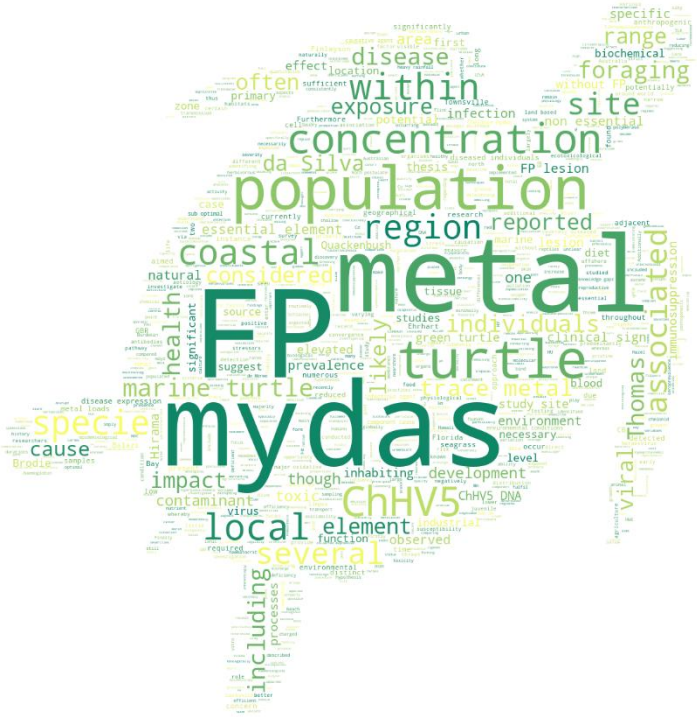
Investigating potential co-factors of Fibropapillomatosis development in *Chelonia mydas* of the Great Barrier Reef

Ph.D. thesis submitted by

Adam Wilkinson

B.Sc., MPhil.



For the degree of

Doctor of Philosophy

In the College of Public Health, Medical and Veterinary Sciences

James Cook University

January 2021

Statement of Access Declaration

I, the undersigned, author of this work, understand that James Cook University will make this thesis available for use within the University Library and, via the Australian Digital Theses network, for use elsewhere. I understand that, as an unpublished work, a thesis has significant protection under the Copyright Act and;

I do wish to place a 12-month embargo on my thesis starting at final acceptance, after which there will be no further restriction on access to this work.

Adam Wilkinson

Date: 15/01/2021

Statement of Sources Declaration

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education.

Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

Adam Wilkinson

Date: 15/01/2021

Electronic Copy Declaration

I, the undersigned, the author of this work, declare that the electronic copy of this thesis provided to the James Cook University Library is an accurate copy of the print thesis submitted, within limits of the technology available.

Adam Wilkinson

Date: 15/01/2021

Declaration of Ethics

The research presented and reported in this thesis was conducted with the approval of the James Cook University Research Ethics Committee and in accordance with the National Statement on Ethical Conduct in Human Research, 2007; Australian Code for the Care and Use of Animals for Scientific Purposes, 2007; and the Queensland Animal Care and Protection Act, 2001. The proposed research methodology received clearance from James Cook University Experimentation Ethics Committee (A2396). This research was conducted under permits granted by the Department of Environment and Science (WISP18586417 and WISP18596817) and the Great Barrier Reef Marine Park Authority (G17/39429.1).

Adam Wilkinson

Date: 15/01/2021

Acknowledgements

From the moment I began my PhD I was learning and learning quick. I thought I was prepared from a research masters, but I was mistaken. What they do not tell you is that a PhD is as much about mastering aspects of admin, health and safety, team leadership, field trip coordination, public affairs, and economics as it is about conducting the research. Throughout my project I accomplished many different things both directly measurable in this thesis but also behind the scenes to make this thesis possible. I obtained a boat licence, upskilled my SCUBA qualifications enrolled in various courses and training to be the most valuable and proficient field operator and leader I could be. I dedicated myself to learn the intricacies common in field work such as safety and risk analysis, team mentorship and coaching and the efficient coordination of dynamic and rapid field processes. By the end of my project, I was proficient as turtle capture, boat handling, blood and scute sampling, gastric lavage, seagrass identification and collection. I developed confidence in laboratory practices, networking, public speaking, and science communication. I invested in professional development to ensure I got the most out of my time as a field manager. I was responsible for all volunteers which were essential to the complex nature of marine turtle research. I conducted all the administration, assisted with training, was responsible for risk assessment of all field activities and maintained all equipment and boat operation. Conducting intensive marine turtle capturing efforts mean plenty of blood, sweat, tears and broken bones! It was unpredictable and very rewarding, as was the entirety of my PhD.

I can honestly say that my PhD journey has been the most difficult and challenging journey of my life. With countless struggles and difficulties both academically and in private, both on a personal level and as a world nation. It is safe to say that I emerge from this milestone with my eyes wide open and ready for whatever this crazy world has to offer up next. 2020 has arguably been the most bizarre year in living memory, for the entire world. With a global pandemic and mass civil unrest on a planet which is on the precipice of catastrophic climate change, it is often hard to maintain the focus and determination required to complete an endeavour so mentally taxing as a PhD and at times it could probably be argued that my sanity was on the line too! I paint an honest picture of my experiences to say that I am happy that I persevered through the tough times because the good times were so much richer and rewarding. I am happy that I am still here at the end of my PhD and I can write this acknowledgements section of my thesis.

First and foremost, I would like to give a massive thank you to Ellen Ariel, my primary supervisor. During my candidature Ellen was awarded official recognition as advisor of the year and I could not agree with this more. She always has her student's best interests at heart and strives to gently guide us through the convoluted and bumpy road of a PhD. From a friendly smile and her supportive nature to firm pep talks and strict deadlines, Ellen always knows what is needed to get you over the finish line. I have learnt a lot about both my subject and other aspects of what it means to be a research scientist. This growth is largely attributable to Ellen but a heartfelt thank you also goes out to my other supervisors, Graham Burgess, Stephanie Duce, Hilary Vanderven for their continued support and advice on study design, field techniques and data analysis direction. Without a solid team of experienced advisors this project would have been significantly harder.

I would like to give a special mention to Dr. Jon Brodie, who sadly passed away towards the end of my candidature. He contributed a lifetime to science, collaborating with countless researchers and working directly on the determination of policy and management of the GBR. I had the fortune to first meet Jon during my master's and was very pleased when he agreed to join my Ph.D. advisory panel. During the time spent working on my project Jon contributed invaluable knowledge and insight into numerous aspects of the topic. He will be missed. along a similar vein, I would like to give a special thank you to Dr. Jason van de Merwe for stepping in at the final hour to provide proficient support in the areas of my panel which were left lacking. Jason did a fantastic job in providing expertise in the ecotoxicological study of metals and was essential when it came to feedback on the drafted thesis.

Next, I would like to give a big thank you to all the crucial volunteer support which I received from the great people of the JCU Turtle Health Research group and my fellow Ph.D. students, who helped me throughout my candidature with everything from thesis editing, data entry, laboratory work and field operations. Catching and sampling of marine turtles is a mammoth task which would have been impossible without a large team of personnel. I would like to give a special thank you to the following people: Wytamma Wirth, Rebecca Diggins, Sara Kophamel, Duan March, Karina Jones, Alicia MacLaine, Narges Mashkour, Lily Donnelly, Edith Shum, Bethany Smith, and Daniel Gonzalez-Paredes. Though, again I would like to thank any and everyone who volunteered their time to help, I really appreciate it.

I would like to give a big heart felt thank you to the Indigenous Ranger groups which I had the privilege to work alongside throughout my project. A special thank you goes to Gudjuda

Reference Group and particularly Edward Smallwood, Bendavow, Diane, Tracey, Sheryl and most of all, Uncle Jim. Thanks for welcoming me and offering your wealth of knowledge, experience, and culture. Most of all, thank you to the traditional owners of the land, past, present, and future for allowing me to conduct my research on the land and in the sea of your country.

Thank you to Dr. Ian Bell (DES) and Dr. Colette Thomas from (SEED) for field assistance throughout certain aspects of sample collection. Particularly, thank you for assisting Sara collect samples from the Howick's green turtle population. This data was invaluable to my project. Also, thank you for sharing your knowledge and experience which influenced my study design and field work. Additionally, thank you to Colette for lending her time to edit parts of my thesis writing and for being so kind as to lend her environmental metals data for reference and comparison in Chapter 3.

I would like to extend a big thank you to all the admin and lab tech staff that helped me throughout. A big thank you goes to Tina, Sherie and Linda for forever helping me to navigate and conquer all the necessary paperwork and ordering of specialised reagents, it would have surely driven me insane without your assistance! Also, a massive thank you to everyone involved with the Doctoral Cohort Program, especially Melissa Crowe and Diana Mendez. I feel very privileged to have been able to be a part of the program. It was a unique resource that I know brought that extra dimension to the project that, for me, made a good Ph.D. experience into a great one. I would also like to give a big thank you to Greg and Glen from Boating and Diving, JCU. You made working with the boats, gear and the planning and implementation of field trips as simple as possible and provided confident support throughout my frequent field seasons. A big thank you to JCU and the APA for providing my scholarship.

A massive thank you to Mathias Ackermann and everyone from The University of Zurich for welcoming and supporting me with my international visit and collaboration. It was a great honour to be invited to attend and work at the institute alongside such renowned scientists in various fields. A big thank you to Mathias for working with me on several occasions, both in Switzerland and Australia, to implement the ELISA protocol, which you helped to develop, on my serum samples.

Finally, I would like to give a big and ongoing thank you to all my friends and family both domestic and abroad for their continued and unwavering support throughout this long and

arduous academic journey. It has been one hell of a trek, but the end is here. Thank you all for helping me to cling on to the last of my sanity and make it this far. I appreciate you all.

I am thankful to all of those acknowledged here and everyone I have no doubt overlooked. I thank anyone who had the patience to work with or listen to me. I am thankful to a lot of people but most of all I am thankful to be have completed this thesis!

Statement of contribution of others

Nature of assistance	Contribution	Title, name, and affiliation of co-contributor
Intellectual support	Conceptual idea, supervision, and data analysis	**A/Prof Ellen Ariel** College of Public Health, Medical and Veterinary Sciences, James Cook University, Australia. **Dr Graham Burgess** College of Public Health, Medical and Veterinary Sciences, James Cook University, Australia. **Dr Jon Brodie** ARC Centre of Excellence for Coral Reef Studies, James Cook University, Australia. **Dr Jason van de Merwe** Australian Rivers Institute, Griffith University, Australia. **Dr Hillary Vanderven** College of Public Health, Medical and Veterinary Sciences, James Cook University, Australia. **Dr Stephanie Duce** College of Science and Engineering, James Cook University, Australia. **A/Prof Scott Heron** College of Science and Engineering, James Cook University, Australia.
	Publication Co-Authors	**A/Prof Ellen Ariel** College of Public Health, Medical and Veterinary Sciences, James Cook University, Australia. **Dr Graham Burgess** College of Public Health, Medical and Veterinary Sciences, James Cook University, Australia. **Dr Jon Brodie** ARC Centre of Excellence for Coral Reef Studies, James Cook University, Australia. **Dr Jason van de Merwe** Australian Rivers Institute, Griffith University, Australia. **Dr Hillary Vanderven** College of Public Health, Medical and Veterinary Sciences, James Cook University, Australia. **Prof. Mathias Ackermann** Institute of Virology, University of Zurich, Switzerland. **Dr. Colette Thomas** Great Barrier Reef Marine Park Authority, Australia **Dr. Thierry Work** United States Geological Society, Hawaii USA
	Mentoring and Peer Support	**Doctoral Cohort Program** Division of Tropical Health and Medicine, James

		Cook University, Australia
	Statistical Support	**Rhondda Jones** Division of Tropical Health and Medicine, James Cook University, Australia
	Editorial Support	**Wytamma Wirth** College of Public Health, Medical and Veterinary Sciences, James Cook University, Australia. **Rebecca Diggins** College of Public Health, Medical and Veterinary Sciences, James Cook University, Australia.
Data Collection	Field Work	**A/Prof Ellen Ariel** College of Public Health, Medical and Veterinary Sciences, James Cook University, Australia. **Dr. Colette Thomas** Great Barrier Reef Marine Park Authority, Australia **Dr Ian Bell** Department of Environment and Science, Queensland Government, Australia **Sara Kophamel** College of Public Health, Medical and Veterinary Sciences, James Cook University, Australia. **Rebecca Diggins** College of Public Health, Medical and Veterinary Sciences, James Cook University, Australia. **Jessica Grimm** College of Public Health, Medical and Veterinary Sciences, James Cook University, Australia. **Duan March** College of Public Health, Medical and Veterinary Sciences, James Cook University, Australia. **Edith Shum** College of Public Health, Medical and Veterinary Sciences, James Cook University, Australia. **Gudjuda Reference Group** Home hill, Australia.
	Permits	**A2396** Animals Ethics Committee, James Cook University, Australia. **WISP18586417** Department of Environment and Science, Queensland Government, Australia. **WISP18596817** Department of Environment and Science, Queensland Government, Australia. **G17/39429.1** Great Barrier Reef Marine Park Authority, Australia.
	Laboratory Support	**Dr. Hillary Vanderven** College of Public Health, Medical and Veterinary Sciences, James Cook University, Australia.

		Prof. Mathias Ackermann Institute of Virology, University of Zurich, Switzerland. **Dr. Wytamma Wirth** College of Public Health, Medical and Veterinary Sciences, James Cook University, Australia. **Yi Hu** Advanced Analytical Centre, James Cook University, Australia.
Financial support	Stipend	**James Cook University Postgraduate Research Scholarship** James Cook University, Australia **Doctoral Completion Grant** College of Public Health, Medical and Veterinary Sciences, James Cook University, Australia.
	Research Funding	**WWF 2019** World Wildlife Foundation, Australia. **Higher Degree Research Enhancement Scheme Round 2 2017** College of Public Health, Medical and Veterinary Sciences, James Cook University, Australia. **Higher Degree Research Enhancement Scheme Round 2 2020** College of Public Health, Medical and Veterinary Sciences, James Cook University, Australia.
	Conference Support	**TropWATER Travel Grant 2018** Centre for Tropical Water and Aquatic Ecosystem Research, James Cook University, Australia

Thesis Summary

Background

Marine turtles are long-lived and charismatic but face a plethora of threats, both on a global and local scale. One significant threat is that of disease, particularly the neoplastic and debilitating disease, Fibropapillomatosis (FP). This disease has been reported in all species but has only reached enzootic levels in *Chelonia mydas* (*C. mydas*). Identification of FP was first reported over 70 years ago, and the disease has received attention since then. However, numerous knowledge gaps regarding aetiology are still present. Association is often placed between FP and the marine turtle virus Chelonid alphaherpesvirus-5 (ChHV5), due to regular detection of viral DNA in biopsied FP lesions (and occasionally in normal skin tissue), though definitive causation is yet to be confirmed, due to lack of the ability to grow the virus *in vitro*. While it is likely that a viral infection is associated with the development of FP, in recent years, evidence has suggested that a multifactorial approach is necessary to adequately describe FP disease expression. Previous research has investigated a range of environmental stressors which may play a role by compromising immune function and thus possibly increasing susceptibility to additional infection. Toxic trace metal exposure is potentially one component cause of FP as metal elements are highly persistent and have been found to cause immunosuppression in marine organisms, including marine turtles. Due to the long-lived nature of *C. mydas* and their propensity for strict site fidelity to narrow foraging ranges means that populations which inhabit coastal grounds in proximity to human settlement, urban development and intensive land practices are potentially exposed to high metal concentrations over extended periods. This is of significant concern as metals are known to bioaccumulate in *C. mydas* tissues and forage material (seagrass and macroalgae) at concentrations far greater than the surrounding environment. As *C. mydas* are reptiles and have lungs, they breathe air at the surface and therefore diet is the primary exposure pathway for trace metals exposure (unlike other aquatic animals which may pass water over gills and absorb contaminants through skin and other membranes). To understand *C. mydas* exposure to elevated metal concentrations and susceptibility to FP, investigation into local environmental metal profiles is also required.

This thesis aimed to investigate and describe local metal profiles in ecologically relevant seagrass meadows and local *C. mydas* populations which inhabit those foraging areas. Additionally, I also aimed to survey the seroprevalence of ChHV5-specific antibodies in the same populations to better determine whether association between the virus and FP expression

is present in *C. mydas* of the GBR. I captured and sampled turtles from five study sites (four coastal sites and one offshore control population) of the northern and central GBR regions. Fibropapillomatosis is known to be enzootic to two of the study sites, with no previous FP cases reported at any of the other locations. All coastal sites are within proximity to human settlement with agricultural and industrial land practises conducted in varying intensities within all catchment areas, whereas the control site is remote and is minimally influenced by anthropogenic activity.

