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# Population Structure, Migration and Habitat Ecology of the Green Turtle (*Chelonia mydas*) in the Grand Lagon Sud of New Caledonia

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Tyffen Chloé Read

B. MarSt (Hons) (University of Queensland) (2011)

Submitted in fulfillment of the requirements of the degree of

Doctor of Philosophy

Griffith School of Engineering

Griffith Sciences

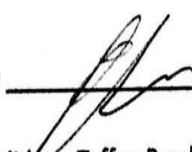
Griffith University Gold Coast Campus

May 2015

## STATEMENT OF ORIGINALITY

This work has not been previously submitted for a degree or diploma in any university.

To the best of my knowledge and belief, this thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

(Signed)   
PhD candidate: Tyffen Read

May 2015

## ACKNOWLEDGEMENT OF PUBLISHED AND UNPUBLISHED PAPERS INCLUDED IN THIS THESIS

THE PAPERS INCLUDED ARE A MIX OF SOLE-AUTHORED AND CO-AUTHORED PAPERS.

Included in this thesis are papers in Chapters 1, 6 and 7 for which I am the sole author.

The bibliographic details (if published or accepted for publication)/status (if prepared or submitted for publication) for these papers are:

**Chapter 1:** Not intended for publication (Read TC)

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Additionally included in this thesis are papers in chapters 2, 3, 4 and 5 which are co-authored with other researchers. My contribution to each co-authored paper is outlined at the front of the relevant chapter. The bibliographic details (if published or accepted for publication)/status (if prepared or submitted for publication) for these papers including all authors, are:

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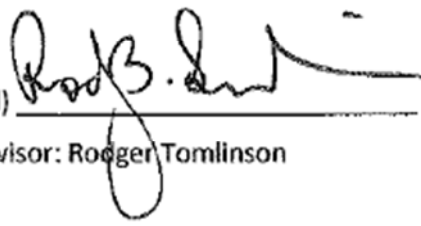
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Principal supervisor: Rodger Tomlinson

## GENERAL ABSTRACT

Conservation management of wildlife populations requires a comprehensive understanding of the population structure, movement and ecology of threatened and endangered species. The green turtle, *Chelonia mydas*, is a species found in both tropical and subtropical waters worldwide and since 1982 is listed as endangered on the IUCN red list of threatened species. While *C. mydas* is afforded protection in many areas of the Pacific it still faces significant threats, both anthropogenic (e.g. by-catch from commercial and recreational fishing, directly targeted as a food source by indigenous groups and boat strikes) and environmental (e.g. climate change, coastal pollution and habitat degradation). These threats are real for *C. mydas* in New Caledonia (NC), however strategies to mitigate the threats are lacking as vital information on the *C. mydas* population structure, movement patterns and habitat use in NC is scant. Proof of *C. mydas* migration within the Southwest Pacific, including linkages with NC and other Pacific countries, has been shown however more comprehensive data is needed. Furthermore, preliminary data suggest the Grand Lagon Sud (GLS) in NC provides important foraging grounds for *C. mydas*. However, identifying the spatial extent and habitat value of these foraging grounds is a challenge due to the complexity of the *C. mydas* life cycle and the species tendency for large scale migrations

This study investigated the population, movement and habitat ecology of foraging *C. mydas* in the GLS using a variety of cutting-edge approaches, including long term titanium tagging, genetic analysis for identification of *C. mydas* Management Units (MU), chemical indicators for habitat preferences, conventional methods for dietary analysis and time-depth recorders and satellite telemetry for movement patterns.

Titanium flipper tag recoveries of *C. mydas* tagged in the last 50 years in both New Caledonia and neighbouring countries indicated most of the tag recoveries found in New Caledonia belonged to females from the southern Great Barrier Reef genetic stock. A high percentage of tag recoveries in southern New Caledonia also belonged to individuals from the d'Entrecasteaux rookery north of New

Caledonia. Some females (n=2) even showed fidelity to foraging sites located 1200 km away from the nesting site located in New Caledonia. These data also reveal previously unknown migrations pathways of turtles between the Chesterfield reefs in the centre of the Coral Sea and the southern Great Barrier Reef as well as some migrations of > 4000 km.

Genetic analysis of ~770 bp of the mitochondrial (mt)DNA control region from 164 foraging turtles sampled in the GLS was done. *C. mydas* sampled ranged in size from 48 to 108.4 cm curved carapace length (CCL) and were captured at five different sites within the GLS between September 2012 and December 2013. To provide baseline data for mixed stock analysis, published data from rookeries were also used in addition to a further 105 samples collected at rookeries in the d'Entrecasteaux Islands and Chesterfields Islands in New Caledonia and Malekula Island in Vanuatu. Exact tests of population differentiation and pairwise  $F_{ST}$  estimates to test for differences in mtDNA haplotype frequencies indicated that rookeries in the d'Entrecasteaux Islands and Vanuatu form unique genetic stocks and that the Chesterfield Islands rookeries are linked to the Coral Sea genetic stock. Mixed stock analysis indicated the highest proportion (mean = 0.63) of foraging turtles originate from the d'Entrecasteaux stock.

*C. mydas* (n=21) killed for indigenous tribal ceremonies in the GLS were examined for stomach contents and to provide additional insights regarding interpretation of diet from stable isotopes analysis of skin tissue.  $\delta^{13}C$  and  $\delta^{15}N$  in skin samples ranged from -19.3‰ to -7.3‰ and 2.8‰ to 15.9‰ respectively, indicating a preference for an algal diet. Isotope analysis concur with the stomach contents analysis of which four algae genera contribute 50.4% of the total dry weight. Namely, *Hypnea* (20.1%), *Ulva* (12.4%), *Caulerpa* (9.1%), *Codium* (8.8%). A significant difference was found within the juveniles *C. mydas* caught at the four different sites (One-way ANOVA,  $F = 69.00$ ,  $df = 3$ ,  $P < 0.01$ ). Within the GLS juveniles (N=179) caught at Ouen Island, Uo/Mato islands and Goro had much higher  $\delta^{13}C$  signatures compared to the juveniles caught in the Isle of Pines. These results provide continuing evidence that *C. mydas* feeding patterns differ between localised foraging grounds.

Horizontal and vertical movement for juvenile and adult *C. mydas* in the GLS were examined using a combination of SPOT/SPLASH satellite tags and time-depth recorders. Seven *C. mydas* (43 - 113.5 cm CCL) were tracked via satellite tags for 111 to 221 days. Ten juveniles (41.2 - 61.1 cm CCL) were equipped with time-depth recorders for periods of 14 to 221 days. All satellite tagged individuals exhibited localised movement within the GLS with a mean 95% convex polygon home range for the satellite tagged juveniles of  $54.28 \pm 2.42 \text{ km}^2$ . Eight time-depth recorders were recovered and revealed that individuals with depth tags spent 80% of their time at  $< 5 \text{ m}$  with a maximum depth of 18 m. Juvenile *C. mydas* displayed diel behavior with a higher number of dives during the day and shallower dives during the night. Together these movement data indicate that shallow waters are important for juvenile life history stages of *C. mydas* in the GLS.

Mark recapture efforts were done with 453 foraging turtles tagged with a titanium tag at five locations within the GLS between 2012 and 2013. POPAN population estimate for one foraging ground of the GLS, at Uo Island, indicated  $> 90$  individuals. Recaptures also indicated most foraging *C. mydas* tagged were resident. A gonad interpretation from fifteen *C. mydas* killed by local tribes further suggested individuals were resident as there were no mature follicles observed. Recaptured individuals ( $n = 54$ ) also provided a mean annual growth rate of  $0.3 \pm 0.1 \text{ cm CCL}$  for the GLS.

Combined, the above data enabled identification of the importance of the GLS to the life cycle of *C. mydas* in the South Pacific. Particular strong genetic and habitat linkages occur with the d'Entrecasteaux Islands and the southern GBR in Australia. These linkages suggesting anthropogenic impacts in the GLS will have reciprocal impacts on *C. mydas* both across the entirety of New Caledonia and in neighbouring countries. The GLS provides significant foraging grounds where juvenile *C. mydas* display strong localised residency. Conservation management efforts should recognise the importance of shallow water habitats in the GLS for endangered *C. mydas*. Furthermore, the unusual absence of mature individuals from these shallow water areas and the strong residency of juveniles suggest *C. mydas* within the GLS are susceptible to localised overfishing. Unregulated harvesting of *C. mydas* may have significant long term local as well as wide reaching consequences. Management

strategies for this species in the GLS therefore require not only local but international collaborative efforts.



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*Only a PhD candidate knows the feeling*

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## CHAPTER 1: A GENERAL INTRODUCTION ON GREEN TURTLES (*CHELONIA MYDAS*)

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In this thesis, I investigate the dynamics of a foraging population of the green turtle *Chelonia mydas* found in the south of New Caledonia and the implications for the rest of the South Pacific. The thesis is presented in a series of seven chapters, with chapters 2-5 written as independent manuscripts for publication purposes. The literature cited is presented at the end of each chapter. Chapter 1 provides a general introduction to sea turtles and more specifically to *C.mydas* in the Pacific region. Knowledge gaps are identified and the key aims of this study are presented.

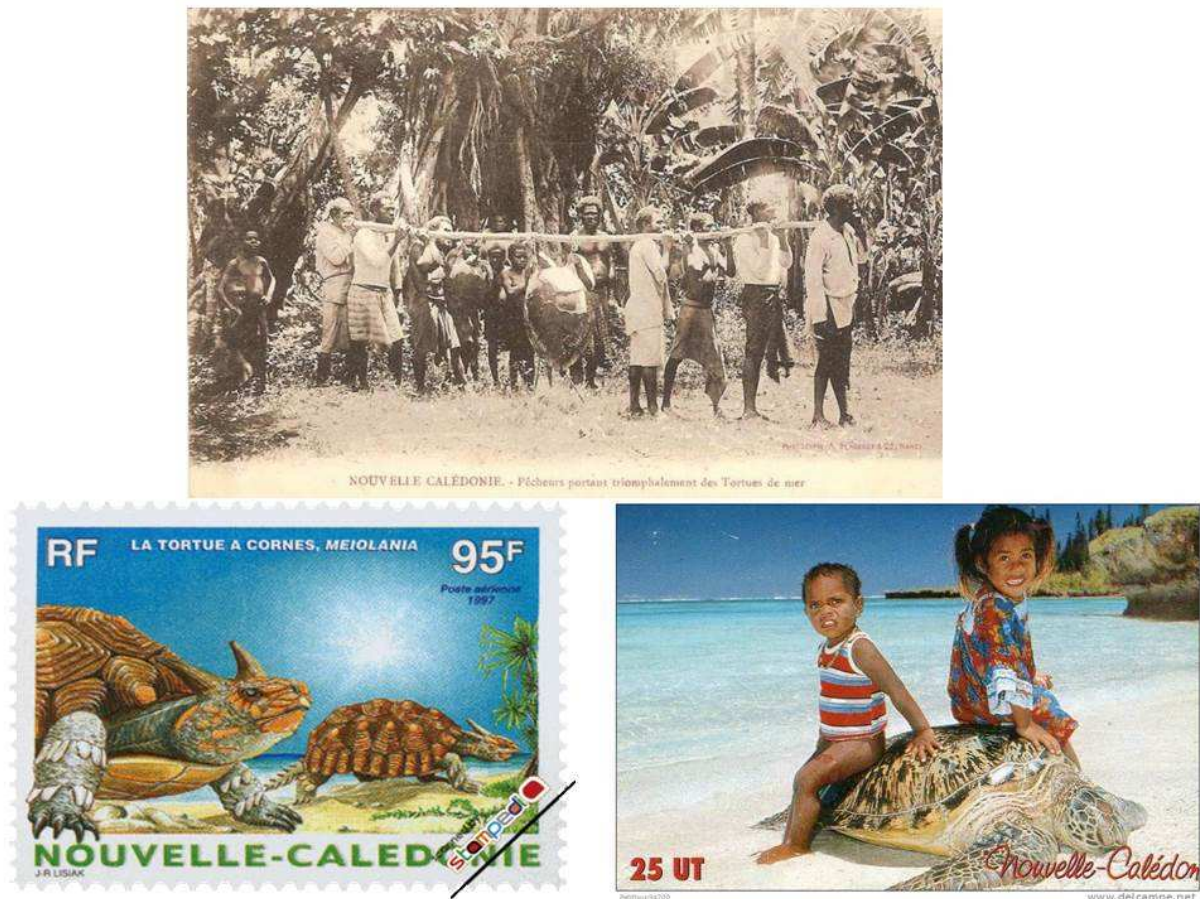
### Endangered species under continual indigenous harvest

Sea turtles are classified into two families. Dermochelidae hosts only one species, the leatherback turtle (*Dermochelys coriacea* Blainville 1816), whereas Cheloniidae include six species which include the loggerhead turtle (*Caretta caretta* Linnaeus 1758), the olive ridley (*Lepidochelys olivacea* Eschscholtz, 1829), the Kemp's ridley (*Lepidochelys kempii* Garman 1880), the hawksbill turtle (*Eretmochelys imbricata* Linnaeus 1766), the flatback turtle (*Natator depressus* Garman 1880) and the green turtle (*Chelonia mydas* Linnaeus 1758). Ongoing debate about the existence of an eighth taxon commonly named the black turtle or Pacific green turtle (*Chelonia agassizzi* Bocourt 1868) continues in the global literature (see Karl and Bowen 1999; Naro-Maciel et al. 2008; Pritchard 1999).

Sea turtles are one of the largest vertebrates occurring in shallow water ecosystems and are a key emblematic species in the South Pacific, however populations have been severely depleted due to decades of anthropogenic pressure. They have been used as an important source of protein and fat for many communities and only human flesh had a higher value (Emory and Gordeon 1975). Sea turtles have been part of indigenous culture for millenaries in the islands of the Pacific (Bass et al. 2011). They are often referred to as sacred, representing a source of life (symbol of fertility) and embodying ancestors (Tippett 1968; Williamson 2013). Turtles have been captured at both nesting and foraging

grounds in order to be used in different ceremonies (deaths, weddings, chiefly inaugurations, yam celebration and peace-making rites) (Figure 1a) where all body parts of the individuals were eaten except the carapace which was often kept for sculptures, ornaments or hooks (Balazs 1983; Emory and Gordeon 1975; Ferdon 1981; Firth 1967; Leblic 1989; Thompson 1940). Men from particular tribes (tribes of the sea and specific families) were the only ones allowed to hunt and butcher turtles and were usually the only ones authorized to eat the meat, although exceptions can be found in some islands where the whole tribe could eat the turtle meat (Firth 1967; Leblic 1989). This pressure on sea turtles is difficult to quantify but other species were brought to extinction during the first human colonization of the islands which suggests sea turtles were also severely depleted. For example, the land crocodile (*Mekosuchus inexpectatus*), a giant running land bird (*Sylviornis neocaledoniae*), a large monitor lizard (*Varanus cf. indicus*) and a horned tortoise (*Meiolania* sp.) (Figure 1b) (De Forges et al. 1998).

Multiple historical records from European explorers describe abundant *Chelonia mydas* populations when they first arrived in the Pacific Ocean. William Dampier recorded large colonies of sea turtles in Indonesia and Western Australia (Masefield 1906) as did James Cook when he sailed to Australia and New Caledonia (Reed 1969). A narrative of the loss of the Chinese junk “Ningpo” on d’Entrecasteaux reefs in New Caledonia report that “They were so numerous in September that the Master turned twenty-seven one morning without wetting his feet” (Chimmo 1856). This was not only the case in the Pacific, Christopher Columbus named an island “Las Tortugas” in the Caribbean because of the large number of turtles (Morrison 1942). In the mid-1980’s, an increase in turtle meat demand worldwide (commercial harvest) and the resulting over harvesting have brought populations of *C. mydas* to extinction in Bermuda, Cayman and Reunion Island (Groombridge and Luxmoore 1989; Parsons 1962). A number of nesting populations are now recovering after decades of harvest (Weber et al. 2014) and some nesting populations have been reported to recover after a being near extinction after a strict total protection was applied like for example at French Frigate Shoals (Hawaii) (Balazs and Chaloupka 2004).



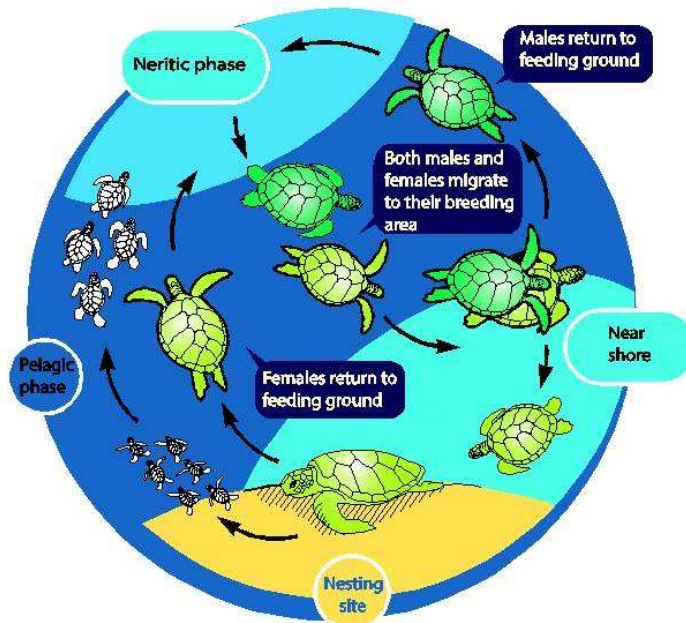
**Figure 1a.** Postcard from New Caledonia- Fisherman proudly carrying their sea turtles (No date). b. Stamp edited in 1997 in New Caledonia representing the extinct horned tortoise (*Meiolania* sp.). c. Telephone card from New Caledonia edited in the 1990's

Changes in the fishing methods are also a contributing factor in the declining numbers of turtles. The switch from spearing or harpooning with primitive gear and wood canoes to motor boats and spear-guns has significantly affected the numbers of turtles successfully caught. Legal harvest remains in some countries around the world and is specifically important in islands nations from the Pacific (Fleming 2001; Fretey 2001; Humber et al. 2014; Humphrey and Salm 1996). The ritual of hunting sea turtles holds a very important place in the hierarchy of a tribe of the sea but also represents a step towards manhood for young traditional hunters. Sea turtle meat is also, for some tribes, the only red meat available. Thus for all these reasons, implementing a strict ban on hunting sea turtles in many islands of the South Pacific is not possible, hence multiple countries are faced with an important challenge: managing an endangered species while traditional fishing still occurs (Figure 1c).

## **Life cycle and ecology *C. mydas***

*C. mydas* is found in tropical and sub-tropical waters around the world (Bowen et al. 1992; Hirth, 1997) and like other sea turtles has a very complex life cycle (Figure 2). They have a phylopatric reproductive behavior where the adults return to the same region where they were born to reproduce (Bowen et al. 1992; Mortimer and Portier 1989; Norman et al. 1994) and this pattern is repeated from generation to generation (Bustard 1972; FitzSimmons et al. 1997). Mating usually occurs in waters adjacent to the nesting beach but also as far hundreds of km's away (Limpus 2009). This means that both males and females migrate from their foraging grounds to reproduce near their hatching place. It has been found that multiple paternities are common and the sperm from different partners is stored by the female during the mating season (Ireland et al. 2003). This is a reproductive strategy hypothesised to (1) avoid reduced hatching success and/or (2) reduce the risk of laying a clutch of eggs that could be genetically unfit (Pearse and Avise 2001). Without sperm storage, females would have to mate between each oviposition (Bakst 1978; Hattan and Gist 1975). Females belonging to the Great Barrier Reef genetic groups (north GBR and south GBR) nest approximately five times per nesting season, and about two weeks apart (Limpus 2009; Limpus et al. 1984; Miller 1985). The average number of eggs per clutch in that region of the world is between 112 and 115.2 with a nest depth averaging 69.2 cm (Limpus et al. 1984; Limpus and Walter 1980). Incubation periods vary from five to eight weeks depending on sand temperature and after they hatch, hatchlings run to the sea and swim towards the open ocean (reviewed in Spotila 2004). The hatchlings then disperse to pelagic waters for approximately 5-6 years (Limpus and Chaloupka 1997; Limpus et al. 1994).





**Figure 2.** Lifecycle of *C. mydas* (@OEIL)

All sea turtles have temperature-dependent sex determination (TSD) meaning that the temperature during the middle third of incubation affects the sex ratio of the offspring (Mrosovsky and Yntema 1980; Standora and Spotila 1985; Yntema and Mrosovsky 1982). Like in other species of reptiles, warm temperatures produce a bias in the number of females (Booth 2006; Shine 1999). The pivotal temperature (the temperature at which a 1:1 sex ratio is produced) varies for each rookery and between species. The pivotal temperature for *C. mydas* at Heron Island is 27.6°C (Limpus 2009).

By using stable isotope analysis (SIA), it was found that the individuals spend 5-10 years in oceanic habitats as omnivores (Reich et al. 2007) where they feed on crustaceans, jellyfish and ctenophores (Arthur et al. 2008; Bolten 2003), this period is called the “lost years” because the hatchlings location and ecology is poorly understood (Reich et al. 2007). After the “lost years”, juveniles come back to coastal waters at approximately 40 to 50 cm (Arthur et al. 2008; Limpus 2009; Limpus and Limpus 2000) where they shift their diet towards more plant based material (Arthur et al. 2008). The diet of sub-adult and adult *C. mydas* consists mostly of turtle grass (*Thalassia testudinum*) in the Caribbean

but is more diverse in Australia and includes sea grass (*Halodule*, *Halophila* and *Zostera* spp.), algae (*Gracilaria*, *Hypnea* and *Polysiphonia* spp.), mangroves and the fruit and leaves of *Avicennia marina* (Arthur et al. 2009; Limpus and Limpus 2000). SIA of animals in foraging grounds as new recruits can indicate a previous feeding location (Reich et al. 2007). Indeed, “spatially discrete food webs” are isotopically distinct and called ‘isoscapes’ (Seminoff et al. 2012). The diet of green turtles is based on availability of different resources within each isoscape (Garnett et al. 1985) but selection between food sources available in each isoscape has been proven (Bjorndal 1985; Brand-Gardner et al. 1999; Fuentes et al. 2006). In Brazil, Tokelau (South Central Pacific), Hawaii and the Torres Strait (Australia), algae was dominant in the stomach contents (Balazs 1980; Balazs 1983; Bjorndal, 1985; Garnett and Murray 1981), however diet has not been investigated in many regions throughout the south Pacific including New Caledonia. Most turtles sampled worldwide do not feed only on a single dietary item as algae species, for example, they are usually found alongside other dietary sources such as sea grass (Arthur et al. 2008; Bjorndal 1997; Brand-Gardner et al. 1999). In some cases it is possible that sub-adult and adults *C. mydas* also consume greater quantities of animal material, such as ascidians, sponges, ctenophores, jellyfish and crustaceans than expected for herbivorous animals but these prey are digested a lot quicker than plant based material (Arthur et al. 2008; Brand-Gardner et al. 1999). This higher digestability could explain why the diet of sub-adult and adult *C. mydas* is labeled as herbivorous in many studies (Limpus 1997) rather than omnivorous (Heithaus et al. 2002). When they reach sexual maturity, aged between 25 and 50 years old and depending on food availability (Chaloupka et al. 2004), they start to migrate back to the region where they were born in order to reproduce (Carr 1967). These migrations last for weeks at a time, depending on the distance between the nesting and foraging sites but the individuals come back to their foraging site (Broderick et al. 2007; Limpus et al. 1992; Mortimer and Carr 1987). The life cycle also has a geographical complexity: studies have shown that *C. mydas* can migrate 100-1000’s of km’s to reach their nesting beach and thus can cross international waters (Cheng 2000; Luschi et al. 1996; Read et al. 2014). Identifying the movements between foraging and nesting grounds is therefore essential for local population

management. This has implications for stock management, and the need to identify the different populations and to have an extensive knowledge of the different life-history stages (pelagic, oceanic and neritic) for each population is critical (Arthur et al. 2008; Bolten 2003; Bowen et al. 2005). This data is lacking for many regions in the South Pacific.

*C. mydas* foraging grounds are labeled as “mixed stock” because individuals from different genetic backgrounds are found at one location (Lahanas et al. 1998). A recent hypothesis was developed relating the effect of passive drift of the dispersal of juveniles towards their future foraging ground (Hays et al. 2010). In addition, it was shown that individuals do not use one specific migration path to get from their feeding ground to their nesting beach and that adults found at one feeding ground can come from different nesting beaches ranging from less than 1000 km away to as far as 2600 km away (Limpus 2009). Females do not nest every year and as such the interval between migrations is usually between three and five years depending on the amount of food available at their foraging areas (Limpus 2009). Very high fidelity to nesting sites has been shown by tag returns (Bustard 1972; Limpus 1992) but also using genetics (Bowen et al. 1992; Norman et al. 1994).

The analysis of maternally inherited DNA (mtDNA) has been used in many studies (Bjorndal et al. 2005; Lahanas et al. 1998) in order to classify the differences between rookeries or nesting aggregations (Bjorndal et al. 2005). This analysis is useful to identify individual Management Units (MU) (Dethmers et al. 2006). This method was extensively applied to study the ecology of sea turtles (Averse 2007; Bowen et al. 1992) and some results can be explained by natal homing (Carr and Ogren 1960) as turtles come back to their nesting beach and thus this “limits the gene flow among rookeries” (Bjorndal et al. 2005). However further work is required in order to fully comprehend the genetics flow of that species. A study that looked primarily at mtDNA genotypes from 15 major rookeries of *C. mydas* from around the world found very little overlap and exchange between rookeries, thus identifying subpopulations within each rookery or group of rookeries (Bowen et al. 1992). This study also highlighted a phylogenetic split between the individuals found in the Atlantic-Mediterranean and the Indian-Pacific oceans (Bowen et al. 1992) which is still being studied to this day (Bourjea et al. 2007).

Currently, thirty genetically identifiable genetic stocks are found in the Australasian region and of them, seven can be found in Australia (Dethmers et al. 2006; FitzSimmons and Limpus 2015) however those in New Caledonia are virtually unknown.

The ecology of *C. mydas* in the Pacific region is particularly complex to study with an average of 25 000 islands spread over 10 000 km<sup>2</sup> and the fact that “sea turtles are a shared resource” among the Pacific islands due to their migrations between foraging and nesting sites (Balazs 1982; Pritchard 1979). Very recent records are also available relating migrations from Vanuatu to New Caledonia and from New Caledonia to Australia and Papua New Guinea, although this region is severely lacking in data (Read et al. 2014 see chapter 2). 96% of central Pacific individuals migrate westward and seem to forage particularly in Fiji (Craig et al. 2004). *C. mydas* released in French Polynesia made their way to Fiji with some individuals also making their way to New Caledonia (Anon 1980; Balazs et al. 1994).

## **Current threats and conservation**

The highest rate of natural predation for hatchlings is on the beach and in the shallows, from crabs, fishes, sharks and sea birds (Gyuris, 1994; Limpus et al. 1984; Witherington and Salmon, 1992; Witzell 1981). However, *C. mydas* have been listed as Endangered since 1986 on the International Union for Conservation of Nature’s red list due to a reported loss of 48% to 67% in the number of mature nesting females annually over three generations (IUCN 2010) even if multiple rookeries show signs of recovery (Broderick et al. 2006; Weber et al. 2014). This important drop in numbers at a regional level is likely due to a number of anthropogenic impacts on both the nesting and foraging grounds of this species. Unintentional bycatch and habitat degradation (of both nesting and feeding grounds) have been identified as major threats (Horikoshi et al. 1994) but another important threat is the intentional harvest of eggs, nesting females during nesting (Chaloupka 2001; Seminoff 2002) and the harvest of individuals in foraging grounds. In 1986, the government of New Caledonia implemented a closed hunting season for sea turtles from the 1<sup>st</sup> of November to the 31<sup>st</sup> of March and banned egg collecting and merchandising of any turtle material. Since 2006, sea turtles are protected all year

round in the North and South Provinces of New Caledonia but permits can be obtained for traditional ceremonies. Between 2006 and 2010, 151 permits were delivered and 228 turtles were officially killed in the North Province. In the South Province, from 2006 and 2012, 70 permits were emitted for 354 turtles. No size limitations are specified on the permits thus large mature individuals are targeted. Oral historical records identified small nesting coastal beaches all over the country that are no longer sustaining any turtle activity as the individuals plus their eggs were both collected.

Six sites were added to the World Heritage List in New Caledonia under the name *The Lagoons of New Caledonia: Reef Diversity and Associated Ecosystems* on 7 July 2008. The Lagoons were listed under three UNESCO categories: 1. Superlative natural phenomena or natural beauty. 2. Ongoing biological and ecological processes. 3. Biological diversity and 4. Threatened species (Menu and Hebert 2006). Since then New Caledonia has been identified as having multiple key biodiversity areas (KBA) for both *C. mydas* and *Caretta caretta* and these KBAs represent global priorities for conservation (Bass et al. 2011). Research priorities for sea turtles in the 21<sup>st</sup> century have been addressed recently with a fifth category identified regarding the species global conservation strategy, i.e. under what conditions (ecological, environmental, social and political) can consumptive use of sea turtles be sustained? (Hamann et al. 2010). This critical question requires a thorough investigation and this study tackles this question in southern New Caledonia.

In the Pacific Ocean, the number of large individuals of *C. mydas* has declined significantly in many countries due to overharvesting by the 1990's (Limpus 1997) and while further data are unavailable there has been no cessation in anthropogenic pressure. Many Pacific islands are faced with a near impossible task to manage an endangered species which is still being actively hunted as part of traditional indigenous culture. It was calculated that > 500 individuals *C. mydas* are legally hunted every year for traditional purposes in New Caledonia (Humber et al. 2014) and the illegal take is very difficult to quantify. A thorough investigation of the movement patterns, ecology, the current population status for *C. mydas* in New Caledonia is severely lacking. This thesis attempts to tackle these gaps with a particular focus on the region of southern New Caledonia. The Tribal Council for the Environ-

ment (CCCE) for southern New Caledonia partly funded this thesis thus showing a willingness to better understand and manage this resource. The general aim of this thesis is to provide essential background information in order to put in place a raft of long-term actions for the conservation of this emblematic species in the region and will hopefully help in the integration of the local populations for the preservation of this resource.

## **Objectives of the thesis**

Marine turtles spend most of their lives in coastal habitats but this life stage is one of the least studied. This study provides new insight into the ecology of this species in the South Pacific and fundamental knowledge that can be used for future studies and for the local and large scale management of *C. mydas* in New Caledonia.

Chapter 1 offers the background necessary to understand the content discussed in all the following chapters, and identifies the threats that affect the *C. mydas* population worldwide and in New Caledonia.

Chapter 2 provides a review of the data collected in the last 50 years identifying multiple migrations of *C. mydas* to and from New Caledonia thus crossing the Coral Sea. This chapter is the basis for most hypotheses that are tested in the following chapters as a literature review could be done on the population of *C. mydas* in New Caledonia itself. The objective of this chapter is to better understand the migration movements of *C. mydas* within the South Pacific region.

In the 3<sup>rd</sup> chapter, a Mixed Stock Analysis (MSA) was done on individuals caught in a large foraging ground which has been added to the World Heritage List in 2008 (GLS) but is used as a hunting ground by the local populations. This is the first in-water study done on sea turtles in New Caledonia. This chapter reinforces and contrasts the results found in the second chapter showing the beneficial use of a multi-faceted approach in order to study a species that has such a complex and wide-spread life cycle. The objective of this chapter is to gather additional information on the dispersal of hatchlings within the Coral Sea.

This 4<sup>th</sup> chapter used traditional ceremonies in which turtles are killed in order to gather information that would otherwise be unobtainable. A Stable Isotopes Analysis (SIA) was done on individuals caught foraging in the GLS and compared the results with direct stomach content examination of individuals caught in that same area. The aim of this chapter is to identify the prey items of *C. mydas* in the GLS and compare the results to other tropical foraging grounds. The documentation and protection of the feeding habitat of a species is a key conservation concern.

The 5<sup>th</sup> chapter uses all the individuals caught during the progress of this thesis to better understand the population dynamics of the area. Multiple equipment (different brands, different models) were applied to *C. mydas* foraging in order to answer multiple questions relating to their behavior. It was also done this way so the different equipment available in New Caledonia could be compared and to make an informed decision on what works best for future studies in remote areas. The objective of this chapter is to provide a general understanding of the use of the feeding habitat by *C. mydas* in the GLS.

Chapter 6 includes all the data collected that could not be added to the chapters for publications: mark-recapture data, growth rates, gonad examinations, population estimates and the first record of an individual found with a fibropapilloma tumor in the study area.

Chapter 7 summarises the general findings of the preceding chapters reviewed as a whole, identified gaps, future research prospects and what should be the next course of actions in order protect *C. mydas* in the region.

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## CHAPTER 2 BIBLIOGRAPHIC DETAILS

**Title:** Migrations of green turtles (*Chelonia mydas*) between nesting and foraging grounds across the Coral Sea

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### Statement of contribution to co-authored published paper

This chapter includes a co-authored paper. The bibliographic details (if published or accepted for publication)/status (if prepared or submitted for publication) of the co-authored paper, including all authors, are:


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My contribution to the paper involved the preliminary analysis and categorisation of the data into a usable format and providing direction on the scope and structure of the analysis. The data was provided by one of the co-author Dr Col Limpus.


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Georges Petro is deceased and thus unable to sign this statement.

