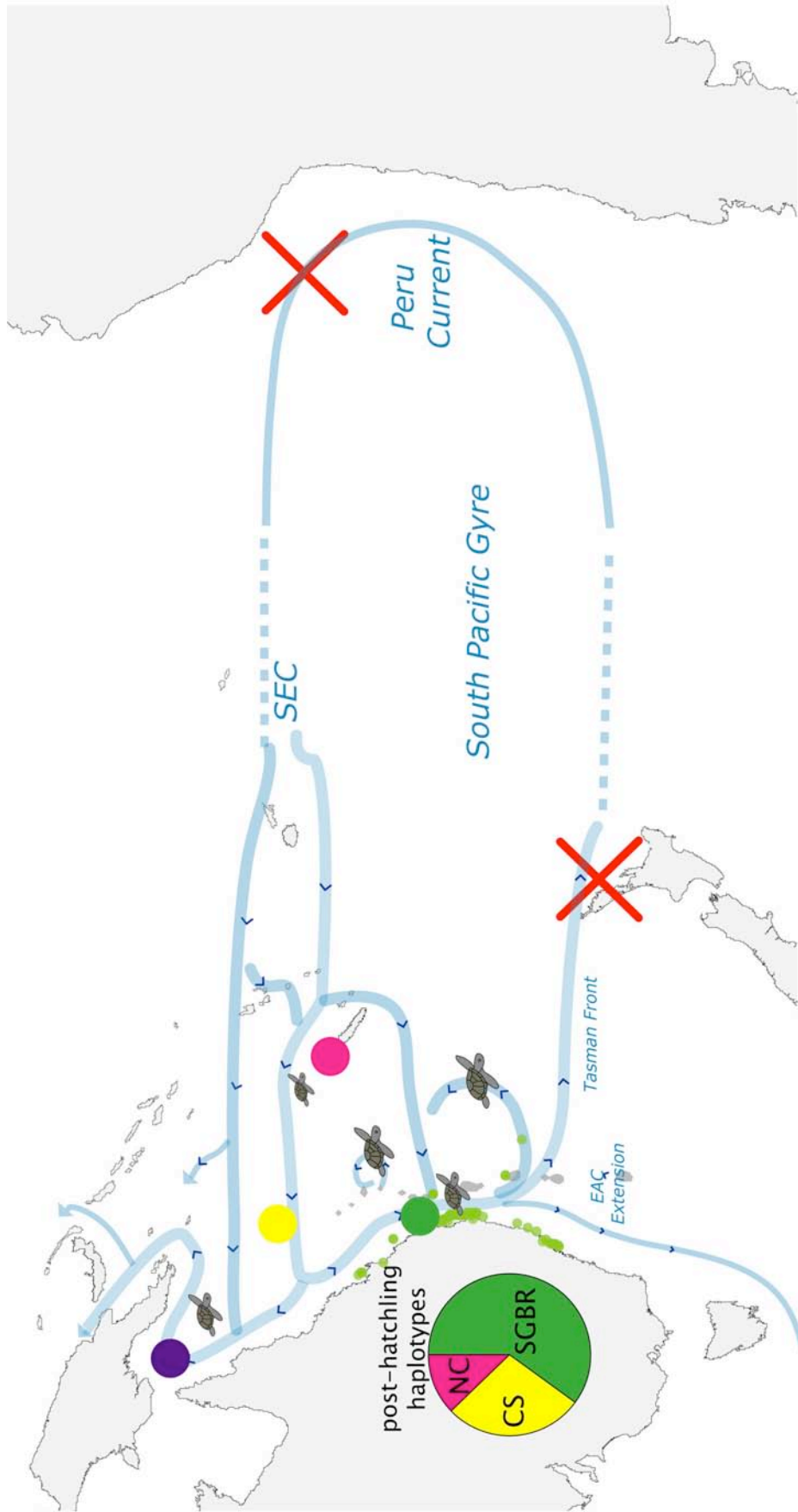


Figure 7.2. Model of the migration route undertaken by post-hatchling green turtles (*Chelonia mydas*) from southwest Pacific Ocean rookeries established in this study. The migration routes are demonstrated by an absence of the post-hatchling's in the southeast Pacific Ocean, and their presence in southwest Pacific offshore waters and through mtDNA analyses.