Environmental metal exposure within local *C. mydas* foraging grounds of the Great Barrier Reef (GBR)

Seagrass species tend to bioaccumulate metals at concentrations greater than that detected in the surrounding environment. Toxic metal exposure is a significant threat to marine turtles inhabiting and foraging in coastal seagrass meadows and are of particular concern in local Bays of the Great Barrier Reef (GBR) as numerous sources of metal contamination are located within the region. Little is known regarding ecotoxicological impacts of environmental metal loads on seagrass or *C. mydas* and thus in chapter 3 I aimed to investigate and describe seagrass metal loads in three coastal sites and one offshore control site located in the Northern and Central GBR. Primary seagrass forage of *C. mydas* was identified and samples collected from foraging sites before and after the 2018/2019 wet season and multivariate differences in metal profiles were investigated between sites and sampling events. Most metals investigated were higher at one or more coastal sites when compared to the control site and Cadmium (Cd), Cobalt (Co), Iron (Fe) and Manganese (Mn) were found to be higher at all coastal sites and total metal profiles were dissimilar between sampling events. Principle Component Analysis (PCA) found that metal profiles in coastal sites were like one another, but all were distinctly different from that of the control site (confidence ellipses did not intercept). Coastal foraging sites are influenced by land-based contaminants which enter the coastal zone via river discharge during periods of heavy rainfall and influences sites closest to the source. Bioavailablility of metal elements are determined by complex interactions and processes which are largely unknown but association between elevated metal loads and turtle disease warrants further investigation to better understand the impact of environmental contaminants on ecologically important seagrass and associated macrograzers.

Local *C. mydas* blood and scute metal profiles along the GBR

Marine turtles face numerous anthropogenic threats, including that of chemical contaminant exposure. Ecotoxicological impact of toxic metals is a global issue facing *C. mydas* in coastal

sites where they reside and forage. Local investigation of *C. mydas* short-term blood metal profiles is an emerging field while very little research has been conducted on scute (carapace) metal loads as potential indicators of long-term exposure. In chapter 4 I aimed to investigate and describe *C. mydas* blood and scute metal profiles in coastal and offshore populations of the GBR. This was achieved by analysing blood and scute material sampled from local *C. mydas* populations in five field sites, for a suite of ecologically relevant metal elements. By applying Principal Component Analysis and comparing coastal sample data to that of reference intervals (diagnostic approach indicative of natural baseline metal concentrations from an ecologically relevant population) derived from the control site, insight was gleaned on local metal profiles of each population. Blood metal concentrations in turtles, from coastal sites, were typically elevated when compared with concentrations recorded in the offshore population (Howick Island Group, HWK). Scute metal profiles were similar in Cockle Bay (CB), Upstart Bay (UB) and Edgecumbe Bay (EB), all of which were distinct from that of Toolakea beach (TLK). Some elements were reported at similar concentrations in blood and scutes, but most were higher in scute samples, indicative of accumulation over time. Coastal *C. mydas* populations may be at risk of toxic effects from metals such as Co, which was consistently found to be at high concentrations magnitudes above the reference intervals derived from the control site, Howick's Island Group (HWK). Little is known about the distribution and toxic effects of element concentrations in marine turtles and further investigation is required to better understand the potential adverse effects of such contaminants on species that inhabit heavily influenced environments in proximity to urban settlement.

Chelonid alphaherpesvirus-5 exposure in local *C. mydas* populations of the GBR

Green turtles (*Chelonia mydas*) are an iconic species which are considered globally as being critically endangered and face significant threats such as the debilitating neoplastic disease, Fibropapillomatosis. The herpesvirus, Chelonid alphaherpesvirus-5 (ChHV5) has been associated with FP. Sero-diagnostic approaches have detected evidence of past exposure in turtles without FP by detecting the presence of anti-ChHV5 antibodies in presumably recovered animals. In chapter 5 I aimed to survey ChHV5 exposure by measuring seroprevalence of ChHV5-specific antibodies in local *C. mydas* populations of five study areas of the Great Barrier Reef, two of which have historically been reported to include turtles with FP. The aim of this study was achieved by applying an ELISA for detecting two turtle antibodies (7s IgY

and 5.7s IgY) specific to ChHV5 glycoproteins (antigen). Seroprevalence ranged from 10 - 45%, with no significant difference ($p = > 0.05$) for either IgY type between all study sites sampled. 30% samples from 2010, were reported as positive for FP, and had higher seroprevalence, for 7s (20%) and 5.7s (15%), though no significant differences were found between these individuals and turtles without FP (collected in 2010 and in the current candidature). Homogenous exposure to ChHV5 across the region is indicative of additional component causes that are necessary for FP disease expression. For instance, ChHV5 may not be the sole infectious agent responsible for FP development. This suggestion is supported by the contrast in prevalence homogeneity of ChHV5 (similar between sites) and FP (inconsistent prevalence within the region). Other potential causes are likely to be environmental co-factors and a multifactorial approach should be considered in future work investigating FP aetiological knowledge gaps.

Summary of thesis findings

I found that environmental metal loads were comparable in seagrass samples collected in coastal sites and were detected at elevated concentrations relative to data for the offshore control site. In *C. mydas* blood a similar pattern was observed, whereby coastal metal concentrations were similar between sites and elevated when compared to the control site. Most metals were within region-specific references intervals previously published. Scute metal concentrations tended to be higher when compared to blood, indicating that long term bioaccumulation likely occurred. Some element concentrations (Co, Cu and Fe) were similar between blood and scutes, suggesting homeostasis may have been reached for such essential elements. When surveying the same coastal and control sites as the ecotoxicological aspects of this thesis, seroprevalence of ChHV5-specific antibodies was similar between all study sites and suggested that viral exposure may be ubiquitous throughout the region and therefore is unlikely to be the sole causative agent of FP.

VILLA, C. A., FLINT, M., BELL, I., HOF, C., LIMPUS, C. J. & GAUS, C. 2017. Trace element reference intervals in the blood of healthy green sea turtles to evaluate exposure of coastal populations. *Environmental Pollution,* 220, Part B**,** 1465-1476.

TABLE OF CONTENT

List of tables

List of figures

List of abbreviations

Abbreviation	Definition
ΔOD	Delta Optical Density
ΔOD450	Delta Optical Density at 450 nm
χ^2	Chi-square independence test
AAC	Advanced Analytical Centre
Al	Aluminium
BAF	Bioaccumulation Factor
BCA	Bicinchoninic acid Assay
Ca	Calcium
CB	Cockle Bay
Cc	*Caretta caretta*
CCL	Curved Carapace Length
Cd	Cadmium
cGBR	Central Great Barrier Reef
ChHV1	Chelonid alphaherpesvirus 1 (
ChHV5	Chelonid alphaherpesvirus 5
ChHV6	Chelonid alphaherpesvirus 6
CLV	Cleveland Bay
Cm	*Chelonia mydas*
Co	Cobalt
Cos2	Cosine 2 (Quality of Association)
Cs	*Cymodocea serulatta*
CSe	Coral Sea
Cu	Copper
DRA	Disease Risk Assessment
EB	Edgecumbe Bay
EBa	EB archived
EBp	EB present
ELISA	Enzyme Linked Immunosorbent Assay
Fe	Iron
FP	Fibropapillomatosis
GBR	Great Barrier Reef
GBRMPA	Great Barrier Reef Marine Park Authority
GBRWHA	Great Barrier Reef World Heritage Area
GST	Glutathione S-Transferase
H_2O_2	Hydrogen peroxide
H_3PO_4	Phosphoric acid
Hg	Mercury
HNO_3	Nitric acid
Hu	*Halodule uninervis*
HV	Herpes virus
HWK	Howick Island Group
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical emission spectrometry

Ig	Immunoglobulin
IUCN	International Union for Conservation of Nature
K	Potassium
Lk	*Lepidochelys kempii*
Lo	*Lepidochelys olivacea*
LOD	Limit of Detection
Mg	Magnesium
Mn	Manganese
MOI	Multiplicity of Infection
NC	New Caledonia
nGBR	Norther GBR
Ni	Nickel
Pb	Lead
PBS	Phosphate Buffer Solution
PCA	Principle Component Analysis
PCR	Polymerase Chain Reaction
POP	Persistent Organic Pollutants
ppm	Parts per million
RI	Reference Interval
ROS	Reactive Oxygen Species
sGBR	Southern GBR
Sr	Strontium
TLK	Toolakea Beach
U	Uranium
UB	Upstart Bay
WS	Wet Season
Zn	Zinc

List of appendices

CHAPTER 1 - Thesis pre-amble

1.1. Marine turtle background

Marine turtles are a unique, ancient and iconic animal, with seven extant species (Frazier, 2003), including *Chelonia mydas* (green turtle), which is the species of focus in this thesis. However, populations around the world consistently undergo decline, at varying rates (Jones et al., 2016). Marine turtles are long lived and inhabit a range of different habitats throughout their life history, including the natal beach and the coastal foraging zones, after a pelagic existence as post-hatchlings (Musick and Limpus, 1997, Bolten, 2003). *Chelonia mydas* spend much of their long-life inhabiting shallow coastal environments, often adjacent to intensive anthropogenic activity. As juveniles, newly recruited to coastal foraging grounds, individuals demonstrate strict site fidelity, inhabiting narrow geographical ranges of approximately 2 km^2 (Hazel et al., 2013). Such characteristics potentially make the green turtle a suitable indicator of local environmental health and may provide early warning of sub-optimal environmental conditions through regular monitoring (Flint et al., 2019, Gaus et al., 2019). However, while *C. mydas* are considered keystone species and are well studied, knowledge gaps are still present. One significant threat to marine turtles that requires further study is that of ecotoxicological implications of chemical contaminants (Hamann et al., 2010), such as toxic trace metal concentrations, on health and susceptibility to disease.

1.2. The role of diet on green turtle susceptibility to toxic metal exposure

Chelonia mydas are reptiles, have impermeable skin and breathe air, which means that the primary metal exposure pathway is through diet (Villa et al., 2017, Work et al., 2020). Coastal dwelling juveniles are considered to have a predominantly herbivorous diet following the ontogenetic diet shift that occurs at the end of their pelagic existence when they recruit into the neritic zone (Morais et al., 2014, Vélez-Rubio et al., 2016). Australian populations often forage primarily on seagrass or red algae species (Thomas et al., 2020), depending on available species within local foraging sites. Within the study region of this thesis, the preferred seagrass species is *Halodule uninervis* (HU), a strap like colonising species which commonly grows in shallow coastal meadows of the Great Barrier Reef (GBR) (Thomas et al., 2020). *Halodule uninervis* has previously been reported as an ideal bioindicator of local environmental health (Bu-Olayan and Thomas, 2016), and is considered an efficient bioaccumulator of certain metals (Thangaradjou et al., 2013, Thomas et al., 2020). This property is beneficial for maintaining

good water quality in the local environment, but not necessarily ideal for *C. mydas,* who are themselves susceptible to bioaccumulation of contaminants and rely on such seagrass for forage (Caurant et al., 1999, Cortés-Gómez et al., 2017, Nicolau et al., 2017, Ross et al., 2017). Several studies have been conducted to survey metal concentrations within coastal seagrass meadows (Schlacher-Hoenlinger and Schlacher, 1998, Haynes, 2001, Brodie et al., 2019), but few have attempted to specifically investigate green turtle exposure to metals (Thomas et al., 2020).

1.3. Trace metal concentrations as significant environmental threats

Trace metals can be divided into two distinct categories when looking at impacts on marine organisms: 1) essential (crucial for biochemical processes), such as Cu, Fe and Mn, and 2) non-essential elements (Cd, Pb and Co). Some trace metals are naturally geoavailable in the environment, with distinct differences in availability seen between coastal and offshore regions (Thomas et al., 2020). Within the coastal zone, adjacent to human populations, a plethora of land-based metal loads are routinely detected at foraging sites of local green turtle populations (Villa et al., 2017, Thomas et al., 2020). Such metals originate from industrial practices, (including mining and metal refining), agriculture and urban development (Brodie et al., 2017). One of several exposure pathways that these contaminants may enter the marine environment is by first leaching from soils and sediment to rivers and into estuaries and the coastal zone (Komoroske et al., 2012, Brodie et al., 2017). This influx of contaminants is most significant during periods of heavy rainfall, such as in the annual wet season (November – March) in north Queensland, the study region of interest to this thesis. Both essential and non-essential metals can have detrimental effects on marine organisms, though non-essential elements are considered potentially toxic at very low concentrations, while essential elements can be toxic above and below optimal concentration ranges (Villa et al., 2017). Numerous metals have been reported to impact *C. mydas* in various ways, such as reduced reproductive success and disrupting endocrine processes, which may lead to additional impacts to individuals and populations (da Silva et al., 2016). Additionally, oxidative stress, through the production of reactive oxygen species (ROS), can impact biomolecular processes and may cause tissue damage (Halliwell and Gutteridge, 2015). Oxidative stress has been associated with immunosuppression (Valyi-Nagy and Dermody, 2005), and as a co-factor in viral infections (Beck et al., 2000).

1.4. Fibropapillomatosis in *Chelonia mydas*

Chelonia mydas are predominantly herbivorous marine reptiles found in the majority of tropical and subtropical regions of the world (Garnett et al., 1985, Pritchard et al., 1997). Threats faced are diverse and depend largely on local climate and intensiveness of industrial land use on a regional scale. One threat that is of significant concern to populations globally is that of disease (Hamann et al., 2010). There are several known diseases that affect *C. mydas* (George, 2017), with fibropapillomatosis (FP) being considered the most notable. FP is an enzootic neoplastic and debilitating disease that is most often detected in *C. mydas* (Jones et al., 2016, Page-Karjian, 2019), but cases have been observed in other sea turtle species. Characterised by growth of large visual lesions from soft tissue areas such as the neck, eyes and flippers (Herbst, 1997, Work et al., 2004), FP is reported, in varying prevalence rates, throughout the natural geographical range of *C. mydas* (Jones et al., 2016). Although FP was first reported in 1938 (Smith and Coates, 1938), there are still many knowledge gaps in the pathogenesis, aetiology and impacts of this disease (Jones et al., 2016). Regarding FP causation, associated causative agents have not been conclusively identified, though at present, researchers consider an alphaherpesvirus, Chelonid alpha-herpesvirus 5 (ChHV5), as the most likely primary infectious agent associated with the disease (Herbst et al., 1995, Quackenbush et al., 2001, Page-Karjian, 2019). Essential criteria necessary to fulfil Koch's postulate was not possible due to difficulty in culturing the virus *in vitro* (Herbst, 1994, Herbst and Klein, 1995, Lu et al., 1999, Work et al., 2009, Ackermann et al., 2012). However, advancements were recently made whereby ChHV5 was successfully cultured on C. mydas tissue rafts (Work et al., 2017). The working hypothesis of ChHV5 as the main infectious agent associated with FP is maintained because ChHV5 DNA is routinely detected within FP lesion biopsies, but not as often in skin samples from turtles without FP (Quackenbush et al., 1998, Lackovich et al., 1999, Page-Karjian et al., 2012, Page-Karjian et al., 2015). Additionally, ChHV5 DNA was detected in blood samples from a wild *C. mydas* USA population using quantitative polymerase chain reaction (qPCR) (Page-Karjian et al., 2020). Positive qPCR results in animals without FP suggest that ChHV5 infection is active, but subclinical (without visible clinical signs) (Page-Karjian et al., 2015). ChHV5 DNA molecular detection is not the only diagnostic tool for identifying viral exposure. Serodiagnostic testing via Enzyme Linked Immunosorbent Assays is an understudied approach that can be implemented to gain insight into prior infection by detecting ChHV5-specific antibodies in green turtle serum samples (Page-Karjian et al., 2020, Work et al., 2020). By detecting the ChHV5 viral footprint, this approach provides the

opportunity to better survey distribution and prevalence of the virus compared to what direct methods of viral detection can achieve. This protocol has recently been used to measure seroprevalence of ChHV5 antibodies in green turtle populations in Hawaii and Florida (Work et al., 2020) and North Carolina (Page-Karjian et al., 2020), where it was found that turtles with, and some without FP, clinical signs were seropositive for ChHV5-specific antibodies (Work et al., 2020). This implied viral exposure in individuals without clinical signs, suggesting that additional factors may influence the FP lesion development, and a multifactorial approach to this topic of research should therefore be considered (Herbst, 1994, Greenblatt et al., 2004, Jones et al., 2016, Page-Karjian, 2019, Page-Karjian et al., 2020).

1.5. Immunosuppression and environmental co-factors.

Turtles with FP lesions are commonly reported to be immunosuppressed (reduced immune function) (Work et al., 2001). However, it is unclear as to whether this condition is causative, or is an effect of the disease (Santos et al., 2010). Though it is likely that, in the case of FP, immunosuppression increases susceptibility to infection (Work et al., 2001), and could possibly influence FP disease expression. Sub-optimal environmental conditions may induce immunosuppression (da Silva et al., 2016), thus providing an opportunity for reactivation of ChHV5 viral replication and development of lesions (da Silva et al., 2016). Environmental co-factors that could potentially cause immunosuppression are biotoxins (Landsberg et al., 1999, Arthur et al., 2006), extreme UV (Duffy et al., 2018), organic contaminants and elevated trace metal exposure (da Silva et al., 2016, Villa et al., 2017).