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## CHAPTER 2: MIGRATIONS OF GREEN TURTLES (*CHELONIA MYDAS*) BETWEEN NESTING AND FORAGING GROUNDS ACROSS THE CORAL SEA

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### Abstract

Marine megafauna tend to migrate vast distances, often crossing national borders and pose a significant challenge to managers. This challenge is particularly acute in the Pacific, which contains numerous small island nations and thousands of kilometers of continental margins. The green sea turtle, *Chelonia mydas*, is one such megafauna that is endangered in Pacific waters due to the overexploitation of eggs and adults for human consumption. Data from long-term tagging programs in Queensland (Australia) and New Caledonia were analysed to investigate the migrations by *C. mydas* across the Coral Sea between their nesting site and their feeding grounds. A review of data collected over the last 50 years by different projects identified multiple migrations of *C. mydas* to and from New Caledonia (n=97) and indicate that turtles foraging in New Caledonia nest in the Great Barrier Reef (Australia) and vice versa. Several explanations exist for turtles exhibiting this energetically costly movement pattern from breeding to distant foraging grounds (1200-2680 km away) despite viable foraging habitat being available in the local vicinity. These include hatchling drift, oceanic movements and food abundance predictability. Most of the international tag recoveries in New Caledonia belonged to females from the south Great Barrier Reef genetic stock. Some females (n=2) even showed fidelity to foraging sites located 1200 km away from the nesting site located in New Caledonia. This study also reveals previously unknown migrations pathways of turtles within the Coral Sea.

**Keywords:** titanium tag, population, movement

## Introduction

Human disturbance is triggering unprecedented and mounting biodiversity losses on a global scale, fuelling concerns over species extinctions and the degradation of important habitats (Mora et al. 2011). Many charismatic and top-level marine fauna have registered dramatic declines in the last decade (Troëng and Rankin 2005; Schipper et al. 2008). Anthropogenic stressors on marine ecosystems are likely to increase as almost all biodiversity hotspots around the world are expected to at least double their human populations within the next 50 to 100 years (Cincotta et al. 2000). Therefore, the identification of spatial and temporal patterns of abundance, reproduction, demography and capacity for resilience to impacts (including exploitation) is critical for managing the conservation of marine megafauna species including turtles.

The green turtle, *Chelonia mydas*, is a circumglobal species classified as endangered on the International Union for Conservation of Nature (IUCN) Red List due primarily to declines from overexploitation of eggs and adult females at nesting beaches, and juveniles and adults in foraging areas (IUCN 2010). Additional pressures on *C. mydas* populations come from incidental mortality in marine fisheries and degradation of marine and nesting habitats (IUCN 2010). Impacts on stocks are exacerbated by the species slow growth, late onset of sexual maturity and low survivorship of hatchlings (Hirth 1997). Sea turtles are highly migratory and upon reaching sexual maturity utilise broadly separated dispersed neritic foraging grounds and limited localised nesting areas that drive regional distribution patterns (Hirth 1997; Limpus et al. 1992). Migrations, often over hundreds of kilometers, are undertaken every few years by both males and females by most sea turtles (Benson et al. 2007; Carr 1986; Mortimer and Portier 1986). Mature females commonly return from foraging grounds to the region of their natal beach (Meylan et al. 1990). Species which cover vast distances across international waters, pose a significant challenge for managers. Consequently, identifying migratory paths between nesting and foraging grounds is important for effective transboundary conservation strategies at both the local sub-population level and the regional population level (Dethmers et al. 2011; Pendoley et al. 2014; Shillinger et al. 2008).

In the last decade there has been an exponential increase in innovative tracking technologies enabling identification of the migratory pathways of marine turtles based on a small number of individuals (Godley et al. 2008). While these technologies are very useful they lack the ability to identify long-term (over decades) patterns of movement across a large number of individuals. An alternative method is the mark and recapture of individuals using flipper tags (Limpus 1992; Meylan 1982). While this method is often intensive it enables the mark and identification of potentially hundreds to thousands of individuals and the identification of large-scale movements if individuals are recaptured at separated nesting beaches or foraging grounds. Furthermore, in conjunction with effort estimates, mark-recapture may enable researchers to derive coarse population estimates (Heppel et al. 1996; Limpus et al. 2003).

Previous studies have shown long-range migrations by *C. mydas* worldwide (Balazs et al. 1994; Cheng 2000; Luschi et al. 1996), however documented examples from the South Pacific are scant but have to date demonstrated the record movement for this species (3880 km). This was attributed to an individual tagged as an immature female at Clack Reef (Australia) and found nesting 17 years later on Wotje Atoll (Marshall Islands) (Limpus et al. 2009). While there is sufficient evidence to suggest substantial movements in the southwest Pacific, hypothesised to be driven by site-fidelity (Limpus et al. 1992), most of these examples are based on satellite telemetry or mark-recapture of only a few individuals (Anon 1980; Balazs et al. 1994; Balazs et al. 1995; Limpus et al. 2009; Craig 1994). A small number of females have been found to travel from a rookery at Scilly atoll in French Polynesia to multiple distant feeding grounds (<2000 km) in Fiji, New Caledonia, Tonga, Vanuatu and Wallis (Balazs et al. 1995) and from the American Samoa to Fiji (Balazs et al. 1994; Craig 1994). However, between Australia and New Caledonia, numerous tagging campaigns of *C. mydas* have been undertaken with over 80,000 individuals tagged in Australia since 1964, thus providing the potential to identify extensive migratory patterns in the Coral Sea. In this study we used multiple long-term databases on the tagging and recapture of *C. mydas* on the east coast of Australia and in New Caledonia, to (1) determine spatial migratory patterns of tagged *C. mydas* across the Coral Sea to and

from New Caledonia, (2) identify temporal patterns of migration, and (3) quantify the patterns of connectivity between foraging and beach nesting areas using both mark-recapture and complimentary examples from satellite telemetry.

## **Materials and methods**

### **Ethics statement**

This research was executed in accordance with GBRMPA/State Marine Park permit G00/240, and G09/25033.1 and New Caledonian permit 2011-2751/GNC and a Griffith University animal care and ethics approval ENG/01/12/AEC.

### **Study sites**

Our study focused on the recapture of tagged *C. mydas* at foraging grounds and nesting beaches between 1972 and 2011 across the spatial extent of the Coral Sea (Figure 1). Australia and New Caledonia border the east and west boundaries of the Coral Sea respectively, while to the north the sea is bordered by the south coast of Eastern New Guinea, the Solomon Islands and Vanuatu.

The study area included four key locations along the east coast of Queensland (QLD), Australia and multiple locations in New Caledonia (NC). These being 1) reef foraging areas within Torres Strait, and the Bramble Cay nesting beach; 2) nesting beaches in the northern GBR (nGBR) including Raine Island, Moulter Cay No.7 and No. 8 Sandbanks and reef and seagrass foraging areas including Clack Island reef and Green Island reef, 3) coral cays of the southern GBR (sGBR) including Heron Island, Northwest Island, Wreck Island, Lady Musgrave Island and Hoskyn Island in the Capricorn-Bunker Groups and Swain Reef's Cays and associated coral reef foraging areas and coastal pastures in Repulse Bay and Shoalwater Bay, 4) the seagrass pastures of Moreton Bay in southeast QLD; and 5) two nesting locations in the islands north of New Caledonia: d'Entrecasteaux atolls and Chesterfields reefs plus multiple feeding grounds around the main island of New Caledonia.

## **Capture and tagging efforts**

We used recapture data from several long-term tagging programs in QLD and New Caledonia (see acknowledgments for tagging programs). In Australia, these tagging campaigns of *C. mydas* since 1964 have resulted in over 80 000 tagged individuals and over 4000 tagged individuals in New Caledonia. Due to the differences in longevity of different *C. mydas* tagging programs, capture and tagging efforts occurred disproportionally among the study locations as tagging efforts were first initiated in Australia, twenty-seven years prior to tagging efforts in New Caledonia (Limpus 2009). Furthermore, to provide a more comprehensive understanding of the dynamics and ecology of *C. mydas*, juveniles and males, which are very rarely found ashore, were also tagged as part of this study starting in 1974 (Limpus 2009; Limpus and Reed 1985). Few migrating *C. mydas* have been tracked via satellite telemetry for their post-breeding migrations within the Coral Sea region. A female was equipped with a satellite tag after nesting at Bamboo Bay, in Vanuatu in 2011 and followed to its feeding area to provide additional information on potential migrations between feeding and nesting grounds in the Coral Sea.

## **Tagging**

*C. mydas* were captured using different methods depending on their activity in foraging grounds or on nesting beaches. In-water turtles were captured by rodeo method using a small boat or by hand in the shallows (Limpus and Read 1985) and by hand for turtles nesting on land. Prior to tagging, standard measurements of the midline curved carapace length (CCL), and gender, when possible, were recorded. In New Caledonia, all individuals recorded in the database were nesting females tagged on the beach.

Pre-1980, external monel tags with a unique identification number were applied to the anterior fin of captured turtles but due to corrosion tag loss was important. The issue was overcome by using a self-piercing, self-locking titanium identification tag in the front flipper immediately adjacent to the first large scale on the proximal rear edge, close to the axilla (Limpus 1992). Probability of loss after nine

years of the tag being applied was reduced from 1 to 0.667 ( $\pm 0.533$ ) by switching from Monel No. 49 tags to titanium tags No.2 (Limpus 1992). The position at which the tags was applied was also tested and position 3 (closer to the axilla) decreases the probability of loss compared to position 1 (at the tip of the front flipper) and position 2 (in the middle of the front flipper) (Limpus 1992). Double tagging was implemented as some tag loss occurred during agonistic interactions at courting but also due to the environment (probably due to digging, encountered rocks and branches, plus crawling) (Schofield et al 2007a; Schofield et al. 2007b; Limpus 1992). Satellite telemetry was also used on a single individual in Vanuatu to test the potential connectivity with other countries within the Coral Sea and to explore future titanium tagging sites.

## **Movements**

The movements of tagged *C. mydas* between individual locations were recorded through the reported recaptures of individuals either within ongoing tagging efforts in Queensland, New Caledonia and other regions in the south-west Pacific or via fishermen and local people. Identification tags enabled the verification of individual movements both spatially and temporally.

## **Statistical analyses**

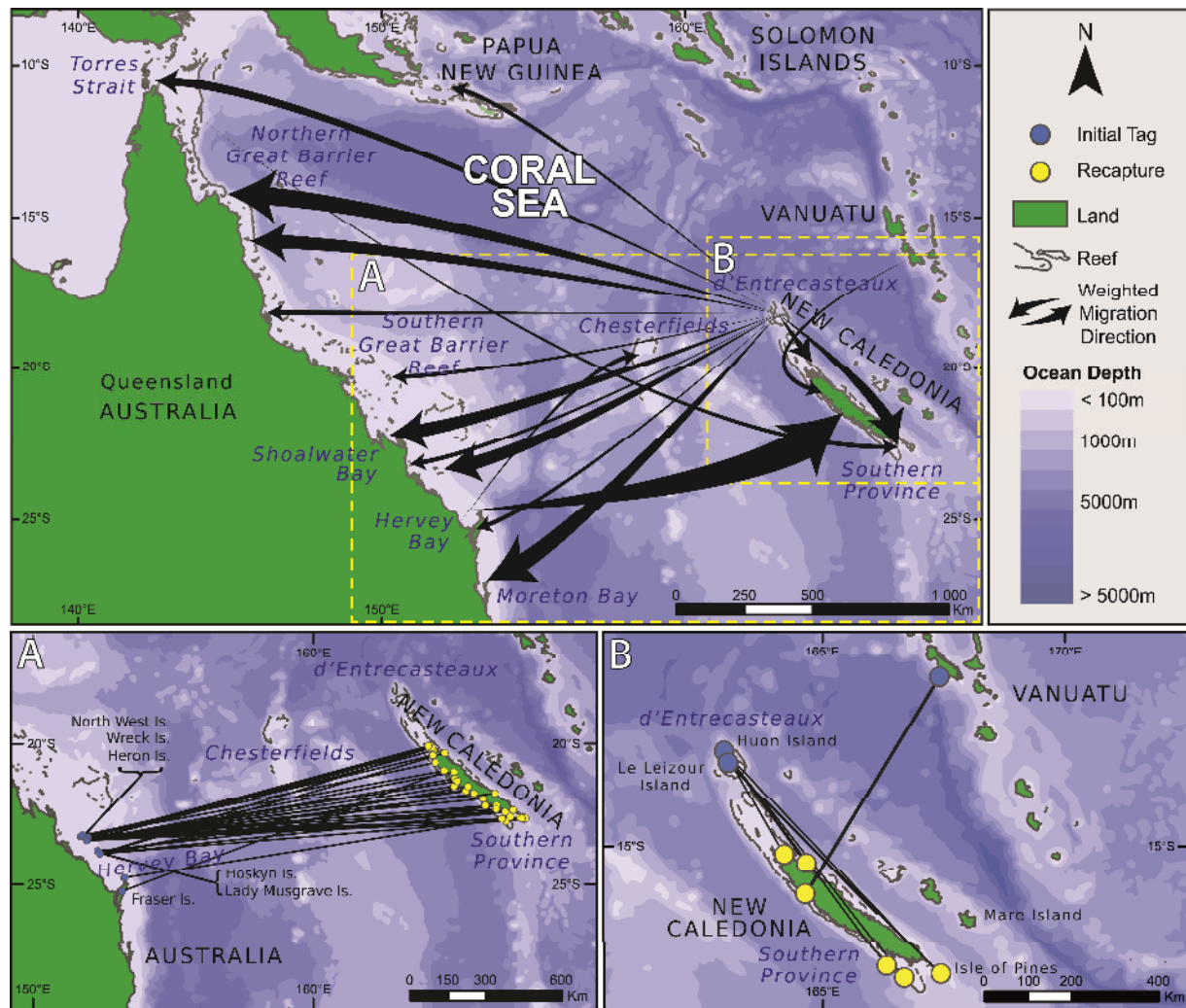
All analyses were completed using Statgraphics. Significance was determined as 0.05. The minimum linear distance between tag and recapture locations was determined using Google Earth and used to identify the extent of movement by individual turtles within the Coral Sea population. Homoscedasticity of tag recoveries (having equal variance) was verified using Bartlett's test and the mean difference in curved carapace length (CCL) between Australia and New Caledonia was compared using a t-test. The nesting beach release point and the feeding ground of the satellite tagged turtle in Vanuatu were used as single capture and recapture locations.



## Results

The major breeding aggregations of *C. mydas* in the south western Pacific region are known to represent independent genetic stocks or management units (Bowen et al. 1992; Dethmers et al. 2011). In Australia, seven different breeding stocks have been identified to this day: southern Great Barrier Reef (sGBR), Coral Sea, northern GBR (nGBR), Gulf of Carpentaria, Ashmore Reefs, Scott Reef and the Northwest Shelf (Bowen et al. 1992; Dethmers et al. 2011; Dutton et al. 2002, Moritz et al. 2002; Norman et al. 1994). Based on tag recovery data analysed in the present study, *C. mydas* foraging within New Caledonian waters originate from at least four independent genetic stocks breeding in at least four different countries: New Caledonian stock (n = 49); Australian stocks (sGBR (n = 45), nGBR (n = 2) stocks) and probably an independent stock in Vanuatu (n = 1). No individuals from the Australian Coral Sea stock have been identified foraging in New Caledonia.

*C. mydas* tagged while nesting in New Caledonia have been recaptured as foraging turtles in three countries: New Caledonia, Australia and Papua New Guinea. A total of 4700 individuals were tagged at d'Entrecasteaux atolls (New Caledonia), resulted in only a 1% post-nesting migration tag recovery. Females (n = 46) nesting at d'Entrecasteaux atolls were found in feeding grounds all along the Queensland coast (n = 37) up to Papua New Guinea (n = 1) but also in New Caledonian waters (n = 8) (Figure 1A). One female tagged in Australia was reported nesting the same year on an island of the Chesterfield atolls (Figure 1B).



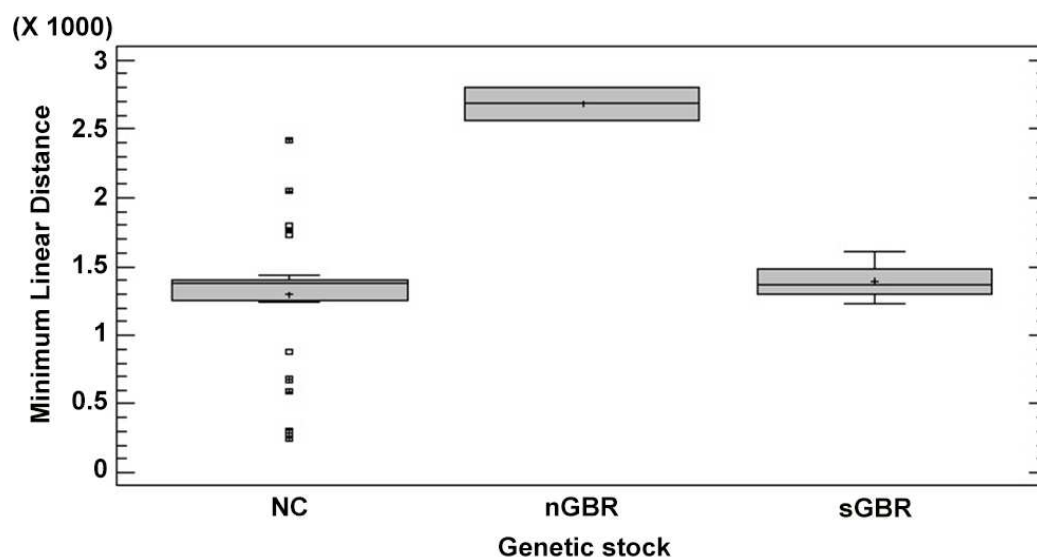
**Figure 1.** Trajectory maps obtained by the tag recoveries ( $n = 93$ ) and satellite tracking of *C. mydas* in the Coral Sea ( $n=1$ )

Less than 0.1% of the *C. mydas* tagged in Australia were recovered in New Caledonian foraging area. Females ( $n = 45$ ) tagged at nesting beaches in the Great Barrier Reef were found in feeding grounds in New Caledonia illustrating reciprocal movements across the Coral Sea in both a westerly and easterly direction (Figure 1). One female (“Bamboo Lady”) was equipped with a satellite tag in Vanuatu while nesting and came to New Caledonia to forage (Figure 1B). Distances traveled between foraging and nesting grounds were significantly different (Kruskal-Wallis test,  $p = 0.03$ ), the longest being between NC and nGBR (2680 km) (Figure 2). Here we made an assumption that all individuals were caught in their feeding ground as they were either in a known feeding ground or hunted in coastal waters by local tribes. The mean time for tag recoveries was not significantly different between females belonging to NC and sGBR genetic stocks (F test,  $p > 0.15$ ) (Figure 3). The minimum time for a tag recovery in this study was 19 days and the maximum was 10585 days with a

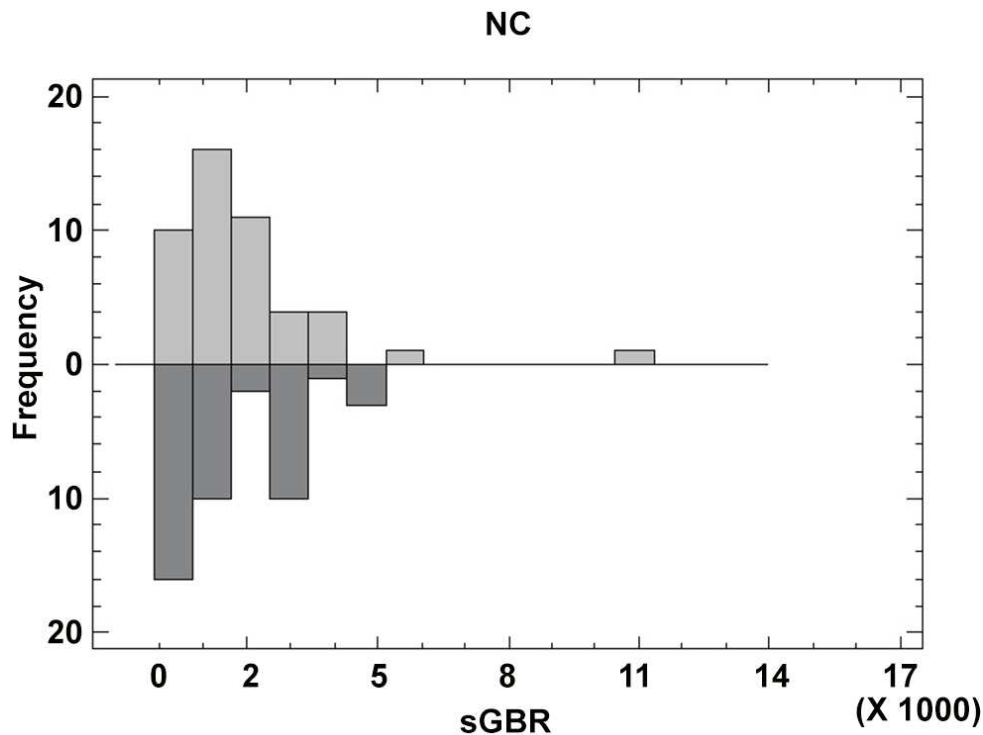
mean of  $1756 \pm 162$  days. Means were not able to be determined for nGBR and Vanuatu due to low number of recaptures (respectively  $n=2$  and  $n=1$ ). However, satellite telemetry revealed it took 12 days for the turtle to travel from Vanuatu to its feeding area and the tags from the only two females from nGBR were recovered in New Caledonia 584 and 1265 days after initial tagging. Two females tagged at their feeding grounds in Australia, reported nesting in New Caledonia were recaptured at their initial capture site (Table 1). Both females from the d'Entrecasteaux rookery, however, were recaptured at separate locations in Australia. One forages in Moreton Bay while the second occurred in Shoalwater Bay (Figure 4).

**Table 1.** Case histories illustrating fidelity of *C. mydas* to feeding areas in Coral Sea

Tag No.	Behaviour	History
K 25986	Feeding	Moreton Bay in 1999, sub-pubescent CCL:102
	Nesting	Huon Island (NC) in 2001, first breeding season
	Feeding	Moreton Bay in 2011 CCL: 106.1
K43255	Feeding	SWB sub-pubescent 2001 CCL: 88.1
	Nesting	Fabre Island (NC) in 2010
	Feeding	SWB Jun2012 non breeding adult CCL: 93.1

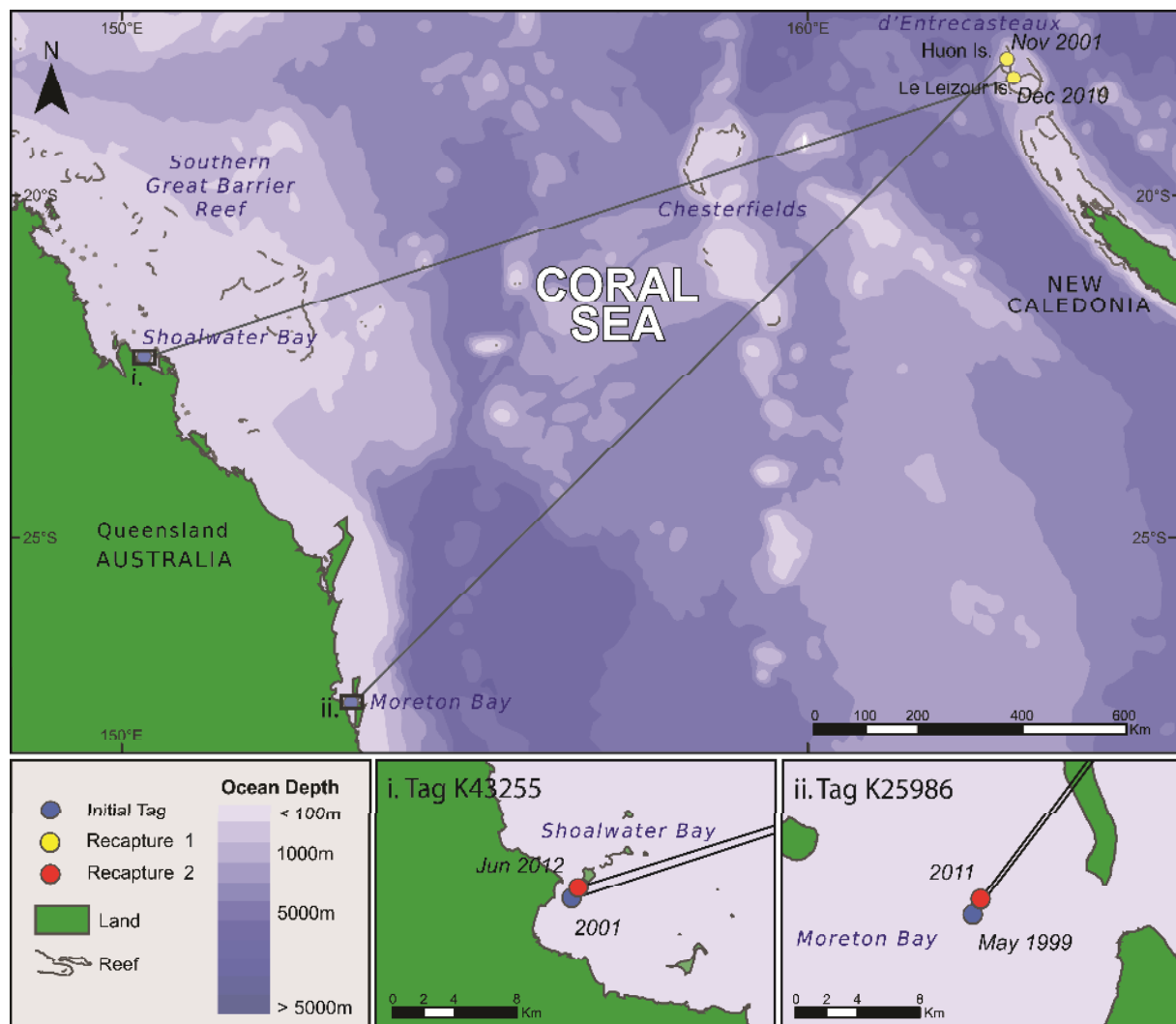


**Figure 2.** Mean linear minimum distance (km) ( $\pm$  SE) from initial tagging locations in the Coral Sea; nGBR ( $n = 2$ ), sGBR ( $n = 39$ ) and NC ( $n = 45$ )



**Figure 3.** Mean time (days) from initial tagging locations in the Coral Sea; sGBR (n = 31) and NC (n = 58)

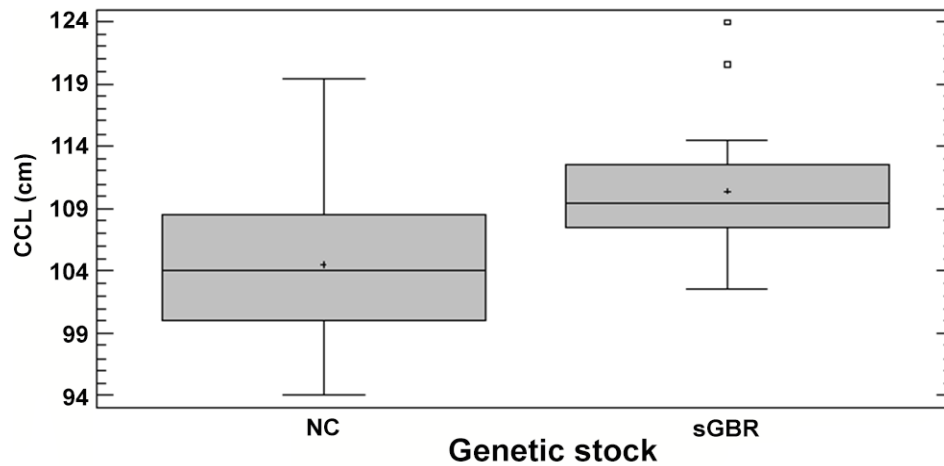
Females were either tagged during a nesting event or recovered at their nesting site and their CCL was recorded during this time (Figure 5). The mean CCL for females recorded in this study nesting at D'Entrecasteaux atolls is 104.5 cm (SE± 2.4) compared to a mean CCL of 110.4 cm (SE± 3.3) for females nesting in Australia (recorded in this study). No significant difference was found between the CCL of females recorded in this study nesting at d'Entrecasteaux atolls and the CCL of all females reported nesting at d'Entrecasteaux (t-test,  $p = 0.7$ ). A significant difference was found between the size of individuals nesting in NC and individuals nesting in sGBR, and known to travel across the Coral Sea (we excluded individuals that were known to nest in NC and recaptured at their feeding site in NC,  $n=6$ ) (t-test,  $p=0.00$ ). The individual from Vanuatu was removed from the analysis (which had a CCL of 102 cm) and the two individuals from nGBR were also excluded (did not have their CCL recorded). The distribution of the size recorded from each female at its nesting site is uneven due to a paucity of female size data (Figure 6). Six percent of the individuals tagged in Australia and found in New Caledonia were males ( $n=4$ ). They were excluded from all analysis due to their low numbers but table 2 recapitulates the data that was collected for these individuals.



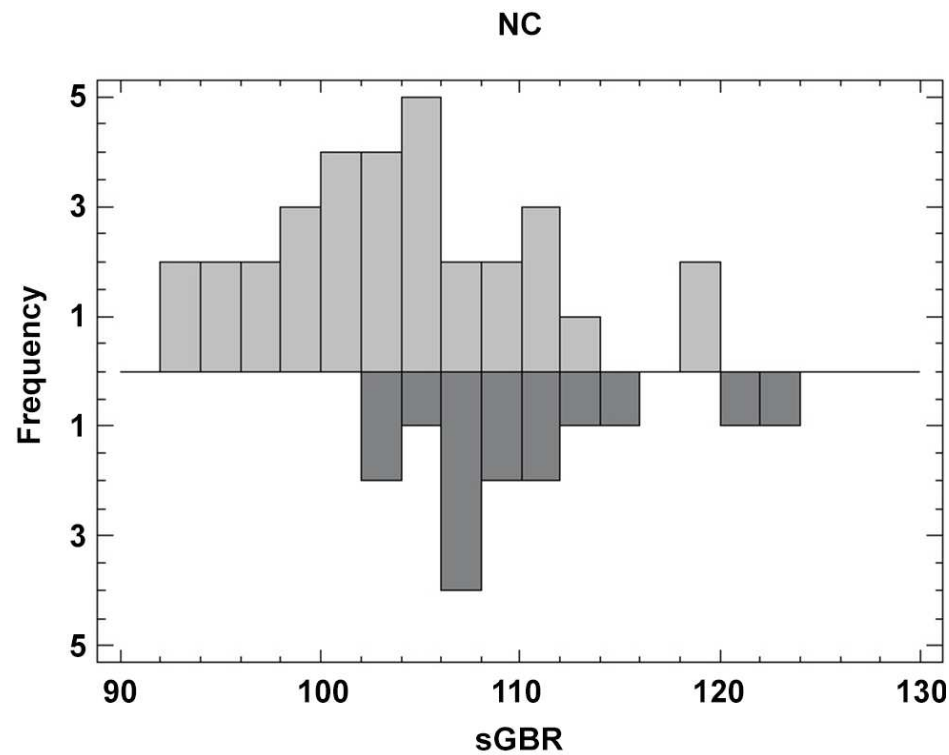
**Figure 4.** Foraging site fidelity recorded by two females *C. mydas* originally tagged in Australia, found nesting in New Caledonia and recaptured in subsequent years back at their respective feeding grounds

**Table 2.** Case histories illustrating male *C. mydas* migration across the Coral Sea

PTAG	TAG	CCL (cm)	PLACE	NPLACE	EDAYS	MinDistance (km)
T	15584	N/A	NC:PUETEGE RF,Merlet	sGBR:CBG:HERON RF	3795	1580
T	78838	N/A	NC:BALABIO	sGBR:HERON ISLAND	4410	1335
X	22681	98,0	NC:MIDWEST MB:MORETON	sGBR:HERON ISLAND NC:ILE HUON-ON	580	N/A
T	78636	N/A	RODEO	BEACH	4552	1419



**Figure 5.** Mean CCL (cm) ( $\pm$  SE) at two nesting areas in the Coral Sea; sGBR (n = 15) and NC (n = 32)



**Figure 6.** Distribution of the mean CCL (cm) at two nesting areas in the Coral Sea; sGBR (n = 15) and NC (n = 32)

## Discussion

Long-term databases on the tagging and recapture of *C. mydas* on the east coast of Australia and in New Caledonia revealed multiple migrations across the Coral Sea showing heterogeneous patterns in connectivity between *C. mydas* nesting and feeding grounds across the Coral Sea. Despite low tag recovery, migratory paths spanned the entirety of the Coral Sea with considerable longevity between mark and recaptures periods.

## **Low tag recovery**

Low tag recoveries could be explained by several factors. Firstly, a very high dispersal rate in the South Pacific. The individuals found nesting in New Caledonia are likely to be foraging on every reef and seagrass pasture within the Southwest Pacific region, thus making it difficult to recapture them (or be informed of their recapture) from areas with low human populations. Secondly, very high loss rate of tags. If a turtle loses its tag then when it is encountered again it is not seen as a recapture. However, there are much higher rates of tag recoveries at dedicated tagging–recapture study sites in Australia of breeding *C. mydas* returning for nesting in subsequent years (Limpus et al. 2002) and in foraging areas (Chaloupka et al. 2004; Limpus et al. 2005). Thirdly, high anthropogenic impact: the low proportion of recaptures could be explained by high numbers being killed for food consumption, as bycatch or by pollution; and finally low percentage of tag return. People are often reluctant to return tags to the appropriate authorities as it is prohibited to hunt turtles in New Caledonia since 2009 (although exceptions are made for traditional purposes).