The heterogeneity that exists amongst green turtle rookeries in the southwest Pacific allowed mixed stock analysis to be conducted in order to determine the stock structure of the post-hatchling green turtles that become stranded along Australia's east coast. These post-hatchlings were found to be a mixture of animals from the southern Great Barrier Reef, Coral Sea and New Caledonian rookeries, thereby confirming the role of the East Australian Current in transportation of post-hatchlings from offshore rookeries (Coral Sea and New Caledonia) and the southern Great Barrier Reef rookeries.

The conclusion that post-hatchling loggerhead and green turtles undertake different migratory routes is further supported by the different sizes that juveniles of the two species recruit into coastal waters. The considerably smaller neritic recruitment size of juvenile green turtles compared to juvenile loggerhead turtles, suggests that the green turtle's oceanic post-hatchling stage is shorter than the loggerhead's, which would indicate that a shorter distance is travelled by green post-hatchlings. Although the size differences in recruitment could be attributed to a number of factors that affect growth rates (e.g. diet, temperature and physiology), the diet investigation did not find any significant differences between the two species. With no significant difference in diet, and the size difference at recruitment to the neritic zone being approximately 30 cm, it seems reasonable to assume that the green post-hatchlings are recruiting to the neritic habitat earlier, and at a younger age than loggerhead post-hatchlings (Limpus *et al.*, 1994a, 1994b).

The present study found limited evidence of the movements of post-hatchling green turtles from the northern Great Barrier Reef rookeries. Although there are no stranding records in this region for post-hatchling green turtles (Chapter 2), the mixed stock analysis (Chapter 4) suggests that hatchlings emerging from rookeries in the northern Great Barrier Reef are not becoming associated with the EAC's flow path. Based on the evidence that post-hatchling green turtles are associated with warm water gyres in the Coral and Tasman seas, it could be assumed that post-hatchlings from the northern Great Barrier Reef have a similar association with the large warm water, offshore gyres that exist in the northern Coral Sea (Gulf of Papua). These northern Coral Sea gyres are also likely to be the habitat of post-hatchlings that have emerged from offshore rookeries and are directed northwards at the SEC's bifurcation point or by its northward flowing jets. Post-hatchling green turtles in the northern Coral Sea may also be transported into the Solomon Sea and northern Vanuatu region before returning to coastal feeding grounds via the SEC.

South western Pacific post-hatchling behaviour in a global context

The findings presented in this thesis corroborate with previous investigations on the migrations of post-hatchling loggerhead turtles, with the conclusion that post-hatchling of this species in the southwest Pacific undertake trans-oceanic migrations. This study has provided the first description of the different migratory routes undertaken by post-hatchling loggerhead and green turtles from the same geographical region.

The differences in the spatial distribution of post-hatchling loggerhead and green turtles in the southwest Pacific are paralleled in the northwest Atlantic. Whereas it is known that loggerhead post-hatchlings undertake trans-oceanic migrations in the northwest Atlantic, there are no occurrences of green post-hatchlings at the oceanic locations where loggerhead

post-hatchlings occur. The difference in neritic recruitment size of green and loggerhead turtles in the southwest Pacific has also been observed in the western Atlantic where green turtles recruit at smaller sizes (26.7 cm SCL, Guseman and Ehrhart, 1990, equivalent to ~29.7 cm CCL, Teas, 1993), than loggerheads (46 cm CCL, Bjorndal *et al.*, 2000, 2001). The similarities in the spatial distribution between the two regions suggests that the migrations undertaken by green and loggerhead post-hatchlings from northwest Atlantic rookeries are comparable to those undertaken from southwest Pacific rookeries.