1.6. Thesis aims and direction

In this thesis, I aimed to investigate links between FP prevalence and trace metal concentration in the diet and body of *C. mydas* in local populations within the central and northern GBR regions. The study sites investigated throughout this these can be separated into three geographical regions (Figure 1.1). Firstly, the control site, Howick Island Group (HWK), is a remote offshore site located at the northern end of the GBR on the east coast of Australia. The *C. mydas* population that resides there is often studied as one that is minimally impacted by anthropogenic contamination, and data collected here is considered near-baseline concentrations (Villa et al., 2017, Bell et al., 2019, Villa et al., 2019, Thomas et al., 2020). The near pristine nature of the region makes HWK an important area of reference for research investigating health and ecotoxicological aspects in coastal *C. mydas* populations of the GBR. The remaining two regions are both coastal, with the first one being the Townsville region,

which encompasses two study sites, Toolakea Beach (TLK) and Cockle Bay (CB). Both sites are adjacent to heavily populated areas of Townville city. CB is near the international Port of Townsville whereas TLK is 30 km north of the city. Within the region of these study sites a range of industrial and agriculture land uses occur (including sugar cane farming and metal refining) with some freshwater runoff occurring during periods of heavy rainfall, entering the coastal environment via the Ross River discharge. Finally, the Burdekin region to the south of Townsville includes Upstart Bay (UB) and Edgecumbe Bay (EB). Unlike Townsville, this region is somewhat less populated, though numerous land practices (particularly agriculture and beef grazing) occur within this catchment area. Within UB is the outflow of the Burdekin River which is a significant source of land based contaminants to UB, CB and EB (Brodie et al., 2017). FP has been found to be enzootic in *C. mydas* populations inhabiting CB and EB, with no reported cases of the disease at any of the other sites described here (Jones et al., 2020).

Three distinct aims were addressed and were as followed. Firstly, I aimed to investigate potential metal exposure of local populations by sampling representative seagrass species that constitute a major fraction of local C. *mydas* diet. Secondly, I aimed to measure and compare short (blood) and long term (scute) metal loads in local *C. mydas* to better understand turtle health and to quantify any elevated metal concentrations of concern. Finally, I aimed to survey ChHV5 antibody seroprevalence in individuals, with and without FP clinical signs, to better understand viral exposure and susceptibility of populations to increased FP development.

Figure 1.1 Map of the five study areas where *C. mydas* were captured and sampled throughout this PhD project. Each site is colour coordinated between the national overview and localised maps. The respective localised maps are displayed in the order of sites from north to south. The order is as follows, The Howick Group of islands and reefs (HWK), Toolakea beach (TLK), Cockle Bay (CB), Upstart Bay (UB) and Edgecumbe Bay (EB). Toolakea and CB appear overlapped in the national overview but are geographically distinct on the local scale.

1.7. References

ACKERMANN, M., KORIABINE, M., HARTMANN-FRITSCH, F., DE JONG, P. J., LEWIS, T. D., SCHETLE, N., WORK, T. M., DAGENAIS, J., BALAZS, G. H. & LEONG, J.-A. C. 2012. The Genome of Chelonid Herpesvirus 5 Harbors Atypical Genes. *PLOS ONE,* 7**,** e46623.

ARTHUR, K., SHAW, G., LIMPUS, C. & UDY, J. 2006. A review of the potential role of tumour-promoting compounds produced by Lyngbya majuscula in marine turtle fibropapillomatosis. *African Journal of Marine Science,* 28**,** 441-446.

BECK, M. A., HANDY, J. & LEVANDER, O. A. 2000. The Role of Oxidative Stress in Viral Infections. *Annals of the New York Academy of Sciences,* 917**,** 906-912.

BELL, I. P., MEAGER, J., VAN DE MERWE, J. P. & MADDEN HOF, C. A. 2019. Green turtle (Chelonia mydas) population demographics at three chemically distinct foraging areas in the northern Great Barrier Reef. *Science of The Total Environment,* 652**,** 1040-1050.

BOLTEN, A. B. 2003. Variation in sea turtle life history patterns: neritic vs. oceanic developmental stages. *In:* LUTZ, P. L., MUSICK, J. & WYNEKEN, J. (eds.) *The biology of sea turtles.* Boca Raton: CRC Press.

BRODIE, J., GRECH, A., PRESSEY, B., DAY, J., DALE, A. P., MORRISON, T. & WENGER, A. 2019. The Future of the Great Barrier Reef: The Water Quality Imperative. *In:* WOLANSKI, E., DAY, J. W., ELLIOTT, M. & RAMACHANDRAN, R. (eds.) *Coasts and Estuaries.* Elsevier.

BRODIE, J. E., LEWIS, S. E., COLLIER, C. J., WOOLDRIDGE, S., BAINBRIDGE, Z. T., WATERHOUSE, J., RASHEED, M. A., HONCHIN, C., HOLMES, G. & FABRICIUS, K. 2017. Setting ecologically relevant targets for river pollutant loads to meet marine water quality requirements for the Great Barrier Reef, Australia: A preliminary methodology and analysis. *Ocean & Coastal Management,* 143**,** 136-147.

BU-OLAYAN, A. & THOMAS, B. 2016. Accumulation and translocation of trace metals in Halodule uninervis, in the Kuwait coast. *Biosciences Biotechnology Research Asia,* 7**,** 25-32.

CAURANT, F., BUSTAMANTE, P., BORDES, M. & MIRAMAND, P. 1999. Bioaccumulation of Cadmium, Copper and Zinc in some Tissues of Three Species of Marine Turtles Stranded Along the French Atlantic Coasts. *Marine Pollution Bulletin,* 38**,** 1085-1091.

CORTÉS-GÓMEZ, A. A., ROMERO, D. & GIRONDOT, M. 2017. The current situation of inorganic elements in marine turtles: A general review and meta-analysis. *Environmental Pollution,* 229**,** 567-585.

DA SILVA, C. C., KLEIN, R. D., BARCAROLLI, I. F. & BIANCHINI, A. 2016. Metal contamination as a possible etiology of fibropapillomatosis in juvenile female green sea turtles *Chelonia mydas* from the southern Atlantic Ocean. *Aquatic Toxicology,* 170**,** 42-51.

DUFFY, D. J., SCHNITZLER, C., KARPINSKI, L., THOMAS, R., WHILDE, J., EASTMAN, C., YANG, C., KRSTIC, A., ROLLINSON, D., ZIRKELBACH, B., YETSKO, K., BURKHALTER, B. & MARTINDALE, M. Q. 2018. Sea turtle fibropapilloma tumors share genomic drivers and therapeutic vulnerabilities with human cancers. *Communications Biology,* 1**,** 63.

FLINT, M., BRAND, A.-F., BELL, I. P. & MADDEN HOF, C. A. 2019. Monitoring the health of green turtles in northern Queensland post catastrophic events. *Science of The Total Environment,* 660**,** 586-592.

FRAZIER, J. 2003. Prehistoric and ancient historic interactions between humans and marine turtles. *The biology of sea turtles,* 2**,** 1-38.

GARNETT, S., PRICE, I. & SCOTT, F. 1985. The Diet of the Green Turtle, Chelonia Mydas (L.), In Torres Strait. *Wildlife Research,* 12**,** 103-112.

GAUS, C., VILLA, C. A., DOGRUER, G., HEFFERNAN, A., VIJAYASARATHY, S., LIN, C.-Y., FLINT, M., HOF, C. M. & BELL, I. 2019. Evaluating internal exposure of sea turtles as model species for identifying regional chemical threats in nearshore habitats of the Great Barrier Reef. *Science of The Total Environment,* 658**,** 732-743.

GEORGE, R. H. 2017. Health Problems and Diseases of Sea Turtles. *In:* LUTZ, P. L. & MUSICK, J. A. (eds.) *The Biology of Sea Turtles, Volume I.* Florida, USA: CRC Press.

GREENBLATT, R. J., QUACKENBUSH, S. L., CASEY, R. N., ROVNAK, J., BALAZS, G. H., WORK, T. M., CASEY, J. W. & SUTTON, C. A. 2004. Genomic variation of the fibropapilloma-associated marine turtle herpesvirus across seven geographic areas and three host species. *J Virol,* 79.

HALLIWELL, B. & GUTTERIDGE, J. M. 2015. *Free radicals in biology and medicine*, Oxford University Press, USA.

HAMANN, M., GODFREY, M., SEMINOFF, J., ARTHUR, K., BARATA, P., BJORNDAL, K., BOLTEN, A., BRODERICK, A., CAMPBELL, L. & CARRERAS, C. 2010. Global research priorities for sea turtles: informing management and conservation in the 21st century. *Endangered Species Research,* 11**,** 245-269.

HAYNES, D. B. 2001. *Pesticide and heavy metal concentrations in Great Barrier Reef sediment, seagrass and dugongs (Dugong dugon).* Ph.D., University of Queensland.

HAZEL, J., HAMANN, M. & LAWLER, I. R. 2013. Home range of immature green turtles tracked at an offshore tropical reef using automated passive acoustic technology. *Marine Biology,* 160**,** 617-627.

HERBST, L. H. 1994. Fibropapillomatosis of marine turtles. *Ann Rev Fish Dis,* 4**,** 389-425.

HERBST, L. H. 1997. *The etiology and pathogenesis of green turtle fibropapillomatosis.* Ph.D., University of Florida, USA.

HERBST, L. H., JACOBSON, E. R., MORETTI, R., BROWN, T., SUNDBERG, J. P. & KLEIN, P. A. 1995. Experimental transmission of green turtle fibropapillomatosis using cell-free tumor extracts. *Dis Aquat Organ,* 22**,** 1-12.

HERBST, L. H. & KLEIN, P. A. 1995. Green turtle fibropapillomatosis: challenges to assessing the role of environmental cofactors. *Environmental Health Perspectives* 103**,** 27-30.

JONES, K., ARIEL, E., BURGESS, G. & READ, M. 2016. A review of fibropapillomatosis in Green turtles (*Chelonia mydas*). *The Veterinary Journal,* 212**,** 48-57.

JONES, K., BURGESS, G., BUDD, A. M., HUERLIMANN, R., MASHKOUR, N. & ARIEL, E. 2020. Molecular evidence for horizontal transmission of chelonid alphaherpesvirus 5 at green turtle (Chelonia mydas) foraging grounds in Queensland, Australia. *PLOS ONE,* 15**,** e0227268.

KOMOROSKE, L. M., LEWISON, R. L., SEMINOFF, J. A., DEUSTCHMAN, D. D. & DEHEYN, D. D. 2012. Trace metals in an urbanized estuarine sea turtle food web in San Diego Bay, CA. *Science of The Total Environment,* 417-418**,** 108-116.

LACKOVICH, J. K., BROWN, D. R., HOMER, B. L., GARBER, R. L., MADER, D. R., MORETTI, R. H., PATTERSON, A. D., HERBST, L. H., OROS, J., JACOBSON, E. R., CURRY, S. S. & KLEIN, P. A. 1999. Association of herpesvirus with fibropapillomatosis of the green turtle Chelonia mydas and the loggerhead turtle Caretta caretta in Florida. *Dis Aquat Organ,* 37**,** 89-97.

LANDSBERG, J. H., BALAZS, G. H., STEIDINGER, K. A., BADEN, D. G., WORK, T. M. & RUSSELL, D. J. 1999. The Potential Role of Natural Tumor Promoters in Marine Turtle Fibropapillomatosis. *Journal of Aquatic Animal Health,* 11**,** 199-210.

LU, Y., NERURKAR, V. R., AGUIRRE, A. A., WORK, T. M., BALAZS, G. H. & YANAGIHARA, R. 1999. Establishment and characterization of 13 cell lines from a green turtle (Chelonia mydas) with fibropapillomas. *In Vitro Cellular & Developmental Biology - Animal,* 35**,** 389-393.

MORAIS, R. A., DOS SANTOS, R. G., LONGO, G. O., YOSHIDA, E. T. E., STAHELIN, G. D. & HORTA, P. A. 2014. Direct Evidence for Gradual Ontogenetic Dietary Shift in the Green Turtle, Chelonia mydas. *Chelonian Conservation and Biology,* 13**,** 260-266.

MUSICK, J. A. & LIMPUS, C. J. 1997. Habitat utilization and migration in juvenile sea turtles. *The biology of sea turtles,* 1**,** 137-163.

NICOLAU, L., MONTEIRO, S. S., PEREIRA, A. T., MARÇALO, A., FERREIRA, M., TORRES, J., VINGADA, J. & EIRA, C. 2017. Trace elements in loggerhead turtles (*Caretta caretta*) stranded in mainland Portugal: Bioaccumulation and tissue distribution. *Chemosphere,* 179**,** 120-126.

PAGE-KARJIAN, A. 2019. Fibropapillomatosis in Marine Turtles. *In:* MILLER, E. R., LAMBERSKI, N. & CALLE, P. P. (eds.) *Fowler's Zoo and Wild Animal Medicine Current Therapy.* St. Louis, Missouri, USA: WB Saunders.

PAGE-KARJIAN, A., NORTON, T. M., RITCHIE, B., BROWN, C., MANCIA, C., JACKWOOD, M. & GOTTDENKER, N. L. 2015. Quantifying chelonid herpesvirus 5 in symptomatic and asymptomatic rehabilitating green sea turtles. *Endangered Species Research,* 28**,** 135-146.

PAGE-KARJIAN, A., SERRANO, M. E., CARTZENDAFNER, J., MORGAN, A., RITCHIE, B. W., GREGORY, C. R., MCNEILL, J. B., PERRAULT, J. R., CHRISTIANSEN, E. F. & HARMS, C. A. 2020. Molecular Assessment of Chelonid Alphaherpesvirus 5 Infection in Tumor-Free Green (Chelonia mydas) and Loggerhead (Caretta caretta) Sea Turtles in North Carolina, USA, 2015–2019. *Animals,* 10**,** 1964.

PAGE-KARJIAN, A., TORRES, F., ZHANG, J., RIVERA, S., DIEZ, C., MOORE, P. A., MOORE, D. & BROWN, C. 2012. Presence of chelonid fibropapilloma-associated herpesvirus in tumored and non-tumored green turtles, as detected by polymerase chain reaction, in endemic and non-endemic aggregations, Puerto Rico. *SpringerPlus,* 1**,** 35.

PRITCHARD, P., LUTZ, P. & MUSICK, J. 1997. Evolution, phylogeny, and current status. *In:* LUTZ, P. L. & MUSICK, J. A. (eds.) *The biology of sea turtles.* London, England: CRC Press.

QUACKENBUSH, S. L., CASEY, R. N., MURCEK, R. J., PAUL, T. A., WORK, T. M., LIMPUS, C. J., CHAVES, A., DU TOIT, L., PEREZ, J. V., AGUIRRE, A. A., SPRAKER, T. R., HORROCKS, J. A., VERMEER, L. A., BALAZS, G. H. & CASEY, J. W. 2001. Quantitative analysis of herpesvirus sequences from normal tissue and fibropapillomas of marine turtles with real-time PCR. *Virology,* 287**,** 105-111.

QUACKENBUSH, S. L., WORK, T. M., BALAZS, G. H., CASEY, R. N., ROVNAK, J., CHAVES, A., DUTOIT, L., BAINES, J. D., PARRISH, C. R., BOWSER, P. R. & CASEY, J. W. 1998. Three closely related herpesviruses are associated with fibropapillomatosis in marine turtles. *Virol,* 246**,** 392-399.

ROSS, D. A. N., GUZMÁN, H. M., POTVIN, C. & VAN HINSBERG, V. J. 2017. A review of toxic metal contamination in marine turtle tissues and its implications for human health. *Regional Studies in Marine Science,* 15**,** 1-9.

SANTOS, R. G., MARTINS, A. S., TOREZANI, E., BAPTISTOTTE, C., FARIAS, J. N., HORTA, P. A., WORK, T. M. & BALAZS, G. H. 2010. Relationship between fibropapillomatosis and environmental quality: a case study with Chelonia mydas off Brazil. *Diseases of Aquatic Organisms,* 89**,** 87-95.

SCHLACHER-HOENLINGER, M. A. & SCHLACHER, T. A. 1998. Accumulation, contamination, and seasonal variability of trace metals in the coastal zone – patterns in a seagrass meadow from the Mediterranean. *Marine Biology,* 131**,** 401-410.

SMITH, G. & COATES, C. 1938. Fibro-epithelial growths of the skin in large marine turtles, Chelonia mydas (Linnaeus). *Zoologica,* 23**,** 93-98.

THANGARADJOU, T., RAJA, S., SUBHASHINI, P., NOBI, E. P. & DILIPAN, E. 2013. Heavy metal enrichment in the seagrasses of Lakshadweep group of islands—A multivariate statistical analysis. *Environmental Monitoring and Assessment,* 185**,** 673-685.

THOMAS, C., BENNETT, W., GARCIA, C., SIMMONDS, A., HONCHIN, C., TURNER, R., HOF, C. & BELL, I. 2020. Coastal bays and coral cays: Multi-element study of *Chelonia mydas* forage in the Great Barrier Reef (2015–2017). *Science of The Total Environment***,** 140042.

VALYI-NAGY, T. & DERMODY, T. S. 2005. Role of oxidative damage in the pathogenesis of viral infections of the nervous system. *Histology and histopathology,* 20**,** 957-967.

VÉLEZ-RUBIO, G. M., CARDONA, L., LÓPEZ-MENDILAHARSU, M., MARTÍNEZ SOUZA, G., CARRANZA, A., GONZÁLEZ-PAREDES, D. & TOMÁS, J. 2016. Ontogenetic dietary changes of green turtles (Chelonia mydas) in the temperate southwestern Atlantic. *Marine Biology,* 163**,** 57.

VILLA, C., BELL, I., HOF, C. M., LIMPUS, C. & GAUS, C. 2019. Elucidating temporal trends in trace element exposure of green turtles (*Chelonia mydas*) using the toxicokinetic differences of blood and scute samples. *Science of the Total Environment,* 651**,** 2450-2459.