## **Post –nesting trends**

The different species of sea turtles are dissimilar in many ways, but one behavior they have in common is that they return to the area where they hatched in order to reproduce, a phase often referred to as “natal homing” (Carr 1967). A way to study this period is to tag turtles as they are leaving the beach after nesting and study their migration back to their feeding grounds. Many studies have looked at different hypothesis on how certain taxa migrate (Akesson and Hedenström 2007; Freake et al. 2006; Southwood and Avens 2010) and many specifically on sea turtles (Hays et al. 2002; Luschi et al. 2007; Papi et al. 1995) but very little is known on why and what pathways do they use. Here we try to provide records to understand the underlying patterns of *C. mydas* dispersion in the South Pacific by looking at the tag recoveries geographically. Tag recoveries from females tagged at d’Entrecasteaux atolls were found all along the QLD coast, in PNG and also around the main island of New Caledonia. We found a very clear west-ward trend for post-nesting migration when nesting oc-

curred in New Caledonia. This has been reported previously from the central South Pacific where 96% of individuals in the study migrated westward and more specifically to Fiji (Craig et al. 2004). The explanation given was that Fiji has large areas of seagrass and algae. No record has been found of *C. mydas* migrating from New Caledonia to Fiji but two individuals have also been recorded to travel from French Polynesia to New Caledonia, thus going past Fiji (Balazs et al. 1995). Within the same study, five individuals were tracked from French Polynesia to Fiji (Balazs et al. 1995). More factors are likely at play to explain the observed westward trend besides the abundance of food resources. Moreover, three females known to nest in New Caledonia were found feeding on Heron Reef, while 23 females known to nest on Heron Island were found in New Caledonia. Turtles born on Heron Island therefore seem to have enough forage near their nesting grounds so why travel 1300 km to feed, with increased energy demands associated with long migration. Taken together, these findings lend strength to the hypothesis that more cues are used by sea turtles to choose their feeding grounds than just the abundance and proximity of food sources. Several species from the family Salmonidae have a similar pattern of natal-homing (Lohmann et al. 2008, Ward 1921) but their migration seems to be explained by a feeding pattern and a trail of pheromones left by descending smolt that triggers the migration (Nordeng 1977), which is not proven in sea turtles. The composition of foraging aggregations seems to be also influenced by currents and the Earth's geomagnetic field (Bass et al. 2006; Lohmann et al. 2006) but not all individuals choose to settle in the exact same way, otherwise individuals coming from the same rookery and born in the same year at one rookery would all be found in one feeding ground, which is not the case (Lahanas et al. 1998; Limpus et al. 2003; Limpus et al. 2005). A recent hypothesis is that foraging site selection reflects passive drift experienced by hatchlings thus the adult's movements seems to be directed by constant currents from breeding sites (Hays et al. 2010). The North Caledonian Jet (NJC) and the South Caledonian Jet (SCJ) both have a western direction thus possibly pushing hatchlings towards Australia. However, this does not explain the eastern trend of turtles known for nesting in Australia and found feeding in New Caledonian waters as these currents now act as restraints (Girard et al. 2006). This pattern of ocean



crossing is also found in loggerhead turtles (*Caretta caretta*). This was proven genetically and by tag recoveries, showing individuals feeding in Australia and belonging to multiple rookeries in the South Pacific (including New Caledonia) (Boyle et al. 2009; Limpus 1989). Tag recoveries have also showed *C. caretta* foraging in feeding grounds spread in the Pacific Ocean and nesting in Australia (Limpus et al. 1992). Once again, it is indicated that the migration is not due to a lack of resources but rather an intricate pattern during the “lost years”. Together these results indicate that other significant factors are yet to be identified in order to fully understand the components of recruitment and migration patterns in sea turtles and more specifically for *C. mydas*.

## **Stocks**

Females that come to nest at d’Entrecasteaux atolls, the nGBR nesting beaches and the sGBR nesting beaches are from independent genetic stocks (Bowen and Karl 2007; Dethmers et al. 2006). There are many sea turtles studies that demonstrate that the turtles nesting at one beach migrate from numerous widely dispersed foraging sites and that turtles living in any one foraging area will have originated from multiple genetic stocks (Bowen and Karl 2007; Dethmers et al. 2006; Luschi et al. 2007; Moritz et al. 2002). However, based on available tag recoveries, the foraging *C. mydas* population of New Caledonia is dominated by turtles from the sGBR stock. Seventeen percent of post-nesting migration tag recoveries from *C. mydas* tagged while nesting in New Caledonia has been recorded from New Caledonian waters and ninety-five percent of recaptured foraging green turtles in New Caledonia that came from Australian nesting beaches come from the sGBR. Only two individuals from the nGBR were recovered in New Caledonia. Knowing that the nGBR and the sGBR populations are genetically distinct, this data shows that the resident populations of *C. mydas* in New Caledonia have a higher percentage of individuals belonging to the sGBR than the nGBR genetic group. This correlates with the data collected in Australia, where the frequency of tag recoveries originating from the sGBR genetic stock increases along eastern Australia south from Torres Strait (9°S) to central New South Wales (33°S) (Limpus and Reed 1985). In the results it was indicated that

*C. mydas* foraging within New Caledonian waters originate from at least four independent genetic stocks breeding in at least four different countries: New Caledonian stock (n = 47); Australian stocks (sGBR (n = 45), nGBR (n = 2) stocks) and probably an independent stock in Vanuatu (n = 1). Mitochondrial DNA from females nesting at Chesterfield atolls has not been tested yet. It is highly probable that it will add a second independent stock in New Caledonia as the two rookeries are separated by more than 500 km (Bowen and Karl 2007).

It was noted that no individuals from the Australian Coral Sea stock (Dethmers et al. 2001; Limpus 2009) have been identified foraging in New Caledonia. This should be investigated further along with the data originating from French Polynesia (Balazs et al. 1995). Two individuals were tagged while nesting at Scilly Island in French Polynesia only to be recovered foraging in New Caledonia. This data adds a fifth independent genetic stock found in *C. mydas* foraging in New Caledonia and indicates that the genetic diversity of the sea turtle population in the Coral Sea is yet to be fully understood.

### **Distance travelled and timing between mark and recapture**

The low number of individuals originating from the nGBR can be explained by the distance that has to be travelled (> 2000 km) and the energetic cost that they incur (Hamann et al. 2002). Many studies report post-nesting migrations of *C. mydas* in the range of 10's of km to 1500 km in (Lahanas et al. 1998; Limpus, Bell and Miller 1995; Limpus et al. 1992; Papi et al. 1995; Troëng and Rankin 2005) but recorded migrations over 2000 km are also part of the ecology of this species in the Pacific (Anon 1980; Balazs 1976; Carr 1975; Dethmers et al. 2006; Hays et al. 2002; Koch, Carr and Ehrenfeld 1969; Limpus et al. 2005; Limpus and Reed 1985; Limpus et al. 1992; Luschi et al. 1996; Luschi et al. 1998; Mortimer and Carr 1987; Seminoff et al. 2008). Our findings are broadly in accord with the global patterns of migration distances for adult Cheloniid turtles and "similar to that predicted for equivalent-sized marine mammals and fish" (Hays and Scott 2013).

Mean time between initial tagging and tag recovery is not significantly different for the individuals that belong to the New Caledonian and sGBR genetic stock. This can be explained by the fact that a

large proportion of tag recoveries from both countries come from hunters and members of the public who report stranded turtles. Here we are reporting on how long it takes to recover a tag not how long it takes a turtles to travel between its foraging and breeding grounds. More field work is needed in New Caledonia and other South Pacific Islands to narrow the mean time of recapture between foraging and nesting grounds to calculate precisely the time frame needed for these individuals to cover those distances and look at the interval between nesting at d'Entrecasteaux atolls. The time for tag recovery in New Caledonia can partly be explained by the lack of an organised program that is necessary to reach remote tribes and educate local populations on the purpose of those tags. All tag recoveries from individuals found feeding in New Caledonia and tagged in Australia were done by fishermen that hunted these turtles for food. It is highly probable that many more females undertake that migration, yet tags are not returned and the data therefore does not reflect the true dynamics of *C. mydas* in the Coral Sea.

### **Feeding site fidelity**

From the individuals known to nest in New Caledonia, two were recaptured at a later date back in their original tagging area at their feeding site in Australia. As showed in the results, the first individual (K25986) was originally tagged in Moreton Bay (MB) in 1999 and recaptured in that same Bay in 2011. The second individual (K43255) was caught at Shoalwater Bay (SWB) in 2001 and recaptured at the same location in 2012. This shows some fidelity of *C. mydas* females to their foraging grounds, even though their nesting site and their foraging site are separated by 1200 km. This behavior has been recorded elsewhere but with shorter distances (in the order of ten to hundreds of km) (Broderick et al. 2007; Limpus et al. 1992).

### **Size**

The significant difference found in CCL between females originating from foraging grounds in Australia versus adults caught on New Caledonian feeding grounds is in concordance with other studies. *C.*

*mydas* living in different locations have different sizes (Limpus et al. 1994; Limpus et al. 2005; Limpus and Reed 1985). These differences can be explained by foraging-ground-dependent growth rates (Limpus et al. 1994). Because *C. mydas* may have a high fidelity to their foraging site (as shown above), their size could reflect the quality of their feeding grounds. The mean CCL for females used in this study recorded nesting at d'Entrecasteaux atolls is not significantly different to the mean of all recorded females nesting on these atolls (Read 2012). In this study, the mean size for females at their nesting grounds in the SGBR is 110.4 cm compared to the historical data giving a mean CCL of 107.0 cm for females nesting at Heron Island (representative of the sGBR) (Limpus et al. 1984). Studies are needed in New Caledonia in order to calculate growth rates at feeding sites.

### **Male turtles**

Out of all of the tags recovered in New Caledonia and belonging to individuals originally tagged in Australia, only 6% (n= 4) belonged to males. At SWB, the mean sex ratio (female: male) for adults caught is 1:1.78 (Limpus et al. 2005) compared to 1:0.80 if we look at four different feeding grounds within the GBR (Limpus et al. 1984). As sea turtles have temperature-dependent sex determination (TSD), skewed sex ratios in a population over a period over time can lead to the disappearance of a population (Hays et al. 2010). It is known that males also migrate to the area where they were born in order to reproduce (FitzSimmons et al. 1997a; FitzSimmons et al. 1997b) and a study reported that the breeding periodicity for male sea turtles is 2.6 times more often than females (Hays et al. 2010). Three of the males in the database were caught by fisherman in their NC foraging area, and one of the males was encountered on the beach at Huon Island (d'Entrecasteaux atolls) during nesting season. This individual appeared to have been basking (Whittow and Balazs 1982). Tagging programs typically focus on females as large number of individuals can easily be tagged on the beach during nesting season. However, tagging males (or juveniles) is important if we are to fully understand population dynamics in *C. mydas* and devise effective management and conservation programs. This

is all the more important as understanding the fate of juvenile life history stages is an important determinant of population changes in sea turtles (Crouse et al. 1987).

### **New trajectories**

As reported in the results, two migrations paths were unraveled within this study. The first ever recorded turtle migration between Australia and the Chesterfield Atolls (New Caledonia). QA 14889 was originally tagged by the Queensland Turtle Research program in western Harvey Bay (Booral) in Queensland on the 30<sup>th</sup> of April 2011. It was seen nesting on Bampton Island (Chesterfield Atolls) on the 19<sup>th</sup> of November 2011. It has been reported that the Chesterfield Atolls are an area of importance for sub-adults and adult male tiger sharks that move between the GBR and New Caledonia (Werry et al. 2014). More research in that area is needed in order to test the hypothesis that these reciprocal movements of this top level predator may reflect on *C. mydas* migrations. Secondly, the post-nesting migration of a female “Bamboo Lady” from Bamboo Bay in Vanuatu to its feeding ground in Voh (New Caledonia) is the first recorded migration of sea turtle between those two countries. New trajectories of megafauna in the Coral Sea are being recorded now that these secluded areas are starting to being investigated.

### **Conclusion**

Most of the tags recovered from *C. mydas* individuals in New Caledonia belonged to turtles known to nest in the sGBR of Australia. New migrations paths were uncovered for *C. mydas* in the Pacific region between the Chesterfield Atolls (in New Caledonia) to Australia and Vanuatu to New Caledonia. This study reinforces that *C. mydas* travel long distances (> 2000 km) between their feeding and nesting grounds in the Coral Sea. The low percentage of tag recoveries, however, needs to be better explained. Is this just due to a lack of tag returns or do the numbers of recaptures reflect the actual importance of migrations throughout the Coral Sea (i.e., with low tag recoveries explained by the lack of capacity at the regional scale)? Findings reported here demonstrate the need for a comprehensive

tag recovery program in New Caledonia. Most of all, this study confirms that sea turtle conservation is not a localised management problem, but rather an international issue and management activities need to be devised and implemented at a larger scale: in this instance across the Coral Sea.

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## CHAPTER 3 BIBLIOGRAPHIC DETAILS

**Title:** Mixed stock analysis of a resident green turtle, *Chelonia mydas*, population in New Caledonia links rookeries in the South Pacific

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## Statement of contribution to co-authored published paper

This chapter includes a co-authored paper. The bibliographic details (if published or accepted for publication)/status (if prepared or submitted for publication) of the co-authored paper, including all authors, are:

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My contribution to the paper involved, most data collection, the preliminary analysis and categorisation of the data into a usable format and providing direction on the scope and structure of the analysis. Some of data was also provided by some of co-authors.

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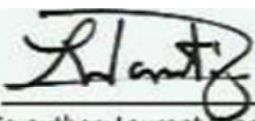
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## CHAPTER 3: MIXED STOCK ANALYSIS OF A RESIDENT GREEN TURTLE, *CHELONIA MYDAS*, POPULATION IN NEW CALEDONIA LINKS ROOKERIES IN THE SOUTH PACIFIC

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### Abstract

Migratory species are known to pose a challenge for conservation because it is essential to understand their complex life history in order to implement efficient conservation actions. In New Caledonia, large seagrass habitats in the Grand Lagon Sud (GLS) are home to resident green turtles (*Chelonia mydas*) of unknown origins. To assess the stock composition in the GLS, 164 foraging turtles were sampled for genetic analysis of ~770 base pairs of the mitochondrial DNA (mtDNA) control region.

Foraging turtles ranging in size from 48.0 to 108.4 cm curved carapace length were captured at five different sites within the GLS between September 2012 and December 2013. To provide baseline data for mixed stock analysis, published data from rookeries were used in addition to 105 samples collected at rookeries in the d'Entrecasteaux Islands and Chesterfield Islands in New Caledonia and at Malekula Island in Vanuatu. Exact tests of population differentiation and pairwise  $F_{ST}$  estimates were used to test for differences in mtDNA haplotype frequencies. These analyses indicated that rookeries in the d'Entrecasteaux Islands and Vanuatu form unique management units and that the Chesterfield Islands rookeries are linked to the Coral Sea management unit. Mixed stock analysis indicated the highest proportion (mean = 0.63) of foraging turtles originate from the d'Entrecasteaux stock.

The larger contribution is estimated to be from a large rookery from New Caledonia, but smaller contributions are suggested from other rookeries in the South Pacific. Marine conservation policies in New Caledonia need to consider the links between the foraging and nesting populations of *C. mydas* in New Caledonia and other rookeries and foraging grounds in the Coral Sea.

**Key words:** mtDNA, sea turtle, marine conservation, marine migratory species, management unit, Cheloniidae

## Introduction

Recruitment and dispersal patterns of species in marine environments are complex processes, often influenced by species-specific responses to features of both nearshore and pelagic environments. For large, highly migratory marine species, dependence on oceanic currents for dispersal varies and affects the connectivity among populations, recruitment patterns, links between foraging and breeding areas and genetic diversity. Long-distance migrations are usually required to travel between foraging and breeding grounds as seen for humpback whales (*Megaptera novaeangliae*), which migrate thousands of kilometres to reach suitable habitat for mating and calving before returning to the high latitude waters where they feed with their newborn calves (Dawbin 1966; Olavarria et al. 2007). Fur seals (*Arctocephalus gazelle* and *Callorhinus ursinus*) migrate hundreds or thousands of kilometres between their colonies and foraging grounds during pup-rearing period (Bonadonna et al. 2001; Ream et al. 2005). Like humpback whales, they show strong fidelity to specific foraging areas year after year and use gyres and currents for these migrations (Ream et al. 2005; Hoffman et al. 2006). In contrast, no parental care is provided by tiger sharks (*Galeocerdo cuvier*), and recruitment patterns from pupping area to foraging grounds can include migrations that exceed a thousand kilometres (Driggers et al. 2008; Werry et al. 2014). For migratory marine species that do not rear their young, the influence of oceanic currents is more important in the dispersal of young as it determines the areas where the juveniles will find sufficient prey such as within upwelling nutrient-rich water as seen for tiger sharks (Gilbes et al. 1996).

Sea turtles have a complex life cycle that includes multiple phases within neritic and pelagic habitats (Bolten 2003b). An epipelagic phase for post-hatchlings ranges from five to ten years (Zug et al. 1995; Zug et al. 2002; Goshe et al. 2010), after which juvenile turtles recruit to coastal waters (Limpus et al. 1994; Limpus and Chaloupka 1997; Limpus and Limpus 2003). Exactly how the juveniles choose their foraging grounds is unknown but multiple studies have reported that foraging site selection may reflect the passive drift in ocean currents experienced by hatchlings (Hamann et al. 2011; Okuyama



*et al.* 2011), as well as other processes such as temperature and prey items (Mansfield *et al.* 2014). When sexual maturity is reached, individuals periodically leave their foraging grounds to undergo breeding migrations before returning to their selected feeding grounds (Limpus *et al.* 1992; Hays *et al.* 2014; Read *et al.* 2014). The natal homing hypothesis for sea turtles (Carr 1967), where breeding turtles return to the region of their birth to breed, has been confirmed by multiple genetic studies for both females (Meylan *et al.* 1990; Bowen *et al.* 1992; Norman *et al.* 1994; Jensen *et al.* 2013a) and males (FitzSimmons *et al.* 1997) and this leads to distinct breeding populations. Studies at different foraging grounds show that multiple genetic stocks are present at any one site (Limpus *et al.* 1992; Bowen *et al.* 1996; Lahanas *et al.* 1998; Limpus 2009). For green turtles (*Chelonia mydas*) in the Indo-Pacific, 30 distinct genetic populations were recognised by FitzSimmons and Limpus (2014) based on mtDNA data. These populations can be referred to as distinct 'genetic stocks' or 'management units' (MUs) (Moritz 1994), which may encompass one or multiple rookeries and multiple foraging grounds. Hence, to effectively protect this threatened species it is important to identify the connectivity between specific rookeries and foraging grounds.

A better understanding of the patterns of connectivity and gene flow is fundamental for the conservation of green turtles in the Coral Sea. The conservation status of green turtles in the South Pacific Islands remains unclear, and although some historical data on distribution are available (Pritchard 1971, 1978, 1979, 1982a, 1982b; 1987; Pritchard *et al.* 1983; Geermans and Farago 1993; Guinea 1993; Hirth 1993) large regions are yet to be studied and minimal long-term data exist. Hunting of foraging and nesting sea turtles has been part of traditional cultures for millennia (Balazs 1982a, 1982b; Allen 2007). Since 2008 in New Caledonia, the harvest of turtles has been limited to special ceremonies and the impact of that take is unknown. A recent study reports that <10 000 individuals a year are being legally harvested in Australia and >500 in New Caledonia (Humber *et al.* 2014), and to assess the impact of that hunting, additional data on population abundance, connectivity and dynamics are needed. Only three genetic studies of green turtles have included New Caledonia turtles and these report that the d'Entrecasteaux rookeries (known as the western New

Caledonia stock; FitzSimmons and Limpus 2014) are a genetically distinct breeding population (Dethmers *et al.* 2006; Jensen 2010; Dutton *et al.* 2014). Nesting females from New Caledonia have been recorded at feeding grounds in Australia and nesting females from Australia have been recorded at feeding grounds in New Caledonia (Limpus *et al.* 1992; Dethmers *et al.* 2010; Read *et al.* 2014). Additionally, mixed stock analyses using genetic data also indicate that some green turtles foraging along the east coast of Australia originate from New Caledonia (Jensen *et al.* in press). Large foraging grounds for green turtles are found around New Caledonia, but no genetic studies have been done to determine the origins of the foraging turtles.

In this study we characterise the mitochondrial DNA (mtDNA) haplotype diversity at unsampled green turtle rookeries in the Chesterfield Islands, which are halfway between the Great Barrier Reef (GBR) and New Caledonia, and at Vanuatu and we increase the sample size for the d'Entrecasteaux rookery to determine if these are unique MUs. These data are used along with published data to conduct a mixed stock analysis of green turtles sampled at a major foraging ground in New Caledonia at Grand Lagon Sud (GLS) to determine the populations of origin.

This information is needed to understand recruitment patterns of green turtles in the region and determine the impacts of regional harvests. Understanding the population dynamics of green turtles in New Caledonia is the first step towards comprehending what is required for sustainable, traditional use of an iconic species.

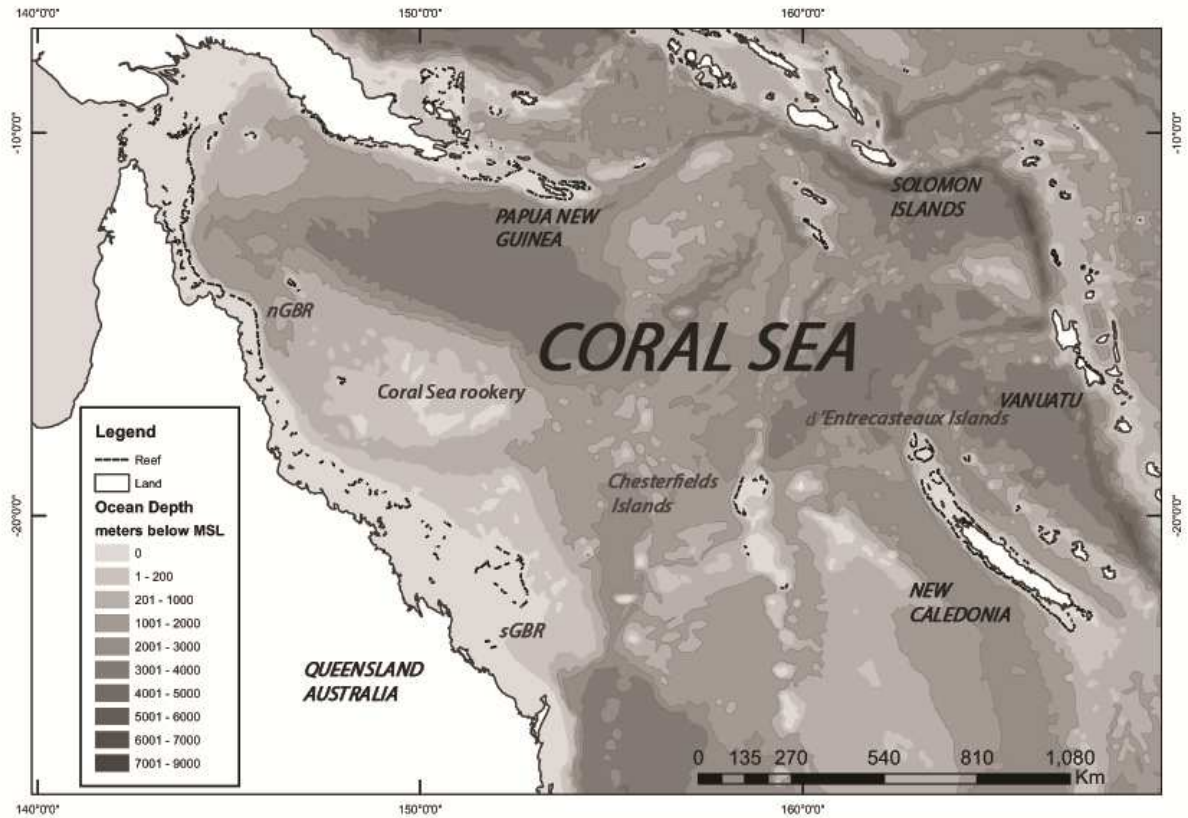
## **Materials and methods**

### **Study area and sampling**

Nesting green turtles were sampled at the two main clusters of rookeries in New Caledonia and one rookery in Vanuatu. The largest breeding population in New Caledonia is located at the d'Entrecasteaux Islands, 180 km north of the tip of New Caledonia, and it has an estimated population size of 1000–5000 nesting females per year (Mounier 2007; Read 2012). These islands are separated from the mainland by 'Le Grand Passage', a channel 600m deep (Fig. 1). Nesting occurs on

Huon, Surprise, Fabre and LeLeizour islands. Previous sampling at d'Entrecasteaux Islands was limited (Dethmers *et al.* 2006; Dutton *et al.* 2014) so additional samples ( $n = 25$ ) were obtained. The second largest breeding population in New Caledonia is at the Chesterfield Islands, located 550 km northwest of the main island of New Caledonia and it has an estimated population size of 100–1000 nesting females per year (Fonfreyde 2012) (Fig. 1). Nesting occurs on Avon, Bampton, Longue, Loop, Mouillage, Passage, Reynard and Skeleton islands. Samples from the Chesterfield Islands ( $n = 49$ ) are the first samples taken for genetic studies (islands are separated by less than 70 km). Additionally, samples ( $n = 31$ ) were collected from a rookery on the west coast of Malekula Island, Vanuatu, which is located ~1000 km from the Chesterfield Islands and 550 km from the d'Entrecasteaux Islands.

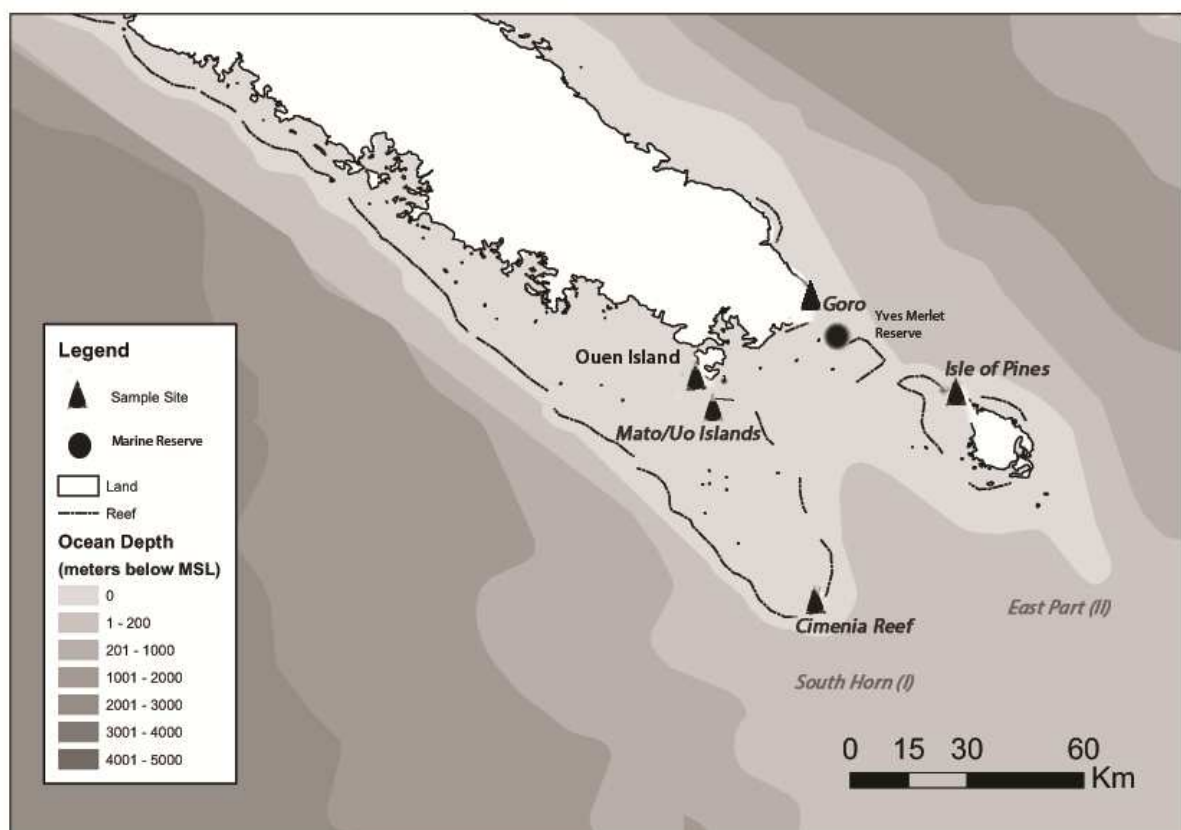
Foraging green turtles were sampled ( $n = 164$ ) from the GLS (S22°00, E167°00), which supports important foraging grounds for green turtles (Fig. 2). It is one of six areas of New Caledonia added to the World Heritage List in 2008 for its reef diversity and associated ecosystems (Menu and Hebert 2006). Grand Lagon Sud has an area of 314 500 ha and is composed of two zones that are geographically distinct: the east part (I) and the south horn (II) (Fig. 2). The first zone is characterised by its reef diversity, the presence of the Isle of Pines with fringing reefs and the only sanctuary (Reserve Merlet) of the GLS in which no human activity is allowed. The south horn has a barrier reef but contains a less diverse reef habitat (Andréfouët and Torres-Pulliza 2004). Both zones (I and II) are known for their extensive seagrass and algal communities, especially on the Cimenia Reef (at the tip of the south horn) (Fig. 2).



**Figure 1.** Coral Sea study area showing green turtle rookeries sampled at the Chesterfield Islands and d'Entrecasteaux Islands in New Caledonia, and Vanuatu and the location of foraging regions in the southern Great Barrier Reef (sGBR) and the northern Great Barrier Reef (nGBR)

Five foraging areas (separated by less than 70 km) were selected after conducting pilot studies in the area based on tribal knowledge that was shared by the tribes of Goro, Ile Ouen and Isle of Pines (Fig. 2). Mato and Uo islands (site 1; S22°33.210, E166°47.220) are two small vegetated islands surrounded by large sandy areas, reef platforms and patches of seagrass beds. Goro (site 2; S22°17.000, E167°05.990) is located in the south tip of New Caledonia where large seagrass beds are found in several bays. Ouen Island (site 3; S22°25.340, E166°48.360) delimits the south border of the Woodin channel halfway between Noumea and Isle of Pines. East of this island are two large reefs, U and Niagi, which have large seagrass beds where the sampling was conducted. These two reefs are essential for the survival of the 80 people who live on the island. Cimenia Reef (site 4; S22°59.240, E166°59.480) is composed of a triple barrier reef and forms the southernmost reef system off

New Caledonia. In between each reef are large seagrass and algal beds. Isle of Pines (site 5; S22°34.340, E167°26.460) is 195 km<sup>2</sup> and is the southernmost island in New Caledonia, with a human population estimated to be 2000 inhabitants. It is the only recorded nesting site for green turtles in the GLS, with an estimated one to ten females a year in 1996, but no reports since (C. J. Limpus, pers. comm.). Foraging habitat throughout the GLS is used by tribes of the GLS to harvest the turtles needed for various celebrations.



**Figure 2.** Sampling sites for foraging *Chelonia mydas* in the Grand Lagoon Sud, off the southern end of the main island of New Caledonia

All resident foraging turtles were captured by the turtle rodeo method (Limpus and Reed 1985).

Additional samples ( $n = 49$ ) were collected from individuals harvested for tribal ceremonies.

All live turtles were tagged with a titanium tag (Limpus 1992), measured for curved carapace length ( $CCL \pm 0.1$  cm), sexed when possible (i.e. pubescent, subadult and adult males with longer tails), and skin samples (0.5 cm<sup>2</sup>) were collected from the rear flipper and placed in absolute ethanol before

releasing the turtles. Individuals were divided into three size classes according to Limpus *et al.* (1994) and Limpus and Chaloupka (1997): juveniles were 20–65 cm CCL, sub-adults were >65–90 cm CCL and adults were >90 cm CCL. Samples were collected in 2012 and 2013 under New Caledonian permits 1139–2012/ARR/DENV and 2012–3253/GNC and a Griffith University animal care and ethics permit ENG/01/12/AEC. Preserved samples were exported from New Caledonia under CITES FR1398800027-E and imported into Australia with AQIS permit IP1209572.

## **Molecular methods**

DNA extraction was done using a salting out method (Jensen *et al.* 2013a) and run on 1% agarose gels to check quantity and quality of the extractions. Samples that did not yield adequate DNA were re-extracted using a DNA extraction kit (Invitrogen, Waltham, MA USA). Polymerase chain reactions (PCR) were used to amplify an >800 base pair (bp) segment of the mtDNA control region using primers LtSeaT (GCATTGGTCTTGTAACCAAAG), which was modified from LTEi9, and H950 g (AGTCTCGGATTAGGGGTTTG) of Abreu-Grobois *et al.* (2006) before cropping it to a ~770 bp segment for analysis. Polymerase chain reaction amplifications were done in 25 µL volumes containing 5–50 ng of template DNA, 1 x reaction buffer, 0.5 mM of each primer and 1 unit of Taq polymerase (MyTaq, Bioline, Alexandria, NSW, Australia). Polymerase chain reaction conditions were as follows: 3 min at 95°C, followed by 35 cycles of 45 s at 95°C, 45 s at the annealing temperature and 45 s at 72°C, with a final extension for 1 min at 72°C. Annealing temperature for the first two cycles was 50°C, followed by two cycles at 51°C and 31 cycles 52°C. Polymerase chain reaction products were sent to Macrogen (South Korea) for purification and sequencing of forward and reverse strands. Sequences were imported in Geneious Pro 7.0.2 ([www.geneious.com](http://www.geneious.com)) for alignment and checked manually. Haplotypes were identified by running a BLAST search against known green turtle haplotypes on GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>).