In the northern Pacific Ocean post-hatchling loggerhead turtles have also been shown to undertake trans-oceanic migrations along the path of the North Pacific gyre (Bowen *et al.*, 1995). As is the case in the southern Pacific, there are no reports of post-hatchling green turtles occurring amongst the oceanic aggregations of loggerhead post-hatchlings. Based on this spatial distribution it is possible that green and loggerhead post-hatchlings in the northern Pacific Ocean are undertaking different migration routes similar to those found in the southern Pacific Ocean. No studies have yet reported on the population structure of turtles in the coastal feeding habitats of the north-western Pacific. Such an investigation may be expected to yield differences in the recruitment sizes of juvenile green and loggerhead turtles in coastal habitats that parallel those found in the northwest Atlantic and southwest Pacific.

The present study included, the only loggerhead populations for which the migratory routes of their post-hatchlings are known are for rookeries in the western side of ocean basins, i.e. north-western Atlantic Ocean (Mexico, south-eastern USA), and north-western (Japan) and south-western Pacific Ocean. Since oceanic crossings have been documented for all of these populations, it could be inferred that undertaking trans-oceanic migrations is the 'inherent nature' of all loggerhead post-hatchlings. However, it would be misleading to project this scenario across all populations, and I propose that this behaviour is a feature only of particular loggerhead turtle populations, i.e. those in the western aspect of ocean basins. Evidence suggesting 'trans-oceanic migrations' are not the norm in all loggerhead post-hatchlings is primarily based on a lack of evidence that exists for other populations. The size class structure and geographical distribution of the oceanic aggregations of loggerhead post-hatchlings, in relation to major currents and rookeries, provided the initial evidence for the trans-oceanic crossing being undertaken by post-hatchlings in the northern Atlantic and Pacific oceans. However, the lack of any equivalent aggregations for other populations of loggerhead turtles may indicate that the trans-oceanic migrations may be undertaken only by these populations.

I propose that the reason the western loggerhead turtle populations undertake oceanic crossings is resource driven. The migratory route taken by the post-hatchling loggerheads emerging from western rookeries results in their transportation to oceanic areas of high productivity. For example, the up-welling off the Peruvian and Californian continental shelves are the most biologically productive up-welling systems in the world and would provide a good food source for oceanic loggerhead post-hatchlings. It may be then, that the oceanic traversal undertaken by pelagic post-hatchlings is not an 'innate need to cross the ocean', but instead behaviour designed to take advantage of high value food resources. If this is the case, loggerhead hatchlings emerging from rookeries on the eastern side of the Atlantic Ocean (i.e. West Africa, Mediterranean Sea), where these regions of higher productivity are, will not embark on trans-oceanic migrations but instead move to more

localised feeding zones. Thus, it could then be predicted that the loggerhead post-hatchling aggregations at the Azores and Madeira archipelagos would be a mix of animals from western Atlantic and Mediterranean rookeries.

The origin of the pelagic loggerhead turtle aggregations at the Azores and Madeira was investigated with genetic techniques by Bowen *et al.* (1996), who concluded that all of the turtles sampled were from northwest Atlantic rookeries. However, seven out of the seventeen haplotypes found at the pelagic feeding grounds had not been found at rookeries, and this strongly suggests that the nesting beach data used in Bowen's study did not adequately represent the contributing rookeries. The inadequacy of the baseline file is highlighted by having genetic information from only one eastern Atlantic loggerhead rookery (Greece). Since this investigation, the Mediterranean populations have received more attention and studies have found that there is genetic distinction amongst the Mediterranean rookeries (Laurent *et al.*, 1998), although there are still loggerhead turtle populations in the Mediterranean (e.g. Greece, Turkey and Libya) and along Africa's northwest coast (Dodd, 1988; Ehrhart *et al.*, 2003) which have not been genetically studied.