VILLA, C. A., FLINT, M., BELL, I., HOF, C., LIMPUS, C. J. & GAUS, C. 2017. Trace element reference intervals in the blood of healthy green sea turtles to evaluate exposure of coastal populations. *Environmental Pollution,* 220, Part B**,** 1465-1476.

WORK, T. M., BALAZS, G. H., RAMEYER, R. A. & MORRIS, R. A. 2004. Retrospective pathology survey of green turtles Chelonia mydas with fibropapillomatosis in the Hawaiian islands, 1993–2003. *Dis Aquat Organ,* 62**,** 163-176.

WORK, T. M., DAGENAIS, J., BALAZS, G. H., SCHUMACHER, J., LEWIS, T. D., LEONG, J. C., CASEY, R. N. & CASEY, J. W. 2009. In vitro biology of fibropapilloma-associated herpesvirus and host cells in Hawaiian green turtles (Chelonia mydas). *J Gen Virol,* 90**,** 1943-1950.

WORK, T. M., DAGENAIS, J., WEATHERBY, T. M., BALAZS, G. H. & ACKERMANN, M. 2017. In-vitro replication of Chelonid herpesvirus 5 in organotypic skin cultures from Hawaiian green turtles (*Chelonia mydas*). *Journal of Virology***,** e00404-17.

WORK, T. M., DAGENAIS, J., WILLIMANN, A., BALAZS, G., MANSFIELD, K. & ACKERMANN, M. 2020. Differences in Antibody Responses against Chelonid Alphaherpesvirus 5 (ChHV5) Suggest Differences in Virus Biology in ChHV5-Seropositive Green Turtles from Hawaii and ChHV5-Seropositive Green Turtles from Florida. *Journal of Virology,* 94**,** e01658-19.

WORK, T. M., RAMEYER, R. A., BALAZS, G. H., CRAY, C. & CHANG, S. P. 2001. Immune status of free-ranging green turtles with fibropapillomatosis from Hawaii. *Journal of Wildlife Diseases,* 37**,** 574-581.

CHAPTER 2 - Contextualising the research

2.1. Introduction

Marine turtles are unique reptiles, comprised of seven species, all of which are currently listed on the International Union for Conservation of Nature (IUCN) Red list of Endangered species with, several considered critically endangered, particularly, leatherback (*Dermochelys coriacea*), hawksbill (*Eretmochelys imbricate*) and kemps ridley turtles (*Lepidochelys kempii*). Loggerhead (*Caretta caretta*) and olive ridley turtles (*Lepidochelys olivacea*) are vulnerable to decline and the flatback turtle (*Natator depressus*), are currently listed as data deficient (Seminoff, 2004). However, increasing conservation efforts aimed at reducing the impact of numerous threats have aided the recovery of some major turtle populations (Chaloupka et al., 2008).

In this thesis, focus is placed on *Chelonia mydas* (green turtle), a species listed as endangered that is predominantly a herbivorous marine reptile found in the majority of tropical and subtropical regions (Garnett et al., 1985, Pritchard et al., 1997). *C. mydas* spend much of their long life inhabiting shallow, accessible coastal environments (Hamann et al., 2002), which allows research and attention from interested parties. Additionally, as a charismatic species, public interest is significant and conservation efforts are high (Frazier, 2005). Finally, *C. mydas* hold strong cultural and societal importance in many remote coastal subsistence communities that rely on *C. mydas* for sustenance and trade (Barrios-Garrido et al., 2002, Barrios-Garrido et al., 2017, Hancock et al., 2017, Barrios-Garrido et al., 2020). All such attributes make *C. mydas* a flagship marine species of significant global importance.

2.2. C. mydas biology and conservation

2.2.1. C. mydas life cycle

C. mydas are considered transient for a significant proportion of their life history. For instance, as post-hatchlings, turtles spend the first 5 – 15 years of adolescence in the pelagic zone of the open ocean, transported by current systems. Limited data is available for this phase of life and it is often referred to colloquially as “the lost years” (Hamann et al., 2002), due to the logistical and geographical challenges of locating and studying post-hatchlings. Once sexually mature, females migrate to natal regions (within proximity to mother nesting beach) to lay eggs every few years (Chaloupka et al., 2004, Broderick et al., 2007, Schofield et al., 2010), during which females may circumvent significant distances (up to approximately 4000 km) (Cheng, 2000,

Cerritelli et al., 2018, Pilcher et al., 2020, Shimada et al., 2020). Between these two distinct transient phases, juvenile *C. mydas* migrate inshore to a neritic, sheltered environment, where they will remain until sexual maturity is reached. This coastal or nearshore zone is a complex environment, influenced by a plethora of processes and pressures, both natural and anthropogenic, not encountered in the open ocean.

C. mydas show strict, long-term, site fidelity to their neritic, inshore habitats foraging grounds (Musick and Limpus, 1997, Shimada et al., 2016), regardless of habitat health and water quality condition (Hazel et al., 2013). Strict site fidelity is when individual turtles within a wider population maintain narrow geographical ranges as small as 2 km^2 (Hazel et al., 2013). *C. mydas* are long lived r-strategists (large number of potential offspring and no parental care) that have late maturity (~30 years), which when combined with traits such as strict habitat fidelity, can leave *C. mydas* susceptible to persistent threats and potential stressors that affect the regions in which they live.

2.2.2. C. mydas diet

Previous research suggests that *C. mydas* experience an abrupt ontogenetic shift in diet when recruitment to the neritic zone occurs (Reich et al., 2007, Arthur and Balazs, 2008, Boyle and Limpus, 2008). While data is limited, it is possible that an onset of significant changes in diet and thus gut biome, in conjunction with abrupt changes in environmental conditions when recruiting to coastal sites, may induce a stress response, which may play a role in the susceptibility of newly recruited juveniles to infection and disease expression. However, through stomach content analysis and stable isotope analysis (SIA) it has been observed that such abrupt shifts are less likely in this species. The theory that a gradual foraging habit shift is more likely (Carman et al., 2012, Morais et al., 2014, Vélez-Rubio et al., 2016). Furthermore, carnivorous components have been observed in numerous *C. mydas* forage samples from different age grounds and regions worldwide (Cardona et al., 2009, Burkholder et al., 2011, Carman et al., 2012).

Once ontogenetic dietary shift occurs, *C. mydas* are considered both selective and opportunistic foragers, which likely target preferred species but also adapt their diet dependent on availability at specific foraging grounds (Arthur and Balazs, 2008). This is supported by the findings which indicated that *C. mydas* have strong dietary preference and may actively avoid consuming species with sub-optimal nutrient availability (Forbes, 1996) . However, Forbes (1996) also

suggested that diet selection is predominantly determined by availability of preferred food material. In contradiction, local *C. mydas* may have a strong dietary preference for specific seagrass species and may only ingest algal species when seagrass biomass is scarce or absent (Mortimer, 1982). Variation at the foraged food species level occurs between foraging sites but also between individual feeding behaviour within foraging areas (Diego and Richard, 2007, Carrión-Cortez et al., 2010, Burkholder et al., 2011, Lemons et al., 2011, Reisser et al., 2013, González Carman et al., 2014, Santos et al., 2015).

C. mydas diet within coastal foraging sites of the GBR has previously been observed to be composed of predominantly seagrass species, such as *Halodule uninervis* (*H. uninervis*). Some variation was observed when comparing coastal populations to that of an offshore population, which also periodically foraged on red algae species (Bell et al., 2019). Biomass density of seagrass was found to be significantly greater and more temporally consistent within the coastal sites, when compared to the offshore foraging area (Bell et al., 2019). Macroalgal (Coles and Long, 2000) and seagrass abundance (Lee Long et al., 1993) is variable both spatially and temporally within the offshore site which supports the finding of a periodic dietary shift between material in this *C. mydas* population. Such observations on *C. mydas* diets and the comparisons between coastal and offshore foraging site composition further supports the potential that feeding behaviour varies between sites based on food availability.

Many seagrass species (including *H. uninervis* and *C. serrulata*) are long lived and slow to succession, making them ideal bioindicators and sentinels for monitoring and detecting the early exposure to possible anthropogenic stresses in the surrounding environment (Schlacher-Hoenlinger and Schlacher, 1998, Pergent-Martini and Pergent, 2000, Orth et al., 2006, Orlando-Bonaca et al., 2015). However, *C. mydas* are particularly at risk of ingesting high concentrations of trace element contaminants, in part, due to the survival adaptations of common coastal seagrass species to metal elements. Such long-lived seagrass species are known to have considerable metal bioaccumulation capacity and binding affinity (Malea and Haritonidis, 1999, Bonanno and Di Martino, 2016, Bonanno and Orlando-Bonaca, 2018). Seagrass meadows are often located in areas subject to natural and anthropogenic impacts, including elevated metal concentrations (Aguirre et al., 1994, Sindermann, 2005). *C. mydas* demonstrably rely on such meadows as viable foraging sites and thus the possibility of high exposure to elevated metal concentrations is apparent for populations inhabiting such foraging grounds. Furthermore, metal concentrations are regarded to be equal in above and below

ground organs of seagrass species, under consistent exposure conditions. Some seagrass species likely apply a mixed tolerance approach to trace element exposure by accumulating and storing toxicants in roots (below ground). This is a common approach in other rooted species and likely aims to minimise any negative effects on photosynthetic apparatus (above ground organs) (Cardwell et al., 2002, Fritioff and Greger, 2006, Reboreda and Caçador, 2007, Willis et al., 2010). Simultaneously, utilising this strategy may also allow species to shed elevated metal concentrations through the expulsion of leaves. By actively mobilising unwanted metals from the roots to shoots the plant may remove the presence of contaminants entirely via high leaf turnovers (Malea and Haritonidis, 1999).

2.2.3. C. mydas immune function and how it compares to mammals

Reptiles are considered an important evolutionary link between ectothermic fish and amphibians and endothermic mammals and birds (Zimmerman et al., 2010a). Like mammals, reptiles mount both innate and adaptive immune response to the presence of pathogens. The innate immune system consists of several rapid non-specific responses to pathogens, such as the production of heterophilic granulomas. As a non-specific response, previous exposure to a particular antigen is not necessary for a reliable defence to be mounted (Zimmerman et al., 2010a).

One distinct difference between mammalian and reptilian immunology is the influence of temperature. As ectotherms, the reptilian immune response is closely linked to ambient environmental temperatures and seasonal variation plays a significant role in the efficiency of a reptile's immune response (Zimmerman et al., 2010a). Such variations often elicit shifts in behaviour, in response to temperature. For instance, fever plays a role in the inflammatory immune response of both endotherms and ectotherms, but instead of a physiological shift in internal temperatures, seen in endotherms (Conti et al., 2004), reptiles must rely on behavioural changes such as moving to warmer waters or basking.

Similarities also exist between mammalian and reptilian adaptive immune functions. Both groups possess adaptive humoral and cell mediated strategies to defend against infection (Pitchappan and Muthukkaruppan, 1977, Burnham et al., 2005, Zimmerman et al., 2010a). If the innate immune system is unable to limit the spread of an infection, aspects of the adaptive system will assist in neutralising any remaining pathogen and to build a memory of past infections to allow more rapid response to possible future re-infection. Cell-mediated immunity

in reptiles consists of at least four types of lymphocyte: B cells, helper T cells (TH cells), natural killer T cells and cytotoxic T cells (TC cells) (Pitchappan and Muthukkaruppan, 1977, Burnham et al., 2005). The function of TC cells is to induce apoptosis (self-destruction) in host cells which are infected by bacteria or viruses to efficiently destroy the pathogen (Zimmerman et al., 2010a). On the other hand, the main role of TH cells is to modulate other immune cells and regulate the production of antigen-specific antibodies within the humoral immune response (Zimmerman et al., 2010a). However, it is worth noting that the exact roll of both TH and TC cells in marine turtles is currently unknown (Zimmerman et al., 2010a). In mammals, TH cells regulate antibody production by interacting with B cells, another type of lymphocyte. Reptilian B cells are stimulated by the presence of viral antigens and unlike mammalian B cells, certain B cells in reptiles may be phagocytic (Zimmerman et al., 2010b).

Mammalian antibodies (immunoglobulins) are made up of two heavy and two light polypeptide chains, with variable regions at the terminal ends forming the antigen-binding site (Zimmerman et al., 2010a). Antibodies (in mammals and reptiles) are categorised into classes, which are based on isotype of the heavy chain. There are five known isotypes of reptilian antibody (Marchalonis et al., 1969, Deza et al., 2009, Wei et al., 2009, Gambón-Deza et al., 2012, Magadán-Mompó et al., 2013), with each isotype fulfilling different functions in the immune defence (Natarajan and Muthukkaruppan, 1985). The IgM isotype in mammals is produced first upon infection followed by IgG. Marine turtle isotype, IgY is the IgG equivalent isotype in reptiles. However, *C. mydas* IgYs are comprised of asymmetrical heavy chains, which is a noticeable difference to the structure of IgM and IgG (Meddings et al., 2014, Work et al., 2015). There are two known types of IgY in *C. mydas*, 7s and 5.7s IgYs (Work et al., 2015). While the exact roles of each IgY is unknown, evidence suggests that 7s IgY is produced first within a few weeks of initial infection and 5.7s IgY is likely produced upon chronic infection (Work et al., 2015). Isotype switching is an example of immune response maturation, whereby B cells producing 7s IgY transition to primarily producing 5.7s IgY while maintaining the antigen-specificity necessary for efficient binding. Isotype switching allows a change in immunological function necessary for adaptive immunity (Zimmerman et al., 2010a). The latent phase between initial infection and the primary antibody response is prolonged in reptiles when compared to mammals and birds, but that phase is shortened upon secondary exposure to the same antigen (Work et al., 2001, Snoeijs et al., 2007). Furthermore, upon re-infection, antibody binding

affinity and titres tend to increase in mammals (Coico and Sunshine, 2015). However, this does not appear to be the case in reptiles (Grey, 1963).

While *C. mydas* immune function is largely an emerging field of study, there is indication that, like other animals, immune responses are negatively impacted by stress. As a response to stress, the adrenal gland secretes steroids such as corticosterone which can suppress humoral and adaptive immune function (Santos et al., 2017), in turn reducing antigen detection and binding efficiency (George, 2017). A wide range of factors can induce a stress response. Stressors can be environmental (such as extreme temperature, salinity and UV light exposure), nutritional (habitat loss and contamination) or physiological (injury, infection) (George, 2017). In addition to a stress response, immunosuppression can be induced directly by several threats, particularly sub-optimal environmental conditions (Balazs and Pooley, 1991).

2.2.4. Threats to C. mydas

Common stressors that *C. mydas* experience include (but are not limited to) the ingestion of marine debris (such as microplastics) (Bjorndal et al., 1994, Clukey et al., 2018), boating collisions (Bjorndal, 1995, Herbst and Klein, 1995, Van Houtan et al., 2010) and a wide range of chemical contaminants (Van de Merwe, 2008, Grillitsch and Schiesari, 2010, Hamann et al., 2010, Santos et al., 2010, van de Merwe et al., 2010). Potential environmental and climatic factors may also impact population success, especially when considering that nesting beach distribution is largely centred around the equator (Pike, 2013) and significant increases in water and air temperatures, driven by changes in climate, have the potential to limit the natural bioclimatic range of successful reproduction and egg development in these regions (Pike, 2013). Furthermore, as the coastal zone is a shallow environment, extremes such as elevated sea temperature and UV exposure have the potential to significantly affect *C. mydas*. Some threats and impacts have received more focus than others. A comprehensive list of turtle threats and research priorities to better manage and conserve global turtle populations was compiled by Hamann *et al*. (Hamann et al., 2010), with disease and non-organic pollution, the two threats focussed on in this thesis, prioritised highly. Most recently, a framework for the recovery of marine turtle populations of Australia was implemented, with the aim to identify and minimise anthropogenic impacts on local populations, in order to provide the opportunity for conservation efforts to better improve the status of these species (Energy, 2017).

2.2.5. Disease in C. mydas

Disease, as a topic in marine turtles, is an emerging field, but is considered an important stressor on marine turtles (Foley et al., 2005, Flint et al., 2010), and is identified among the top 20 research priorities in marine turtle research and conservation (Hamann et al., 2010). Knowledge on marine turtle-specific diseases is particularly limited due to complexities in studying aetiology and transmission (Mashkour et al., 2018), with different aspects of health and disease receiving varying levels of focus. Currently, investigation into viral diseases specific to *C. mydas* is lacking, with only a few known infections. Known marine turtle associated herpesviruses are herpesvirus respiratory disease, grey-patch disease (causes by Chelonid herpesvirus-1,ChHV1) and lung, eye and trachea disease (caused by ChHV6) (George, 2017, Rao et al., 2020).

Various health assessment methods used in other animals have been validated in *C. mydas* in the past to assess health and aid in rehabilitation (Herbst and Jacobson, 2002). Limitations were present in this approach and successful diagnosis of the cause of some turtle diseases is often lacking (Reséndiz and Lara-Uc, 2018). In leu of a reliable protocol, wildlife disease investigations called for a more structured, evidence based and multidisciplinary approach to better manage and reduce the risk of disease. Such a method can be found in the wildlife disease risk assessment (DRA) that was most recently published by the World Organisation for Animal Health and the IUCN species survival commission in 2014 (Jakob-Hoff et al., 2014). However, the proposed DRA was meant to inform causative diagnosis of diseases in numerous endangered species (Jakob-Hoff et al., 2014). Due to the unique life history, biology and the difficulties faced in capturing marine turtles, particularly in remote areas, modifications were deemed necessary. A one health approach has recently been proposed, whereby aspects of marine turtle, human and environmental health are all considered to be impacted and therefore, significant areas of study when diagnosing wildlife disease. Such a one health approach has recently been structured and published to specifically address the diagnosis and management of marine turtle diseases (Mashkour et al., 2020). Aspects of such a species-specific DRA have been considered throughout this thesis, whereby emphasis is placed the potential impact of anthropogenically influenced environmental stressors on the development of one particular marine turtle disease, fibropapillomatosis.