## Statistical analysis

ARLEQUIN ver. 3.5.1.2 (Schneider *et al.* 2000) was used to estimate haplotype and nucleotide diversity, run exact tests and test whether pairwise  $F_{ST}$  estimates were significantly different from zero using mtDNA haplotype frequency data from the sampled rookeries in New Caledonia and green turtle management units including: Aru, NorthWest Shelf, Ashmore, Coburg, Gulf of Carpentaria, southern Great Barrier Reef (sGBR), northern Great Barrier Reef (nGBR), northern Papua New Guinea, Vanuatu, Coral Sea, Marshall Islands, Yap, Palau, American Samoa and French Polynesia (Dethmers *et al.* 2006; Dutton *et al.* 2014; Jensen *et al.* in press). BAYES software (Pella and Masuda 2001) was used to estimate the proportional contributions of green turtle stocks in the Indo-Pacific to the studied foraging ground. An initial mixed stock analysis (MSA) was done using data from a shorter sequence fragment (~384 bp) that were available (Dethmers *et al.* 2006) for 30 Indo-Pacific stocks (FitzSimmons and Limpus 2014). Unlikely contributors (mean = 0 and upper 97.5 CI < 0.02) were excluded from the final analysis, which used ~770 bp sequences from seven rookeries (Table 1). Seven chains were run using 20 000 Markov chain Monte Carlo (MCMC) steps (burn-in of 10 000 runs) to calculate the posterior distribution. To test that the posterior probability distribution of the chains had converged (shrink factor >1.2), the Gelman and Rubin shrink factor diagnostic was applied. The final MSA was run twice, once with uniform priors and the second time with population size entered as weighted prior under the assumption that larger rookeries provide larger contributions to foraging grounds. Population size was derived from Department of Environment and Heritage (2005) for all rookeries except those at d'Entrecasteaux and Chesterfield islands, which were derived from Fonfreyde (2012) and Read (2012) (Table 1). Individuals with orphan haplotypes (haplotypes not found at any rookeries) were removed automatically by the program as being uninformative.

**Table 1.** Haplotype frequency for Indo-Pacific *Chelonia mydas* management units (genetic stocks; rookeries of the Coral Sea and Chesterfields stock shown separately) and from a foraging ground at the Grand Lagon Sud of New Caledonia, including estimated total population size of nesting females and sample size (N). Haplotype designations indicate previous and new (CmP) names and GenBank references for the sequences

				Management Units													Foraging ground
				Location	nGBR	Coral Sea	Chesterfields	sGBR	d'Entrecasteaux	PNG	Aru	Vanuatu	Marshall Islands	Micronesia	Am. Samoa	French Polynesia	Location
				Datasource	a	a	b	a	a,b,c	a	a	b	c	a,c	c	c	Stage
				Pop size	24300	300	2500	6600	7500	800	1000	200	350	750	70	350	Year
old	Long	Genbank ID	Reference	N	81	48	49	102	64	18	28	31	128	538	17	9	2012-2014
B1 <sup>1</sup>	CmP44.1	KF311751	Dutton et al .2014b		46	-	-	-	11	-	-	-	-	-	-	-	19
B7 <sup>1</sup>	CmP98.1	FJ917199	Dutton et al. 2009		8	-	-	-	-	-	-	-	-	-	-	-	3
B4 <sup>1</sup>	CmP81.1	KJ502610	Jensen et al. 2015		7	-	-	-	-	-	-	-	-	-	-	-	-
CmP171	CmP171.1	KJ502631	Jensen et al. 2015		1	-	-	-	-	-	-	-	-	-	-	-	-
CmP108	CmP108.1	KJ502585	Jensen et al. 2015		2	-	-	-	-	-	-	-	-	-	-	-	-
J2 <sup>1</sup>	CmP68.1	KJ502591	Jensen et al. 2015		2	-	-	1	-	-	-	-	-	-	-	-	-
C12 <sup>1</sup>	CmP84.1	KJ502630	Jensen et al. 2015		4	-	-	-	-	-	-	-	-	-	-	-	-
A2 <sup>1</sup>	CmP47.1	KF311753	Dutton et al 2014b		2	36	30	93	9	-	-	1	-	-	1	-	45
B1 <sup>1</sup>	CmP44.4	KJ502638	Jensen et al. 2015		1	-	-	-	-	-	-	-	-	-	-	-	-
B3 <sup>1</sup>	CmP80.1	KF311760	Dutton et al. 2014b		3	9	12	8	14	-	-	-	-	-	-	-	32
B1 <sup>1</sup>	CmP44.5	KJ502605	Jensen et al. 2015		1	-	-	-	-	-	-	-	-	-	-	-	-
B6 <sup>1</sup>	CmP134.1	KJ502639	Jensen et al. 2015		1	-	-	-	-	-	-	-	-	-	-	-	-
CmP169	CmP169.1	KJ502608	Jensen et al. 2015		1	-	-	-	-	-	-	-	-	-	-	-	-
CmP170	CmP170.1	KJ502609	Jensen et al. 2015		2	-	-	-	-	-	-	-	-	-	-	-	-
CmP180	CmP180.1	KJ502640	Jensen et al. 2015		-	-	2	-	-	-	-	-	-	-	-	-	-
CmP193	CmP193.1	KJ502635	Jensen et al. 2015		-	-	1	-	-	-	-	-	-	-	-	-	-
CmP168	CmP168.1	KJ502617	Jensen et al. 2015		-	1	-	-	-	-	-	-	-	-	-	-	-
CmP164	CmP164.1	KJ502594	Jensen et al. 2015		-	-	1	-	-	-	-	-	-	-	-	-	-
C1 <sup>1</sup>	CmP83.1	KJ502566	Jensen et al. 2015		-	1	-	-	-	-	-	-	-	-	-	-	-
C3 <sup>1</sup>	CmP49.1	AB819808	Hamabata et al. 2014		-	1	-	-	1	1	-	-	-	-	-	-	3
A2 <sup>1</sup>	CmP47.5	KJ502578	Jensen et al. 2015		-	-	1	-	1	-	-	-	-	-	-	-	-



CmP173	CmP173.1	KF311766	Dutton et al. 2014b	-	-	-	-	2	-	-	-	-	-	-	-	-
CmP117	CmP117.1	KF311763	Dutton et al. 2014b	-	-	-	-	1	-	-	-	-	-	-	-	1
CmP160	CmP160.1	KF311765	Dutton et al. 2014b	-	-	-	-	1	-	-	-	-	-	-	-	1
B1 <sup>1</sup>	CmP44.2	KF311752	Dutton et al. 2014b	-	-	-	-	2	-	-	-	-	-	-	-	4
C13 <sup>1</sup>	CmP85.1	KF311761	Dutton et al. 2014b	-	-	-	-	18	-	-	-	-	-	-	-	23
CmP31	CmP31.1	KF311748	Dutton et al. 2014b	-	-	2	-	2	-	-	-	-	-	-	-	-
CmP174	CmP174.1	KJ502612	Jensen et al. 2015	-	-	-	-	1	-	-	-	-	-	-	-	6
CmP118	CmP118.1	KP856705	This study	-	-	-	-	1	-	-	-	-	-	-	-	-
A3 <sup>1</sup>	CmP20.1	AB819806	Hamabata et al. 2014	-	-	-	-	-	16	1	3	91	276	-	-	3
C7 <sup>1</sup>	CmP88.1	KJ502619	Jensen et al. 2015	-	-	-	-	-	1	-	-	-	-	-	-	-
C14 <sup>1</sup>	CmP91.1	KF311762	Dutton et al. 2014b	-	-	-	-	-	-	27	21	-	1	-	-	5
A2 <sup>1</sup>	CmP47.2	KJ502604	Jensen et al. 2015	-	-	-	-	-	-	-	5	-	-	-	-	-
A2 <sup>1</sup>	CmP47.3	KJ502593	Jensen et al. 2015	-	-	-	-	-	-	-	1	-	-	-	-	-
CmPo2	CmP65.1	KF311756	Dutton et al. 2014b	-	-	-	-	-	-	-	-	1	-	4	8	7
CmP56	CmP56.1	KF311754	Dutton et al. 2014b	-	-	-	-	-	-	-	-	-	-	-	1	-
CmI2, CMJ9	CmP32.1	KF311749	Dutton et al. 2014b	-	-	-	-	-	-	-	-	12	84	-	-	-
E2 <sup>1</sup>	CmP61.1	KF311755	Dutton et al. 2014b	-	-	-	-	-	-	-	-	1	76	-	-	-
A4 <sup>1</sup>	CmP77.1	KF311759	Dutton et al. 2014b	-	-	-	-	-	-	-	-	1	68	-	-	-
A3 <sup>1</sup>	CmP20.2	KF311744	Dutton et al. 2014b	-	-	-	-	-	-	-	-	-	2	-	-	-
A3 <sup>1</sup>	CmP20.3	KF311745	Dutton et al. 2014b	-	-	-	-	-	-	-	-	-	10	-	-	-
A3 <sup>1</sup>	CmP20.4	KF311746	Dutton et al. 2014b	-	-	-	-	-	-	-	-	-	16	-	-	-
CMJ5	CmP123.1	KF311764	Dutton et al. 2014b	-	-	-	-	-	-	-	-	-	2	-	-	-
A6	CmP67.1	KF311758	Dutton et al. 2014b	-	-	-	-	-	-	-	-	-	2	-	-	-
CmPo2	CmP65.2	KF311757	Dutton et al. 2014b	-	-	-	-	-	-	-	-	-	-	2	-	-
CMJ3	CmP22.1	KF311747	Dutton et al. 2014b	-	-	-	-	-	-	-	-	22	1	10	-	2
																orphan haplo- types
A7 <sup>1</sup>	CmP55.1	KJ502596	Jensen et al. 2015	-	-	-	-	-	-	-	-	-	-	-	-	3
CmP34	CmP34.1	KJ502581	Jensen et al. 2015	-	-	-	-	-	-	-	-	-	-	-	-	2
CmP49	CmP49.5	KM923921	Jensen et al. 2015	-	-	-	-	-	-	-	-	-	-	-	-	1
CmP85	CmP85.2	KP663713	This study	-	-	-	-	-	-	-	-	-	-	-	-	2
CmP97	CmP97.1	FJ917198	Dutton et al. unpublished	-	-	-	-	-	-	-	-	-	-	-	-	2

Datasource; a=Jensen et al. In Press, b=Read et al. 2015, c=Dutton et al. 2014, d=Hamabata et al. 2014

## Results

Sequence data were obtained from an ~770 bp fragment, which included most of the mitochondrial control region, for 105 nesting turtles and 164 turtles at the GLS foraging grounds. The size of the individuals sampled from the Chesterfields, d'Entrecasteaux and Vanuatu rookeries varied from 95.6 -112.9 cm CCL, 95-115.5 cm CCL and 80-106 cm CCL, respectively.

### Genetic differentiation of rookeries

The d'Entrecasteaux rookery displayed 13 haplotypes including three common haplotypes that together accounted for 67% of samples. The Chesterfield rookery revealed eight haplotypes with CmP47.1 accounting for 61% of the sample. Five haplotypes were found from the Vanuatu rookery with CmP91.1 being found in 68% of the individuals sampled. One new haplotype (CmP118.1) was found at d'Entrecasteaux rookery (GeneBank ID KP856705). Estimates of haplotype diversity at the three rookeries were highest at d'Entrecasteaux ( $h=0.84 \pm 0.024$ ) and similar between Chesterfield and Vanuatu ( $h=0.57 \pm 0.065$  and  $0.52 \pm 0.096$ , respectively). Estimates of nucleotide diversity were similar for d'Entrecasteaux ( $p=0.024 \pm 0.012$ ), the Chesterfields ( $p = 0.017 \pm 0.009$ ) and Vanuatu ( $p=0.023 \pm 0.012$ ).

Exact tests for haplotype frequency differentiation showed that mtDNA frequencies at the Chesterfields rookery were significantly different to those of the d'Entrecasteaux rookery ( $P < 0.0001$ ), but not different to those of the Coral Sea rookery ( $P = 0.21$ ) (Table 2). Similarly, the pairwise  $F_{ST}$  value for the Chesterfields and d'Entrecasteaux rookeries was significantly different from zero ( $F_{ST} = 0.24$ ,  $P < 0.0001$ ), but this was not so for the estimate between the Chesterfields and Coral Sea rookeries ( $F_{ST} = < 0.01$ ,  $P < 0.0001$ ). The d'Entrecasteaux rookery was significantly differentiated from the Coral Sea rookeries (exact test  $P < 0.0001$ ,  $F_{ST} = 0.29$ ,  $P < 0.0001$ ), and from a combined Chesterfields–Coral Sea sample (exact test  $P < 0.0001$ ;  $F_{ST} = 0.25$ ,  $P < 0.0001$ ) (Table 2). The Vanuatu sample was significantly different to the d'Entrecasteaux and combined Chesterfields–Coral Sea sample ( $F_{ST} = 0.33$ ,  $P < 0.0001$  and  $F_{ST} = 0.51$ ,  $P < 0.0001$ , respectively). The

d'Entrecasteaux, Chesterfields and Vanuatu rookeries were significantly differentiated ( $P < 0.0001$ ) from all other Indo-Pacific stocks, located up to ~4700 km away, as presented in Dethmers *et al.* (2006) and Dutton *et al.* (2014). The Vanuatu MU was significantly different to the Aru, Indonesia MU ( $F_{ST} = 0.35$ ,  $P < 0.0001$ ), although they share the CmP91.1 haplotype at relatively high frequency.

**Table 2.**  $F_{ST}$  values for pairwise comparisons of selected *Chelonia mydas* genetic stocks; abbreviations are: nGBR, northern Great Barrier Reef and sGBR, southern Great Barrier Reef and n.s =  $P > 0.05$ ; \* $P < 0.01$ ; \*\* $P < 0.001$

Population pairwise FST's	d'Entrecasteaux	Chesterfields	Coral Sea	sGBR	Aru	Vanuatu
d'Entrecasteaux						
Chesterfields	0.18**					
Coral Sea	0.26**	> 0.01 n.s				
sGBR	0.46**	0.16**	0.06*			
Aru	0.47**	0.63**	0.73**	0.86**		
Vanuatu	0.30**	0.44**	0.53**	0.72**	0.13**	
nGBR	0.16**	0.36**	0.44**	0.59**	0.55**	0.40**

### Mixed stock analysis of the GLS foraging ground

The majority of individuals sampled in this study were juveniles ( $n = 120$ ) as well as 26 sub-adults and 18 adults. In total, 19 haplotypes were found among the individuals sampled in the GLS ( $n = 164$ ) (Table 1). The four most common haplotypes found were CmP47.1 (28%), CmP80.1 (20%), CmP85.1 (14%) and CmP44.1 (11%). Four orphan haplotypes that were previously observed in foraging grounds by Jensen *et al.* (in press) were found in the GLS and these were removed automatically by Bayes for the analysis: CmP49.5 ( $n = 1$ ), CmP97.1 ( $n = 2$ ), CmP55.1 ( $n = 3$ ) and CmP34.1 ( $n = 2$ ). In total, 'orphan' haplotypes made up 6% of foraging samples. One new haplotype (CmP85.2; GenBank ID KP663713) was found at two locations in the GLS (Goro and Ouen islands) and was also removed from the analysis as an orphan haplotype. Exact test of genetic differentiation indicated that the GLS foraging ground has significantly different haplotype frequencies than either the d'Entrecasteaux or Chesterfields breeding areas ( $P = 0.008$  and  $< 0.0001$ , respectively).

The initial MSA based on the ~384 bp data from Indo-Pacific MUs suggested that the Aru stock had an estimated contribution of 21% (95% CI = 0.00–0.51) with uniform priors and 5% (95% CI = 0.00–0.28) with weighted priors. However, Aru was excluded from the final analyses because the Vanuatu

rookery shares the same common haplotype (CmP91.1) and is a more likely contributor. The Commonwealth of Northern Mariana Islands, which originally had a suggested contribution of 1.8% (95% CI = 0.00–0.21; uniform prior) was also excluded from the analysis as CmP20.1 was found in three individuals in the GLS but this haplotype was also recorded in PNG, Vanuatu, Marshall Islands and Micronesia; all stocks closer to the GLS. The final analysis included 11 MUs (Table 1). Mixed stock analyses showed that the d'Entrecasteaux MU was the most likely contributor to the foraging grounds in the GLS, with an estimated mean contribution of 65% with uniform priors (95% CI = 0.49–0.87) and 61% (95% CI = 0.45–0.77) with weighted priors that account for rookery size (Table 3). This rookery was the only one in which the 95%CI did not include zero. The second most likely source population was the sGBR stock, which had an estimated contribution of 24% (95% CI = 0.00–0.40) with uniform priors and to 24% (95% CI = 0.00–0.39) with weighted priors (Table 3). Other contributions of  $\leq 5\%$  were estimated for the MUs of the Chesterfields–Coral Sea (4–5%; 95% CI = 0.00–0.39 with a uniform prior and 0.00–0.37 with a weighted prior), French Polynesia (3–4%; 95% CI = 0.0–0.1 with a uniform prior and 95% CI = 0.0–0.09 with a weighted prior), nGBR (1–4%; 95% CI = 0.00 to <0.1, with a uniform prior and 95%CI = 0.00–0.12 with a weighted prior) and Vanuatu (3%; 95% CI = 0.00–0.07 for both priors) (Table 3).

**Table 3.** Estimates of the rookery origin of green turtles foraging in New Caledonia based on mixed stock analysis using either uniform prior or priors weighted by estimated population size of each rookery. Abbreviations are nGBR, northern Great Barrier Reef and sGBR, southern Great Barrier Reef

Rookeries	Weighted priors				Uniform priors			
	MEAN	SD	0.025	0.975	MEAN	SD	0.025	0.975
d'Entrecasteaux	0.76	0.1	0.59	0.96	0.77	0.1	0.59	0.95
sGBR	0.14	0.09	0	0.3	0.12	0.1	0	0.29
nGBR	<0.01	0.01	0	0.42	<0.01	<0.01	0	<0.1
Chesterfields + Coral Sea	0.02	0.06	0	0.33	0.02	0.02	0.05	0.21
French Polynesia	0.04	0.03	0	0.11	0.05	0.02	0	0.1
Vanuatu	0.02	0.02	0	0.06	0.01	0.02	0	0.06

## Discussion

### Identification of MUs

This study produced unexpected results showing that the two largest green turtles rookeries in New Caledonia are not part of the same MU and that the nesting population in the Chesterfield Islands is more closely linked with other rookeries in the Coral Sea. The d'Entrecasteaux nesting population, located north of the main island of New Caledonia, was identified as a separate MU from all other sampled populations in the Indo-Pacific (Dethmers *et al.* 2006; Dutton *et al.* 2014). Our increased sample size supported the original designation of the d'Entrecasteaux breeding area as an independent MU. Sampling had not been conducted at the nesting beaches in the Chesterfield Islands, which are ~465 km from the d'Entrecasteaux Islands, until this study. Surprisingly, the results indicate that the Chesterfield Islands nesting population is distinct from the d'Entrecasteaux MU, but not from the Coral Sea MU, which is ~800 km away. It is not known whether the link between the rookeries in the Chesterfield Islands and Coral Sea is due to ongoing or historic gene flow. Limited tagging of nesting females from the Coral Sea rookery and no tagging at the Chesterfield rookery, until this study, precludes the ability to document ongoing gene flow. Genetic structure and significant divergence have been recorded at multiple scales with distant rookeries (>1000 km) being part of the same MU (Dethmers *et al.* 2006; FitzSimmons and Limpus 2014), in contrast to nearby nesting beaches (<500 km) being recorded as different MUs (Dethmers *et al.* 2006; Cheng *et al.* 2008; Nishizawa *et al.* 2011). Vanuatu was shown to form a separate green turtle MU from the d'Entrecasteaux and Chesterfield–Coral Sea MUs. A large number (over 80) of green turtle nesting beaches have been reported for Vanuatu. The one sampled in this study at Bamboo Bay, Malekula Islands, is believed to be the largest, with ~25–50 females nesting annually. Other reported nesting sites are scattered up to 400 km from Bamboo Bay. Given that this study has identified Vanuatu as a unique MU, it is important that there be further sampling of other rookeries in Vanuatu to determine whether they are part of the same MU or are distinct.

## GLS foraging grounds

The largest contributor to the green turtles foraging in the GLS is the d'Entrecasteaux MU, regardless of whether rookery size was accounted for or not. This is the largest rookery in New Caledonia, with a recorded mean of 564 nesting females a night and it is located ~500 km north of the GLS (Read 2012). In contrast, the large (estimated 2800 adult females; Department of Environment and Heritage 2005; Fonfreyde 2012) Chesterfields–Coral Sea MU, located ~1000 km to the north-west, was estimated to have a low (4–5%) contribution to the GLS foraging population, and the confidence interval included zero. Instead, the second largest (>20%) estimated contributor was the sGBR, located ~1650 km across the Coral Sea. It is difficult to accurately determine the contribution of the Coral Sea rookery to the GLS due to the high genetic similarity (shared haplotypes Cmp47.1 and Cmp80.1) between the Coral Sea and sGBR populations. Smaller contributions from other MUs were suggested as providing foraging turtles to the GLS, thus connecting it further to multiple rookeries in the Coral Sea, but the estimates of minor contributions are unreliable, given the uncertainty (including zero).

The possibility of an Aru contribution to the GLS feeding ground (located >4000 km away) was based on the presence of haplotype Cmp91.1 in five individuals. This haplotype characterises (96%) samples from green turtles in the Aru, Indonesia MU, and had only been observed at very low frequencies in rookeries in Malaysia, Western Australia and the Gulf of Carpentaria (Dethmers *et al.* 2006) before this study, which recorded it at high frequency in the Vanuatu population. Thus, we can hypothesise that the individuals recorded in the GLS might, in fact, belong to the Vanuatu MU.

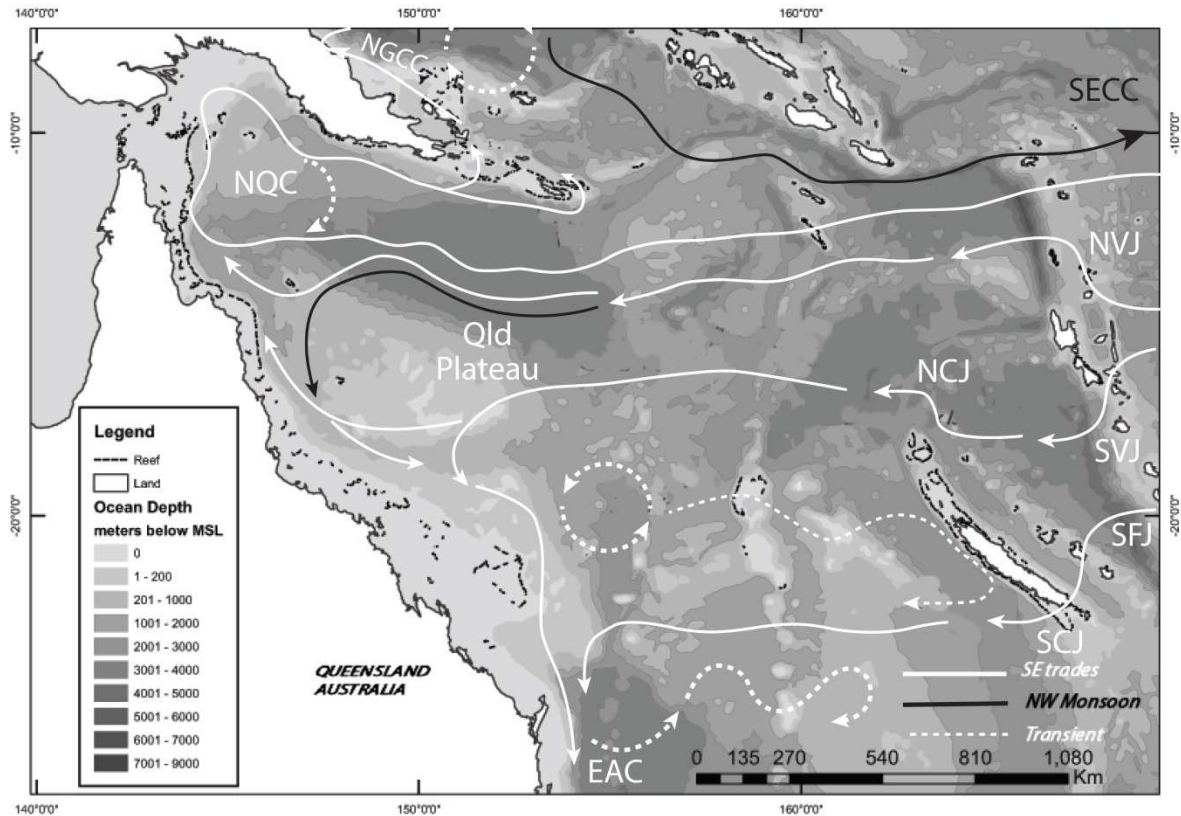
Since 1964, >40 000 individuals have been tagged in the sGBR and a similar number have been tagged in the nGBR, compared to 4700 tagged in New Caledonia (Limpus 2009; Read *et al.* 2014). Tag recovery data established that turtles from GBR rookeries use feeding grounds in New Caledonia (Limpus 2009; Read *et al.* 2014). Most (51%) of the tag recoveries in New Caledonian waters have been from the d'Entrecasteaux nesting areas and 46% from sGBR nesting turtles, and only two tag recoveries (4%) have come from nGBR rookeries (Read *et al.* 2014). The large discrepancy between

the tag recovery data and the MSA results for the estimated contribution of the sGBR is likely a sampling artefact of unequal tagging effort. Post-nesting migrations of nesting green turtles at the Coral Sea rookery ( $n = 3270$  tagged; C. J. Limpus, pers. comm.) encompass foraging areas in northern New South Wales, Papua New Guinea and throughout the GBR, but none to date have been recorded in New Caledonia (Limpus 2009). The small estimated contribution from French Polynesia has been documented previously by tag recoveries (Balazs *et al.* 1995; Read *et al.* 2014), but no tag recoveries have been made from the other possible contributors. Very recently, an individual was found nesting in the Chesterfield Islands bearing a tag from a foraging ground in Hervey Bay, in the central Queensland coast (Read *et al.* 2014), thus adding this rookery to records of breeding migrations of individuals between New Caledonia and Australia as reported in other studies (Limpus 1992; Read *et al.* 2014). Additionally, a satellite tagged nesting female from Vanuatu, that had the Cmp91.1 haplotype (K. MacKay, unpubl. data) travelled to a foraging ground in New Caledonia (Read *et al.* 2014), thus strengthening the hypotheses that the Vanuatu rookery is providing turtles with the 'Aru' haplotype to the GLS. Five of the observed haplotypes in the GLS were orphans, not yet identified from any rookery. A large rookery in New Caledonia was not tested within this study (Beautemps-Baupré, Island of Ouvéa; Pritchard 1987) and many sand cays in the region could be potential nesting areas for green turtles (C. J. Limpus, pers. comm.), thus a gap in the genetic sampling exists.

Origins of juvenile marine turtles at feeding grounds are largely the result of oceanic currents that facilitate the dispersal of post-hatchlings to epipelagic foraging areas and their journey back to benthic feeding grounds (Bolten 2003a; Okuyama *et al.* 2011; Mansfield *et al.* 2014). Sub-adult and adult turtles may show strong fidelity to specific foraging grounds throughout their life, as seen in the GBR (Limpus *et al.* 1992), but the possibility exists that green turtles in New Caledonia could shift foraging grounds as they develop. For at least the juvenile turtles at the GLS foraging grounds, the expectation is that d'Entrecasteaux post-hatchlings would be pushed to the west with the North Caledonian jet, which is part of the south equatorial current (SEC), which splits after it reaches the

Queensland continental shelf (Ganachaud *et al.* 2007). The effect of this current on post-hatchling dispersal is suggested by a genetic study to determine the origins of green turtles foraging along the Queensland coast (Jensen *et al.* in press). For juvenile (35–65 cm CCL) turtles, contributions of 4.5–13.8% were estimated from the d’Entrecasteaux MU, at foraging grounds located from –20°S and further south, but no contributions were indicated at foraging grounds at –14°S and further north. Southwards travel along the Queensland coast would also occur for sGBR post-hatchlings, which are transported south and east in the east Australian current, as suggested for loggerhead (*Caretta caretta*) and green turtles (Limpus *et al.* 1992; Boyle *et al.* 2009), although the extent to which green turtles travel eastwards in this current before turning north is unknown and would be influenced by the northern Tasman currents (Ridgway and Dunn 2003). Possible other sources for GLS foraging turtles were the nGBR, Chesterfields–Coral Sea, Vanuatu and French Polynesia stocks, all of which had estimated mean contributions estimated at  $\leq 5\%$ . Post-hatchling turtles from Vanuatu and the Chesterfields–Coral Sea would likely be carried westwards by the north Vanuatu jet, North Caledonia jet or south Vanuatu jet (Ganachaud *et al.* 2007) towards Australia to cycle counter-clockwise back towards New Caledonia, or they may travel in more localised eddies in the region. Westwards travel in the SEC could explain the contribution from French Polynesia, but it is also possible that these individuals could have come from American Samoa based on haplotype frequencies (Dutton *et al.* 2014). Even though the nGBR is the largest rookery in the South Pacific (Limpus 2009), it is also situated >4000 km from the GLS, so a combination of distance, current patterns and post-pelagic orientation is likely contributing to the estimated low representation of the nGBR in the GLS. Oceanic currents would take many of the post-hatchling nGBR turtles north within the New Guinea coastal current (Fig. 3), as was observed in the distribution of prawn and lobster larvae originating from northern Torres Strait and located south of Papua New Guinea (MacFarlane and Guinea 1980). This is due to the location of the nGBR rookeries, which are north of where the SEC splits as it approaches the east Australian coast (Ganachaud *et al.* 2007). Further studies are needed to understand the migration patterns of post-hatchlings during their epipelagic phase in the Coral Sea.





**Figure 3.** Ocean currents in the Coral Sea abbreviated as follows: EAC, east Australian current, NQC: north Queensland current, NCJ: north Caledonian jet, SCJ: south Caledonian jet, SFJ: south Fiji jet, SVJ: south Vanuatu jet, NVJ: north Vanuatu jet, SECC: south Equatorial countercurrent, NGCC: New Guinean coastal current

## Conservation implications

The findings of this study are important for the conservation of green turtles in the South Pacific, both in terms of informing conservation efforts in New Caledonia and in emphasising the need to link management actions with other countries. We have recorded that one of the largest feeding grounds in New Caledonia is populated mostly from the largest breeding area in New Caledonia. From a management perspective there is the advantage that both of these areas are World Heritage sites and located in the same country. Consequently, we discovered an important recruitment process at the regional (country) scale, which has fundamental management implications for a species that often has cross-boundary population dynamics in the Indo-Pacific (FitzSimmons and Limpus 2014). Added

protection was granted when the d'Entrecasteaux Islands became a national park in 2013 (SMMPM 2013). The confirmation of this rookery as a separate MU and the field data collected over the years at this breeding area should help us understand the conservation threats due to overharvesting even though many gaps in our knowledge need to be addressed at nesting beaches (e.g. nesting census, emergence success, breeding periodicity) and at the foraging grounds (population demographics). Additional foraging grounds (other than the GLS) were identified in New Caledonia by tag recovery data from traditional hunters (Read *et al.* 2014), but studying post-nesting migrations should be a priority. Contributions from the d'Entrecasteaux MU to the aggregations of foraging green turtles off eastern Australia additionally means that these turtles are susceptible to unquantified levels of Indigenous harvest at some of their Queensland foraging grounds.

The Chesterfield Islands are a very important breeding area for green turtles in the Coral Sea, by its central but remote geographical location that links it to the Coral Sea MU, and the presence of shared haplotypes suggested it may have functioned as a stepping stone between the GBR and New Caledonia MUs. The link between the Chesterfields and Coral Sea could be due to ongoing gene flow between rookeries or may reflect historical gene flow from colonisation as sea levels have changed and made new nesting areas available. The Coral Sea stock is estimated to have only a couple of hundred individuals nesting per year (Limpus 2009) but the Chesterfield Islands rookery has an estimated 100–1000 nesting females per year (Fonfreyde 2012). Additional tagging studies are needed to investigate the possibility of ongoing gene flow between these rookeries.

The MSA results indicate that the Indigenous tribes of the GLS harvest turtles that mostly originate from a protected rookery in New Caledonia, possibly making it easier to implement conservation strategies at a local scale, but some targeted turtles come from various international rookeries, suggesting that international collaboration needs to be implemented between the island nations of the South Pacific. This scenario can be positive in terms of conservation and management of the resource. Localised migrations between breeding and feeding grounds have also been reported in the

Gulf of Carpentaria, which is being managed with the involvement of the local Indigenous communities (Kennett *et al.* 2004). The management of a MU within one country has a direct impact compared with a scattered MU with multiple stakeholders. In New Caledonia, large individuals are being targeted for ceremonies and the number of adults in the GLS foraging ground is low, thus hunters have to go further from their tribes to find the adults (T. C. Read, pers. comm.). We recommend that community-based turtle management strategies be established that consider the impacts of harvest on the source populations, particularly to prevent the loss of mature adult green turtles from this World Heritage area.

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## CHAPTER 4 BIBLIOGRAPHIC DETAILS

**Title:** A two-method approach to identify the foraging patterns of *Chelonia mydas* in New Caledonia

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### Statement of contribution to co-authored published paper

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My contribution to the paper involved, all data collection, the preliminary analysis and categorisation of the data into a usable format and providing direction on the scope and structure of the analysis.

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## CHAPTER 4: A TWO-METHOD APPROACH TO IDENTIFY THE FORAGING PATTERNS OF *CHELONIA MYDAS* IN NEW CALEDONIA

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### Abstract

Opportunities to look at entire stomach contents of marine megafauna are scarce as it is a lethal method. Here we used stable isotopes analysis (n= 179) combined with identification of entire gut contents from green turtles (*Chelonia mydas*) (n=21) slaughtered for tribal ceremonies from the Grand Lagon Sud, a World Heritage Area located in the south of New Caledonia.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in skin samples ranged from -19.3‰ to -7.3‰ and 2.8‰ to 15.9‰ respectively. These results indicate a preference for an algal diet and concur with stomach contents analysis of which four algae genera contribute 50.4% of the total dry weight: *Hypnea* (20.1%), *Ulva* (12.4%), *Caulerpa* (9.1%), *Codium* (8.8%). A significant difference in  $\delta^{13}\text{C}$  was found within the juveniles caught at the four different sites. The juveniles caught at Ouen Island, Uo/Mato Islands and Goro had much higher  $\delta^{13}\text{C}$  signatures compared to the juveniles caught in the Isle of Pines, thus indicating a potential difference in trophic level. These results provide continuing evidence of differing feeding patterns for *C. mydas* between foraging grounds and the need for intra-specific studies in each foraging ground in order to implement an efficient management of the species.