In addition, the occurrence of very small post-hatchlings (e.g. 9 cm CCL, Bolten, *et al.* 1998) at the Azores could indicate that eastern populations are represented at these feeding grounds. These smaller turtles are more likely to have originated from west African populations as, based on surface flow rates through the Strait of Gibraltar and swimming capabilities of loggerhead turtles smaller than 40 cm SCL (Revelles *et al.*, 2006), are unlikely to successfully exit the Mediterranean against the eastward flowing current. Therefore, based on present information, it can not be dismissed that eastern Atlantic rookeries are represented at the oceanic feeding grounds used by the western Atlantic loggerhead post-hatchlings. If more comprehensive data on the potential contributing rookeries were included in a baseline file and a mixed stock analysis was repeated, it is highly likely that eastern stocks would be recognised as partial contributors to the oceanic loggerhead feeding population at the Azores and Madeira archipelagos.

This difference in regional migratory behaviour that I propose amongst loggerhead post-hatchlings from different populations, may lend an explanation for the difference in sizes of neritic recruitment that occur between eastern and western Atlantic Ocean populations. Populations from the eastern side are smaller at recruitment 32 cm CCL in the Mediterranean (Laurent *et al.*, 1998) and 44 cm in the Gulf of California compared with 46cm in NW Atlantic (Bjorndal *et al.*, 2000) and 67 cm CCL in Australia (Limpus and Limpus, 2003a). Post-hatchlings on the eastern side do not travel as far and therefore may spend a shorter time in the oceanic zones, and hence recruit into neritic habitats at a smaller size. Since it is not completely known what additional factors determine the time that post-hatchlings stays in the oceanic habitat this speculation can not be tested.

Why are there differences between post-hatchling loggerhead and green turtles?

The present research has shown that loggerhead and green post-hatchlings in the southwest Pacific overlap in both temporal and trophic dimensions, i.e., as hatchlings they are emerging from their nests at the same time, and as post-hatchlings they share the same foraging strategies. Therefore, the different migratory routes they undertake may be a

mechanism that has evolved to reduce food competition on the spatial dimension between the oceanic life history stages of these two species.

Further examples of sea turtle behaviour that act as a mechanism for niche separation can be found in both the oceanic and coastal habitats. In the oceanic habitat, differences exist between the foraging behaviours of post-hatchling green and leatherback (*Dermochelys coriacea*) turtles, in terms of differences in prey category, prey size and foraging depth (Salmon *et al.*, 2004). Furthermore, satellite telemetry studies have highlighted another example of niche separation behaviour in the oceanic zone, where differences exist between the feeding locations and foraging behaviours of olive ridley and loggerhead turtles in the North Pacific Ocean (Polovina *et al.*, 2004). Niche separating behaviour is also evident in the coastal habitat, where species with overlapping habitats have evolved very different feeding regimes, e.g. loggerhead turtles have developed enlarged jaws for the consumption of hard-shelled molluscs and crustaceans, green turtles have gut characteristics that deal with a predominately herbivorous diet, and a hawksbill's diet is dominated by sponges.

Alternatively it may be that the migratory routes undertaken by post-hatchling green and loggerhead turtles are based on temperature preferences. Post-hatchlings that associate with eddies of the Coral and Tasman seas off Australia's east coast, are in a warm water habitat where mean monthly temperatures range between 20°C to 26°C (Tanner, 2006). In comparison, post-hatchlings that are associated with the South Pacific Ocean's subtropical gyre will encounter cooler waters, where temperatures can be less than 20°C (Mendelsohn and Schwing, 2002). The evidence derived from spatial distribution of post-hatchlings in the South Pacific Ocean (Chapter 2) found that green post-hatchlings migrate within the warmer habitat and loggerhead post-hatchlings migrate within the cooler habitat. These patterns are consistent with the overall temperature preferences of these species. Green turtles are considered a tropical species, whereas loggerhead turtles are more temperate in their distribution and can withstand colder temperatures (Witherington and Ehrhart, 1989).

What mechanisms are involved in post-hatchling migration route variations?

The stranding data presented in Chapter 2 has shown that post-hatchling loggerhead and green turtles are initially associated with the EAC as it flows southwards along Australia's east coast. After the EAC swings away from the coast, the post-hatchlings of the two species must at some point exhibit behavioral differences that result in loggerhead post-hatchlings maintaining an association with the South Pacific subtropical gyre and green post-hatchlings taking up occupancy in the local offshore gyres.