2.3. Fibropapillomatosis

Fibropapillomatosis (FP) is a disease that is believed to be associated with a virus (Quackenbush et al., 1998, Lackovich et al., 1999, Lu et al., 2000, Quackenbush et al., 2001, Page-Karjian et al., 2012, Rodenbusch et al., 2014, Page-Karjian et al., 2015), though a definitive link is yet to be determined. The first recorded case of FP was over 70 years ago, in 1938 by Smith and Coates (1938), in a captive *C. mydas* from Florida, USA. While this disease has received continued attention since then, aspects of the disease remain cryptic (Jones et al., 2016), with limited data on transmission and aetiology. FP is a neoplastic disease, identified in all seven species of marine turtle, but is particularly prevalent in *C. mydas* (Herbst, 1994, Quackenbush et al., 2001, Foley et al., 2005, Duarte et al., 2012), only reaching panzootic (widespread globally) proportions in this species (Williams et al., 1994).

Clinical FP signs are often characterised by the presence of external fibroepithelial lesions (tumours) in soft tissue areas, including the eyes, neck, base of the tail (Mascarenhas and Iverson, 2008). In severe cases, internal lesions can be observed in areas including the mouth, heart, liver, and kidneys (Mascarenhas and Iverson, 2008). Such lesions are pathognomonic to the disease and are easily identified (Jones et al., 2016). Lesions have been recorded in sizes ranging from 0.1 to 30 cm in diameter and the colour is dependent on the local tissue pigment (Herbst, 1994). Depending on size, frequency and location, lesions may have debilitating effects by inhibiting feeding and mobility, or hindering visual faculties (Balazs, 1991, Chaloupka and Balazs, 2005). Similarly, internal lesions can cause organ failure, including cardiac dysfunction, buoyancy issues and respiration compromise (Smith and Coates, 1938, Balazs, 1986, Herbst, 1994, Quackenbush et al., 1998). Turtles observed in the field with FP often move noticeably slower than clinically healthy individuals (Anecdotal evidence) and likely suffer lethargy and energy deficits from inefficient foraging or direct energy demands of lesion presence (Hirama et al., 2014).

2.3.1. Fibropapillomatosis epidemiology

Prevalence of FP in *C. mydas* populations has been reported with vast variability (between 1% and 90%) around the world (Herbst, 1994). The identification of turtles with lesions is considered sporadic, but with elevated occurrence in some populations (Quackenbush et al., 2001). Present in all major global ocean basins, FP is described as having a circumtropical distribution (Herbst, 1994, Aguirre and Lutz, 2004). In *C. mydas*, the disease has previously

been recorded in populations from regions, such as the USA, Australia, and Brazil (Baptistotte et al., 2001, Chaloupka et al., 2009, Rossi et al., 2016, Jones et al., 2020, Work et al., 2020). The disease expression in FP is likely dependant on several component causes, whereby several factors related to turtle physiology, life history and environmental conditions are required to form a sufficient cause (or a causal pathway) for the development of FP. For instance, *C. mydas* with FP are most commonly juvenile or sub-adult individuals inhabiting nearshore habitats within the catchments of large human populations and areas with low hydrodynamic regimes, such as lagoons or bays (Balazs, 1991, Ehrhart, 1991, Herbst, 1994, Limpus and Miller, 1994, Herbst and Klein, 1995, Hirama et al., 2014). The apparent trend observed in life stage of afflicted individuals may be indicative of affected turtles dying and therefore being removed from the population or of turtles with FP recovering with immunity acquired necessary for protection as adult animals (Van Houtan et al., 2010). Furthermore, the convergence of turtles with FP has been reported at several locations around the world. Whereby, such individuals are captured in narrow geographical ranges (hotspots), at significantly higher prevalence than the remainder of the local population. These phenomena have been reported at sites in Australia (Jones et al., 2020) (including two sites within the current thesis) and in at least one population of the USA (Ehrhart, 1991). In Florida, significant differences in FP prevalence (0 – 50%) was observed in *C. mydas* inhabiting foraging grounds only 1 km apart (Ehrhart, 1991). Little is known about what may cause convergence of turtles with clinical signs, though several hypotheses may be applied. For instance, local hydrodynamic processes may transport debilitated individuals to one specific location or turtles in that area may be exposed to sub-optimal environmental conditions and stressors, not present in other areas within the same foraging location. While data is deficient in this area, strict foraging fidelity seen in *C. mydas* (Hazel et al., 2013) may play a role in this convergence of FP cases.

2.3.2. Potential health impacts of FP

The full extent of the health effects of FP remain unclear. FP impacts both biochemical and haematological parameters of turtles (Aguirre et al., 1995, Work and Balazs, 1999, Aguirre and Balazs, 2000, Swimmer, 2000). Signs of elevated stress, lymphocytopenia, neutrophilia, monocytosis, and hyperglobulinaemia have been observed in *C. mydas* with FP (Day et al., 2010, da Silva et al., 2016). While oral lesions are a common clinical sign of FP in *C. mydas* in Hawaii (Aguirre et al., 2002) and are rarely observed in Florida (Hirama and Ehrhart, 2007), such growths have not been identified in individuals at any other location, including within

Australian populations (Limpus et al., 2015). Furthermore, in Florida, Hirama *et al*. (Hirama et al., 2014) observed that haematocrit (volume of red blood cells to total blood volume), haemoglobin concentration, and total protein were negatively correlated with severity of FP lesion presence. Similarly, *C. mydas* in Hawaiian populations with severe lesion development were anaemic and hypoproteinemic (Aguirre et al., 1995, Work and Balazs, 1999).

In addition, other theories have suggested that *C. mydas* foraging dive durations could be significantly reduced due to severity of FP in individuals (Hirama et al., 2014). A lower haematocrit and haemoglobin concentration may suggest that moderately and severely diseased individuals are at risk of having lower oxygen-carrying capacity than mildly affected and non-diseased individuals. This lack of efficient oxygenation would likely cause a feedback loop, whereby diminished oxygenation could inhibit swimming efficiency, reducing dive times. Sub-optimal dive durations would logically amount to reduced foraging time. This, in turn, would suggest lower energy levels (an increase in lethargy) in moderate and severely diseased turtles when compared to healthy (non-diseased) individuals, which would likely manifest as a longer time to reach breeding condition, reducing reproductive output of diseased individuals over a lifetime. Hypoproteinemia in severely diseased individuals would in fact imply nutrient deficiency (Hirama et al., 2014). Such deficiency would suggest inefficient food intake or failure to store sufficient nutrients from ingested food, or a combination of both stressors (Hirama et al., 2014). Furthermore, immunosuppression in turtles with FP has been observed (Cray et al., 2001). However, it is less clear whether immunosuppression is a cause or an effect of the infection and/or disease expression (Herbst, 1994).

While FP has been studied since its discovery in 1938, several aspects of disease aetiology remain unanswered. Factors associated with disease expression are currently unknown, though several hypotheses have been postulated. As variation between populations, both globally and locally, is often observed in FP prevalence, an association with an infectious agent may be most plausible (Jones et al., 2016). The current hypothesis that receives the most attention suggests that lesion development may be associated with a marine turtle specific herpesvirus, the chelonid alphaherpesvirus 5 (ChHV5).

2.3.3. ChHV5

The seemingly contagious nature of FP transmission in marine turtles, when comparing individuals of the same population and between distinct study sites, lead researchers to believe

that FP was likely associated with an infectious agent (Herbst, 1994). Earlier researchers implicated a range of viruses, including papillomavirus (Herbst, 1994) and several herpesviruses (HVs), as the causative agents of FP (Jacobson et al., 1991, Herbst, 1994). Quackenbush et al. (1998) first associated herpesvirus with tumours in sea turtles by amplifying the highly conserved DNA polymerase gene of ChHV5/ HV from FP lesions. Since this discovery, numerous molecular techniques, including quantitative PCR, have been implemented to measure potential association of ChHV5 DNA with FP lesions in other *C. mydas* populations, and most FP tumours analysed were positive for ChHV5 DNA (Lackovich et al., 1999, Quackenbush et al., 2001). In these studies, positive detection of ChHV5 DNA in turtles without FP was rare. Due to the consistent association with diseased turtles, ChHV5 became associated as a potential primary causative agent of FP, warranting further investigation into the pathogenesis and transmission of the virus. Recent studies, with more sensitive testing capabilities, have had more success in amplifying ChHV5 DNA in tissue samples from turtles without FP (Page-Karjian et al., 2012, Page-Karjian et al., 2015, Page-Karjian et al., 2020). Such findings provide further evidence to support the likelihood of early and/or latent viral infections in clinically healthy *C. mydas* (Page-Karjian et al., 2017). In other words, the absence of visible FP lesions does not necessarily imply lack of viral infection. Rather, ChHV5 is likely one of several component causes (co-factors) required for FP expression in *C. mydas*.

Even though ChHV5 has long been associated as a primary aetiological agent of FP, successful culturing of this virus has proven difficult, until recently (Work et al., 2017). Thus, Koch's postulates for determining whether ChHV5 is the primary causative agent of FP, have not currently been met (Lackovich et al., 1999, Lu et al., 1999, Coberley et al., 2001, Work et al., 2009, Work et al., 2017). In contrast, when approaching the subject of causation from an epidemiological standpoint, ChHV5 largely meets the Bradford-Hill's criteria of causation principles (Bradford, 1965), and while ChHV5 is likely a necessary cause (a component cause necessary in any sufficient disease expression), there are other component causes necessary that may be absent in *C. mydas* infected with ChHV5, but without clinical signs of FP. It is recommended that more focus be placed on an epidemiological approach toward identifying the extent of association between ChHV5 and other potential associated component causes to FP. For instance, recent investigation has found a papillomavirus (CmPV) associated with a significant proportion of FP lesions sampled (Mashkour et al., 2018). This discovery warrants

further effort to survey the distribution and prevalence of this virus in local populations to better determine the extent of association with FP.

Previous research into ChHV5 phylogeny suggests that the virus co-evolved with marine turtles for up to 300 million years, diverging from reptilian and mammalian alpha-herpesvirus' prior to the genetic separation of the latter two subgroups (Herbst et al., 2004), likely making it specific to marine turtles (Jones et al., 2016). A total of four phylogeographical groups of ChHV5 have been identified around the world (Eastern Pacific, Western Atlantic / Eastern Caribbean, mid-west Pacific and Atlantic), with an unknown number of local viral variations present within each group. Four distinct ChHV5 variants (haplotypes) were detected in Floridian populations (Ene et al., 2005), six in Brazil (Rodenbusch et al., 2014) and at least six in Australian *C. mydas* (Jones et al., 2020). Such regional differences suggest that geographical area plays a significant role in ChHV5 phylogeny. Certain variants are often more prevalent within a region than others, and largely depends on locality and possibly genetic stock of the host turtle (Jones et al., 2016, Jones et al., 2020). It is currently unknown whether aspects such as virulence, transmissibility and infectivity differs between viral variants (Jones et al., 2016), and this may further confound investigation into ChHV5 association with FP in some areas.

ChHV5 has not yet been identified in pelagic juveniles; therefore, it is speculated that individuals are not exposed to the virus until neritic zone recruitment (Herbst, 1994, Patrício et al., 2012). These new recruits are faced with a plethora of stressors associated with, but not limited to, long distance migration, adaptive changes to new environmental conditions, shifts in diet and increases in population density (Jones et al., 2016). It is possible that these factors, when combined, could cause a decline in immune system efficiency, leading to increased susceptibility to ChHV5 infection and FP development in juveniles (Jones et al., 2016).

Previous studies have discussed potential transmission pathways of ChHV5, including parasitic infections (Dailey and Morris, 1995, Aguirre et al., 1998, Greenblatt et al., 2004). Herpesviruses are known to be transmitted between individuals through contamination of bodily fluids such as mucus, blood, and saliva (Herbst, 1994, Davison, 2002, Ene et al., 2005, Patrício et al., 2012). Furthermore, herpesviruses have been observed to survive in salt water for up to two weeks before the viral proteins degrade (Curry et al., 2000). Foraging grounds, where there are large numbers of turtles, are thought to be the most likely areas where disease

transmission occurs (Herbst et al., 1999, Ene et al., 2005). However, the presence of ChHV5 does not guarantee lesion development (Page-Karjian et al., 2020, Work et al., 2020).

2.3.4. Investigating the ChHV5 footprint

While detection of ChHV5 DNA is an invaluable diagnostic tool, indicative of current lytic infection, other methods are available for the study of viral infection. One established alternative to viral DNA amplification is to measure antibodies against the virus. By detecting reactions between specific ChHV5 antigens and antibodies against ChHV5, in *C. mydas* samples, previous infection may be inferred (Work et al., 2020). If both techniques were applied simultaneously, a more robust and reliable detection of viral infection by extending the window of detection, by applying serodiagnostic tools such as Enzyme Linked Immunosorbent Assays (ELISA), as opposed to only identifying current infections.

Protocols implemented in ELISA are sensitive and have long been used in human (Engvall and Perlmann, 1972, Cooper et al., 1989, Sulkanen et al., 1998) and animal immunology (Cooper et al., 1989, Lambrecht et al., 2007, Kornacka et al., 2016), though, until recently (Work et al., 2020), no such protocol was available to measure seroprevalence of ChHV5 antibodies in *C. mydas*. The application of ELISA in serum samples collected from distinct populations in Florida and Hawaii found that up to 60% of clinically healthy (no visible FP lesions) reacted in the ChHV5 ELISA (Work et al., 2020). Additionally, by detecting particular types of antibodies, Work et al. (2020) were able to glean greater understanding of ChHV5 seroprevalence, both spatially and temporally. Where this protocol may be limited is in our current understanding of *C. mydas* immune function and antibody production, though advancement in this area of study has been seen in recent years (Work et al., 2015).

2.3.5. Environmental stressors and FP

As described previously, ChHV5 appears to be specific to marine turtles and viral infection is not exclusive to animals with FP clinical signs. Additionally, evidence suggests that the emergence of FP in marine turtles is likely due to modern day extrinsic environmental factors (Jones et al., 2016). High site fidelity and long-life are both defining life history traits of *C. mydas* (Hamann et al., 2002, Hazel et al., 2013). Due to these characteristics, turtles are likely to remain or return to specific localities regardless of unfavourable environmental changes that may have occurred. As a result, any unfavourable conditions present would likely have negative effects on *C. mydas* populations (Hawkes et al., 2009, Poloczanska et al., 2009,

GBRMPA, 2014). A range of environmental conditions including elevated water (Lafferty et al., 2004) and air temperatures (Herbst et al., 1995), ultraviolet radiation (Duffy et al., 2018) and toxic algae blooms (Landsberg et al., 1999) have been suggested as potential FP tumour promoters, though definitive environmental co-factors have not been identified. Marine pollution is considered a significant factor in the marked decline in marine turtle populations (Gramentz, 1988, Anan et al., 2001, Maffucci et al., 2005). However, the toxic effects of contaminants are not well understood (Camacho et al., 2013a). In 2010 therefore, marine pollution impacts, in addition to FP, were listed among 20 research priorities in the marine turtle conservation field (Hamann et al., 2010).

Environmental contamination can be defined as the presence of a hazardous substance(s), a contaminant (most commonly a manmade chemical), that is not naturally present within a given environment or ecosystem. Environmental contamination is often confused with environmental pollution, which is when contamination of the environment reaches a point where natural processes are significantly altered, in an adverse way (Muralikrishna and Manickam, 2017). Environmental contaminants are one category of focus in determining FP aetiology (Storelli et al., 2005, Camacho et al., 2013a, Ley-Quiñónez et al., 2013) and are often categorised as being organic or inorganic.

Persistent organic pollutants (POPs) used as insecticides, fungicides and pharmaceuticals are one example of commonly detected organic contaminants reported in coastal sites adjacent to human settlement and land use (Hermanussen et al., 2006b, Van de Merwe, 2008, Camacho et al., 2013a, Keller et al., 2014). Due to an inability to break down in the environment and high lipophilicity which POPs exhibit (Baird and Cann, 2005), persistent exposure is considered a significant concern (Van de Merwe, 2008). However, while POPs are still produced and applied both legally and illegally, the use of POPs largely ceased in the mid-twentieth century after the significant adverse effects on the environment were made clear (Carson, 1962). In 2001 this was further advanced when a global treaty, the Stockholm Convention on Persistent Organic Pollutants, was signed by world governments to minimise the impact of persistent chemicals on human health and the environment by ceasing or restricting the production, exportation, and application of specific POPs (Lallas, 2001). While POPs are potentially toxic to *C. mydas* as endocrine disrupters, evidence suggests that POPs and other endocrine disrupting chemicals are not associated as possible aetiological co-factors of FP (Keller et al., 2014). Thus, the scope of this thesis does not cover this category of contaminants.