**Key words:** sea turtle, stable isotopes analysis, digestive track examination

### Introduction

The importance of dual protection of endangered species such as *Chelonia mydas* as well as its habitat is self-evident when “two major causes of a species extinction are hunting and destruction of natural habitat” (Darin 2000, Hoekstra et al. 2005). Identifying critical habitats such as the foraging habitat and associated feeding patterns of endangered species is important for implementing an effective conservation strategy. In the past, foraging patterns of migratory marine megafauna were



challenging to identify and studies were usually done with a small number of individuals (Erftemeijer et al. 1993; Lick and Piatkowski 1998) or over long-term periods (Fernandez et al. 2009; Marsh et al. 1982) and were mostly achieved with stranded animals (Alonso et al. 1999, Santos et al. 1999, Santos et al. 2001). Nonetheless these studies have been essential to understanding many feeding patterns as animal welfare law developed (Ealey 1954; Gans and Pooley 1976; Konishi et al. 2009; Lagler 1943). The sacrifice or significant injury of individuals for research is often no longer acceptable, particularly for emblematic, rare and species endangered, thus alternative methods have emerged to study feeding and diets (e.g. regurgitating sea kraits *Laticauda* spp.: Brischoux et al. 2007; Séret et al. 2008) and the use of biopsy sampling needles for internal tissue sampling (Gyuris and Limpus, 1986). In regards to marine turtles, gastric lavages have been used in many studies in order to investigate the recently ingested food (Brand-Gardner et al. 1999; Forbes and Limpus 1993; Fuentes et al. 2006). Stable isotope analysis has emerged as an alternative non-lethal method for diet analysis in many studies (Peterson and Fry 1987). SIA is used in different types of studies around the world and with different goals. Firstly, “the delineation of diets or trophic relationships and sources of nutrients to individuals or populations, secondly the assessment of the relative contributions of endogenously and exogenously derived nutrients to reproduction in birds that travel to breed, and, more recently, the assignment to origin of migratory individuals” (Hobson 2011).

*C. mydas* have a complex life cycle and have been found, using SIA, to shift diet at different life stages (Arthur et al. 2008). Individuals that have not recruited back to shore yet are known to feed on a wide range of planktonic material found in pelagic waters (Bolten 2003). That pelagic phase has been referred at as “the lost years” as a limited amount of information is available due to the difficulty to study the individuals during that interval of time (Carr 1952; Putman et al. 2013). A study, using SIA on the scutes, was able to determine a timeframe for that phase for *C. mydas* that recruit in the Bahamas (Reich et al. 2007). SIA found in tissue of animals in foraging grounds as new recruits can indicate a previous feeding location (Reich et al. 2007). Spatially explicit predictions of elemental isotope ratios across a geographical area in a particular matrix are called ‘isoscapes’ (Seminoff et al.

2012). The new recruits then integrate an inshore habitat, at about 40 cm and between 5-6 years old (Limpus and Chaloupka 1997), diet changes and they tend to be more herbivorous feeding mostly on seagrass and algae (Bjorndal 1997; Limpus et al. 2005).

Multiple rookeries for *C. mydas* have been impacted in the last century (Frazier 1980; Parsons 1962; Witzell 1994). Habitat loss, with overharvesting are why *C. mydas* is currently on the International Union for the Conservation of Nature red list as an endangered species (IUCN 2010). *C. mydas* are known to migrate from Australia into New Caledonian waters, either to feed or to nest (Limpus et al. 1992; Read et al. 2014). No studies document the trend in numbers of nesting turtles in New Caledonia but records from an aerial survey from February 1980 (Pritchard 1982) and tales of a sailing vessel in September 1856 (Chimmo 1856) indicate a decline in the number of nesting females at d'Entrecasteaux atolls which is, to this day, the most important nesting site for *C. mydas* in New Caledonia (Read 2012). This breeding area has been identified as one of the 30 independent genetic stock of the Indo-Pacific (Dethmers et al. 2006; FitzSimmons and Limpus 2014; Jensen 2010; Read unpublished data) and has been found to be the most likely provider for one the largest feeding grounds for *C. mydas* in New Caledonia, the Grand Lagon Sud (Read et al. submitted). Multiple studies identify the specific prey species on which *C. mydas* feed, once they have recruited to the coastal area, but important differences were reported between study areas (Arthur et al. 2009; Limpus and Limpus 2000; Mortimer 1981). Grazing doesn't occur randomly, the species re-crops areas on which they later feed on to get more digestible, higher protein and lower in lignin leaves (Bjorndal 1980). The diet of herbivores can be modified with time but a short-term change in feeding behavior would seem inefficient as the hindgut microflora would not be adapted to the new type of food to be processed (Bjorndal 1985). In addition, diet selectivity was reported to be based on "availability and/or preference" for marine herbivores and specifically in *C. mydas* (Garnett et al. 1985; Horn 1989; Mortimer 1981; Ross 1985). With these findings and the numerous studies that account for foraging site fidelity for that species (Limpus et al. 1992; Plotkin 2002; Speirs 2002), it can be hypothesised that without a change in the habitat at their foraging site, *C. mydas* are bound to

keep the same feeding patterns if the preferred forage area is available, thus the preferred forage needs to be identified, mapped and protected.

Prior to the present study, no data were available on the foraging patterns of sea turtles in New Caledonia. Here we use the indirect SIA method on foraging individuals and the direct examination of stomach contents from individuals killed for tribal ceremonies in order to address this critical issue. The use of this two-faceted approach highlights the specifics of the diet in the actual study site. Both techniques combined have been used on multiple species (Dehn et al. 2007; Harrigan et al. 1989; Ruiz-Cooley et al. 2006) including *C. mydas* (Burkholder et al. 2011; Cardona et al. 2009; González Carman et al. 2014; Lemons et al. 2011; Williams et al. 2014) but to our knowledge, this study is the first to use SIA and stomach content examinations on *C. mydas* that are used for tribal ceremonies. As such this study will facilitate the understanding of the habitat use and the protection of that species while creating a link between science and indigenous culture.

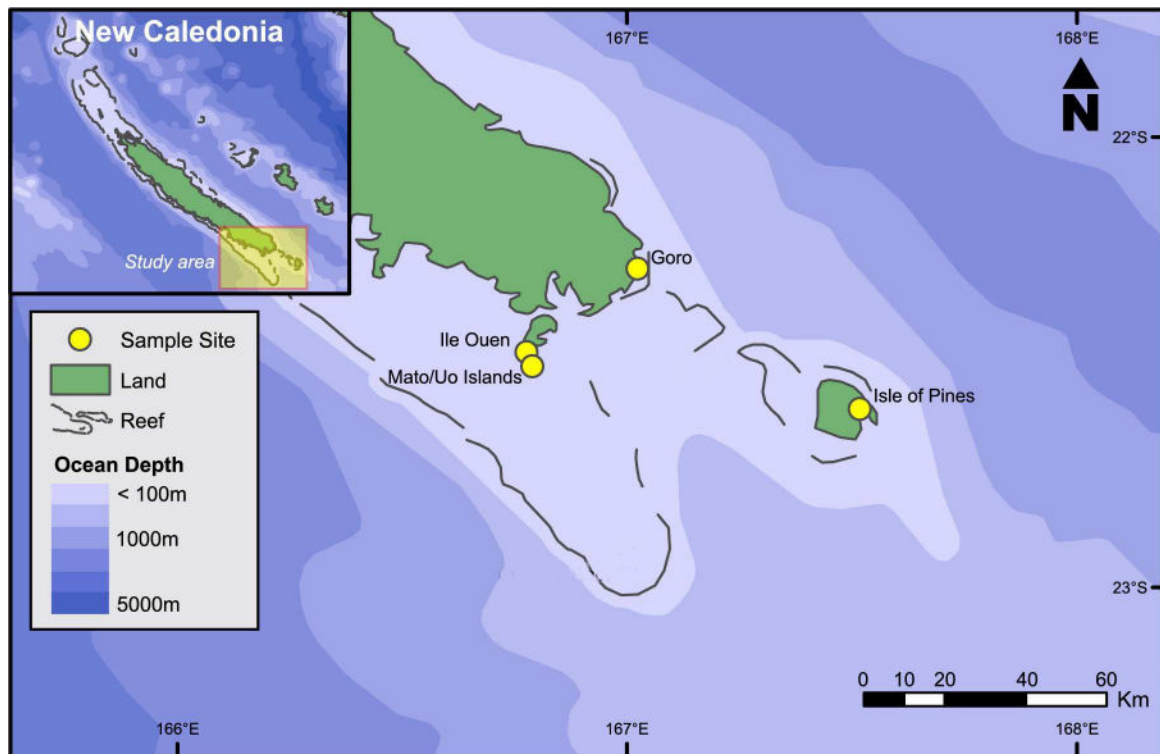
## **Methods**

This study was conducted in the Grand Lagon Sud (GLS) (Figure 1) of New Caledonia (22°30'S, 167°57'E) which supports a recognised foraging grounds for *C. mydas* (Menu and Hebert 2006, Mounier 2007). GLS is a World Heritage site in good health characterized by a large reef diversity and the presence of many endangered species. Sea turtles are a protected species in New Caledonia (N°4-2009/APS) but permits to hunt *C. mydas* are granted for traditional ceremonies. Data collection occurred between August 2012 and March 2014.

### **Turtle capture**

A total of 200 skin samples were examined in this study. One hundred and seventy-nine individuals were captured using the turtle rodeo method (Limpus and Reed 1985) in four areas of the GLS: Goro (n = 68), Ouen Island (n = 47), Uo/Mato Islands (n = 59) and Isle of Pines (n = 26) between August 2012 and March 2013. All individuals caught were tagged with a titanium tag (Limpus 1992),

measured (CCL), sexed when possible using external morphology and a skin sample was taken. The turtles were released in the vicinity of their respective capture sites. Twenty-one *C. mydas* that were caught and killed by tribes in the GLS (turtle rodeo method) for traditional ceremonies Goro (n = 13), Ouen Island (n = 3) and Isle of Pines (n = 5) between March 2013 and March 2014 were also sampled. CCL was recorded when possible. From these tribe killings, 21 stomach contents could be examined.



**Figure 1.** Foraging areas used to sample *C. mydas* feeding patterns in New Caledonia

The individuals were divided into four categories depending on their size according to Limpus et al. (1994) and Limpus and Chaloupka (1997). This included; new recruits (20-65 cm curved carapace length (CCL) with a very white plastron and very low algae presence on the carapace), juveniles (between 20 and 65 cm CCL) with a yellow/dicoloured plastron, sub-adults (65 and 90 cm CCL) and adults (90 cm CCL and plus). The juveniles, sub-adults and adults are presumed to be long term residents at their capture sites.

### Sample collection and preparation

Epidermal tissue ( $\sim 1 \text{ cm}^2$ ) was collected from the hind flipper of *C. mydas* using a scalpel from 179 turtles collected using the first method of capture and 21 turtles caught for tribal ceremonies. All tissue samples were placed in 90% NaCl for storage (see Arthur et al. 2008). Before stable isotopes analysis ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ), they were rinsed with distilled water and dried at  $50^\circ\text{C}$  for 48 hours prior to being ground (with a mortar and pestle) to a fine powder (Diocares 2014). The analysis was undertaken at Griffith University Stable Isotope Analysis Laboratory using a sample preparation system (Sercon Europa EA-GSL) and an isotope ratio mass spectrometer (Sercon Hydra 20-22). Stomach contents were collected from animals ( $n = 21$ ) that were left alive for up to five days before being killed. The samples were collected as the turtles were killed and the carcasses prepared for sharing of the meat among community members.

### **Sample analysis**

Statistical significance was determined as 0.05 and all results are presented as mean  $\pm$  SD. One-way ANOVA or Kruskal-Wallis analysis of variance, when One-way ANOVA assumptions were not satisfied, were used to compare  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ratios recorded in the four size categories in this study. We used multiple studies to identify the possible food source corresponding to the results found (Arthur et al. 2008; Carseldine and Tibbetts 2005; Ceriani et al. 2012; Connolly 2003; Grice et al. 1996; Guest et al. 2004, Hatase et al. 2002; Lemons et al. 2011; Melville and Connolly 2005; Takai et al. 2000, Udy and Dennison 1997; Werry and Lee 2005).

The method used for collecting and storing the stomach contents follows Garnett et al. (1985) except that subsamples were dried at  $70^\circ\text{C}$  for 48 hours (samples were completely dried when removed). Because 9% of the contents were not identifiable and 54% could not be identified to the species level, food species were identified to genus level where possible and aggregated to taxonomic groups as follows: seagrasses and animal material, while algae were grouped by division [Chlorophyta (green algae), Phaeophyta (brown algae) and Rhodophyta (red algae)]. A PERMANOVA was applied to the

data (with area and category as factors) in order to compare the feeding patterns of the different *C. mydas*.

## Results

### Stable isotopes analysis

A total of 200 samples (including both methods of collection) were used in this stable isotopes analysis from four different life stages (Table 1). The CCL of turtles ranged from 38.0 to 114.2 cm ( $n = 188$ ; mean  $\pm$  SD =  $52.1 \pm 13.9$ ). The isotopic signature of  $\delta^{13}\text{C}$  varied significantly across the different life stages sampled (One-way ANOVA,  $F = 9.83$ ,  $df = 3$ ,  $P = <0.03$ ). Juveniles and new recruits were not significantly different but both were significantly lower than the adults (Tukey's HSD). Sub-adults were not significantly different to other life stages (Table 1). No significant differences were found in the isotopic signatures of  $\delta^{15}\text{N}$  between the different life stages tested in this study.

**Table 1.** Carbon and nitrogen stable isotope signature of feeding *C. mydas* collected from different life history stages in New Caledonia

Life stage	n	Epidermal stable isotopic signature			
		$\delta^{13}\text{C}$ (‰)		$\delta^{15}\text{N}$ (‰)	
		Mean	Range	Mean	Range
<b>New recruit</b>	15	-11.6	-14.5 to -8.6	8.9	3.2 to 14.3
<b>Juvenile</b>	159	-12.7	-18 to -7.3	7.8	3.7 to 15.9
<b>Sub-adult</b>	7	-13.6	-18.4 to -7.8	7.9	2.8 to 10.8
<b>Adult</b>	19	-14.5	-19.3 to -8.1	7.9	4.2 to 12.9

The  $\delta^{13}\text{C}$  signatures were not significantly different between sites for the adults (Kruskal-Wallis). This was also the case for sub-adults (Goro had to be removed as only one individual was sampled from that life stage). A significant difference was found within the juveniles caught at the four different sites (One-way ANOVA,  $F = 69.00$ ,  $df = 3$ ,  $P < 0.01$ ). The juveniles caught at Ouen Island, Uo/Mato Islands and Goro had much higher  $\delta^{13}\text{C}$  signatures compared to the juveniles caught in the Isle of Pines. New recruits were only found in one area (Goro), thus no comparison could be done.

The isotopic signature of  $\delta^{15}\text{N}$  between all adults and sub-adults caught were not significantly different between sites (Kruskal-Wallis, respectively  $P = 0.467$  and  $P = 0.319$ ). The  $\delta^{15}\text{N}$  isotopic

signature of juveniles was significantly different between areas (Kruskal-Wallis,  $F = 14.55$ ,  $df = 3$ ,  $P < 0.003$ ). Juveniles from Goro and Uo/Mato had similar isotopic signatures compared to the juveniles caught at Ouen Island but juveniles caught at Isle of Pines were not significantly different to the other sites (Tukey's HSD).

## Stomach contents

Five plant genera contribute the most (91.7%) to the total dry weight matter. These were algae *Hypnea* (20.1%; Rhodophyta), *Ulva* (12.4%; Chlorophyta), *Caulerpa* (9.1%; Chlorophyta), and two seagrasses *Halodule* (26.9%) and *Cymodocea* (14.5%). Algal species predominated (65%) in comparison to seagrasses (24%), with the balance being from the Kingdom Animalia (6.7%) (Table 2). The analysis of stomach contents indicated significant differences between areas in terms of food content groupings (PERMANOVA,  $F = 3.34$ ,  $df = 5$ ,  $P < 0.001$ ). Ouen Island significantly differs from the other areas with a majority of food items recovered belonging to the seagrass species.

**Table 2.** Composition of *C. mydas* stomach contents analysed from foraging grounds in the GLS (New Caledonia)

Food type	Number of stomachs			Percentage of dry matter			Total
	Total	With >5% DM	With >50% DM	Goro (n=14)	Ouen Island (n=3)	Isle of Pines (n=5)	
<b>Chlorophyta</b>							
Codiales							
<i>Codium</i> sp.	5	4	1	20.5	0	6	8.8
Caulerpales							
<i>Caulerpa serrulata</i>	1	1	0	0.7	0	0	0.2
<i>Caulerpa urvilleana</i>	11	10	1	18.5	0	8.8	9.1
Udotea sp.	1	1	0	0	2.7	0	0.9
Ulvaes							
<i>Ulva</i> sp.	8	5	3	13.7	0	23.6	12.4
Total Chlorophyta	14	9	5	39.7	2.7	38.4	<b>26.9</b>
<b>Rhodophyta</b>							
Gigartinales							
<i>Hypnea</i> sp.	17	10	7	44.0	0	16.2	20.1
Ceramiales							
<i>Ceranium</i> sp.	0	0	0	0	0	tr	tr

Total Rhodophyta	17	10	7	44.0	0	16.2	<b>20.1</b>
<b>Phaeophyta</b>							
Fucales							
<i>Turbinaria ornata</i>	3	3	0	3.1	0	0	1.0
<i>Sargassum lacerifolium</i>	6	6	0	2.7	0	2.6	1.8
Dictyotales							
<i>Dictyota sp.</i>	3	3	0	0.9	0	0	0.3
Total Phaeophyta	8	8	0	7.6	0	2.6	<b>3.4</b>
<b>Total algae</b>	<b>18</b>	<b>1</b>	<b>17</b>	<b>91.3</b>	<b>2.7</b>	<b>57.2</b>	<b>50.4</b>
<b>Seagrass</b>							
<i>Cymodocea serrulata</i>	6	4	2	1.3	14.7	27.4	14.5
<i>Halodule uninervis</i>	5	3	2	0.2	68	12.4	26.9
<b>Total seagrass</b>	<b>7</b>	<b>2</b>	<b>5</b>	<b>1.4</b>	<b>82.7</b>	<b>39.8</b>	<b>41.3</b>
<b>Total plants</b>	<b>21</b>	<b>21</b>	<b>21</b>	<b>92.1</b>	<b>85.4</b>	<b>97.0</b>	<b>91.5</b>
<b>Animals</b>							
Porifera	3	3	0	1.2	8.7	0	3.3
Medusozoa	5	5	0	3.3	7	0	3.4
<b>Total animals</b>	<b>7</b>	<b>8</b>	<b>0</b>	<b>4.5</b>	<b>15.7</b>	<b>0</b>	<b>6.7</b>

## Discussion

### Between life stages

The results show that *C. mydas* predominantly feed on algae even when seagrass is available which contrasts with the results from a study done at Green Island on the Great Barrier Reef reporting a dominance of seagrass (> 81% of volume) as a food type (Fuentes et al. 2006). However a similar pattern has been described in Brand-Gardner et al. (1999) in a study in Moreton Bay (Australia). The low number of stomach contents from juveniles in this study did not allow for differentiation of feeding patterns within life stages but > 50% of the volume of all stomach contents were algae.

$\delta^{15}\text{N}$  signatures were not significantly different between the life stages sampled. A study done in Moreton Bay (Australia) showed that new recruits have significantly higher levels of  $\delta^{15}\text{N}$  compared



to the other life stages (Arthur et al. 2008). This is explained by an ontogenetic shift in diet, juveniles at sea feed on a different array of food (carnivorous) compared to when they have recruited to the coastline (herbivorous) which changes their place in the food web (Minagawa and Wada 1984). This difference was also found in another study done in the Bahamas using scute samples (Reich et al. 2007). The low number of new recruits used in this study and the fact that they were all captured in the same area could possibly explain the different pattern reported in this study. Another hypothesis is the difference in time frame between the retention of stable isotopic signatures in scutes and epidermis samples (Seminoff et al. 2006b). More sampling should be done on new recruits and sub-adults in order to verify these levels of  $\delta^{15}\text{N}$ . On the other hand, the levels of carbon found in epidermal tissue were significantly different between adults and juveniles of the *C. mydas* sampled. As the individuals grow older, the levels of  $\delta^{13}\text{C}$  lowered. This pattern is contrary to the literature. Adults and sub adults used in this study had similar  $\delta^{13}\text{C}$  signatures so it is highly probable that they have been foraging on similar carbon sources for an extended period of time. A pattern of fidelity to a feeding area has been recorded by many studies using the mark-recapture method around the world (Chaloupka et al. 2004; Limpus et al. 1994; Limpus et al. 1992) for *C. mydas* as well as for other species of sea turtles (Broderick et al. 2007; Limpus 1989; Schofield et al. 2010). This hypothesis could explain the results found in NC.

The discrimination level for  $\delta^{13}\text{C}$  is +0.17 ‰ in *C. mydas* epidermal tissue (Seminoff et al. 2006b). Our results indicate that all the individuals caught in this study potentially forage mostly on macroalgae and seagrass (Table 3). These results concur with those reported in many studies regarding *C. mydas* (Lanyon et al. 1989, Forbes 1994, Read et al. 1996, Brand-Gardner et al. 1999, Speirs 2002) and are also verified by the stomach contents used in this study. This shows that the short term feeding (6-13 days) (Brand et al. 1999) examined in the stomach contents concurs with the longer-term SIA results (45 days to several months) (Reich et al. 2007, Seminoff et al. 2006b, Vander Zanden et al. 2014). An SIA should be done in near future on the identified prey items in order to confirm the results.

**Table 3.** List of potential food sources for *C. mydas* in the GLS and their stable isotope ranges from different studies worldwide

Food source	Location	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Source
Seagrass	Moreton Bay ,Australia and San Diego Bay, USA	-15.4 to -6.6	2.2 to 10.4	Arthur et al. 2008; Carseldine and Tibbets 2005; Connolly 2003; Grice et al. 1996; Guest et al. 2004; Melville and Connolly 2005; Lemons et al. 2011; Udy and Dennison 1997
Macroalgae	Moreton Bay ,Australia and San Diego Bay, USA	-20.1 to -15.7	4 to 12.5	Connolly 2003; Guest et al. 2004; Lemons et al. 2011
Mangrove	Moreton Bay ,Australia	-29.0 to -17.0	0.7 to 5.8	Arthur et al. 2008; Melville and Connolly 2003; Werry and Lee 2005
Zooplankton	Japan and Moreton Bay Florida, USA	-21.3 to -14.1	4.8 to 16.3	Arthur et al. 2008; Hatase et al. 2002; Takai et al. 2000
Crustacean and molluscs	San Diego Bay, USA	-17.8 to -15.4	13.8 to 15.8	Ceriani et al. 2012; Lemons et al. 2011
Tunicates and porifera	San Diego Bay, USA	-22.9 to -21.3	2.1 to 3.7	Lemons et al. 2011

## Between areas

The only individuals that had over 80% of their content represented by different seagrass species are the individuals from Ouen Island ( $n = 3$ ). The other individuals mainly feed on multiple species of algae. This result concurs with the  $\delta^{15}\text{N}$  ratios thus showing that the individuals from Uo/Mato Island, Goro and Isle of Pines have a similar feeding pattern. Chlorophyta species are the most preyed on by the individuals of Goro and Isle of Pines. More samples are needed to compare the feeding habits of *C. mydas* in that area.

Results show significant differences between landing areas (ie. tribes) with both SIA and stomach contents but the exact area at which the individuals are caught for the tribal ceremonies is difficult to know. However, the stomach contents from Goro and some individuals from Isle of Pines appear to come from very similar feeding grounds compared to the individuals caught by the tribe of Ouen

Island and other individuals caught for Isle of Pines. A hypothesis for this result could be that they have been hunted in related areas or one same area.

Furthermore, the preference of GLS *C. mydas* for algae has been found in other studies. For instance, *Hypnea* sp. was also a preferred food type for *C. mydas* in Torres Strait (Garnett et al. 1985) which does not support the hypothesis that *C. mydas* feed mostly on sea grass at their foraging site and on algae during travel (Mortimer 1981). The stomach contents from the individuals from Ouen Island were mostly seagrass (> 80%) and a large seagrass pasture was identified where the turtles were caught. This area should be recognized as significant for this species within the region.

Turtles that have been in the foraging grounds longer have lower  $\delta^{13}\text{C}$  compared to new recruits and juveniles. This result is in contrast with the results recorded in a similar study done in Australia (Arthur et al. 2008) which report an opposite trend.

The ingestion of animal material by *C. mydas* has been hypothesised to be incidental in multiple studies (Brand-Gardner et al. 1999; Mortimer 1981; Read and Limpus 2002) and this contrasts with a number of recent studies that report a voluntary ingestion of this food item (Arthur et al. 2007; González Carman et al. 2014; Heithaus et al. 2002; Seminoff et al. 2006a; Williams et al. 2014). In this study, animal material was found in seven stomach contents. More data is needed in order to investigate the likely occurrence of this prey item in the GLS. A certain amount of animal material is frequently found in stomach contents of *C. mydas* and it could be a more important part in the diet of this species than currently understood. Due to the high digestibility of this prey item compared to plant material, the percentages found are usually low compared to sea grass or algae (Arthur et al. 2007; Heithaus et al. 2002). Mixed diet was also reported in a previous study using *C. mydas* caught by indigenous fisheries (André et al. 2005). From the results of the SIA and the stomach content examination, it can be assumed that *C. mydas* in New Caledonia feed on more than one trophic level thus supporting the hypothesis for an omnivorous diet rather than an herbivorous one. However, the use of SIA to decipher dietary complexity in a predator is only possible if dietary items differ appreciably in their isotope values (Jones and Seminoff 2013). This is the first study to investigate the

feeding ecology of *C. mydas* in New Caledonia and our work provides a “current-condition” baseline in that area that is being used for mining. This collaboration with the different tribes is also the first step towards an integrated management of the resource and could be used as an example for the rest of the South Pacific.

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## CHAPTER 5 BIBLIOGRAPHIC DETAILS

**Title:** Horizontal and vertical movement patterns of *Chelonia mydas* in southern New Caledonia

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### Statement of contribution to co-authored published paper

This chapter includes a co-authored paper. The bibliographic details (if published or accepted for publication)/status (if prepared or submitted for publication) of the co-authored paper, including all authors, are:

Read TC, Wantiez L, Werry JM, Keller F, Chateau O, Farman R, Limpus CJ. Horizontal and vertical movement patterns of *Chelonia mydas* in southern New Caledonia.

My contribution to the paper involved, all data collection, the preliminary analysis and categorisation of the data into a usable format and providing direction on the scope and structure of the analysis.

(Signed) \_\_\_\_\_ (Date) 5/05/2015

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## CHAPTER 5: HORIZONTAL AND VERTICAL MOVEMENT PATTERNS OF *CHELONIA MYDAS* IN SOUTHERN NEW CALEDONIA

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### Abstract

Understanding the movement patterns of an endangered population is critical for the implementation of an effective management and conservation strategy. Here we present horizontal and vertical movement for juvenile and adult green turtles (*Chelonia mydas*) (n=16) in the Grand Lagon Sud (GLS) of New Caledonia. We used a combination of satellite tags and time-depth recorders to identify both horizontal and vertical habitat use. Seven turtles, three adults and four juveniles (43-113.5 cm Curved Carapace Length (CCL) were tracked via satellite tags for 111 to 221 days. Time-depth recorders were placed on ten juveniles (41.2 - 61.1 cm CCL) for periods of 14 to 221 days. All satellite tagged individuals exhibited localized movement within the GLS with a mean 95% convex polygon home range of  $54.28 \pm 2.42 \text{ km}^2$ . Individuals with depth tags (n=10) spent 80% of their time at < 5 m with a maximum depth of 18 m. Juvenile *C. mydas* displayed diel behavior with a higher number of dives during the day and shallower dives during the night. Combined these movement data indicate that shallow waters are important for juvenile life-history stages of *C. mydas* in the GLS.

### Introduction

Since 2004, *Chelonia mydas* is listed as an endangered species on the International Union for the Conservation of Nature red list. This is due to a 48-67% decline in the annual number of mature females nesting over three generations (IUCN 2010). This alarming drop in abundance is related to multiple factors that include overharvesting of eggs and females at nesting beaches, overexploitation of individuals at their foraging areas and habitat loss (Limpus 1997). Studies conducted at nesting ground studies represent the majority of the literature on sea turtles and a gap in in-water data has been reported (Bjorndal 1999). *C. mydas* occur in tropical and sub-tropical waters around the globe

(Bowen et al. 1992) and display a complex life cycle strategy. They are known to nest on sandy beaches and hatchlings disperse to pelagic waters for a period of five to six years in the South Pacific (Limpus et al. 1994; Limpus and Chaloupka 1997). Beyond the pelagic phase, juvenile *C. mydas* recruit to neritic waters (Limpus and Chaloupka 1997; Bjorndal et al. 2000; Limpus et al. 2005). The mechanisms which control the choice of recruitment to foraging ground are still to be understood but one hypothesis states that foraging sites reflect passive drift experienced by hatchlings in the pelagic waters (Hays et al. 2010). After this move from oceanic to neritic waters, a shift in diet occurs from omnivorous to a mainly herbivorous regime (Bjorndal 1997; Reich et al. 2007). New recruits to a foraging ground are recognizable by their white plastron and protruding ventral ridges (Limpus et al. 2005; Arthur et al. 2008). Upon sexual maturity, aged between 25 and 50 years old depending on food availability (Chaloupka et al. 2004), both males and females migrate (once every two to five years) back to the area where they were born in order to reproduce. This behavior is often referred to as “natal homing” (Carr 1967; Meylan et al. 1990). These migrations last for months at the time, depending on the distance between the nesting and foraging sites but the individuals often return to their foraging site (Mortimer and Carr 1987; Limpus et al. 1992, 2005; Broderick et al. 2007, Read et al. 2014).

Foraging populations are known to support “mixed stocks” (Lahanas et al. 1998), with individuals found in a foraging area belonging to different genetic stocks (Moritz et al. 2002). Thirty independent genetic stocks for *C. mydas* have been identified in the Western Pacific (Jensen 2010; Fitzsimmons and Limpus 2014). The genetic diversity of multiple foraging grounds of *C. mydas* in the South Pacific has been studied but gaps have been identified (Fitzsimmons and Limpus 2014). A study done in the Grand Lagon Sud (GLS) of New Caledonia estimated that > 60% of the individuals belong to the d’Entrecasteaux rookery (located north of the main island of New Caledonia), 20% were estimated to come from the south Great Barrier Reef (sGBR) genetic stock and multiple smaller contributions ( $\leq$  5%) from other genetic stocks of French Polynesia, north Great Barrier Reef (nGBR) and the Coral Sea (Chapter 3).

Sea turtles are “surfacers” that spend most of their time underwater and surface for gas exchange (Berkson 1966). Their slow reptilian metabolism, their extreme resistance to asphyxial distress and their ability to regulate their buoyancy allows them to control their diving time (Berkson 1966; Hays et al. 2007). Dive patterns of sea turtles have been studied for the last 25 years and increased with the use of Time-Depth Recorders (TDR's) (Hochscheid 2014). Multiple studies have investigated inter-nesting dive patterns of adult revealing that all individuals studied stayed mostly < 25 m (Hays et al. 2002a; Hays et al. 2002b; Hochscheid 2014). A number of studies have investigated the diving behavior of foraging individuals but for short periods ranging from 3 hours to 92 days because of difficulty in recapture (Ballorain et al. 2013; Hazel et al. 2009; Seminoff et al. 2006; Taquet et al. 2006). Results also show a mean < 25 m (Hochscheid 2014). Rice and Balazs (2008) recorded the dive patterns of three adults during their oceanic migrations. Diving was shallow (1-4 m) and of short duration during the day but became deeper (up to 135 m) after sunset. This diel behavior was also recorded in foraging studies that included juveniles and sub-adults *C. mydas* (Ballorain et al. 2013; Ogden et al. 1983; Seminoff and Jones 2006).

The use of satellite tracking has contributed to the study of the ecology of sea turtles and implementation of management strategies worldwide since the 1980's (Godley et al. 2008; Gredzens et al. 2014). Post-nesting migrations of *C. mydas* have been described using this technology in many tropical and sub-tropical waters (Balazs et al. 1994; Cheng 2000) and was also used to describe the navigational ability of this species in open-water (Luschi et al. 1998; Papi et al. 1995). More recently, the satellite tags have been used to calculate the home range of foraging populations (Hart and Fujisaki 2010; Makowski et al. 2006; Seminoff et al. 2002). Satellite tracking of foraging juveniles has been studied in previous studies but for periods ranging from hours to 115 days (Hart and Fujisaki 2010).