One possibility is that post-hatchling loggerhead and green turtles are employing a navigational mechanism to which they respond to ensure the desired migration route outcomes. Among the suite of navigational mechanisms suggested to be used by sea turtles, the most probable mechanisms employed during post-hatchling migrations are geomagnetic cues or water temperature gradients (rationale provided in Chapter 1).

Laboratory experiments indicate that loggerhead hatchlings have a guidance system in which regional magnetic fields function as navigational markers and elicit changes in swimming direction at crucial geographic boundaries (Lohmann *et al.*, 2001). If this ability is

shared by post-hatchling loggerhead and green turtles in the southwest Pacific, and is the mechanism used to determine the migratory routes taken by these species, it would be expected that exposure to certain magnetic fields would reflect predictable differences. For example, if the hatchlings of the two species were exposed to a field that replicates the southwestern corner of the South Pacific gyre, loggerhead hatchlings would be expected to swim in a west-northwest direction and green hatchlings in a northerly direction. If post-hatchling loggerhead turtles do use this navigational system it would be expected that very few loggerheads travelling eastward from New Caledonian rookeries would go northwards at the SEC bifurcation. Instead they would respond to geomagnetic cues to actively seek a path with the southward flowing EAC in order to follow the route conducive to a South Pacific Ocean crossing.

Experiments testing the ability of turtles to detect and respond to magnetic fields have not been conducted in the southwest Pacific. The design of experiments to test whether green and loggerhead hatchlings from southwest Pacific rookeries can distinguish among magnetic fields, and whether different magnetic fields elicited different responses, would be instructive on at least two accounts. First, they would provide an opportunity to further investigate the navigational abilities based on geomagnetic cues, of both post-hatchling loggerhead turtles and post-hatchling green turtles. Secondly, such experiments would provide insight into whether navigation based on responses to regional magnetic fields is the mechanism that drives the different migration routes undertaken by post-hatchling loggerhead and green turtles in the southwest Pacific.

Alternatively, the different migratory routes may not depend on a complex navigational system, but may result from differences in swimming behaviour of the two species. Comparison of swimming behaviour between green and loggerhead hatchlings has shown that they both swim continuously for approximately 24 hours during the swim frenzy period, after which power-stroke rate decreases in both species (Wyneken, 1997). During the swim frenzy, both swimming speeds and power-stroke bout duration vary between the two species. Green hatchlings swim faster than loggerhead hatchlings with average speeds of 1.57 km/hr (Frick, 1976), compared to 1.26 km/hr (Salmon and Wyneken, 1987). The power-stroke bout duration is shorter after the swim frenzy in green hatchlings and shorter at the start of swim frenzy in loggerhead hatchlings (Wyneken, 1991). Variations also exist in the post-frenzy swimming behaviour. Post-hatchling green turtles swim using the power-stroke or dogpaddle (hind flippers only) methods whilst loggerhead post-hatchlings show a limited propensity to swim, preferring to float with their flippers on the carapace, with dogpaddling as the primary swimming method (Witherington, 1994b). Additionally green post-hatchlings have a broader aerobic scope than loggerhead post-hatchlings, and a higher oxygen consumption rate during the swim frenzy. It is not clear how, or even if, the differences that occur in swimming behaviour and physiology between green and loggerhead hatchlings and post-hatchlings might correspond to the variations that are observed between the migration routes that are taken by these species. However, it may be that the greater swimming abilities of the green post-hatchlings provide them with the capacity to direct themselves northward after the EAC leaves the coast in response to decreasing temperatures, whereas loggerhead post-hatchlings, with comparatively reduced swimming capabilities, are unable to remove themselves from this current and consequently undertake trans-Pacific migrations.

Studies conducted by Owens (1980) found that green hatchlings actively selected waters between 29.1°C and 31.0°C when provided with water that ranged in temperature between 26.5°C and 36.8°C. The preference for warmer water, combined with their greater swimming abilities than loggerhead hatchlings, may provide green turtle hatchlings with the mechanisms to ensure an association within the warmer water gyres as opposed to being taken on a cooler water circuit of the South Pacific Ocean.