The modulation of immune response by some inorganic chemical contaminants, including trace metals, such as cadmium (Cd), cobalt (Co) and lead (Pb), likely increases the risk of disease expression in *C. mydas* and other reptiles (Balazs and Pooley, 1991, Guillette et al., 1994, Van Houtan et al., 2014, Work et al., 2014), and may induce latent virus reactivation or increased virulence (Aguirre et al., 1994). In fact, elevated FP prevalence is commonly observed in *C. mydas* populations inhabiting polluted nearshore habitats, adjacent to human settlements (Limpus and Miller, 1990, Herbst, 1994, Herbst and Klein, 1995). Environmental contaminants are a particularly complex area of ecotoxicological study due to the likelihood of dynamic mixture effects, amongst other challenges. Unknown mixtures of any number of chemicals entering the marine environment potentially have synergistic, additive, or antagonistic influence on the effect of each chemical, relative to isolated effects of each chemical individually (Momčilović, 1988, Escher et al., 2008, Magnusson et al., 2010). This area of study is extremely hard to study as new chemicals (particularly organics), with the potential to cause adverse environmental impacts, are introduced frequently via application in a wide range of industries (Storelli et al., 2005, Thomas et al., 2020).

2.4. Trace metal toxicology

2.4.1 Metals in the environment

One branch of chemical contaminant that is of main concern regarding *C. mydas* health and FP aetiology is trace metals, or metal elements that are found in the environment at low concentrations. A plethora of metals and metalloids occur naturally in the environment, either in native or ionic form. The distribution of specific metal concentrations significantly differs based on the geochemical makeup of the study site. For instance, coastal sites, with predominantly muddy substrates are expected to be higher in terrigenous metals (derived from land based metal ores) such as Al and Fe, whereas offshore locations tend to have greater concentrations of naturally occurring marine metals such as strontium (Sr) and uranium (U) (Munksgaard and Livingstone Parry, 2002, Weber et al., 2006, Thomas et al., 2020). In regards, to the presence of local non-essential metal concentrations, local point sources and industrial land activity play a major role in availability. Trace elements and a multitude of other contaminants enter the nearshore habitats through a number of different sources - both natural and anthropogenic (urban, industrial and agricultural; Sindermann, 2005). All non-natural element concentrations are expected to be higher closer to the pollution source (Gaudry et al.,

2007), most often in coastal zones adjacent to human settlement (Villa et al., 2017, Brodie et al., 2019, Vijayasarathy et al., 2019, Thomas et al., 2020).

2.4.2. Essential vs toxic metals

Metals that are necessary for physiological and biochemical cell function are referred to as essential element (e.g. Cu, Fe, Mn and Co) (Chang et al., 1996). Conversely, metals with no natural ecological or biological function are deemed non-essential (e.g. Al, Ni, Cd) (Suzuki and Suzuki, 1996). Both essential and non-essential elements can be toxic to organisms and can have negative effects (in varying severities) on the physiological, biochemical, and immunological processes of vertebrates, including *C. mydas* (da Silva et al., 2016, Villa et al., 2017).

Essential elements, while biologically required, may also pose acute and chronic risk, if ingested at high loads (and likewise if such elements are deficient) (Gaus et al., 2012, da Silva et al., 2016, Villa et al., 2017). As essential elements fulfil roles in numerous biochemical functions, concentrations are typically maintained within narrow ranges controlled by physiological processes. In an optimal situation where, essential metals are consistently at natural concentrations and a turtle is not exposed to elevated concentrations, little variation in these metal loads is anticipated (Aggett et al., 2015, Villa et al., 2017). However, this may not be the case when elements are encountered at elevated concentrations (or at depleted concentrations), which can alter a turtle's ability to maintain homeostasis (i.e., the ability to regulate and maintain a condition within the body at a certain concentration). Definitive studies into species-specific toxic thresholds and contamination levels are currently limited for *C. mydas*, largely due to obvious ethical and logistical restrictions involved in conducting direct toxicity testing on marine turtles. In recent years however, ecotoxicological comparison in trace metal exposure between local *C. mydas* populations has been conducted by collecting baseline health and pollution data from a cohort of *C. mydas* inhabiting a near-pristine, minimally disturbed study site (Villa et al., 2017, Flint et al., 2019, Villa et al., 2019). Furthermore, recent advancements in the culturing of marine turtle cells has allowed for the development and validation of cytotoxic bioassays that allow for species-specific direct *in vitro* analysis of impacts posed to marine turtles (Finlayson et al., 2019, Finlayson et al., 2020), allowing the investigation into comparable toxicological end points (for example, the effect concentrations

associated with mortality of 50% of cells in culture, EC_{50}) (Finlayson et al., 2019, Finlayson et al., 2020).

2.4.3. Accumulation of metals in C. mydas

The bioavailability of all trace metal elements is determined primarily by salinity, pH, oxidative state of mineral components and the redox potential (Guilizzoni, 1991), and is indicative of the efficiency with which metals may enter biosystematics circulation (Van de Merwe, 2008). Ionic uptake in the gastrointestinal system relies on a metal complex being soluble, primarily by it binding to certain ligands in food (Nieboer and Fletcher, 1996). Once absorbed into the system metal ions are then transported to different organs and tissues and bind to biological membranes and other constituents in various ways. For example, positively charged ions often bind to negatively charged membranes and can disrupt natural functions such as transmembrane transport of essential resources (Foulkes, 1996). Tissues and organs which are common sites of metal storage are the blood (van de Merwe et al., 2010, da Silva et al., 2016, Villa et al., 2017, Villa et al., 2019), liver and kidney (Anan et al., 2001, Gaudry et al., 2007, Grillitsch and Schiesari, 2010, Cortés-Gómez et al., 2014, da Silva et al., 2014, Faust et al., 2014, Nicolau et al., 2017).

Some examples of bioaccumulated trace metals in marine turtles can be seen in Table 1, which looks at metal loads from turtles sampled in various countries. Previous studies referenced in the table of concentrations do not include all instances of research into metal loads but are a good representation of work conducted. Also, the range of elements included do not represent all metals which have been measured in marine turtle populations but were chosen here as each element is included in research throughout this thesis.

Table 2.1. Examples of previous studies investigating the bioaccumulation of trace metals (mean ± SD, mg/L (blood) and mg/kg (liver and kidney)) in various marine turtle species (*L. olivacea* = *Lepidochelys olivacea* (Olive ridley), *L. kempii* = *Lepidochelys kempii* (Kemps ridley), *C. caretta* = *Caretta caretta* (Loggerhead) and *C. mydas* = *Chelonia mydas* (Green)) and sample types (blood, liver, and kidney). Data were compiled and modified from the review conducted by Cortés-Gómez et al. (2017).

Country	Species	Pb	Cd	Cu	Mn	Ni	Zn	Reference
BLOOD								
Mexico	*L. olivacea*	0.19 ± 0.03	0.09 ± 0.04	0.47 ± 0.08	-	0.56 ± 0.26	11.68 ± 0.94	(Páez-Osuna et al., 2010)
Mexico	*L. olivacea*	<LOD*	0.55	1.09	2.47	2.17	37.19	(Zavala-Norzagaray et al., 2014)
Mexico	*L. olivacea*	0.02 ± 0.01	0.17 ± 0.10	0.60 ± 0.11	0.59 ± 0.09	0.04 ± 0.02	10.43 ± 4.12	(Cortés-Gómez et al., 2014)
USA	*L. kempii*	0.01	-	0.52	-	-	7.50	(Kenyon et al., 2001)
USA	*L. kempii*	0.03 ± 0.03	0.01 ± 0.005	0.41 ± 0.11	-	-	6.71 ± 4.46	(Wang, 2005)
Cape Verde	*C. mydas*	0.06 ± 0.02	0.29 ± 0.25	1.27 ± 8.46	0.03 ± 0.03	1.41 ± 6.66	4.97 ± 2.9	(Camacho et al., 2013b)
Australia	*C. mydas*	0.02 ± 0.05	0.03 ± 0.09	1.09 ± 0.99	-	-	7.92 ± 0.66	(van de Merwe et al., 2010)
USA	*C. mydas*	1.26 ± 0.22	0.01 ± 0.04	0.75 ± 0.04	0.46 ± 0.09	-	-	(Komoroske et al., 2012)
Brazil	*C. mydas*	0.98 ± 0.15	0.08 ± 0.01	0.95 ± 0.10	-	9.15 ± 1.45	0.66 ± 0.11	(da Silva et al., 2016)
Australia	*C. mydas*	0.02 ± 0.01	0.003 ± 0.00	0.64 ± 0.17	0.06 ± 0.04	0.01 ± 0.007	11.0 ± 2.50	(Villa et al., 2017)
LIVER								
Mexico	*L. olivacea*	<LOD	4.47	9.18	0.025	0.14	11.78	(Gardner et al., 2006)
Mexico	*L. olivacea*	0.11 ± 0.08	82.87 ± 36.65	16.37 ± 10.34	3.36 ± 1.34	0.08 ± 0.05	46.65 ± 16.38	(Cortés-Gómez et al., 2014)
Turkey	*C. caretta*	0.88 ± 0.33	2.71 ± 0.97	0.75 ± 0.22	-	2.89 ± 0.77	-	(Kaska et al., 2004)
Italy	*C. caretta*	0.16 ± 0.05	3.36 ± 1.94	7.69 ± 4.63	-	-	29.3 ± 7.71	(Storelli et al., 2005)
Portugal	*C. caretta*	0.10 ± 0.01	5.03 ± 0.54	5.99 ± 0.48	1.78 ± 0.09	0.14 ± 0.02	24.01 ± 0.94	(Nicolau et al., 2017)
Japan	*C. caretta*	0.08 ± 0.03	9.74 ± 3.37	17.7 ± 8.93	2.18 ± 0.40	<0.03	28.1 ± 4.73	(Sakai et al., 2000b)
Japan	*C. mydas*	0.12	8.0	11.11	1.88	0.06	58.3	(Sakai et al., 2000b)
Japan	*C. mydas*	0.12 ± 0.10	4.55 ± 2.42	34.75 ± 21.5	1.18 ± 0.51	-	21.8 ± 7.65	(Anan et al., 2001)
China	*C. mydas*	0.03 ± 0.01	0.27 ± 0.24	33.25 ± 37.15	4.06 ± 3.45	0.06 ± 0.06	32.22 ± 15.98	(Lam et al., 2004)
Brazil	*C. mydas*	1.12 ± 0.12	1.47 ± 0.22	25.22 ± 3.97	-	-	10.12 ± 0.72	(da Silva et al., 2014)
USA	*C. mydas*	0.10 ± 0.02	0.90 ± 0.12	37.1 ± 7.3	2.31 ± 0.18	0.15 ± 0.03	35.0 ± 3.3	(Faust et al., 2014)
KIDNEY								
Mexico	*L. olivacea*	0.01	20.41	1.65	1.80	0.54	2.32	(Gardner et al., 2006)
Mexico	*L. olivacea*	0.06 ± 0.03	150.9 ± 110.1	1.41 ± 0.68	2.70 ± 1.19	0.07 ± 0.04	40.61 ± 22.61	(Cortés-Gómez et al., 2014)
Turkey	*C. caretta*	1.43 ± 0.76	6.10 ± 3.53	0.75 ± 0.21	-	3.46 ± 0.87	-	(Kaska et al., 2004)
Italy	*C. caretta*	0.12 ± 0.07	8.35 ± 4.83	1.21 ± 0.54	-	-	23.1 ± 4.53	(Storelli et al., 2005)
Portugal	*C. caretta*	0.08 ± 0.03	1.86 ± 0.37	4.72 ± 0.24	2.09 ± 0.13	0.06 ± 0.01	30.5 ± 1.49	(Nicolau et al., 2017)
Japan	*C. caretta*	0.16 ± 0.05	38.3 ± 17.5	1.30 ± 0.25	1.50 ± 0.51	0.22 ± 0.09	25.4 ± 4.39	(Sakai et al., 2000b)

Japan	*C. mydas*	0.07	41.2	1.51	17.17	0.51	34	(Sakai et al., 2000b)
Japan	*C. mydas*	0.29 ± 0.19	51.12 ± 23	2.97 ± 1.46	2.01 ± 0.49	-	60.84 ± 21.96	(Anan et al., 2001)
Brazil	*C. mydas*	1.94 ± 0.14	10.18 ± 0.82	4.39 ± 0.39	-	-	19.54 ± 1.47	(da Silva et al., 2014)
USA	*C. mydas*	0.21 ± 0.11	3.97 ± 0.61	3.24 ± 1.11	1.21 ± 0.17	0.06 ± 0.007	27.9 ± 5.70	(Faust et al., 2014)
MUSCLE								
Mexico	*L. olivacea*	<LOD	0.09	0.25	0.15	0.002	0.25	(Gardner et al., 2006)
Turkey	*C. caretta*	0.48 ± 0.65	0.71 ± 0.68	0.31 ± 0.16	-	2.04 ± 0.74	-	(Kaska et al., 2004)
Italy	*C. caretta*	-	0.07 ± 0.03	0.59 ± 0.41	-	-	27.9 ± 4.85	(Storelli et al., 2005)
Italy	*C. caretta*	<LOD	0.16 ± 0.01	0.48 ± 0.05	0.27 ± 0.05	-	21 ± 2.8	(Andreani et al., 2008)
Portugal	*C. caretta*	0.01 ± 0.00	0.16 ± 0.01	0.55 ± 0.04	0.14 ± 0.01	0.08 ± 0.03	19.79 ± 0.82	(Nicolau et al., 2017)
Japan	*C. caretta*	0.02 ± 0.03	0.06 ± 0.02	0.81 ± 0.27	0.28 ± 0.11	0.08 ± 0.02	25.0 ± 3.49	(Sakai et al., 2000b)
Japan	*C. mydas*	<0.03	0.02	0.25	0.26	0.05	9.67	(Sakai et al., 2000b)
Japan	*C. mydas*	0.02 ± 0.01	0.04 ± 0.03	0.17 ± 0.08	0.09 ± 0.02	-	9.54 ± 3.72	(Anan et al., 2001)
Turkey	*C. mydas*	0.28 ± 0.06	0.29 ± 0.09	0.42 ± 0.17	-	1.94 ± 0.75	-	(Kaska et al., 2004)
FAT								
Mexico	*L. olivacea*	<LOD	0.69	0.83	2.10	0.03	3.70	(Gardner et al., 2006)
Italy	*C. caretta*	0.063 ± 0.031	0.113 ± 0.02	0.446 ± 0.087	0.826 ± 0.13	-	62.1 ± 8.4	(Andreani et al., 2008)
Japan	*C. caretta*	<0.03	0.06 ± 0.03	0.11 ± 0.03	0.12 ± 0.09	<0.03	96.1 ± 18.8	(Sakai et al., 2000b)
Japan	*C. mydas*	<0.03	0.06	0.33	0.2	0.06	51.3	(Sakai et al., 2000b)
China	*C. mydas*	0.08 ± 0.026	<LOD	0.99 ± 0.383	0.19 ± 0.057	0.15 ± 0.047	105 ± 17.48	(Lam et al., 2004)

*LOD = Limit of detection. Below this value (<LOD), concentrations are not quantifiable and thus cannot be reported reliably. LOD is determined by the precision of the analytical instrument used.

There are two distinct categories in which metals can be found in vertebrates: detoxified metals and those elements which are still bioavailable (Lewis and Devereux, 2009). Most trace metals (both essential and non-essential elements) found in excess concentrations will likely be detoxified via long-term storage in tissues and organs where metabolism and re-mobilisation of metals is unlikely (Anan et al., 2001). For instance, in marine turtles some trace elements (Cd, Pb, Zn) are stored long-term in the muscles, liver, and kidneys, while some (Al, Pb and Zn) are also stored in the bone and carapace. Concerning instances where concentrations of certain elements are stored, accumulation is likely the result of detoxification and excretion rates being slower than uptake rates. (Villa et al., 2017). Moreover, due to the long lifespans and extended life history stages of marine turtles, pollutant bioaccumulation is more likely to be quantifiable over the total life span (Lutcavage et al., 1997, Caurant et al., 1999). For

instance, Hg has been found to accumulate at higher concentrations in marine turtles compared to other shorter-lived organisms (Caurant et al., 1994, Lahaye et al., 2006).

Metal uptake rates are often greater than the rate of detoxification and excretion (da Silva et al., 2016, Villa et al., 2017), with bioaccumulation of elevated concentrations becoming inevitable. Bioaccumulation is the gradual increase in concentration of chemical substances over time (Alexander, 1999) and is a particular risk to animals like *C. mydas* which are long lived and demonstrate strict site fidelity (Villa et al., 2017, Thomas et al., 2020). One consistent property of metals is their persistence in aquatic environments. This persistence is due to slow, partial biodegradation through bacterial metabolic pathways (Camacho et al., 2013a). When combined with the characteristics of *C. mydas* life history, the accumulation of these elements is anticipated. Three factors which contribute to bioaccumulation of trace metals are chemical form, concentration, and bioaccessibility (i.e., whether the metal is in dissolved soluble form available for active or passive uptake by plants or animals) of trace elements in coastal habitats are variable on a regional scale, due to several confounding factors. Known factors which have influence include the geochemical composition of the sediment (Thomas et al., 2020) and the distance from contaminant source (Gaudry et al., 2007, Villa et al., 2017, Brodie et al., 2019, Vijayasarathy et al., 2019, Thomas et al., 2020).