It is reported that New Caledonia is an important foraging area for *C. mydas* (Mounier 2007) but no data is actually available to detect any trends on the condition of this foraging population. However the extent of horizontal and vertical movement of *C. mydas* in this region is virtually unknown, yet vital for identifying important areas and critical habitats of this foraging population. In this study, we

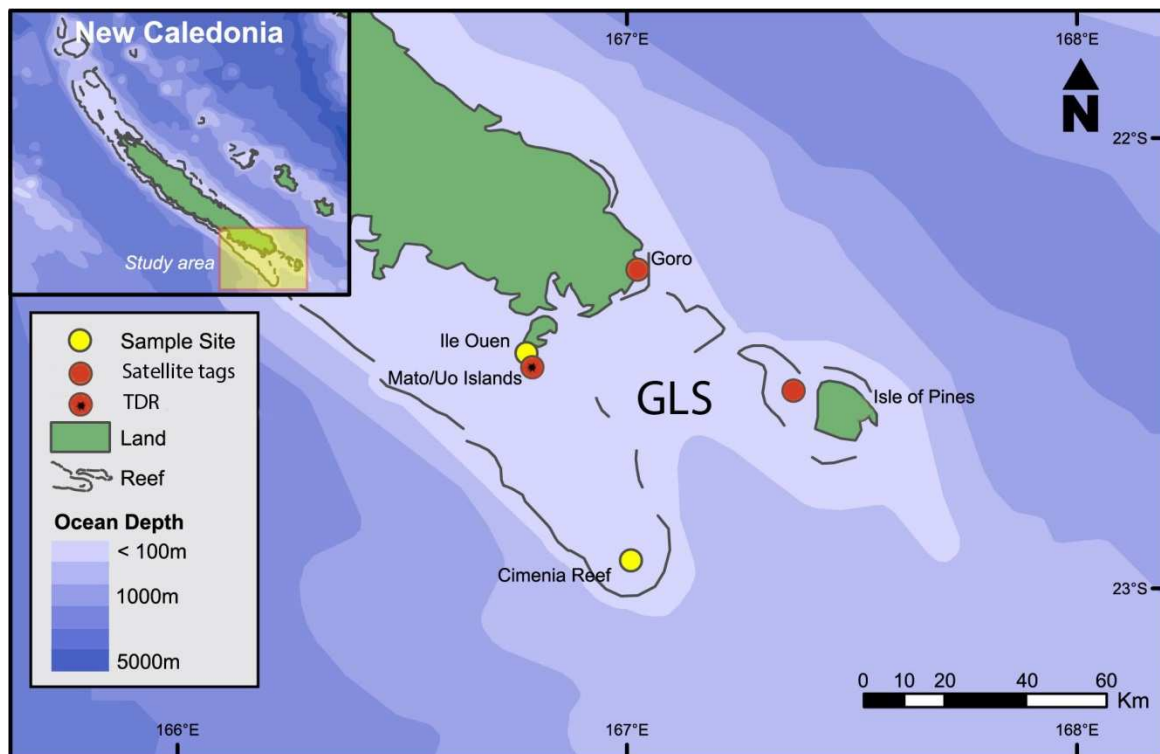
aim to determine the vertical habitat use of juveniles in feeding grounds, identify horizontal spatial habitat use with estimation of the home range of foraging individuals using the kernel density population estimates (KDE), and determine if the new recruits displayed resident behavior. The present paper relates the results of the first in-water study on sea turtles in New Caledonia. Sampling a large foraging area like the GLS and obtaining key demographic parameters, provides key insights on the ecology of the individuals found in that area and help in the design of fishery control regulations for that species that is still targeted by local indigenous populations for their traditional celebrations.

## **Materials and methods**

### **Study area and sampling**

Foraging *C. mydas* were sampled (n=16) from the Grand Lagon Sud (GLS) (S 22°00, E 167°00), one of six areas of New Caledonia added to the World Heritage List in 2008 for its reef diversity and associated ecosystems (Menu and Hebert 2006). The GLS is a seascape in good health characterized by a large reef diversity and the fact it supports important foraging grounds for *C. mydas* and a medium to high density nesting area for loggerhead turtles (*Caretta caretta*) (Mounier 2007) (Figure 1). Five foraging areas were selected after pilot studies in the area based on indigenous tribal knowledge shared by the tribes of Goro, Ouen Island and Isle of Pines (Figure 1). The GLS is used by the tribes to harvest the turtles needed for the traditional celebrations. Mato and Uo Islands (site 1; S 22°33.21", E 166°47.22") are two small vegetated high islands surrounded by large sandy areas, reef flats and patches of seagrass beds. Goro (site 2; S 22°17.00", E 167°05.99") is located at the south tip of New Caledonia where large seagrass beds are found in several bays. Ouen Island (site 3; S 22°25.34", E 166°48.36") delimits the South border of the Woodin channel halfway between Noumea (the capital) and Isle of Pines. Cimenia Reef (site 4; S 22°59.24", E 166°59.48") is composed of a triple barrier reef and forms the southernmost reef system off New Caledonia. In between each reef are large seagrass and algal beds. Isle of Pines (site 5; S 22°34.34", E 167°26.46") is a 195 km<sup>2</sup> island, that is the southernmost inhabited island in New Caledonia and has a human population estimated to be 1969 inhab-

itants in 2009. It is the only recorded nesting site for *C. mydas* in the GLS, with an estimated less than 10 females a year in 1996 but no reports since (Limpus pers. comm.).



**Figure 1.** Sampling sites for foraging *Chelonia mydas* in the Grand Lagoon Sud, off the southern end of the main island of New Caledonia. (n=453 for titanium tagging, n=7 for satellite tags and n= 10 for TDR's)

All resident foraging turtles were captured by the turtle rodeo method (Limpus and Reed 1985) and tagged with a titanium tag (Limpus 1992) and their curved carapace length (CCL) was measured before being released at their capture site.

## Satellite telemetry

At Goro, three new recruits (43-46.3 cm CCL) had F4G 371A tags from Sirtrack equipped with Fast-loc™ technology (Sirtrack Ltd, Havelock North, NZ) attached to their carapace using 2-part epoxy resin (Fiber glass Coatings). Anticipated battery life was 104 days. We set the tag to be active 24h d<sup>-1</sup> and the tags were recovered after 220 days of deployment (Table 1).

At Isle of Pines, three adults (90-113.5 cm CCL) were equipped with SPOT-293A (Wildlife Computers, Redmond, WA, USA). We set the first tag to be active 24h d<sup>-1</sup> and the two others satellite tags were

set to 12h d<sup>-1</sup>. The tags were attached using Rapid Cure 5 minute epoxy from West System. Anticipated battery life was 540 days, the first tag only emitted for 99 days and the second tag emitted for 218 days. One device is still transmitting (Table 1).

At Uo Island, one individual (61.1 cm CCL) was also equipped with a SPLASH10-F-297A tag with Fast-loc™ technology (Wildlife Computers). The anticipated battery life of these tags was 240 days and we set the tag to be active 24 h d<sup>-1</sup> (Table 1). The tag was attached with Devcon Dev-Pak Adhesive Cartridge 5 minute epoxy.

We ensured that each device plus epoxy tube did not exceed 5 % of the turtle's body weight (Figure 2). The tags from Uo Island and Goro were relocated using the rodeo method and the high recapture rate in these areas. Service Argos provides the locations with accuracy as good as  $\pm 350$  m.



**Figure 2.** Juvenile *C. mydas* equipped with a satellite tag in southern New Caledonia

**Table 1.** Summary of the satellite tagging done on *C. mydas* in the GLS

<b>Turtle ID</b>	<b>CCL (cm)</b>	<b>Size class</b>	<b>Area tagged</b>	<b>Date tagged</b>	<b>Last emission</b>	<b>Deployment duration (days)</b>	<b>Number of detections from satellites</b>
NC659	46.3	J	Goro	20/06/2014	21/01/2015	221	2923
NC628	46.3	J	Goro	20/06/2014	20/01/2015	220	1051
NC661	43.0	J	Goro	20/06/2014	20/01/2015	220	483
NC518	113.5	A	IDP	05/11/2013	24/02/2014	111	46
NC799	103.2	A	IDP	16/07/2014	20/02/2015	218	5
NC779	103.0	A	IDP	16/07/2014	still running		
NC073	61.1	J	Uo	16/09/2014	10/12/2014	194	714

## Time-depth recording

At Uo Island, ten individuals (41.2-61.1 cm CCL) were equipped with time depth recorders (TDR), model Mk9 archival tags (Wildlife Computers). The TDR's were placed in a black PVC tube that was attached to the carapace margin of each individual using 2-part epoxy resin (Devcon Dev-Pak Adhesive Cartridge 5 minute epoxy) and they were programmed to record depth, temperature and light level every 20 s. The area was chosen specifically for this experiment due to a very high recapture rate. Deployments varied between 14-221 days (Table 2). We ensured that each device plus epoxy and PVC tube did not exceed 5% of the turtle's body weight (Figure 3). Wildlife Computers-DAP processor 3.0 was used to extract the data from the TDR's. 80% of the TDR's deployed were retrieved using the rodeo method.



**Figure 3.** *C. mydas* equipped with a time depth recorder in southern New Caledonia

## **Data filtering and analysis**

### **Satellite telemetry**

Satellite data points were grouped into location classes (LCs) according to the accuracy of the transmission (highest to lowest accuracy: 3, 2, 1, 0, A, B and Z) (CLS 2008). Error estimate on LC's 3, 2 and 1 are < 1.5 km. The error estimation of a class 0 location is higher than 1.5 km - the error could be 50, 100, 500 km. The accuracy of A and B locations cannot be quantified. We excluded data points that were classed as Z as the adequacy could not be proven. Individual satellite data points for each turtle were plotted and inaccurate outliers were removed from the dataset. Data points that occurred over land were also removed. Home ranges were then defined using the 95% minimum convex polygon analysis tool in OzTrack (<http://oztrack.org/>), and plotted the locations and trajectories of each individual (Figure 4). Location, home range and trajectory shapefiles were exported from Oztrack into ArcGIS 10.2 to calculate home range sizes (using the WGS 1984/PDC Mercator projection), and to map the locations and trajectories of each individual against existing land forms and bathymetry.

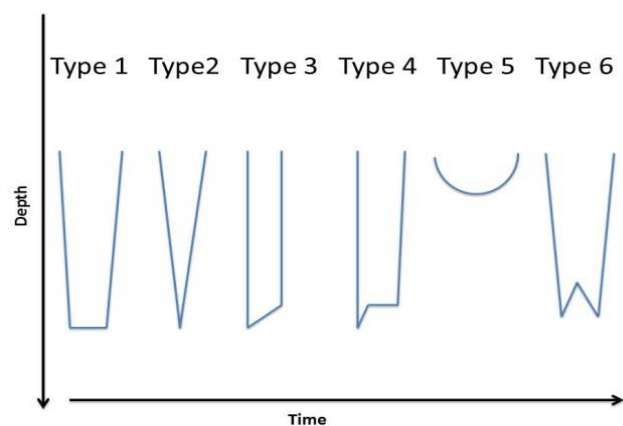


## TDR's

To allow for an accurate measurement, the first 24 hours of sampling for each TDR were excluded from the analysis. This exclusion period has been proven to be an important measure as some turtles showed a different dive pattern in the first hours post-release (Hazel et al. 2009). The possible effect of the tide and moon cycles on the depths recorded was analysed using a PERMANOVA (three factors repeated measures model: turtle, moon and tide). These factors were analysed as the moon and tide are used by the local tribes to hunt for turtles for traditional ceremonies. The differences in depths recorded between day and night were also analysed using a PERMANOVA (two factors model: turtle, day/night). In order to maintain consistency with previous studies, the dives were labelled into six types (Houghton et al. 2002; Minamikawa et al. 1997; Seminoff et al. 2006) (Figure 4). Dive 1 is a U shape dive, descent  $\geq 2$  m followed by a period of  $> 20$  sec at the same depth before returning to the surface. Dive 2 is a V shape dive, descent  $\geq 1$  m followed by an immediate ascent. Dive 3 is a descent, a progressive ascent followed by a straight ascent to the surface. Dive 4 is very similar to Dive 3 but the straight ascent is predated by a period of time at an intermediate depth at the top of a first progressive ascent. Dive 5 is also a U shape dive but  $\leq 1$  m with no stopping. Dive 6 is a W shape, a descent followed by a minor ascension of  $\leq 1$  m than another descent of  $\leq 1$  m before the final ascent to the surface. Dive data was analysed using MT Dive software (Jensen Software Systems, 2006, Laboe, Germany). Individual dives were defined as any depth exceeding 1 m. For each dive, the software calculated the start and end time as well as the maximum depth reached. The software also calculated the post-dive surface time, time spent at the bottom and identified the type of dive. All statistical analysis were performed using Statgraphics, Pimer v6 and Permanova+ for Primer Softwares, and significance assumed if  $P \leq 0.05$ .

**Table 2.** Summary of the results from TDR's deployed on *C. mydas* (n=10) in the GLS

Turtle ID	CCL		Area tagged	Date tagged	Last emis-sion	Deployment duration (days)	Proportional time at depth below surface												
	Size class						0-1 m	1-2 m	2-3 m	3-4 m	4-5 m	5-6 m	6-7 m	7-8 m	8-9 m	9-10 m	>10-15 m	>15 m	
NC102	45.4	J	Uo	30/12/2013	16/09/2014	221	1%	53%	37%	3%	3%	1%	1%	2%	0%	0%	0%	0%	
K90694	54.6	J	Uo	04/06/2014	18/09/2014	105	0%	1%	1%	1%	2%	15%	61%	3%	7%	7%	2%	1%	
NC514	48	J	Uo	30/12/2013	21/03/2014	83	36%	21%	9%	31%	2%	1%	0%	0%	0%	0%	0%	0%	
K90690	41.2	J	Uo	30/12/2013	04/06/2014	142	1%	65%	10%	5%	19%	0%	0%	0%	0%	0%	0%	0%	
NC688	47.4	J	Uo	04/06/2014	16/09/2014	103	0%	2%	52%	33%	1%	1%	1%	0%	0%	0%	3%	6%	
NC073	61.1	J	Uo	04/06/2014	16/09/2014	103	41%	9%	1%	3%	6%	25%	4%	2%	2%	2%	2%	1%	
K90696	43.4	J	Uo	04/06/2014	17/09/2014	14	28%	69%	2%	1%	0%	0%	0%	0%	0%	0%	0%	0%	
NC100	50.4	J	Uo	04/06/2014	18/09/2014	105	53%	37%	0%	0%	1%	7%	0%	0%	0%	0%	0%	0%	
K90695	51.2	J	Uo	04/06/2014	still running														
NC106	52.6	J	Uo	04/06/2014	still running														



**Figure 4.** Types of dives found in the diving behaviour of *C. mydas*

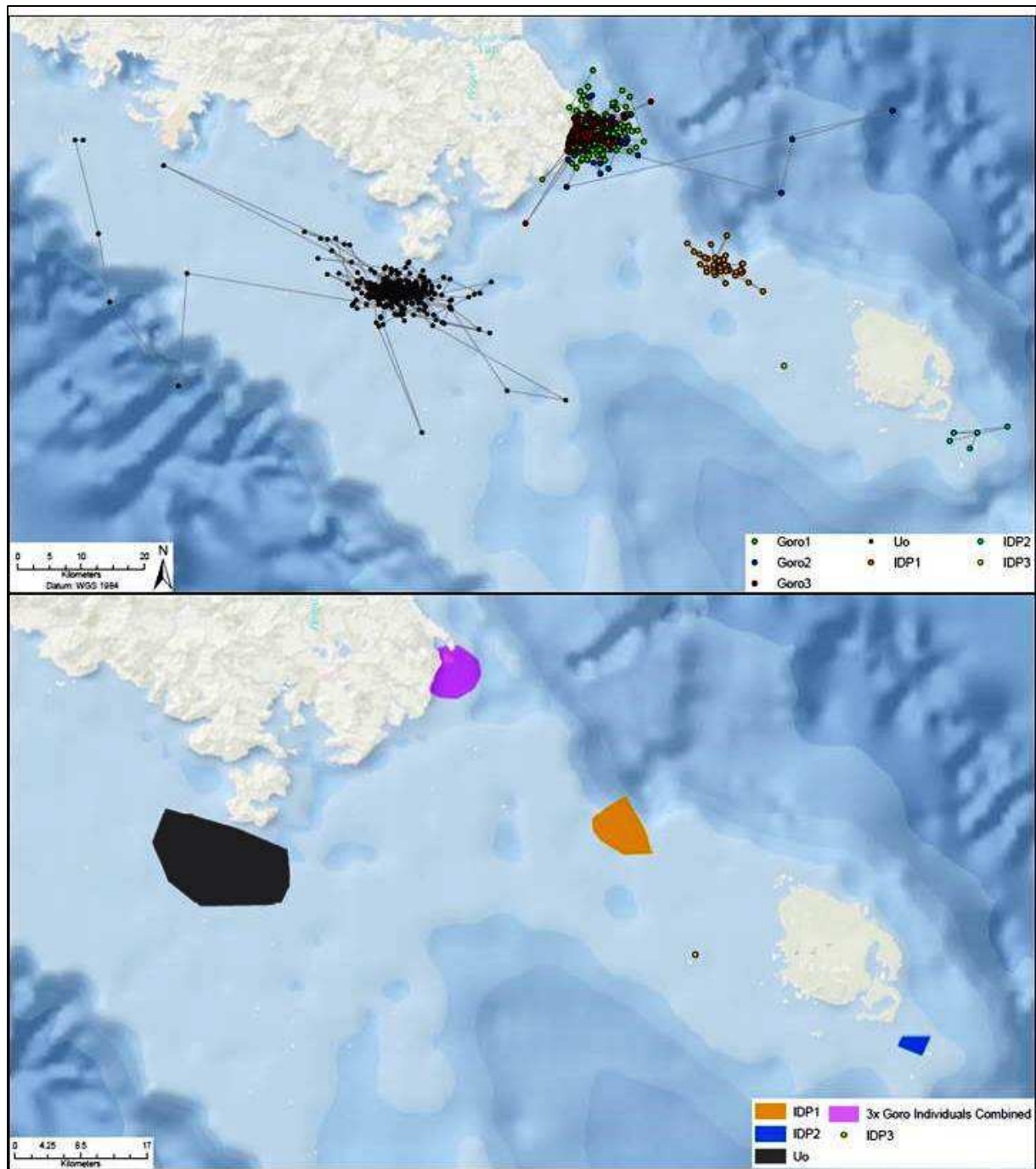
## **Results**

### **Foraging and habitat usage of satellite tagged turtles**

The mean home range for the individuals tagged in the GLS was  $54.28 \pm 2.42 \text{ km}^2$ . Home range for the juveniles equipped with satellite tags at Goro for > 6 months was estimated to be  $35.44 \pm 2.06 \text{ km}^2$  (Figure 5). This home range reports that the individuals did not leave the bay in which they were captured. The first individual tagged at Isle of Pines had a home range of  $38.73 \text{ km}^2$  and the second individual had a home range of  $7.40 \text{ km}^2$  (Figure 5). These individuals also show a fidelity to the area of initial capture. The juvenile tracked at Uo Island had a home range of  $173 \text{ km}^2$  (Figure 5). This large home range encompasses the deeper waters (> 20 m) past the reef flat.

### **Dive depth and dive patterns**

From the ten individuals equipped with TDR's at Uo, eight were recovered and the recaptures were at the initial capture area. The mean study time was 110 days for TDR's and the individuals spent 80% of their time at a depth < 5 m (Table 2). Mean depth was  $1.9 \pm 0.4 \text{ m}$  but significant differences were recorded within individual behaviors (3 factors PERMANOVA  $df = 7$ ,  $F = 456.6$  and  $P < 0.01$ ). The depth pattern was different between individuals for the different moon phases (interaction turtle x moon,  $p < 0.001$ ). No differences between moon phases were found for two individuals but all the other individuals showed different patterns between phases with no detectable trends. The depth patterns was also different between individuals for the different tide phases (interaction turtle x tide,  $P < 0.001$ ).



**Figure 5.** 95% convex polygon of home ranges recorded on *C. mydas* in the south of New Caledonia (IDP1, first individual caught at Isle of Pines; IDP2, second individual caught at Isle of Pines; IDP3, third individual caught at Isle of Pines; Uo, is the only individual tagged at Uo Island)

Two individuals reported differences at all tides and another individual showed differences at all tides except between Low Tide and Rising Tide. The diving pattern at Low Tide and Lowering Tide was different. High Tide for two juveniles showed a difference between Rising Tide and High Tide. Low Tide and High Tide patterns were different to the Lowering Tide pattern for one individual. This pat-

tern was also repeated for a second individual except that Low Tide is replaced by Rising Tide. The last individual showed all different patterns for each tide except Lowering Tide and Low Tide; and Rising Tide and High Tide. No significant interaction ( $p=0.13$ ) was observed between tide and moon factors.

58% of the recorded dives in this study are Type 1 (U dives). Only one individual never showed that Type 1 dive, rather displayed 60% of dives being of Type 2 (V dives) (Table 3). Overall, the second most important type of dive was Type 2 dive representing 30% of recorded dives. The type of dives did not significantly vary with the factor Day/Night (One Way ANOVA  $df = 1$ ,  $F = 13.11$  and  $P < 0.01$ ).

### Dive time

The mean dive time was  $12.1 \pm 2.6$  min and individuals mean ranged from 4–27 min (Table 3). The mean dive time did not vary significantly with the factor Day/Night when all individuals are combined (Two-Way ANOVA  $df = 1$ ,  $F = 0.03$  and  $P > 0.8$ ).

### Temperature

At Uo Island, the mean recorded temperature was  $23.2 \pm 0.6$  °C and temperatures ranged from 18–37° C (Table 4). Significant differences were observed for the mean temperatures (Two-way ANOVA  $df = 3$ ,  $F = 173.8$  and  $P < 0.01$ ). Observed mean for the different seasons is  $25.6 \pm 0.3$  °C for autumn,  $22.2 \pm 0.2$  °C for winter,  $22.1 \pm 0.1$  °C for spring,  $26.2 \pm 0.2$  °C for summer.

**Table 4.** Recorded temperatures by TDR's in the GLS

Turtle ID	CCL (cm)	Size class	Area tagged	Date tagged	Last emission	Temp (°C) mean (min-max)
NC102	45.4	J	Uo	30/12/2013	16/09/2014	23.8 (20-30)
K90694	54.6	J	Uo	04/06/2014	18/09/2014	22.0 (19-25)
NC514	48	J	Uo	30/12/2013	21/03/2014	26.1 (24-30)
K90690	41.2	J	Uo	30/12/2013	04/06/2014	25.6 (21-30)
NC688	47.4	J	Uo	04/06/2014	16/09/2014	22.0 (19-27)
NC073	61.1	J	Uo	04/06/2014	16/09/2014	21.9 (19-26)
K90696	43.4	J	Uo	04/06/2014	17/09/2014	22.1 (20-27)
NC100	50.4	J	Uo	04/06/2014	18/09/2014	22.1 (18-26)

K90695	51.2	J	Uo	04/06/2014	still running
NC106	52.6	J	Uo	04/06/2014	still running

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**Table 3.** Recorded dive types of eight juvenile *C. mydas* in the GLS

Individual	Number of recorded dives	Number of days deployed	Mean dives per day	Type 1	Type 2	Type 3	Type 4	Type 5	Type 6	Mean dive duration (min)	Range (s)
K90690	2875	142	20	1958	453	0	202	0	262	14	40 - 1487
K90694	6018	105	57	2752	2943	0	98	43	182	14	40 - 5460
K90696	4174	14	298	2092	2040	0	23	2	17	4	40 - 1560
NC073	11773	103	114	4419	7125	0	112	9	108	8	40 - 11760
NC100	551	105	5	447	46	0	7	4	47	27	40 - 3780
NC102	4396	221	20	2764	873	0	60	38	661	8	40 - 3000
NC514	1450	83	17	692	278	0	243	36	201	6	40 - 1800
NC688	472	103	0.22	333	74	0	16	25	24	16	40 - 3480

## Discussion

### Home range and residency to feeding grounds

In this study, the home range of the individuals studied was  $> 50 \text{ km}^2$ . The difference between the two adults tagged at Isle of Pines could be explained by the five detections of the second individual. Four individuals (three new recruits of Goro and one adult from Isle of Pines) have very similar home ranges of approximately  $30 \text{ km}^2$ . The very large home range of the juvenile tagged at Uo Island ( $> 170 \text{ km}^2$ ) exceeds home range estimates in the literature. More data in this feeding area is needed in order to be validated.

In American Samoa, home range for adults *C. mydas* averaged  $27 \text{ km}^2$  (95% convex polygon) (Craig et al. 2004) which concurs with the tracking of juveniles in the everglades in Florida (Hart and Fujisaki 2010) but are very different to the  $2 \text{ km}^2$  home range of juveniles at Palm Beach, Florida (Makowski et al. 2006). The foraging range of adults was found to vary between  $0.8\text{--}8.5 \text{ km}^2$  in Repulse Bay (QLD, Australia) (Whiting and Miller 1998). These different home ranges show that the habitat usage depends on the area and the age class. The results from this study are the first home ranges to be calculated for *C. mydas* in New Caledonia. This type of data has been used in multiple countries in order to create or adjust protected areas (Dobbs et al. 2007, Schofield et al. 2013).

All satellite tracking attained in this study showed that the individuals were resident to the area in which they were tagged for the duration of the study. The three new recruits tracked in Goro did not move out of the study area within the study time thus showing short-term fidelity to their original foraging area with, however, some movements outside the reef. Long-term tracking via further satellite tag deployments on these same individuals (still identifiable with the titanium tag) will however help to provide insight into multi-year residency times and identify migrations from these areas. Eight TDR's were retrieved out of ten deployed, again demonstrating that the individuals also exhibited short-term residency in the area of initial capture. Long-term residency of *C. mydas* in their feeding grounds has been shown in multiple studies (Broderick et al. 2007; Chaloupka and Limpus 2005;



Limpus et al. 1992; Read et al. 2014). With the home range being of  $> 30 \text{ km}^2$  for the individuals studied, identifying the extent to which home ranges may overlap will provide important information for marine protected area design in the GLS. The Merlet marine reserve is  $172 \text{ km}^2$  and is the only fully protected area in the GLS (no take, no go zone)(Menu and Hebert 2006). The results show that this area offers protection to the individuals that live within the marine reserve but not to the juvenile *C. mydas* that are resident to the reef on which they were captured in this study.

### **Dive patterns**

The results of the dive patterns showed that the individuals spent most of their time at a depth  $< 5 \text{ m}$ . The maximum depth recorded was  $18 \text{ m}$  for the individuals studied. The reef in that area drops to  $20 \text{ m}$  deep (<http://www.shom.fr/>) but caves are available to the individuals at about  $7\text{-}10 \text{ m}$  (Read pers. comm.). The use of shallow waters by the tagged individuals concur with a similar study done in Moreton Bay, Australia (Hazel et al. 2009). This result is of importance as it indicates that the individuals use the shallow part of the reef flat and may be more prone to interact with anthropogenic activities and more susceptible to indigenous harvesting. Coastal shallow waters have been reported to be a hazardous habitat for juveniles due to the higher percentage of marine debris that can be encountered and ingested (Bjørndal et al. 1994; Bugoni et al. 2001). This higher risk of anthropogenic interactions in coastal habitat was also recorded with the deployment of traditional and professional fishing gear which can impact multiple species (Casale et al. 2010; Mascarenhas et al. 2004; Pandav et al. 1997). But the proximity to the coast also incurs interactions that can be fatal with boat strikes (Bugoni et al. 2001; Casale et al. 2010; Orós et al. 2005). Vessel speed has been found to be an important factor in the level of the injuries sustained by boat strikes (Hazel et al. 2009). In 1992, a new zoning plan in Moreton Bay (QLD, Australia) included Go-Slow areas in order to protect marine mammals and sea turtles (Chilvers et al. 2005).

Type 1 and 2 dive patterns were mostly recorded in the juveniles studied in the GLS. The predominance of U dives in the behavior of adult *C. mydas* has been recorded in multiple studies (Ballorain et

al. 2013; Hochscheid et al. 1999). But in Hochscheid et al. (1999), V dives only accounted for 2% which contrast with Seminoff (2006), where V dives were the most recorded type of dives (53%) and U dives only represented 6% of the total dive patterns. It should be noted that no type 3 dives were recorded in this study. U dives have been correlated to two behaviors. Firstly, bottom resting (Cheng 2009; Rice and Balazs 2008; Seminoff et al. 2006) and secondly, foraging (Hochscheid et al. 1999). V dives have been linked to exploration and orientation (Hochscheid et al. 1999; Seminoff et al. 2006). Thus we can hypothesise that in this foraging area the individuals spend more time resting and feeding rather than actively searching for seagrass beds and that they feed during the day and rest at night. Juveniles *C. mydas* and leatherback turtles (*Dermochelys coriacea*) have been compared in a study investigated the diving ontogeny in Florida (Salmon et al. 2004). Results demonstrated that older *D. coriacea* made deeper dives than younger ones but this pattern was not found in *C. mydas* (Salmon et al. 2004). This difference in behavior could not be established in our study thus more data needs to be collected in order to test this hypothesis in the study area.

### **Environmental factors**

An interaction between tide and depth was found in this study. However, no clear patterns could be isolated. Individual turtles used the tide in different ways. Two individuals had different depths patterns for all four daily tide cycles but the other six individuals indicated a difference in depth and mainly between Rising/Low tide and Rising/High Tide with lower depths at lower tides. Response to tidal movements in foraging individuals has been investigated in the past (Brooks et al. 2009). Results indicated that a significant linear interdependence between turtles and tides but in regards to the displacement of the individuals rather than the depth. Continued tidal transport was hypothesised to be a better indicator of the movements of foraging sea turtles rather than the Selective Tidal Stream Transport which suggests that individuals mainly use the Lowering or Rising Tide for transport (Brooks et al. 2009). These results allow us to hypothesis that juvenile *C. mydas* might use tides on multiple levels, displacement but also depth depending on the bathymetry of the foraging area. An

interaction between the moon cycle and the depth was also recorded but individuals used the moon cycle differently thus no trend could be detected. Night time foraging has been found to be high when the night light was important in a study done at Mayotte Island in the Indian Ocean (Taquet et al. 2006) but each individual in the present study had a different diving pattern and no global effect of the moon nor the tide could be found. This difference could be explained by the bathymetry, the features of the sea floor or the availability of the prey items.

The mean sea temperature recorded in this study was  $23.2 \pm 0.6$  °C. The average yearly sea temperature for Noumea (capital of New Caledonia, located 50 km away from study area) is 25.0 °C but our study was mainly done in winter and the average sea temperature for winter is 23.4 °C which corresponds to the data observed in the study area ([www.seatemperature.org](http://www.seatemperature.org)). The high temperatures recorded in this study ( $> 28$  °C, highest sea temperature recorded) could be explained by basking events at the surface or the individuals being stranded on a reef flat at low tide. This behavior was described in a study on captive immature *C. caretta* (Sapsford and Van der Riet 1979). The range of temperatures found in this study are not near the thermal limits of function for the metabolism of *C. mydas* (Schartz 1978). Cold stunned individuals have been reported at temperatures at  $< 10^{\circ}\text{C}$  and individuals were found to stop feeding at temperatures between  $11\text{--}18^{\circ}\text{C}$  but could be active to a temperature up to  $34^{\circ}\text{C}$  (Mendonca 1983).

### **Dive duration**

Our results indicate a mean dive time of 12.1 min and the longest recorded was 3.2 hrs. These results concur with dive durations recorded in summer (mean of 13.1 min) for juveniles of the same species at Heron Island (Australia) (Southwood et al. 2003).

In a study done in the Cayman Islands on juvenile *C. mydas*, results revealed mean dive durations of 3.8 min for diurnal dives and 11.9 min for nocturnal dives (Blumenthal et al. 2010). The longest rec-

orded dive for *C. mydas* was 5.1 hrs and was recorded on an adult female (Broderick et al. 2007) but other species have been known to dive longer. The record for the longest breath-holding dive for a marine vertebrate was 10.2 hrs and was achieved by an overwintering female *C. caretta* (Broderick et al. 2007).

Highly variable dive patterns in interesting females have been recorded for *C. mydas* in multiple studies (Hochscheid et al. 1999; Hays et al. 2002b) but also in other species of sea turtles (Hamel et al. 2008; Houghton et al. 2002; Sato et al. 1998). This variability in dive duration cannot be explained by a response to the distribution of prey items as feeding does not generally occur within this period (Bjorndal 1985). Thus additional cues are involved in diving patterns which illustrates that a precise examination of each specific population is fundamental to understand and describe the regional activities and allow for local management strategies.

Results show a higher number and shallower dives during the day compared to less but deeper dives at night. A majority of individuals ( $n = 4$ ) showed no significant differences between the depth and the time of day but three individuals are found to significantly dive deeper at night compared to one individual that dived deeper during the day. These deeper and longer dives at night concur with multiple studies done on *C. mydas* (Ogden et al. 1983; Seminoff and Jones 2006) as well as other species of sea turtles (Beavers and Cassano 1996; Van Dam and Diez 1996; Witt et al. 2010).

In summary, the use of satellite tracking and TDR's proved to be a useful method for investigating the movements of *C. mydas* in the GLS. Results showed a use of shallow foraging habitat by juveniles and a mean home range of 50 km<sup>2</sup>. Thus we have demonstrated that assessing habitat usage within a coral reef habitat is an important tool in order to improve the management for this species. The Merlet marine reserve is 172 km<sup>2</sup> (Menu and Hebert 2006). This area offers protection to the individuals that live within the marine reserve but the results found in this study indicate that the juvenile *C. mydas* are resident to the reef on which they were captured. Therefore the protected area does not shelter the rest of the resident population of the GLS. A study reviewing a large number of studies worldwide indicates that the number of individuals are significantly higher in protected areas (Scott

et al. 2012). Further tracking tag deployments are necessary to provide more detailed marine protected area design for the conservation of *C. mydas* within the GLS.