Future research directions

The present study has established that post-hatchling loggerhead and green turtles in the southwest Pacific take different migration routes, and in doing so has provided the foundations for some instructive future research. Founded on a post-hatchling's unfamiliarity with its prospective route, I have proposed that the navigational mechanisms used by post-hatchlings are based on geomagnetic cues and/or water temperature gradients. To explore the validity of these proposals, geomagnetic experiments similar to those outlined by Lohmann *et al.* (2001) could be conducted with hatchling loggerhead and green turtles obtained from southeast Queensland rookeries. The results from such a study will provide informative and comparative data on the navigational abilities of hatchlings of these two species in the southern Pacific Ocean. Additionally, insight will be gained into whether a post-hatchling's response to regional geomagnetic fields could account for loggerhead and green turtles undertaking different migratory routes during their post-hatchling stage.

In exploring the navigation mechanisms it would be worthwhile to explore further the potential role that temperature may have on eliciting swimming responses from hatchling green turtles. Owens' (1980) study found that hatchling green turtles respond to temperature gradients, however this was only shown at the microhabitat scale. Whether a response to temperature gradients can be transcended to larger, ecological, scales is yet to be explored.

This study has suggested that post-hatchling loggerhead turtles originating from rookeries on an ocean's eastern side, may not be undertaking trans-oceanic migrations as has been shown for loggerhead turtles from western rookeries. This hypothesis warrants further investigation and a good starting point would be through further mtDNA analysis of oceanic populations in the Azores and Madeira. Confirmation that loggerhead post-hatchlings of eastern Atlantic origin reside in waters around the Azores and Madeira would provide key evidence that these populations do not undertake trans-oceanic migrations. Furthermore, it would provide insight into the motivation for trans-oceanic migrations undertaken by post-hatchling loggerhead turtles from populations in the western aspect of the oceans' basins.

Implications for conservation and management

Prior to the present study, the ecology of post-hatchlings from Australia's loggerhead and green turtle populations could only be speculated upon based on knowledge derived from northern Pacific and northern Atlantic loggerhead populations. The present study has provided region-specific information for post-hatchlings in the southwest Pacific Ocean, and additionally has provided new insight into post-hatchling ecology and migrations on a global level.

In the context of sea turtle management, information on the migration routes, and hence spatial distribution of the post-hatchling life history stage, ensures that future conservation and management strategies can recognise and account for potential threats. The present study found that post-hatchling loggerhead turtles from Australian populations undertake trans-oceanic migrations. This migratory route takes them to the waters off the coasts of Peru and Chile, where oceanic stage post-hatchlings are documented as being caught in long-line fisheries operating in the Peruvian current. Currently records of turtles that have been captured are reasonably low (n=52) (Alfaro-Shigueto *et al.*, 2004; Donoso *et al.*, 2000; Kelez *et al.*, 2004) in comparison to the thousands that are being caught in the northern Pacific long-line fisheries (Wetherall *et al.*, 1993a). However this number of recorded captures of post-hatchling loggerhead turtles by these fisheries is almost certainly a gross underestimate. The capture of southwest Pacific loggerheads in these fisheries highlights a segment of the life history stage that has the potential to be heavily impacted upon. As such, agencies responsible for the management of Australia's endangered loggerhead turtle populations need to firstly, define acceptable limits to the mortality of loggerhead turtles in the eastern Pacific, and secondly, remain vigilant of by-catch mortality resulting from fishery interactions in the south-eastern Pacific Ocean region.

An inter-governmental approach would provide a means for Australia to encourage and support observer programs operating within the long-line fisheries in south-eastern Pacific waters, thereby ensuring accurate by-catch mortality information is gathered. An inter-governmental approach could also provide an opportunity for the Australian government to encourage the employment of by-catch reduction strategies in the South American fishery operations. Additionally, the development of inter-government relations in regard to the loggerhead turtles in the eastern Pacific could facilitate further research into Australian loggerhead post-hatchlings with the deployment of satellite tags for habitat use information.