C. mydas are primarily exposed to trace elements through diet and ingestion of forage material (Villa et al., 2015). This is supported by findings that have suggested metal load and total body size of green marine turtles is negatively correlated (Sakai et al., 1995, Pople et al., 1998). This link is likely explained by dietary development and the shift to herbivorous foraging as juveniles migrate from pelagic to neritic environments as part of their natural ontogenetic change (Bjorndal et al., 1997, Caurant et al., 1999, Sakai et al., 2000a). *C. mydas* shift from pelagic nurseries (where they are predominantly omnivorous) to the neritic zone (predominantly herbivorous) at approximately 30 cm straight carapace length (SCL) (Musick and Limpus, 1997). Zooplankton, a primary food source for pelagic dwelling turtles, likely contain significantly higher concentrations of metals, due to the process of biomagnification, when compared to seagrass or macroalgae, which both serve as a sub-adult and adult food source (Sakai et al., 1995). Biomagnification refers to when contaminant concentrations increase with each successive trophic level of a food, particularly in persistent chemicals such as trace metals (Connell, 1989, Gray, 2002).

Because of the unavoidable lethality involved in collecting internal tissue samples, an opportunistic and unpredictable approach is often taken to studying metal loads in liver and kidney (and other internal tissues) (Aguirre et al., 1994, Sakai et al., 1995, Caurant et al., 1999), whereby reliance on stranded or euthanised turtles occurs. Blood sampling is considered a viable option for the investigation of metal exposure in turtles due to the short-term nature of blood metal storage (Kenyon et al., 2001, Day et al., 2005, Day et al., 2007, van de Merwe et al., 2010). Other minimally invasive techniques to monitor trace metal concentrations in marine turtles are available, such as lachrymal gland secretions (tears) (Perrault et al., 2019) and through analysis of eggs laid during the nesting season (Ross et al., 2016). These two alternative sampling techniques were not applied in this thesis as funding and permitting scope did not accommodate for such field efforts. Numerous studies have employed blood collection in recent years as means to measure metal exposure on the population level (van de Merwe et al., 2010, Ley-Quiñónez et al., 2013, Villa et al., 2015, da Silva et al., 2016, Villa et al., 2017, Villa et al., 2019), through non-lethal and minimally invasive techniques (Owens and Ruiz, 1980). The short-term snapshot that blood metal analysis provides may limit the application of this method for measuring metal exposure, but such limitations could be minimised by the additional application of sea turtle scute metal analysis (Villa et al., 2019). Keratinised scute that form a turtle's carapace has received attention recently as a possible alternative to the study of metal exposure and storage, offering an additional non-lethal sample set for the long-term analysis of metal loads (Villa et al., 2019).

2.4.4. Health risks posed by elevated trace metal exposure

Toxic metal exposure can induce an array of adverse effects, dependant on the metal studied. For instance, some elements have neurotoxic (Mn, Ni and Al), genotoxic (Cd, Ni), endocrine (hormone) disrupting (Pb, Hg and Cd) or autoimmune effects (Cd, Hg) (Grillitsch and Schiesari, 2010, Schultze et al., 2014). In regards to the study of FP aetiology and the theory that immunosuppression likely plays a role in disease expression, one of the most significant toxic effects of metals is that of oxidative stress, which is associated with a decline in immune function, observed in a range of animals (da Silva et al., 2016). Essential elements such as copper (Cu) and iron (Fe) are critical for optimal organ and metabolic function; however, when excess exposure to such elements occurs, highly reactive oxygen species (ROS) may be formed and can cause cell damage. Aquatic animals, are particularly sensitive to environmental contaminants that invoke increased production of ROS (Lesser, 2006).

Reactive oxygen species are derived from oxygen molecules and have strong oxidising properties (Valdivia et al., 2007). A number of environmental factors, including trace element exposure, can cause elevated ROS production, which overwhelm and interfere with cell and tissue antioxidant efficiency (designed to limited the concentration of ROS) (Monserrat et al., 2007). Such reduction in antioxidant capacity can cause deleterious effects via an oxidative stress condition (Sies, 1985, da Silva et al., 2016). Oxidative damage negatively impacts biomolecular processes of lipids, proteins, and nucleic acids (Halliwell and Gutteridge, 2015). The condition can inevitably cause tissue damage, which may lead to cell death (Valdivia et al., 2007). Moreover, oxidative stress has previously been found as a contributing factor in multiple viral infections (Schwarz, 1996, Beck et al., 2000). ROS are shown to affect and regulate host inflammatory and immune responses. (Valyi-Nagy and Dermody, 2005). Nevertheless, the knowledge and data available on the full deleterious impacts of oxidative stress on marine turtles are lacking (Valdivia et al., 2007, Labrada-Martagón et al., 2011, Perrault, 2014) and, would therefore benefit from further study. In addition to the aforementioned essential elements, non-essential elements such as Cd, Co and Pb have been observed to induce immunosuppression in *C. mydas* (da Silva et al., 2016). While recommended environmental thresholds for many metals are not available, the bioaccumulation risk is particularly concerning for long- lived species such as marine turtles.

Long term storage of excess metals in organs can aid in reducing the concentration of bioavailable elements, though it is not the only method of detoxification. Metal detoxification can also occur in a soluble phase (Rainbow, 2007). Metallothioneins are low molecular weight cytosolic proteins that possess sulphur, resulting in high metal binding affinity (Amiard et al., 2006). These proteins sequester the associated metal by limiting the metabolic availability of the element (Rainbow, 2007). Elevated bioaccumulation of elements and any negative effects will be minimised so long as the rate of detoxification is greater than the rate of uptake (Rainbow, 2002, Marsden and Rainbow, 2004). Uptake and detoxification rates are not uniform rates and thus, when uptake exceeds detoxification, trace element toxic thresholds may be exceeded and thus such metals will begin binding to sites, interfering with normal metabolic processes (Rainbow, 2002, Marsden and Rainbow, 2004). Toxicity onset has the potential to occur at any aqueous concentration if, for instance, the uptake rate changes to exceed detoxification for long enough to allow the available metal concentration to exceed toxicity thresholds.

2.5. The study region

The Great Barrier Reef World Heritage Area (GBRWHA) is considered a region of great importance for a plethora of reasons that span countless industries, species and crucial ecological processes, and is high in biodiversity of both coral and fish species (Fabricius and De'ath, 2000, Cheal et al., 2012). Different ecosystems and habitats of the GBR perform numerous functions, both ecologically (sediment stabilisation) and economically (established fisheries and aquaculture industries) to surrounding regions and communities that inhabit land adjacent to reef (DeVantier et al., 1998). The GBR extends for 2,300 km (an area of approximately 344,440 km^2) from the Torres Strait, North of Australia down the Queensland coast of Australia, within the Coral Sea to Fraser Island, Queensland, at the southern end. The Great Barrier Reef Marine Park Authority (GBRMPA) is the governing body responsible for managing all aspects of the GBRWHA. However, management and conservation as well as resource allocation and law enforcement jurisdiction, is fragmented and is largely dependent on adjacent governing bodies of the adjacent land. Environmental study often focuses on localised areas which are ecologically relevant and may be applicable to the wider system. This is the approach adopted in this thesis, with a large study area comprising several study sites located in the northern and central geographical regions of the GBR catchment area.

2.5.1. Study sites

The study sites investigated throughout this thesis are comprised of four geographically distinct coastal sites and one offshore site (Figure 2.1), located in the waters of the GBR and utilised by *C. mydas* for foraging. The Howick Island Group (HWK) is situated at the northern end of the GBR and is home to a *C. mydas* population that is examined regularly by the Department of Environment and Science, QLD Government, as it provides optimal conditions for the study of different aspects of turtle and environmental health (Bell et al., 2019, Flint et al., 2019, Villa et al., 2019, Thomas et al., 2020). HWK is minimally influenced by anthropogenic activity and therefore is often considered a near-pristine site, with baseline data collection possible, for a range of parameters relevant for health and ecotoxicological study (Flint et al., 2019). The coastal sites can largely be separated into two regions. Toolakea Beach (TLK) and Cockle Bay (CB) are geographically distinct sites located close to Townsville in north Queensland. TLK (19° 08' 36" S, 146° 34' 56" E) is a north-facing beach and is relatively exposed when compared to CB (19º 10 ' 26.7 " S 146 º 49 ' 32.1" E), which is a west-facing sheltered bay on Magnetic Island, adjacent to the mainland. CB is within the confines of the larger area of Cleveland Bay.

Upstart bay (UB) and Edgecumbe Bay (EB) are located south of Townsville within the central GBR region. UB (19° 44 ' 44.4" S 147° 36 ' 03.8 " E) is a site that is located within the Burdekin region and is influenced by freshwater runoff from the Burdekin River. South of UB is EB (20° 6′ 49″ S, 148° 23′ 25″ E), which is a sheltered bay adjacent to town of Bowen.

Figure 2.1. Map of the five study areas where *C. mydas* were captured and sampled in this study. Each site is colour coordinated between the national overview and localised maps. The respective localised

maps are displayed in the order of sites from north to south. The order is as follows, The Howick Group of island and reefs (HWK), Toolakea beach (TLK), Cockle Bay (CB), Upstart Bay (UB) and Edgecumbe Bay (EB). TLK and CB appear overlapped in the national overview but are geographically distinct on the local scale.

2.5.2. C. mydas genetic stocks

C. mydas populations inhabiting the coastal zone of Australia can be divided into nine genetic stocks (Jensen, 2010, Jensen et al., 2016, Jensen et al., 2019). The genetic diversity of Australian *C. mydas* populations is also influenced by international breeding stocks from neighbouring countries such as New Caledonia and Papua New Guinea, where individuals from such breeding stocks share local foraging grounds in Australian waters (Jensen, 2010, Jones et al., 2018, Jensen et al., 2019). A recent study was conducted, by Jones et al. (2018), identifying the origin of turtles inhabiting the same study region as this thesis. Four distinct *C. mydas* genetic stocks (Northern GBR (nGBR), Southern GBR (sGBR), New Caledonian (NC) and Coral sea (CS) stocks) were identified in foraging grounds sampled here, as well as some orphaned haplotypes whose origin could not be determined. *C. mydas* residing in Cockle Bay, Townsville (a study site sampled throughout this thesis) were found to originate predominantly from the sGBR and nGBR breeding stocks (both accounting for a total of 90% of turtles sampled). The origins of turtles inhabiting other sites in this thesis have not yet been analysed but what is known is that the more northerly the study site the more prevalent turtles for the nGBR stock will be and similarly at southerly sites and prevalence of the sGBR breeding stock (Jensen et al., 2016, Jones et al., 2018, Jensen et al., 2019).

2.5.3. Declines in local FP

As previously discussed, FP prevalence is often greater in *C. mydas* populations inhabiting coastal sites adjacent to human populations, where contaminants of concern are most often detected at potentially toxic concentrations (Villa et al., 2017, Jones, 2019, Villa et al., 2019, Thomas et al., 2020). A recent study observed that, while low, prevalence of FP was highest in *C. mydas* captured in the coastal site of Cleveland Bay (2.3%), when compared to UB (1.3%) and the offshore site HWK (0.04%) over a period of three years, between 2014 and 2017 (Bell et al., 2019). Once prevalent in several bays (Limpus et al., 2005, Jones et al., 2016, Jones et al., 2020), total recorded FP case numbers on the GBR, have decreased in recent years. Similar decline is seen in other regions of the world, including in Hawaiian populations (Chaloupka et al., 2009, Work et al., 2020). This abrupt shift in disease prevalence further confounds

epizootiology and pathogenic understanding of the disease in the local study area. Without intensive study it is difficult to conclude whether diseased individuals died and were removed from the population or whether aetiological conditions subsided and the animal recovered (Work et al., 2020).

2.5.4. Local threats and exposure to chemical contaminants

Within the region of this study there are shared anthropogenic threats between sites, which include boat strikes, habitat loss and chemical contamination sources, but risk factors and severity of these shared threats vary, depending on numerous factors specific to the area and human activity pressures undertaken in the region. For instance, as the population of Townsville (approximately 180,000), adjacent to CB, is significantly larger than that of the Burdekin region (approximately 17,700), boat traffic and therefore risk of collision is lower for *C. mydas* inhabiting foraging grounds in UB, as opposed to those adjacent to Townsville.

Other potential threats include specific exposure to influx of land-based chemicals from sources such as agricultural, industrial and urban processes (Gaus et al., 2019). Study sites included in this thesis have some similarities in known sources of potential contaminants. For example, agricultural land practices (predominantly sugar cane and fruit production) are extensive in the catchment adjacent to the study region (excluding HWK). The exposure to contaminants is dependent on which industries are present and which products and processes are applied, in addition to local river flow and coastal current direction, both of which influence transport of contaminants to local environments.

While all coastal sites within this thesis are exposed to point-source contamination of an unknown number of chemical contaminants, concentrations of metals likely vary between sites. Concentrations of some metals (not naturally geoavailable in the region) are dependent on industry point sources. For instance, in north Queensland substantial mining and metal refining activity occurs. Nickel (Ni) and Copper (Cu) is refined in Townsville and legacy mining and refining of Co and Ni occur in the Burdekin catchment, in proximity to Upstart Bay (UB). It can therefore be expected that the coastal zone adjacent to these activities will be more frequently exposed to contaminants from a greater number of sources as sites adjacent to smaller human populations, such as in Edgecumbe Bay (EB), which is adjacent to Bowen, south of Townsville.

However, metal elements are highly persistent and therefore legacy contaminants often remain in local coastal zones for long periods of time, even when original sources of such contaminants cease to exist. Metals often adhere to suspended sediment particulates within the water column and may remain in local areas in undisturbed sediment once settlement occurs (Hermanussen et al., 2006a). If land activity causes sediment to be resuspended (through dredging activity, for example), these once dormant sources of metals may again be remobilised and distributed with prevailing currents, to areas previously un-impacted by such elevation in these metals (Hedge et al., 2009). One local example of this is observed in UB, where particular agricultural land uses, including beef grazing, is associated with significant increases in coastal erosion (Bartley et al., 2015), which is believed to be one source of elevated metal concentrations such as Co and Cd, both of which are considered toxic to marine organisms, including *C. mydas* (da Silva et al., 2016). Significant erosion can lead to re-suspension and dispersal of such elements, to adjacent areas. Influx of suspended sediments is of concern during periods of heavy rainfall where significant flood plumes are likely to be transported from land to estuarine and coastal zones (Haynes, 2001, Lewis et al., 2014). This dynamic makes the prediction of metal exposure of local *C. mydas* difficult without site specific investigation, which is what we aimed to achieve, in part, in this thesis and all work associated with the project.

In addition to the well-studied threats faced by *C. mydas* populations inhabiting coastal foraging grounds, infrequent and unpredictable events may also pose a threat to local *C. mydas*. A mass stranding and mortality event occurred in *C. mydas* inhabiting UB in 2012. It is believed that neurotoxicity, associated with exposure to an unknown contaminant, was likely a significant factor in the morbidity and mortality observed (Gaus et al., 2019). A definitive causative diagnosis was not possible, however, as adequate samples and data was not available from the event. The sporadic and random nature of such snapshot toxic events make it impossible to predict future events, but by studying persistent contamination of known foraging sites, increased understanding and knowledge can be gleaned, and this will form the basis for an efficient and reliable response to any unforeseen environmental impacts that may occur in the future.

2.5.5. Variation in metal exposure between sites

HWK is minimally influenced by anthropogenic activity and therefore is often considered a near-pristine site. Due to this status, *C. mydas* within this population can be used as a baseline

cohort for health and ecotoxicological end points, with data likely representing optimal health ranges (Villa et al., 2017). When such data are directly compared to data from coastal populations, which are often closer to anthropogenic point sources for pollution, natural baseline data allows the detection of any sub-optimal conditions or elevated contaminant concentrations. Furthermore, with such baseline data, insight can be gleaned into susceptibility and risk levels of local populations to specific stressors by calculating reference intervals (RIs) (Villa et al., 2017).

In addition to geographical distance from anthropogenic contaminant point sources, several other factors will influence the variability of bioavailable metal loads found in certain areas and environments. For instance, local substrate composition and grain size plays a significant role in metal profiles of local regions, with coastal sites likely higher in metal-bound fine sediments. Metal bioavailability is greater in reducing sediments such as muds and silts (associated with coastal sites), as opposed to sands and gravels (more commonly isolated from coastal influence) (Thomas et al., 2020). Furthermore, between sites, substrate particle size likely further partitions metal exposure. In UB, average sediment grain size is expected to be smaller than in CB due to transport and settlement of terrigenous and estuarine particulate matter via substantial input from Burdekin River flood plume events (Bainbridge et al., 2012, Delandmeter et al., 2015, Flint et al., 2019), which is a significant source of contaminants to the local marine environment during period of heavy rainfall (Bainbridge et al., 2012). Another factor affecting differences between local metal loads is the physical geography and topography of an area. UB is less sheltered from prevailing south-easterly trade winds and thus any hydrologically driven re-suspension of metal bound sediments will likely be more frequent (Thomas et al., 2020). The impact of sediment resuspension is further influenced by the ecological properties of local seagrass meadows, including size, species composition and ability to accumulate and sequester ecologically relevant metals. All of which play a role in the capacity for sediment stability and thus susceptibility to significant resuspension (Thomas et al., 2020).