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## CHAPTER 6: GLS POPULATION MORPHOMETRICS/DEMOGRAPHICS

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### Abstract

A total of 453 *C. mydas* individuals were caught foraging in the Grand Lagon Sud of New Caledonia, area added to the World Heritage list in 2008. *C. mydas* were captured in five different areas by the rodeo method with the knowledge of the traditional land owners, so females as well as males and juveniles were available for this study (sex was determined when possible (i.e, pubescent and mature males with longer tails)). All individuals were tagged and measured before being released where they were caught. Mean size was  $51.3 \pm 10.4$  cm CCL, of which only five individuals were classified as adults. Mean yearly recapture rate in this specific study area was  $1.9 \pm 0.2$  and the mean growth rate in the GLS was  $0.3 \pm 0.1$  cm but significant differences were found between study areas. The data collected within this study are valuable for the implementation of a conservation strategy for this species still being targeted as a food source and additionally providing data for the understanding of the recruitment patterns in the South Pacific.

### Introduction

Green turtles (*Chelonia mydas*) are an endangered species (IUCN 2010) found in tropical and subtropical waters around the world (Bowen et al. 1992). The number of large individuals of *C. mydas* have declined significantly in many countries of the Pacific Ocean due to overharvesting (Limpus 1997). This species has a very complex life cycle: *C. mydas* are known to nest on sandy beaches and hatchlings disperse to pelagic waters for a period that varies between 5-6 years (Limpus and Chaloupka 1997; Limpus et al. 1994). They then recruit to neritic waters, usually at a size  $\sim 40$  cm curved carapace length (CCL) in Australia and become resident to their foraging area (Limpus and Chaloupka 1997). They also shift their diet from omnivorous to mainly herbivorous (Bjorndal 1997). When they reach sexual maturity, aged between 25 and 50 years old depending on food availability

(Chaloupka et al. 2004) , they start to migrate (once every 2 to 5 years) back to the region where they were born in order to reproduce (Carr 1967). These migrations last for months at the time, depending on the distance between the nesting and foraging sites but the individuals come back to their foraging site (Broderick et al. 2007; Limpus et al. 1992; Mortimer and Carr 1987). And the life cycle also has a geographical complexity: studies have shown that *C. mydas* can migrate 100-1000's of km's to reach their nesting beach and thus can cross international waters (Cheng 2000; Luschi et al. 1996). This has implication for stock management, and the need to identify the different populations and to have an extensive knowledge of the different stages (pelagic, oceanic and neritic) for each population is critical (Arthur et al. 2008; Bolten 2003; Bowen et al. 2005).

Foraging areas are known to be “mixed stocks” (Lahanas et al. 1998), meaning that not all individuals found in one foraging area belong to the same management unit and belong to different genetic stocks (Chapter 3). *C. mydas* are commonly seen in New Caledonia but have been used as a source of protein for millenaries by South Pacific Islands Nations but also by sailors that were on exploration trips or stranded in the area (Pritchard 1982). Sea turtles are protected in New Caledonia but permits are given for specific tribal ceremonies such as the yam celebration or weddings/deaths of chiefs.

The present paper relates some of the results of the first study on the population structure of the foraging population of *C. mydas* in the south of New Caledonia. It is thought that New Caledonia is an important area for foraging *C. mydas* in the South Pacific but no data is actually available to support this. By sampling a large foraging area and obtaining population dynamics and key demographic parameters, it will give us an insight on the ecology of the individuals found in New Caledonia and help in the management of that species that is still being hunted as a food source in our waters.

## **Methods**

### **Mark-recapture**

All resident foraging turtles (n = 453) were captured by the turtle rodeo method (Limpus and Reed 1985) and double tagged with a titanium tag (Limpus 1992), their curved carapace length (CCL) was



measured, sex was determined when possible (i.e, pubescent and mature males with longer tails) before being released at their capture site. Tag loss could not be estimated as no individuals were recorded with a missing tag over the time frame of the study. The individuals were divided into four categories depending on their size according to Limpus et al. (1994) and Limpus and Chaloupka (1997): new recruits were > 20 - 65 cm CCL with two apparent ridges located on their white plastron, juveniles were > 20 - 65 cm CCL with coloured plastron, sub-adults were > 65 - 90 cm CCL and adults were > 90 cm CCL.

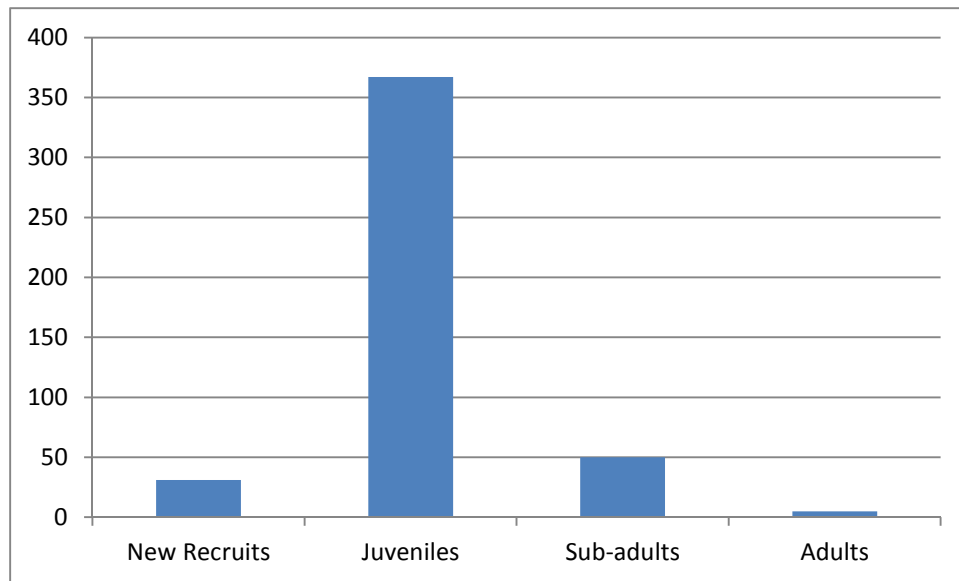
Gonads interpretation were done on individuals (n = 25) hunted by tribes of the GLS for traditional ceremonies during the sampling of chapter 4. Photographs of the reproductive system were taken during the carving of the individuals. Gonads were scored by sex, maturity and breeding status using the methods of Limpus (1992), Limpus and Limpus (2003) and Limpus et al. (2005).

Females were scored in four categories. Being 1a) pubescent immature (compact ovary, no scars and oval oviduct ~10 mm diameter), 2a) preparing for breeding (ovary with enlarged vitellogenic follicles >1 cm diameter), 3a) bred in the previous season (presence of healing corpora lutea (> 3 mm diameter) and enlarged atretic follicles) and 4a) bred in the season before last or up to three years ago (presence of corpora albicantia ~ 3 mm diameter and white radiating connective tissue). Males were scored as 1b) pubescent immature (testis have a solid structure and epididymis not expanded from body wall and 2b) sexually mature (testis are ellipsoidal and epididymis has distinct ridge and raised from body wall). A health assessment was also done on each individual studied.

## **Results**

### **CCL**

The mean size was  $51.3 \pm 10.4$  cm CCL with a range from 37.7 to 113.5 cm CCL (Figure 1). Only five adults were caught and 88% of the individuals caught were juveniles.



**Figure 1.** Class sizes of foraging *C. mydas* caught in the south of New Caledonia

### Recaptures

At Goro, a total of 150 individuals were captured and ten were recaptured in subsequent tagging events. At Ouen Island, 131 individuals were tagged and three individuals were recaptured. At Uo/Mato Islands, 87 individuals were tagged and 42 were recaptured (Table 1). This study area is the only area where we got multiple recaptures of the same individuals. The mean yearly recapture rate in this specific study area was  $1.9 \pm 0.2$ . From the ten individuals equipped with TDR's at Uo, eight were recovered and the recapture was at the initial capture area (within 5 km). One recapture was found on a separate reef (< 10 km away) during a tagging event: NC 080 tagged with a titanium tag at Uo Island was recaptured 18 months later at a second study site: Ouen Island.

**Table 1.** Summary of foraging *C. mydas* captures done in the south of New Caledonia

Study site	Tagging history status	2012	2013	2014
Goro	<b>Primary taggings</b>			
	First time tagged turtles	67	42	26
	<b>Recaptures</b>	4	2	0
	Turtles previously caught in the same area			
	<b>Total turtles</b>	<b>71</b>	<b>44</b>	<b>26</b>
Ouen Island	<b>Primary taggings</b>			
	First time tagged turtles	14	61	63
	<b>Recaptures</b>			
	Turtles previously caught in the same area	2	1	0

	Adult turtles originally tagged at nesting beaches and not recorded in the study area	0	0	1
	Turtles that have changed foraging area	0	0	1
	Total turtles	<b>16</b>	<b>62</b>	<b>65</b>
Cimania reef	<b>Primary taggings</b>			
	First time tagged turtles	N/A	42	N/A
	<b>Recaptures</b>			
	Turtles previously caught in the same area	N/A	0	N/A
	Total turtles			
Ile of Pines	<b>Primary taggings</b>	N/A	<b>42</b>	N/A
	First time tagged turtles			
	<b>Recaptures</b>	N/A	39	15
	Turtles previously caught in the same area			
	Total turtles	N/A	39	15
Uo/Mato islands	<b>Primary taggings</b>			
	First time tagged turtles	54	12	22
	<b>Recaptures</b>			
	Within study recaptures	N/A	22	30
	Total turtles	<b>54</b>	<b>34</b>	<b>52</b>

### Population estimate

The only study area in the GLS with a high number of recaptures was Uo Island (Table 1). Population estimate at Uo Island were calculated using Mark Software (Scharz and Arnason 1996) and the data in table 2.

POPAN Model (Arnason and Scharz 1999) was selected because it was the best fitted for our data and the species studied (POPAN provides a parameterization of the Jolly-Seber model (Schwarz and Arnason 1996) that is particularly robust. The model is based on  $\{ \Phi(\sim 1)p(\sim \text{time})\text{pent}(\sim 1)N(\sim 1) \}$  where  $\Phi$  is apparent survival,  $p$  is the probability of detection and  $\text{pent}$  is the probability of entry. This model's condition of use is that the proportion of marked individuals in a sample must be an unbiased estimate of the proportion of marks in the population and the individuals must be returned to their original capture area tagged and alive. The results show an estimated population of  $96.8 \pm 5.7$  for foraging *C. mydas* at Uo Island.

**Table 2.** Summary of tagging events and recaptures done at Uo Island, GLS

Trip	New tags	New re-captures	Multiple re-captures	Effort (days of sampling)	Number of days between trips	Date
------	----------	-----------------	----------------------	---------------------------	------------------------------	------

1	49	0	0	5		07/09/2012
2	5	11	0	2	123	08/01/2013
3	3	1	0	1	171	28/06/2013
4	3	6	1	2	27	25/07/2013
5	1	9	1	1	158	30/12/2013
6	3	6	1	1	39	07/02/2014
7	5	3	4	1	45	24/03/2014
8	1	6	7	1	72	04/06/2014
9	10	8	10	3	104	16/09/2014
10	0	0	10	3	83	08/12/2014

The number of recaptures did not allow us to calculate population estimates in the other study areas of the GLS.

### Growth rates

A total of 54 juveniles (CCL at initial capture 39.4–62.9 cm) were recaptured in three study sites (Goro, Ouen Island, Uo Island) (Table 3). The mean yearly growth rate of recaptures in the GLS was  $0.4 \pm 0.08$  cm but significant differences were found between study areas (Two Way ANOVA  $df = 2$ ,  $F = 32.78$  and  $P < 0.01$ ). Mean growth rate for Uo Island ( $n = 42$ ) was  $0.2 \pm 0.05$  cm  $yr^{-1}$  which is in a similar range as the mean growth rate for Ouen Island ( $n = 3$ ) of  $0.3 \pm 0.3$  cm  $yr^{-1}$ . The higher mean growth rate was found at Goro ( $n = 9$ ):  $1.4 \pm 0.3$  cm  $yr^{-1}$ .

**Table 3.** Summary of recaptures and growth of *C. mydas* in the GLS

Primary			CCL		Time between capture	Estimated
Tag number	Area	Age	Capture	Recapture	and recapture (months)	growth/year (cm)
NC593	Goro	J	46.6	47	7	0.7
K 90623	Goro	J	42.5	43.7	12	1.2
NC 538	Goro	J	56.5	57.9	10	1.68
NC 526	Goro	J	43.1	46.3	17	2.3
K 90610	Goro	J	45.6	47.3	7	2.9
K 90680	Goro	J	46.3	46.4	4	0.3
NC 653	Goro	J	44.7	45.5	7	1.4
NC 632	Goro	J	45.9	46.8	7	1.2
NC 659	Goro	J	46.3	46.7	7	1.2
NC256	Ouen	J	62.9	62.9	18	0.0
R 26376	Ouen	J	60.7	62.5	22	1.0
R 26399	Ouen	J	57.4	57.4	5	0.0

K 90694	Uo	J	54.6	54.6	24	0.0
NC 071	Uo	J	45	44.6	10	0.0
NC 084	Uo	J	50.2	50.2	10	0.0
NC 090	Uo	J	47.6	47.5	10	0.0
NC 092	Uo	J	43.7	43.3	10	0.0
NC 114	Uo	J	42	42	10	0.0
NC 119	Uo	J	41.1	40.9	10	0.0
NC 122	Uo	J	55	54.6	10	0.0
NC 156	Uo	J	45.8	45.7	10	0.0
NC 159	Uo	J	49	48.9	4	0.0
NC 165	Uo	J	54.5	54.2	15	0.0
NC 294	Uo	J	45.8	45.5	20	0.0
NC 457	Uo	J	48.2	48.2	15	0.0
NC290	Uo	J	44.2	43.8	6	0.0
NC292	Uo	J	42.9	42.8	17	0.0
NC294	Uo	J	45.8	45.3	18	0.0
NC 100	Uo	J	50.4	50.5	24	0.1
K 90690	Uo	J	41.2	41.3	21	0.1
K 90695	Uo	J	51.2	51.3	21	0.1
NC 086	Uo	J	48.9	49.1	24	0.1
NC 094	Uo	J	43.4	43.6	24	0.1
NC 106	Uo	J	52.6	52.8	21	0.1
NC 103	Uo	J	55.2	55.5	27	0.1
NC 102	Uo	J	45.4	45.7	24	0.2
NC 073	Uo	J	61.1	61.5	27	0.2
NC 078	Uo	J	41.9	42.4	27	0.2
NC 088	Uo	J	43.6	43.8	10	0.2
NC 513	Uo	J	39.4	39.6	10	0.2
NC 075	Uo	J	47.1	47.6	24	0.3
NC 096	Uo	J	47	47.7	27	0.3
K 90696	Uo	J	43.4	44.2	27	0.4
NC292	Uo	J	42.9	44	23	0.6
NC 104	Uo	J	53.6	54.6	18	0.7
NC 688	Uo	J	47.4	47.9	9	0.7
NC 459	Uo	J	45.2	46.7	18	1
K 90686	Uo	J	48.7	48.6	4	0.0
K 90688	Uo	J	50.4	50.3	4	0.0
K 90693	Uo	J	47.3	47.3	4	0.0
NC 118	Uo	J	53	53.3	4	1.2
NC 511	Uo	J	51.5	52.1	7	1.2
NC 514	Uo	J	48	48.1	6	0.0
NC 689	Uo	J	42.2	41.8	6	0.0

### Gonad examinations

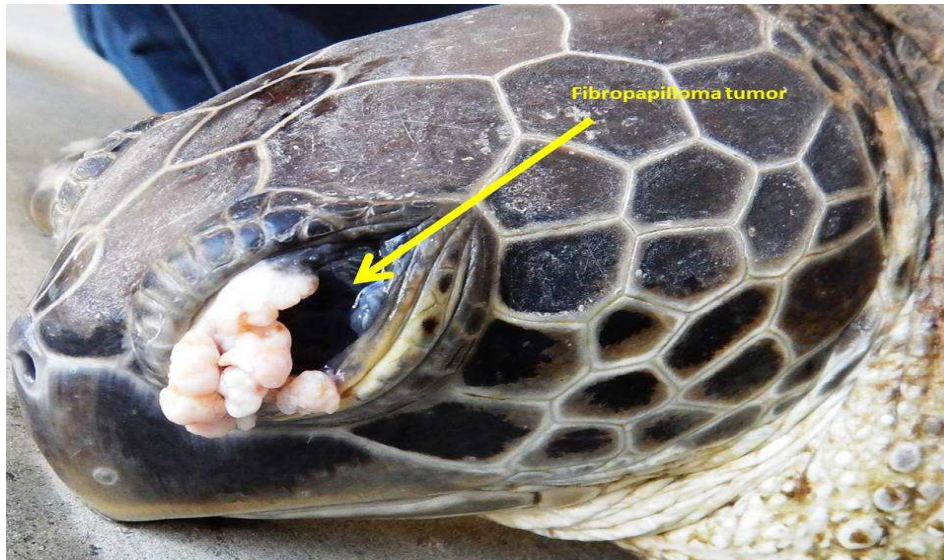
A total of 15 females and 10 males had their gonads examined during this study (Table 4). One female was preparing to breed for the next season, two had bred the last season, four were immature

but most had breed in the past (n = 8). Only one male was a pubescent immature, all the other males examined were sexually mature (n = 9).

**Table 4.** Summary of gonad interpretation of *C. mydas* in the GLS (1a. pubescent immature, 2a. preparing for breeding, 3a. bred previous season, 4a. bred season before last, 1b. pubescent immature and 2b. sexually mature)

Area	CCL (cm)	Sex	Gonads interpretation
Ouen Island	91.2	F	1a
IDP	N/A	F	4a
IDP	N/A	F	4a
Goro (Touaourou )	N/A	F	2a
Goro (Touaourou )	N/A	F	3a
Goro (Touaourou )	96.2	F	1a
Goro (Touaourou )	94.6	F	4a
Goro (Touaourou )	109.4	F	3a
Goro	101.4	F	4a
Goro	105.2	F	4a
IDP	100.6	F	4a
IDP	94.5	F	1a
Ouen Island	103.8	F	4a
Ouen Island	112.0	F	4a
Ouen Island	97.5	F	1a
Goro (Touaourou )	99.9	M	2b
Goro (Waho)	99	M	2b
Goro (Waho)	102	M	2b
Goro (Waho)	96.1	M	2b
Goro	102.2	M	2b
Goro	94.5	M	2b
Goro	101.6	M	2b
IDP	116.5	M	2b
IDP	94.5	M	1b
IDP	104	M	2b

## Health



**Figure 2.** Tumor found on *C. mydas* in the GLS of New Caledonia

The juvenile *C. mydas* (53.5 cm CCL) was found at Ouen Island (22°29'041"S, 166°45'015"E). Gross and microscopic pathology was compatible with fibropapillomatosis. Molecular analyses confirmed the presence of chelonid herpesvirus 5 DNA for F-Sial and MO4 genes.

## Discussion

### Size-class partitionning

The mean CCL for the individuals in this study is approximately 51 cm. This results show a large number of juveniles in this foraging area compared to the other age classes. Less than 1% of the individuals caught for this study were adults ( $n=5$ ). This result is of high significance as the adults are the size class targeted by locals for the traditional ceremonies. A genetic study found that 76% of the individuals foraging in the GLS are estimated to belong to the d'Entrecasteaux rookery, located north of the main island of New Caledonia and 20% are likely to nest in the southern Great Barrier Reef (Chapter 3). No trend can be detected to this day for the d'Entrecasteaux rookery but an upward trend has been identified in the number of nesting females in South East Queensland (Limpus pers. comm.). The large number of nesting females at both these nesting areas do not relate to the low numbers of adults caught within the study area but could explain the high proportion of juveniles. Another po-

tential justification is that adults are concentrating in other foraging grounds in or outside the GLS. Water depth and predator avoidance could also explain the partitioning in habitat use or excessive hunting as the adults are the ones targeted for the traditional celebrations. The Merlet Reserve should be investigated as we hypothesise that a higher number of adults may be found in this protected area as less hunting has occurred in this area.

### **Demographic informations**

When comparing our results to similar size-class individuals recaptured in other studies, it was found that Goro has a similar growth pattern than juveniles in the sGBR with a growth rate of  $1.35 \text{ cm yr}^{-1}$  (Limpus and Walter 1980) but in the Bahamas, a study reported a mean growth rate of  $4.2 \text{ cm yr}^{-1}$  (Bjorndal et al. 2000). This difference between the growth rates in the Atlantic and Pacific Oceans has been recorded in multiple studies (Bjorndal and Bolten 1988, Chaloupka et al. 2004).

Differences in growth rates have been hypothesised to be linked to multiple factors: genotype, sex, habitat quality, water quality, water temperature, food availability, prey items (Bjorndal and Bolten 1988, Boulon and Frazer 1990, Collazo et al. 1992, Chaloupka et al. 2004). A significant inverse correlation between population density and mean annual growth rate was reported in a long-term study (Bjorndal et al. 2000) and this could be a possible explanation for the low growth rate at Uo Island. This foraging ground may have also reached carrying capacity. This scenario was reported in Hawaii by a study that investigated the ecosystem structure and processes at Kaloko, Honokōhau based on three factors: biomass estimates and consumption rates, primary production rates and the effect of sea urchin activity (Wabnitz et al. 2010). A study in the Caribbean calculated the carrying capacity of pastures and indicated that the seagrass *Thalassia testudinum* has a range of 122 to 4439 kg green turtles/ha (Bjorndal et al. 2000). If we used this data as a baseline, the estimated 8 ha of seagrass pasture found at Uo Island thus putting the potential carrying capacity between 96–3544 individuals. The estimated population of Uo Island was calculated to be  $> 90$  individuals thus showing that the



area may indeed be close to the lower limit of the carrying capacity, thus more data is needed in order to explain the low growth rates recorded.

From the recorded growth rates and the size of the animals captured during this study, we can hypothesise that the GLS is home for these foraging individuals for at least 70 years thus inclining for a long term management of this species in the area.

This estimation of population at a foraging ground is the first to be done in New Caledonia and gives a density of 69 *C. mydas* per km<sup>2</sup> on the reef flat of Uo Island but a kilometric abundance index (KAI) was calculated after an aerial survey over 1791 km of New Caledonian waters was done in Jun 2003 (Liardet 2003). Species could not be distinguished thus the KAI relates to a number of sea turtles. The KAI for the GLS was 0.05 individual per km which is much lower density than the results of this study but the aerial survey went mostly over open sea water as our study only looked at one reef flat.

All examined gonads reported that the individuals hunted were from resident turtles of the GLS meaning that the *C. mydas* used for the traditional ceremonies are part of the mixed stock foraging in the study area. Thus from the genetic analysis (Chapter 3), it can be hypothesised that a majority of the individuals killed by the locals belong to the d'Entrecasteaux genetic stock. In 2014, one individual caught by the tribe of Ouen Island had a tag from d'Entrecasteaux (K90659) applied the previous year during nesting season. This is very important information in terms of management of this resource. Only five of the 25 individuals examined were pubescent immature, indicating that mature adults are the most impacted by the local hunting. For New Caledonia and all the other South Pacific islands, we recommend that a size restriction (strict ban on hunting of mature adults) is applied to all permits given for traditional hunting of sea turtles in order to insure the minimum threshold of adults needed for genetic diversity and reproduction. Gonads examinations provided evidence that all adults caught in this study for traditional ceremonies were resident individuals of the foraging area even when they were caught during nesting season thus concurring the low nesting in the area as for decades adults and eggs were collected for the traditional ceremonies and to our knowledge, nesting

of *C. mydas* has not occurred in the GLS for the last 10 years. Not all individuals killed could have their gonads examined as the carving happens simultaneously for many tribes that are geographically distant. We recommend systematic recording of CCL and sex of the individuals killed for traditional purposes for long term monitoring. This would allow for a more comprehensive study of the individuals targeted but would also allow for a more thorough examination of the population dynamics in the area.

Not all individuals carved could have their gonads inspected but sex could be determined by external features. A total of 41 *C. mydas* were examined during the tribal ceremonies and that included 10 males and 25 females.

In the present study, only one individual was found in a different study area than the one it was originally captured in but the two study areas are only separated by less than 10 km. With the home range being of  $> 30 \text{ km}^2$  for the individuals studied, we can hypothesise that this individual has not changed feeding area but the two studies sites are just be overlapping.

New recruits were observed at four of the study areas: Goro ( $n = 25$ ), Ouen Island ( $n = 3$ ), Uo Island ( $n = 3$ ) and Cimenia Reef ( $n = 1$ ).

## **Health**

This is the first record of this disease in the south of New Caledonia. Now that the disease has been identified, future monitoring should record any change in the density of the disease and foraging areas near altered catchments should be checked for the presence and density of the disease. Consumption of individuals with tumors should be avoided (Yasumoto 1998).

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## CHAPTER 7: SUMMARY AND GENERAL DISCUSSION

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### Migration

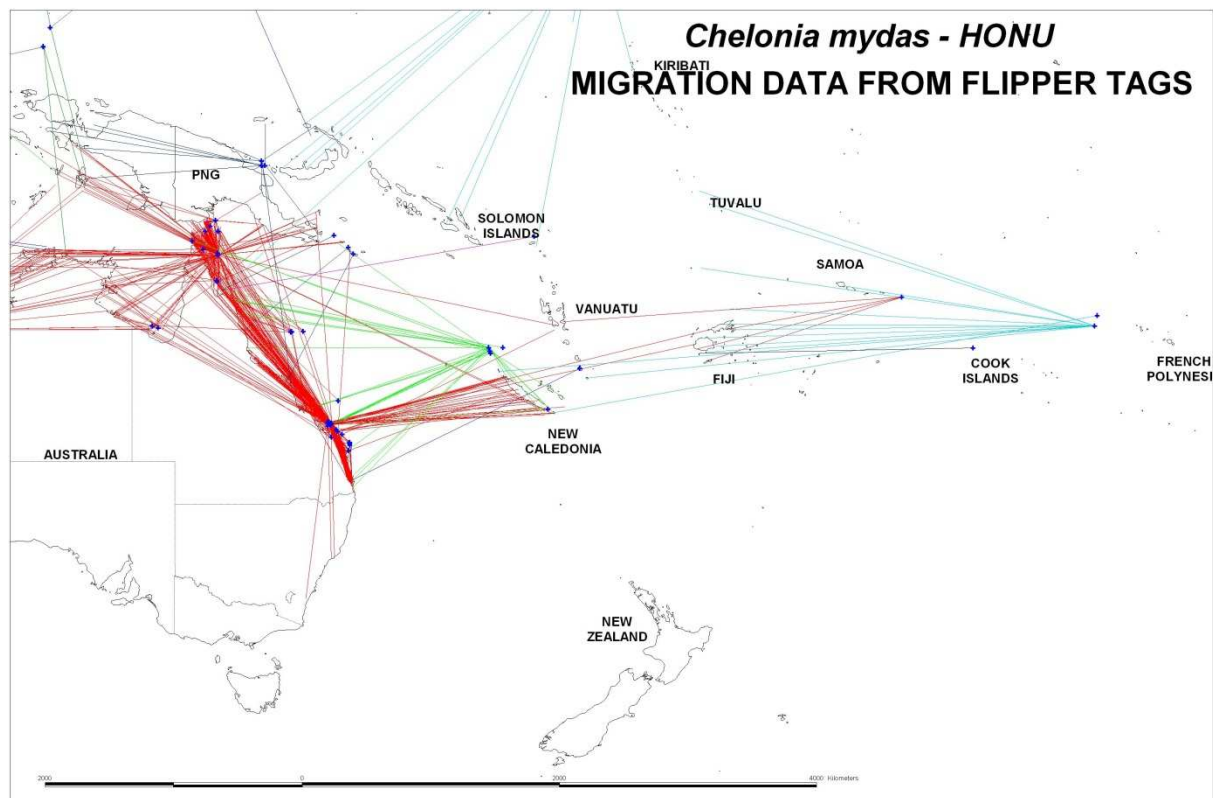
In 2007, 150 *C. mydas* nesting colonies were estimated in the South Pacific and only 10–15 are composed of more than 2,000 individuals (Allen 2007). The largest number of rookeries in the Pacific is located in Queensland (Australia) with over 60 rookeries and an estimated population of 70 000 turtles (EHP 2005; Limpus 2009). The only nesting site of any magnitude in French Polynesia today has an estimated 300–400 annual nesting females (Craig et al. 2004). New Caledonia is the second largest breeding area for *C. mydas* in the South Pacific after Australia with > 25 nesting sites recorded in New Caledonia and three large rookeries (d'Entrecasteaux 5000-10 000, Chesterfields 500-1000, and Beautemps-Baupré 100-500) (Fonfreyde et al. 2012; Mounier 2007; Read 2012).

Multiple studies have shown long-range migrations of *C. mydas* in all tropical waters around the world (Carr and Ogren 1960, Hughes 1974, Meylan 1982) however documented examples from the South Pacific are scant. Tag recoveries have allowed for a small number of females to be documented migrating from Scilly atolls in French Polynesia to Fiji, New Caledonia, Tonga, Vanuatu and Wallis (Balazs et al. 1995). Migrations were also recorded from American Samoa to Fiji (Balazs et al. 1994; Craig 1994). In total, the 26 recaptures of primarily post-nesting turtles from French Polynesia, American Samoa, and Cook Islands showed a similar course of direction and destination: 96% migrated westward after nesting, with 58% going specifically to Fiji (Craig et al. 2004).

The first chapters of this thesis provided data to identify two patterns. Which neighbouring countries *C. mydas* are migrating from to nest in New Caledonia? And where do breeding *C. mydas* from New Caledonia forage? The review of data collected over the last 50 years by different projects identified multiple migrations of *C. mydas* to and from New Caledonia (n = 97) and indicate that most of the tag recoveries made in New Caledonia belonged to females from the d'Entrecasteaux rookery and the south Great Barrier Reef genetic stock (respectively n = 49 and n = 45 out of 97 individuals). Some

females (n = 2) demonstrated fidelity to foraging sites in Australia located 1200 km away from the nesting site located in New Caledonia. This study also reveals a previously undescribed migration from the Chesterfields Atolls to Hervey Bay in Australia.

These large scale migrations across borders and through protected and unprotected areas indicate a need for a regional management plan for this species. This has been prepared by SPREP in the “Pacific Islands regional marine species programme 2013-2017” (SPREP 2012) and objectives have been laid out and now just have to be followed by all members.



**Figure 1.** Breeding migrations of *C. mydas* in the Southwest Pacific identified from flipper tag recoveries. Lines join non-nesting tag recovery sites with the respective nesting sites. Line colours have been chosen to allow easy identification of tag recoveries from the respective nesting areas (Data supplied via three turtle data base managements: SPREP, Qld EHP, Western Australian Parks and Wildlife).

## Genetics

A large scale review of the genetic diversity of *C. mydas* in the Indo-Pacific was done (Fitzsimmons and Limpus 2014) and this study is adding additional data to this important project as data was lacking from this region of the world and it is hypothesised (due to the size of the *C. mydas* rookeries in New Caledonia) that these large rookeries provide important numbers of individuals to the foraging grounds in the rest of the South Pacific. The mixed stock analysis was conducted in the GLS data using mtDNA data from 30 genetic stocks throughout the Western Pacific to estimate the origin of foraging turtles. The results indicated that rookeries at the d'Entrecasteaux Islands and Vanuatu are independent genetic stocks and that the Chesterfield Islands are linked to the Coral Sea genetic stock. The most likely contributors to the GLS are the d'Entrecasteaux rookeries located north of the main island of New Caledonia. The southern Great Barrier Reef (sGBR) population is the second most likely contributor to the GLS, and multiple contributions  $<0.05$  were indicated to derive from rookeries situated as far as 4000 km away in the Coral Sea (French Polynesia, Commonwealth of the Northern Mariana Islands and Vanuatu). Marine conservation policies in New Caledonia need to consider the links between the foraging and nesting populations of *C. mydas* in New Caledonia and other rookeries and foraging grounds in the Coral Sea.

## Habitat use

### Population dynamics

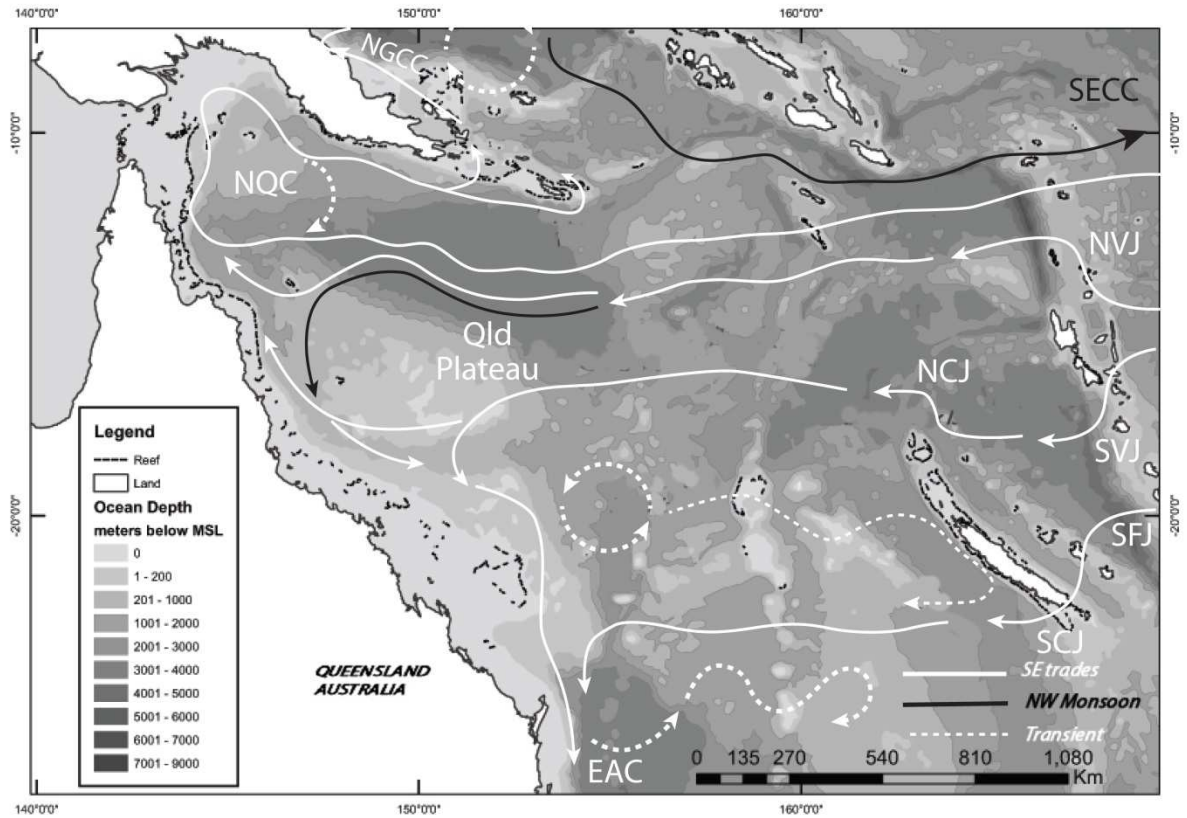
Genetic analysis of the area has showed that 76% of the individuals found in the GLS belong to the d'Entrecasteaux genetic stock (Read et al. Submitted). An increase in the proportion of nesting recruits from that stock has been highlighted in a study done in Australia (Jensen In press). This breeding area has been classified as World Heritage in 2008 (Menu and Hebert 2008) and since, added protection was implemented by the creation of a national park in that zone (SMMPM 2013). A significant inverse correlation between population density and mean annual growth rates of juveniles *C.*



*mydas* was established (Bjorndal et al. 2000). Four hypothesis for the low numbers of adults encountered within the study area. 1: depleted adult population, 2) adults take refuge in deeper waters, 3) differences in micro-habitat use and 4) developmental migration.