The present study has shown that post-hatchling green turtles do not undertake trans-oceanic migrations, thus management issues are retained within the southwest Pacific region for this life stage. Currently, there are no records of post-hatchling green turtles being captured in oceanic fisheries or being directly threatened by other anthropogenic activities anywhere in the southern Pacific, as those that exist for Australian post-hatchling loggerhead turtles.

Investigations into the extended region of the mtDNA for loggerhead turtles did not expose any further resolution between known rookeries. These results support the current designation of the southern Great Barrier Reef rookeries as one management unit. The information obtained from the New Caledonia rookeries shows that this rookery is not genetically distinct from the east Australian management unit. The phylogenies that were constructed provide strong evidence that this is unlikely to change as further resolution that will define intra-rookery structure is unlikely to exist.

Investigations into the extended region of the mtDNA region for green turtles did find that further resolution existed than what has previously been described. This increase in resolution is limited and the phylogeny shows that the resolution between rookeries may not be significantly increased as this species shows reduced natal philopatry in the Pacific Ocean. The results support the retaining of four management units (southern Great Barrier Reef,

northern Great Barrier Reef, Coral Sea and New Caledonia) for southwest Pacific green turtle rookeries.

Post-hatchling loggerhead and green turtles investigated in this study had consumed anthropogenic debris (e.g. plastics, nylon cord). Although the effects can not be directly assessed, it has been shown that post-hatchling turtles do not compensate for the consumption of non-nutritional items, which results in reduced energy and nitrogen uptake (McCauley and Bjorndal, 1999). The recognition of this problem with the qualitative information provided by this study provides an avenue that could be used in public awareness and educational campaigns to highlight pollution issues and its effects on marine life.

Summary

The results presented in this thesis provide convincing evidence that loggerhead and green post-hatchlings from southwest Pacific rookeries occupy offshore waters, where they feed predominately on pelagic organisms of animal origin. Although the initial migratory routes of the two species are influenced by the same offshore currents (i.e. EAC), after they depart the Australian continental shelf the two species take different routes and consequently their oceanic post-hatchling stages are lived at different spatial scales. The spatial and size distribution data and genetic analysis of the post-hatchlings have shown loggerhead turtles undergo trans-Pacific migrations with the South Pacific Ocean subtropical gyre. In contrast, green post-hatchlings do not undergo trans-oceanic migrations, but remain in warm water gyres that exist in the Tasman and Coral seas off the Eastern Australian continental shelf.

The research presented in this thesis provides the first detailed information on green post-hatchling sea turtles for any location, enabling comparison between post-hatchling ecology of different species. This study has provided substantiation to the hypothesis that the post-hatchling stage of different sea turtle species may vary. The migrations found to be taken by loggerhead post-hatchlings are comparable to those undertaken by post-hatchlings from rookeries in the northern Pacific and northern Atlantic oceans. However, through critical review of post-hatchling data this thesis also cautions against assuming trans-oceanic migrations are the norm for post-hatchling loggerhead turtles. Instead trans-oceanic migrations may be resource driven, and consequently the populations on the eastern aspect of the ocean basins may not undertake such extensive migrations.

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Table A.1. Latitudes and longitudes for regional nesting locations referred to in this thesis.

Location	Latitude	Longitude
<i>Bramble Cay</i>	-9.1333	143.8667
<i>Heron island</i>	-23.4330	151.9167
<i>Mon Repos</i>	-24.7983	152.4333
<i>Moreton Bay</i>	-27 17	153 15
<i>Moulter cay</i>	-11.4500	144.0000
<i>No. 7 sandbank</i>	-13.4500	143.9833
<i>No. 8 sandbank</i>	-13.3667	143.9667
<i>Raine Island</i>	-11.6000	144.0200
<i>La Roche Percee</i>	-21.6125	165.4458
<i>Swains Reef</i>	-21.8000	152.4167
<i>Wreck Island</i>	-23.3333	151.9500
<i>Wreck Rock</i>	-24.3167	151.9667

Attached as electronic copy with thesis.