### **Developmental area**

Due to the low recapture rate ( $n = 5$ ) and the high numbers of new recruits foraging in Goro ( $n = 24$ ), we hypothesised that Goro was a developmental area. Goro is located on the tip of the main island of New Caledonia and is crossed by the South Caledonian Jet (SCJ) which could be bringing the pelagic juveniles into the coastal areas (Figure 2). The three new recruits that were tracked with satellite telemetry did not move from their original tagging area even though they were studied for approximately six months. The first record of immature East Pacific *C. mydas* using multiple foraging grounds was described in the Baja California Peninsula in 2010 (Senko et al. 2010). This type of behavior was not recorded within this study. The data collected in this study could not allow testing for the hypothesis that Goro was indeed a developmental area or part of a multiple foraging ground behaviour. One turtle (K90680) was recaptured in the same area at which it was initially tagged but four months later, the plastron was pale yellow and covered green algal growths. These changes were recorded in individuals in Shoalwater Bay (Limpus et al. 2005) and were also reported in loggerhead turtles (*Caretta caretta*) (Limpus and Limpus 2003a) suggesting Goro may have alternative functions for new recruits (i.e: port of entry for new recruits into the lagoon of the GLS).



**Figure 2.** Currents in the Coral Sea explaining parts of the results in this study on *Chelonia mydas* (EAC, east Australian current, NQC: north Queensland current, NCJ: north Caledonian jet, SCJ: south Caledonian jet, SFJ: south Fiji jet, SVJ: south Vanuatu jet, NVJ: north Vanuatu jet, SECC: south Equatorial countercurrent, SGCC: south Guinean countercurrent)

More capture-recapture is therefore needed in the other four study sites in order to calculate population estimates for these foraging areas and provide a comprehensive estimation of the population of foraging individuals in the GLS. The “Reserve Merlet” should be sampled (capture-mark-recapture) in order to compare the dynamics of this area to the rest of the GLS as we hypothesized that this is where a higher concentration of adults might be found.

## Feeding

Opportunities to look at entire stomach contents of marine megafauna, to examine multiple feeding events, are scarce as it is a fatal method. We used *C. mydas* killed for tribal ceremonies in New Caledonia to ground truth the results from the stable isotopes analysis run simultaneously. The samples were collected from three different tribes that hunt in the GLS. The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in skin samples in this study ranged from -19.3‰ to -7.3‰ and 2.8‰ to 15.9‰ respectively. These results report a

preference for algal diet and concur with the stomach contents analysis. Four genera contribute the most (73.4%) to the total dry weight matter: algae, *Hypnea* (34.4%), *Caulerpa* (11%), and *Ulva* (11%) and the seagrass *Halodule* (17%). These results provide more evidence that *C. mydas* feeding patterns differ from one foraging ground to another.

The GLS, and more specifically Goro, is under important mining pressure. The identification of the different prey items of *C. mydas* is a useful tool and mapping and monitoring of the specific algal and seagrass beds should be a priority in the near future.

### **Implications for *C. mydas* conservation and management**

This study has shown that New Caledonia is not only an important nesting area for *C. mydas* in the Coral Sea but also sustains a genetically diverse foraging population. Sea turtles are a shared resource (Balazs 1982) and this study highlights the need for a regional management plan for *C. mydas* in the South Pacific as results have linked New Caledonia to multiple countries. Including, Australia (titanium tags and genetics), French Polynesia (titanium tags and genetics), Vanuatu (titanium tag and genetics), Commonwealth of the Northern Mariana Islands (genetics) and Papua New Guinea (titanium tags).

However, the most interesting result for management is that > 60% of these individuals belong to a New Caledonian genetic stock. The d'Entrecasteaux rookery is under the Fisheries Department of the Government of New Caledonia as the GLS is under the governance of the South Province.

The data collected in this study can be a baseline for all future foraging grounds studies in New Caledonia but can be used also by other South Pacific Islands that have the same issues (conservation and traditional fishing). I recommend that a turtle symposium is organized in the near future in order to discuss measures that could be put in place to ensure the survival of this endangered species as well as traditional customs at a regional level.

## Future research

This project focused on *C. mydas* but two other species were identified foraging within the study area (*Caretta caretta* and *Eretmochelys imbricata*) additionally New Caledonia has been identified as the second most important area after Australia for the east Pacific genetic stock of *C. caretta* (Boyle et al. 2009; Limpus and Limpus 2003b). The science should also be addressed for these species.

## More in the GLS

In-water studies should be continued in the GLS as multiple elements of the population dynamics would also be instrumental for grasping the entire set of factors that control the demographics of this species in the area. For example, size classes/size at first breeding, survivorship and age duration. The sampling done in the GLS should also be expanded to the rest of New Caledonia as *C. mydas* is consumed all over the mainland and associated islands. One of the key demographic tools to study foraging sea turtles is investigating the sex ratio of a population. This step was not possible during this study but is essential to understand the full dynamics of this population. This should be a priority in the future in-water studies done in the area.

Long-term monitoring of feeding and nesting grounds has been essential to the understanding and management of this species around the world (Balazs and Chaloupka 2004; Hawkes et al. 2005, Troëng and Rankin 2005; Limpus 2009). This project should be the base layer for future work in this area under important mining pressure. These long living individuals require a continuing investment and regional durable management plan. Now that the genera *Hypnea*, *Caulerpa*, *Ulva* and *Halodule* have been identified as important to *C. mydas* in the GLS, an updated catalog of the marine benthic plants should be done and the health of these should be investigated and compared to historical data collected in the region (Garrigue and Tsuda 1988). Protection of these habitats will be crucial for the future of this species in the GLS.

## **Reserve Merlet**

The only marine park in the GLS is the “Yves Merlet Special Marine Reserve” and all activities are strictly prohibited in this area. It was established in 1970 and is relatively undisturbed but enforcement from the mainland is difficult as a traditional rite of passage through the reserve is tolerated (SPREP 1985). The 172 km<sup>2</sup> zone “constitutes a representative sample of New Caledonia’s marine heritage and is a valuable area for biological and ecological studies on reef lagoon’s systems” (SPREP 1985). No permits were delivered to this study in order to assess the turtle population in that area. The effectiveness of this marine park on the conservation of this species should be investigated as a study done in New Caledonia on multiple commercial fishes showed that the areas surrounding the marine parks were positively affected by the no-fishing zone but were not protecting the whole population (Chateau and Wantiez 2009). A study done on satellite tracking projects that followed the movement of *C. mydas* in all tropical and sub-tropical waters found that adults are significantly aggregated in Marine Protected Areas (Scott et al. 2012). This was also found in a study done on *C. caretta* in Greece (Schofield et al. 2013). In Australia, the ecology of sea turtles was considered in the design of the zoning of the Great Barrier Reef Marine Park (Dobbs et al. 2007). This integration of the research into the management is a valuable example and should be followed in all Marine Parks planning.

## **D’Entrecasteaux and Chesterfield Atolls**

D’Entrecasteaux breeding area has been identified as the most likely contributor to the GLS foraging population of *C. mydas* and some nesting individuals from that rookery were recovered in different feeding grounds located in Papua New Guinea, Australia (Torres Strait, nGBR, sGBR, Shoalwater Bay, Hervey Bay and Moreton Bay) and additional ones in New Caledonia (Read et al. 2014). An important information that is missing is where are all the tagged turtle (n = 4700) from that area as only 1% of the tags applied have been recovered. Satellite tagging of nesting females in the area would allow us to gather some additional much needed information on this rookery and on the feeding ground

used in this management unit. A deficiency in any trend detection for the nesting population is a gap that should be addressed.

The Chesterfields breeding area has been linked to the Coral Sea genetic stock but are estimated to contribute at a very minimal percentage to the population of the GLS (2%). This rookery was estimated at 100-1000 nesting individuals per year (Fonfreyde et al. 2012) but only one individual has been assigned to a feeding ground in Australia (Hervey Bay) (Read et al. 2014). More data is needed on this important rookery in order to assess the importance of this rookery in the South Pacific and how it contributes to foraging grounds in the region.

### **Aerial surveys**

Approximately 500 turtles were caught during this study in only five feeding areas but the GLS is made of hundreds of reefs that are suitable for foraging turtles. The GLS extends over an area of 314 500 hectares and investigating population estimates will require years of turtle rodeo. Aerial surveys could be the solution for an effective abundance estimate but studies have shown that the numbers provided by such study are usually well under the real numbers (Marsh and Sinclair 1989) but it shows a broad picture of the distribution of sea turtles in the area. New technology (use of Unmanned Aerial Vehicle) could be used in order to do some population estimates in remote islands that are difficult to access and large areas at a lower costs and with a better accuracy (Jones IV et al. 2006; Hodgson et al. 2013). Recently, this method was used successfully on marine mammals (Hodgson et al. 2013; Maire et al. 2013; Mejias et al. 2013) and is very promising for sea turtles.

### **Genetic gap**

One major rookery was not described in New Caledonia, the Beautemps-Baupré rookery (Ouvéa, Loyalty Islands), which needs to be genetically investigated in order to provide a better understanding of the recruitment patterns in that part of the world. Gaps in the genetic description of many rookeries of the South Pacific have been identified (Fitzsimmons and Limpus 2014) but with a global

action plan these gaps could be addressed in a near future. This is an important part of the process to study the possible ongoing gene flow that may be happening in the areas of the world where climate change including rising sea water levels influences the natural processes (Fuentes et al. 2010; Hawkes et al. 2009; Hays et al. 2010).

## **Recovery plan**

The Recovery Plan for Marine Turtles in Australia was done in 2003 and was established as a five years strategy (Baker and al. 2003). Five different habitats were identified in the life cycle of sea turtles: the natal beach, the mating area, the inter-nesting habitat, the feeding area and the pelagic waters. Within those habitats, six objectives were defined by a team of experts. 1) Reduce sea turtle mortality and when appropriate increase natural survivorship (including integrative management with Aboriginal and Torres Strait Islander communities), 2) Monitor the populations by developing programs and protocols, 3) Manage factors affecting nesting, 4) Identify and protect key habitats, 5) Communicate and educate stakeholders, 6) Support and maintain international collaborations, 7) A similar recovery plan was done for the U.S Pacific Islands (PSTR 1998) and nine objectives were reported as fundamental to the conservation of *C. mydas* in this region of the world. 1) Stop the direct harvest of adults and eggs (education and penalties), 2) Eradicate FP, 3) Reduce incidental mortality (by-catch), 4) Assess population size and status, 5) Identify management units through DNA analysis, 6) Support international management with neighboring countries, 7) Identify and protect keys areas, 8) Manage the effects of development on key areas, 9) Eradicate non-native predators.

Further studies need to be done in the South Pacific in order to fully understand all the aspects of the ecology and physiology of sea turtles in that region of the world but the take home message is that no conservation plans will be successful without an integrative management of this shared resource with the local tribes (Kennett et al. 2004a, Kennett et al. 2004b) and an international partnership between all the countries that are part of the life cycle of these endangered animals. A similar long-

term recovery plan to the ones cited above but including sustainable traditional hunting should be set-up in the rest of the South Pacific and more specifically in New Caledonia.

## Conclusion

This study provides new migratory evidence for *C. mydas* in the South Pacific, thus strengthening that sea turtles are indeed a shared resource and should be managed at a regional level. The migration data was consolidated by the genetic analysis of the foraging individuals of the GLS, and offering evidence of a possible recruitment in New Caledonia from rookeries located up to > 4000 km away. One new haplotype was entered into GenBank (CmP 118.1) from the d'Entrecasteaux breeding area and the Chesterfields breeding area was linked to the Coral Sea genetic stock. This new information regarding the Coral Sea breeding area provides new data for the understanding of the recruitment patterns of this genetic stock within the South Pacific. The genetic analysis also provided crucial information at a local scale. The largest genetic stock found in the GLS is the d'Entrecasteaux genetic stock, located 400 km from the study area and within New Caledonian waters. The findings above are crucial for a larger scale protection of this migratory specie but *C. mydas* are known to spend most of their time at their foraging grounds. Investigations within the GLS have demonstrated that juveniles are resident to foraging areas for periods ranging from 72–827 days. More data is needed in order to establish the fidelity patterns on a longer term (years to decades) for this population. Home range has been calculated for three juveniles and exceeds 30 km<sup>2</sup>. This is the first home range calculation for foraging sea turtles in New Caledonia. The examination of the habitat use revealed a predominance of U dives, indicating feeding and resting behaviours but also shorter and shallower dives during the day compared to longer and deeper dives at night. Prey items have been identified and algae represent the larger proportion of the diet of the individuals in the GLS, more specifically *Hypnea sp.*

This study is the first study to investigate foraging turtles in New Caledonia. Consequently, the data collected within the multiple chapters of this thesis will hopefully be used for the management of



this emblematic species that holds a particular significant place in the Pacific culture. The more we understand about the ecology, the more chances we have to ensure the preservation of both the cultural aspect of this species and the species itself.

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## TERMINOLOGY AND ABBREVIATION

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List of terms and abbreviations used within this thesis:

ANOVA	-	Analysis of variance
Bp	-	Base pair
CCCE	-	Tribal Council for the Environment
CCL	-	Curved carapace length
CNMI	-	Commonwealth of Northern Mariana Islands
Cs	-	Coral Sea
DENV	-	Departement of Environment of the South Province
EAC	-	East Australian Current
FP	-	Fibropapilloma
GLS	-	Grand Lagon Sud
IUCN	-	International Union for the Conservation of Nature
KAI	-	Kilometric Abundance index
KBA	-	Key biodiversity area
KDE	-	Kernel density estimation
Km	-	Kilometres
LCs	-	Location classes
MB	-	Moreton Bay
MSA	-	Mixed stock analysis
mtDNA	-	Mitochondrial Deoxyribonucleic acid
N/A	-	Not available
NGCC	-	New Guinea coastal current
NC	-	New Caledonia
NCJ	-	North Caledonian jet
nGBR	-	Northern Great Barrier Reef
NQC	-	North Queensland current
NR	-	New recruits
PERMANOVA	-	Permuted multivariate analysis of variance
PNG	-	Papua New Guinea
QLD	-	Queensland
SD	-	Standard deviation
SECC	-	South Equatorial countercurrent
SFJ	-	South Fiji jet
sGBR	-	Southern Great Barrier Reef
SIA	-	Stable isotopes analysis
SMMPM	-	Fisheries Department of New Caledonia
Sp.		Species (single)
Spp.	-	Species (plural)
SVJ	-	South Vanuatu jet
SWB	-	Shoalwater Bay
TDR	-	Time-depth recorder
TSD	-	Temperature-dependent sex determination

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## APPENDIX 1: LIST OF PRESENTATIONS

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### Conference presentations

- **Tyffen C. Read**, Laurent Wantiez, Colin J. Limpus and Jonathan Werry (2012) Etude la population des tortues vertes résidant dans le Grand Lagon Sud. Doctoriales 2012 UNC, Noumea, New Caledonia
- **Tyffen C. Read**, Colin J. Limpus, Laurent Wantiez, and Jonathan Werry (2013) Migration of green turtles (*C. mydas*) between nesting and feeding grounds across the Coral Sea. 33<sup>rd</sup> International Sea Turtle Symposium, Baltimore, Maryland, USA
- **Tyffen C. Read**, Nancy N. FitzSimmons, Michael Jensen, Laurent Wantiez, Olivier Chateau, Jonathan Werry, Richard Farman and Colin J. Limpus (2014). Genetic structure of the resident *C. mydas* population of the Grand Lagon Sud, New Caledonia. 34<sup>th</sup> International Sea Turtle Symposium, New Orleans, Louisiana, USA
- **Tyffen C. Read**, Nancy N. FitzSimmons, Laurent Wantiez, Olivier Chateau, Florent Keller, Jonathan Werry, Richard Farman and Colin J. Limpus (2014). Genetic structure of the resident *C. mydas* population in New Caledonia: strong links to multiple rookeries in the Coral Sea. 2<sup>nd</sup> Bi-annual Australian Sea Turtle Symposium, Perth, Western Australia
- **Tyffen C. Read**, Laurent Wantiez, Olivier Chateau, Florent Keller, Jonathan Werry, Richard Farman and Colin J. Limpus (2015). Using a multi-method approach to study the foraging patterns of *Chelonia mydas*: a case study in New Caledonia. 35<sup>th</sup> International Sea Turtle Symposium, Dalaman, Turkey
- **Tyffen C. Read**, Laurent Wantiez, Olivier Chateau, Florent Keller, Jonathan Werry, Richard Farman and Colin J. Limpus (2015). Population structure, migration and habitat ecology of the green turtle (*Chelonia mydas*) in the Grand Lagon Sud of New Caledonia. 2<sup>nd</sup> Bi-annual French Sea Turtle Symposium, Paris, France
- **Tyffen C. Read** (February 2016). Horizontal and vertical movement patterns of *Chelonia mydas* in southern New Caledonia. 36<sup>th</sup> International Sea Turtle Symposium, Lima, Peru



## APPENDIX 2: MEDIA REPORTS



Figure 1. Article in the 32<sup>nd</sup> journal from Vale NC in December 2011

■ **L'invité du jeudi.** Tyffen Read, spécialiste des tortues marines

# Apprentie chercheuse

Lorsqu'elle aura terminé sa thèse, Tyffen Read deviendra la première Calédonienne spécialiste des tortues marines. Guidée par sa passion des océans et grâce à des rencontres décisives, cette jeune fille de 25 ans s'impose doucement, mais sûrement, dans l'univers scientifique en Océanie.



**A 25 ans, Tyffen Read est la première Calédonienne spécialiste des tortues marines. Elle soutiendra sa thèse en mars 2015.**

Elle n'a que 25 ans mais son parcours est déjà remarquable. En quelques années, Tyffen Read est devenue la spécialiste des tortues vertes de Calédonie. Elle en a même fait son sujet de doctorat, débuté l'an dernier. « Je ne me serais jamais imaginé faire un jour une thèse, avoue cette Calédonienne au caractère bien trempé. Quand j'étais au lycée, j'étais plutôt indécise. Si j'ai choisi la filière scientifique, c'est uniquement pour travailler sur les tortues », avoue-t-elle en souriant. « D'où lui vient cette passion ? » « Je ne sais pas vraiment, ça a toujours été comme ça, répond-elle en se plongeant dans ses souvenirs.

**« Parfois, il y a des choses incroyables qui arrivent. Et tout se goupille bien ! »**

Ça aurait pu être les dauphins, comme toutes les filles. Mais non, c'était les tortues. « Les passions sont difficilement explicables.

**Sirène.** En cherchant bien, quelques souvenirs lui remontent en mémoire. « Ma mère travaillait au ponton de Dumbéa. On l'appelait la sirène. Je passais tous mes week-ends là-bas, à regarder les poissons. J'adorais ! » Elle sait à peine marcher qu'elle sauve des bébés tortues égarés, en les ramenant à l'aquarium. « Je ne m'en souviens pas, c'est ma mère qui me l'a raconté mais j'imagine qu'inconsciemment, ça a joué un rôle. »

Les années passent mais sa passion reste intacte. Au lycée, elle se démène pour combler ses lacunes en sciences et profite des cours d'anglais, qu'elle maîtrise grâce à ses origines australiennes, pour bosser ses maths. « J'étais une élève plutôt moyenne, avec une forte tendance à travailler uniquement ce qui me plaisait. »

Le lendemain du Bac, alors qu'elle enchaîne avec un petit travail de traduction pour le WWF, elle fait une rencontre qui va tout changer. Elle sympathise avec Colin Limpus, le grand ponte australien des tortues marines.

**Cursus.** Deux mois plus tard, elle s'envole à Brisbane pour effectuer un stage avec lui. « En fait, j'ai commencé le terrain avant de rentrer à l'Université. » Quelques mois avant, elle avait décroché une bourse d'étude de la province Sud pour suivre un cursus de biologie marine. Et comme par hasard, elle s'était inscrite à l'université de Brisbane. « Parfois, il y a des choses incroyables qui arrivent. Et tout se goupille bien. »

La chance ne fait pas tout. Grâce à sa persévérance, Tyffen Read décroche ses premiers diplômes et termine son cursus universitaire par un stage à la Roche Percée. Pendant plusieurs mois, elle fait l'essuie-glace sur la plage en attendant que les tortues pondent. Les nuits sont courtes mais elle s'accroche et obtient finalement son diplôme. Forte de cette expérience et toujours avec le soutien de son « maître », elle négocie le sujet de thèse de ses rêves : « l'étude des tortues vertes dans le grand lagon Sud calédonien ». Lorsqu'elle n'est pas sur le lagon, elle analyse ces résultats dans son bureau situé à l'Aquarium de Nouméa. Un travail qu'elle réalise sous la tutelle de plusieurs professeurs australiens et d'un professeur de l'université de Nouvelle-Calédonie. Après avoir prélevé des échantillons sur les tortues, l'apprentie chercheuse s'envolera dimanche pour l'Australie, afin de réaliser les études génétiques et de décrypter leur ADN. Objectif : percer le secret de son animal totem.

Virginie Grizon

**Figure 2.** Press article in the local journal « Les Nouvelles Calédoniennes » from the 29th of August 2013

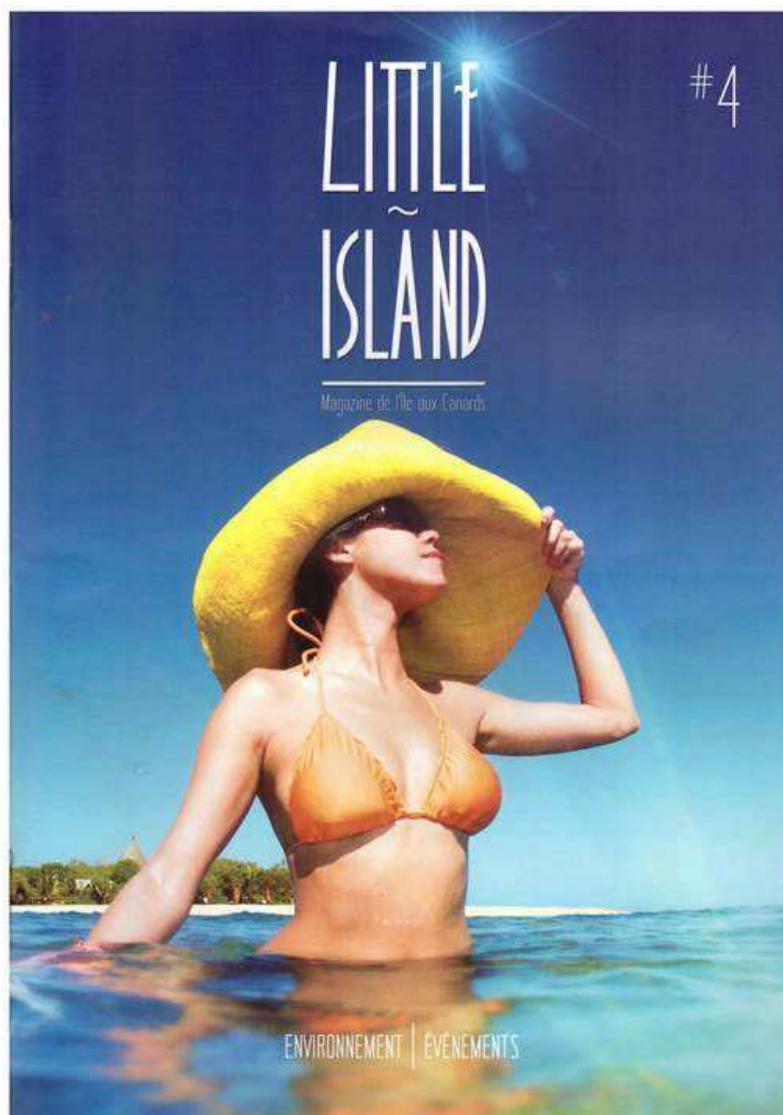


Figure 3. Press article in the magazine “OEIL” from November 2013





**Figure 4.** TV episode of “Demain c’est nous” aired on November 2013 on NCTV



## Of turtles and men

From the beginning of humanity, the turtles have fascinated human beings. A respected and untouchable animal for some people, it is coveted by others. Many turtles are now protected by the Washington Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Let's discover the life cycle and behaviour of one of the ten oldest animal species in the world...

### Origins

The history of turtles goes back more than 230 million years. The turtles are still here, having survived many climatic crises! Over the last two centuries, the extinction threat has been growing alarmingly. "Currently, explains Tyfflen Read, researcher and turtle expert at the 'Aquarium des lagon' (Lagoon Aquarium), the major problem worldwide in the sea is the great amount of plastic waste and long line fishing, which are real dangers for turtles, as well as the destruction of the egg-laying sites." On a global level, it is estimated that the Green Turtle population has decreased by 90% over the past half century. In Caledonia, no figures are available regarding the turtle population which would allow for an analysis of this issue.

### Turtles in human minds

In the Pacific region, the turtle is a major component of culture, which carries a strong symbolic meaning. "In the Kanak custom, says the scientist, the turtle is both a taboo and a totem. It is the symbol of the regeneration of the elders." An ancestral animal and living fossil, the turtle makes the children dream of an era filled with huge dinosaurs... It scarcely changed since then but thanks to its incredible adaptability, it has been surviving for several million years!

### The turtle, a great traveller

Sea turtles are both faithful to their land and a migrating animal... Just after their birth, the little turtles scatter in the open sea. When they reach sexual maturity, they come back every 3 to 5 years on their birth site to lay their eggs. "Green Turtles and Loggerhead Turtles can travel more than 4,000 km between the reefs where they feed and their egg-laying site. Therefore, there are no Caledonian turtles as such, as some of them actually come from Polynesia, Australia and other Pacific islands." Tyfflen Read has been spending the last three years studying turtles in the Great Southern Lagoon. "What we would like to set up is a census of the turtle population because, until now, this has never been done in the lagoon." As a disciple of Dr Colin Limpus, an Australian turtle expert, Tyfflen Read works to preserve the species. "There are 30 genetic groups of Green Turtles known in the Western part of the Pacific, she indicates. Here, we identified 7 species, among which the *Entrecasteaux Turtle*, a genetic group endemic to the country." Around Ducks Island, we are able to observe Green Turtles, Loggerhead Turtles and Hawksbill Turtles, attracted by the food they find in the generous sea grass beds and by its very healthy reef.

### Reproduction and egg-laying: an intense step

Female turtles do not hesitate to travel thousands of kilometres to get back to their birth place. Why do they travel so far? According to specialists, when they reach the sea, baby turtles store the earth's magnetic fields, registering some sort of GPS landmarks they will use to come back once adults. "Many hypotheses have been put forward on this issue, indicating the researcher, the most convincing being that they instinctively know that their birth place fulfils the conditions necessary for a successful egg-laying." This step requires lots of energy for the turtles which fast during the whole travel until they are back to their feeding site. After this great journey, the females which are ready to lay their eggs slowly reach the beach and sweep the sand to settle in. Then, they dig a deep vase-shaped nest to lay their eggs. Approximately 65 days later, the little turtles start coming out, looking for the brightest point in the horizon before starting their dangerous journey to reach the sea.

### NATURE DOES THINGS WELL. WHAT IF WE LET IT?

*Small guide of precautions to take in order not to disturb the turtles*

- Do not intervene and keep away from females laying their eggs.
- Do not dig the nests.
- Do not intervene while the baby turtles are progressing towards the sea.
- Do not change the ambient brightness.

### DREAMER'S WORDS


When you get close to a turtle, it will systematically turn on the side, presenting its shell as if it was a shield. It is a self-defence mechanism. To avoid being bitten on the belly. Turtles really each have their own personality, from the more timid to the very cool one which remains unimpressed. Observing them often makes you understand part of their history, from a cut in the shell (mark of a boat propeller) to missing shell scales, result of a shark bite... Turtles hold some kind of magic power which attracts us. I think they have something to do with our origins.


When you see a turtle in the water, you just can't help... admiring it! But you have to remain at your place and avoid bothering them. We are lucky they are protected in our lagoon...



Figure 5. Press article in the magazine "The little Island" from December 2014







## Thalassa





Nouvelle-Calédonie,  
les gardiens du lagon

**Diffusion :**  
Vendredi 9 Janvier à 20h45

**Durée :** 115 min


**Genre :**  
Magazine

Tous publics / 16:9 / Stéréo / VOST / Sous-titrage Malentendant

 J'aime 0
  Tweet 0
  +1 0
 

## Magazine

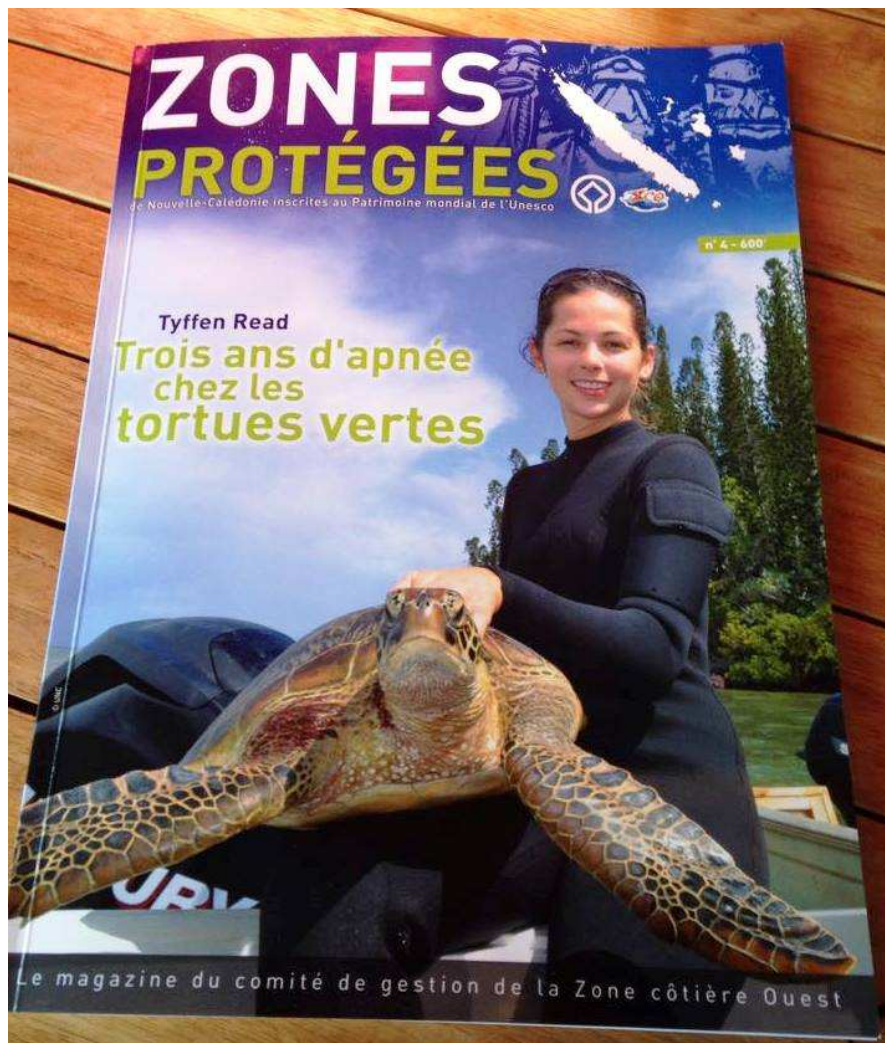
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 Présentateur : **Georges Pernoud**

**Sommaire**

Depuis 2008, le lagon de Nouvelle-Calédonie est inscrit au patrimoine mondial de l'humanité par l'Unesco. Cet écrin préservé regorge d'une nature foisonnante. Tortues, requins, dugongs y vivent paisiblement le long de la deuxième plus longue barrière de corail au monde après celle d'Australie. Des hommes et des femmes se battent pour que ce patrimoine ne disparaisse pas.

**Figure 6.** Extract from a TV magazine showcasing “Thalassa” aired on national TV in January 2015



**Figure 7.** Cover of the 4<sup>th</sup> magazine “Zones protégées” edited in October 2015