2.6. Thesis aims and objectives

Outlined above is a story of concern, potential, and uncertainty. FP is a disease that is debilitating and visually shocking. Advancement has been made in reptilian and marine turtle immunology and the knowledge base on FP and associated aetiological variables is ever

growing. There is great potential that someday definitive diagnosis of the causative co-factors of the disease will be met, but currently, knowledge gaps are ever present. This thesis aims to address some of those gaps and focused on three distinct aspects of the topic at hand (Figure 2.2). Firstly, I aimed to investigate metal exposure within local *C. mydas* populations by measuring a suite of ecologically relevant elements in *C. mydas* preferred seagrass species. Secondly, I aimed to look at recent metal loads in local *C. mydas* of the GBR. By measuring metal concentrations in blood and scute samples, it was possible to gain insight into short- and long-term exposure of a population to environmental contaminants. Finally, I aimed to conduct the first serological survey in Australia to map the seroprevalence of ChHV5 in local *C. mydas* populations. By applying ELISA to *C. mydas* serum, it was possible to detect ChHV5-specific antibodies and thus provide valuable data on critical epizootiological questions for understanding the role and association of the virus to FP. In the discussion chapter at the end of this thesis (Chapter 6) I synthesise all findings to investigate whether any links or associations were detected between ecological metal concentrations and the occurrence of FP in local populations.

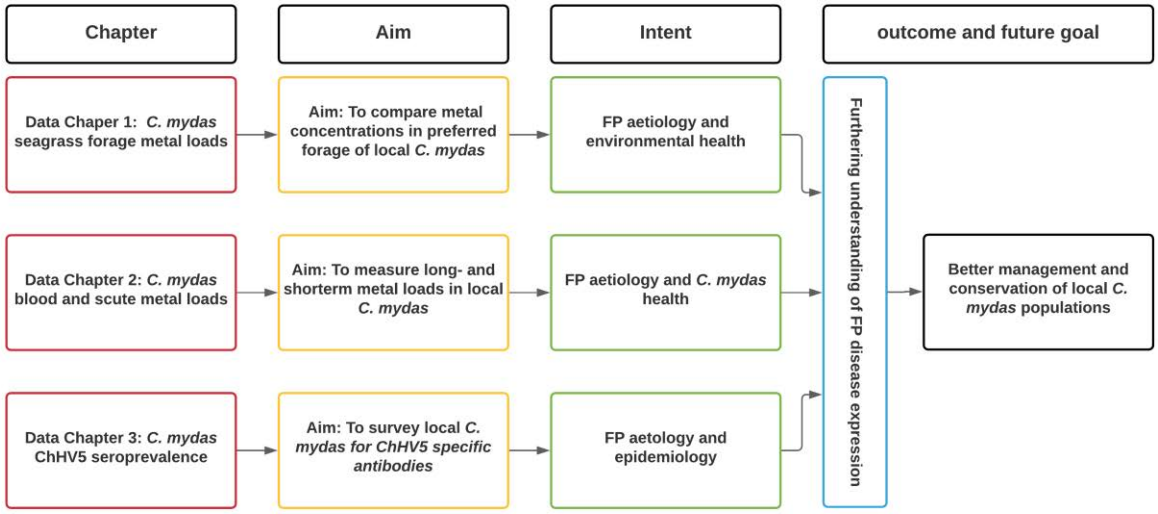


Figure 2.2. A flow chart outlining the aims and intent of each experimental chapter of this thesis and how data from each adds to the knowledgebase and understanding of aspects of the topic of FP disease aetiology and epidemiology.

2.7. References

AGGETT, P., NORDBERG, G. F. & NORDBERG, M. 2015. Essential Metals: Assessing Risks from Deficiency and Toxicity. *In:* NORDBERG, G. F., FOWLER, B. A. &

NORDBERG, M. (eds.) *Handbook on the Toxicology of Metals (Fourth Edition).* San Diego: Academic Press.
AGUIRRE, A. A. & BALAZS, G. H. 2000. Blood Biochemistry Values of Green Turtles, Chelonia Mydas, With and Without Fibropapillomatosis. *Comparative Haematology International,* 10**,** 132-137.
AGUIRRE, A. A., BALAZS, G. H., SPRAKER, T. R. & GROSS, T. S. 1995. Adrenal and hematological responses to stress in juvenile green turtles (chelonia mydas) with and without fibropapillomas. *Physiol Zool,* 68**,** 831-854.
AGUIRRE, A. A., BALAZS, G. H., SPRAKER, T. R., MURAKAWA, S. K. K. & ZIMMERMAN, B. 2002. Pathology of Oropharyngeal Fibropapillomatosis in Green Turtles Chelonia mydas. *Journal of Aquatic Animal Health,* 14**,** 298-304.
AGUIRRE, A. A., BALAZS, G. H., ZIMMERMAN, B. & GALEY, F. D. 1994. Organic contaminants and trace metals in the tissues of green turtles (*Chelonia mydas*) afflicted with fibropapillomas in the Hawaiian islands. *Marine Pollution Bulletin,* 28**,** 109–114.
AGUIRRE, A. A. & LUTZ, P. L. 2004. Marine Turtles as Sentinels of Ecosystem Health: Is Fibropapillomatosis an Indicator? *EcoHealth,* 1**,** 275–283.
AGUIRRE, A. A., SPRAKER, T. R., BALAZS, G. H. & ZIMMERMAN, B. 1998. Spirorchidiasis and Fibropapillomatosis in green turtles from the hawaiian islands. *Journal of Wildlife Diseases,* 34**,** 91-98.
ALEXANDER, D. E. 1999. Bioaccumulation, bioconcentration, biomagnification. *Environmental Geology***,** 43-44.
AMIARD, J. C., AMIARD-TRIQUET, C., BARKA, S., PELLERIN, J. & RAINBOW, P. S. 2006. Metallothioneins in aquatic invertebrates: Their role in metal detoxification and their use as biomarkers. *Aquatic Toxicology,* 76**,** 160-202.
ANAN, Y., KUNITO, T., WATANABE, I., SAKAI, H. & TANABE, S. 2001. Trace element accumulation in hawksbill turtles (Eretmochelys imbricata) and green turtles (Chelonia mydas) from Yaeyama Islands, Japan. *Environmental Toxicology and Chemistry,* 20**,** 2802-2814.
ANDREANI, G., SANTORO, M., COTTIGNOLI, S., FABBRI, M., CARPENÈ, E. & ISANI, G. 2008. Metal distribution and metallothionein in loggerhead (Caretta caretta) and green (Chelonia mydas) sea turtles. *Science of The Total Environment,* 390**,** 287-294.
ARTHUR, K. E. & BALAZS, G. H. 2008. A Comparison of Immature Green Turtle (Chelonia mydas) Diets among Seven Sites in the Main Hawaiian Islands. *Pacific Science,* 62**,** 205-217.
BAINBRIDGE, Z. T., WOLANSKI, E., ÁLVAREZ-ROMERO, J. G., LEWIS, S. E. & BRODIE, J. E. 2012. Fine sediment and nutrient dynamics related to particle size and floc formation in a Burdekin River flood plume, Australia. *Marine Pollution Bulletin,* 65**,** 236-248.
BAIRD, C. & CANN, M. 2005. *Environmental Chemistry,* New York, W.H. Freeman and Company.
BALAZS, G. & POOLEY, S. Research plan for marine turtle fibropapilloma: results of a December

1990 workshop. . NOAA-TMNMFS-SWFC-156., 1991 Hawaii, USA. Department of Commerce, National Oceanographic and Atmospheric Administration.
BALAZS, G. H. 1986. Fibropapillomas in Hawaiian green turtles. *Marine Turtle Newsletter,* 39**,** 1-3.

BALAZS, G. H. 1991. Current status of fibropapillomas in the Hawaiian green turtle,*Chelonia mydas*. *Research Plan for Marine Turtle Fibropapilloma (Balazs GH, Pooley SG, eds). Report No. NOAA-TM-NMFS-SWFSC-156. Honolulu: US Department of Commerce, National Oceanographic and Atmospheric Administration, National Marine Fisheries Service***,** 47-57.

BAPTISTOTTE, C., SCALFONE, J., GALLO, B., SANTOS, A. D., CASTILHOS, J. D., LIMA, E., BELLINI, C. & BARATA, P. 2001. Prevalence of sea turtle fibropapillomatosis in Brazil. *In:* COYNE, M. S. & CLARKE, R. D. (eds.) *Proceedings the 21 Annual Symposium on Sea Turtle Biology and Conservation.* NAOO Tech Memo NMFS-SEFSC-528.

BARRIOS-GARRIDO, H., ESPINOZA-RODRÍGUEZ, N., ROJAS-CAÑIZALES, D., PALMAR, J., WILDERMANN, N., MONTIEL-VILLALOBOS, M. G. & HAMANN, M. 2017. Trade of marine turtles along the Southwestern Coast of the Gulf of Venezuela. *Marine Biodiversity Records,* 10**,** 15.

BARRIOS-GARRIDO, H., MARCHENA, M., MEDINA, A., MEJÍA, K., NAVA, A. & RINCÓN, J. Descriptive analysis of the fishing arts that affect the marine turtles in the Gulf of Venezuela. Proceedings of the 22nd annual Symposium on Sea Turtle Biology and Conservation. Miami, Florida, USA, 2002.

BARRIOS-GARRIDO, H. A., MONTIEL-VILLALOBOS, M. G., PALMAR, J. & RODRÍGUEZ-CLARK, K. M. 2020. Wayuú capture of green turtles, Chelonia mydas, in the Gulf of Venezuela: A major Caribbean artisanal turtle fishery. *Ocean & Coastal Management,* 188**,** 105123.

BARTLEY, R., CROKE, J., BAINBRIDGE, Z. T., AUSTIN, J. M. & KUHNERT, P. M. 2015. Combining contemporary and long-term erosion rates to target erosion hot-spots in the Great Barrier Reef, Australia. *Anthropocene,* 10**,** 1-12.

BECK, M. A., HANDY, J. & LEVANDER, O. A. 2000. The Role of Oxidative Stress in Viral Infections. *Annals of the New York Academy of Sciences,* 917**,** 906-912.

BELL, I. P., MEAGER, J., VAN DE MERWE, J. P. & MADDEN HOF, C. A. 2019. Green turtle (Chelonia mydas) population demographics at three chemically distinct foraging areas in the northern Great Barrier Reef. *Science of The Total Environment,* 652**,** 1040-1050.

BJORNDAL, K. A. Biology and conservation of sea turtles. World conference on sea turtle conservation, 1995 Washington D.C., USA. Smithsonian Institution Press Washington.

BJORNDAL, K. A., BOLTEN, A. B. & LAGUEUX, C. J. 1994. Ingestion of marine debris by juvenile sea turtles in coastal Florida habitats. *Marine Pollution Bulletin,* 28**,** 154-158.

BJORNDAL, K. A., LUTZ, P. & MUSICK, J. 1997. Foraging ecology and nutrition of sea turtles. *The biology of sea turtles,* 1**,** 199-231.

BONANNO, G. & DI MARTINO, V. 2016. Seagrass *Cymodocea nodosa* as a trace element biomonitor: Bioaccumulation patterns and biomonitoring uses. *Journal of Geochemical Exploration,* 169**,** 43-49.

BONANNO, G. & ORLANDO-BONACA, M. 2018. Trace elements in Mediterranean seagrasses and macroalgae. A review. *Science of The Total Environment,* 618**,** 1152-1159.

BOYLE, M. C. & LIMPUS, C. J. 2008. The stomach contents of post-hatchling green and loggerhead sea turtles in the southwest Pacific: an insight into habitat association. *Marine Biology,* 155**,** 233-241.

BRADFORD, A. 1965. Association or causation. *Proc R Soc Med,* 58**,** 295-300.
BRODERICK, A. C., COYNE, M. S., FULLER, W. J., GLEN, F. & GODLEY, B. J. 2007. Fidelity and over-wintering of sea turtles. *Proceedings of the Royal Society B: Biological Sciences,* 274**,** 1533-1539.
BRODIE, J., GRECH, A., PRESSEY, B., DAY, J., DALE, A. P., MORRISON, T. & WENGER, A. 2019. The Future of the Great Barrier Reef: The Water Quality Imperative. *In:* WOLANSKI, E., DAY, J. W., ELLIOTT, M. & RAMACHANDRAN, R. (eds.) *Coasts and Estuaries.* Elsevier.
BURKHOLDER, D., HEITHAUS, M., THOMSON, J. & FOURQUREAN, J. 2011. Diversity in trophic interactions of green sea turtles *Chelonia mydas* on a relatively pristine coastal foraging ground. *Marine Ecology Progress Series,* 439**,** 277-293.
BURNHAM, D. K., KEALL, S. N., NELSON, N. J. & DAUGHERTY, C. H. 2005. T cell function in tuatara (Sphenodon punctatus). *Comparative Immunology, Microbiology and Infectious Diseases,* 28**,** 213-222.
CAMACHO, M., LUZARDO, O. P., BOADA, L. D., LÓPEZ JURADO, L. F., MEDINA, M., ZUMBADO, M. & ORÓS, J. 2013a. Potential adverse health effects of persistent organic pollutants on sea turtles: Evidences from a cross-sectional study on Cape Verde loggerhead sea turtles. *Science of The Total Environment,* 458–460**,** 283-289.
CAMACHO, M., ORÓS, J., BOADA, L. D., ZACCARONI, A., SILVI, M., FORMIGARO, C., LÓPEZ, P., ZUMBADO, M. & LUZARDO, O. P. 2013b. Potential adverse effects of inorganic pollutants on clinical parameters of loggerhead sea turtles (*Caretta caretta*): Results from a nesting colony from Cape Verde, West Africa. *Marine Environmental Research,* 92**,** 15-22.
CARDONA, L., AGUILAR, A. & PAZOS, L. 2009. Delayed ontogenic dietary shift and high levels of omnivory in green turtles (Chelonia mydas) from the NW coast of Africa. *Marine Biology,* 156**,** 1487-1495.
CARDWELL, A. J., HAWKER, D. W. & GREENWAY, M. 2002. Metal accumulation in aquatic macrophytes from southeast Queensland, Australia. *Chemosphere,* 48**,** 653-663.
CARMAN, V. G., FALABELLA, V., MAXWELL, S., ALBAREDA, D., CAMPAGNA, C. & MIANZAN, H. 2012. Revisiting the ontogenetic shift paradigm: The case of juvenile green turtles in the SW Atlantic. *Journal of Experimental Marine Biology and Ecology,* 429**,** 64-72.
CARRIÓN-CORTEZ, J. A., ZÁRATE, P. & SEMINOFF, J. A. 2010. Feeding ecology of the green sea turtle (Chelonia mydas) in the Galapagos Islands. *Journal of the Marine Biological Association of the United Kingdom,* 90**,** 1005-1013.
CARSON, R. 1962. *Silent spring,* Boston, Massachusettes, USA, Houghton Mifflin Harcourt.
CAURANT, F., AMIARD, J.-C., AMIARD-TRIQUET, C. & SAURIAU, P.-G. 1994. Ecological and biological factors controlling the concentrations of trace elements (As, Cd, Cu, Hg, Se, Zn) in delphinids Globicephala melas from the North Atlantic Ocean. *Marine Ecology Progress Series,* 103**,** 207-219.
CAURANT, F., BUSTAMANTE, P., BORDES, M. & MIRAMAND, P. 1999. Bioaccumulation of Cadmium, Copper and Zinc in some Tissues of Three Species of Marine Turtles Stranded Along the French Atlantic Coasts. *Marine Pollution Bulletin,* 38**,** 1085-1091.
CERRITELLI, G., BIANCO, G., SANTINI, G., BRODERICK, A. C., GODLEY, B. J., HAYS, G. C., LUSCHI, P. & ÅKESSON, S. 2018. Assessing reliance on vector

navigation in the long-distance oceanic migrations of green sea turtles. *Behavioral Ecology,* 30**,** 68-79.
CHALOUPKA, M. & BALAZS, G. 2005. Modelling the effect of fibropapilloma disease on the somatic growth dynamics of Hawaiian green sea turtles. *Marine Biology,* 147**,** 1251–1260.
CHALOUPKA, M., BALAZS, G. H. & WORK, T. M. 2009. Rise and fall over 26 years of a marine epizootic in Hawaiian green sea turtles. *J Wildl Dis,* 45**,** 1138-1142.
CHALOUPKA, M., BJORNDAL, K. A., BALAZS, G. H., BOLTEN, A. B., EHRHART, L. M., LIMPUS, C. J., SUGANUMA, H., TROËNG, S. & YAMAGUCHI, M. 2008. Encouraging outlook for recovery of a once severely exploited marine megaherbivore. *Global Ecology and Biogeography,* 17**,** 297-304.
CHALOUPKA, M., LIMPUS, C. & MILLER, J. 2004. Green turtle somatic growth dynamics in a spatially disjunct Great Barrier Reef metapopulation. *Coral Reefs,* 23**,** 325-335.
CHANG, L. W., MAGOS, L. & SUZUKI, T. 1996. *Toxicology of metals,* New York, USA, Lewis Publishers.
CHEAL, A., EMSLIE, M., MILLER, I. & SWEATMAN, H. 2012. The distribution of herbivorous fishes on the Great Barrier Reef. *Marine Biology,* 159**,** 1143-1154.
CHENG, I. J. 2000. Post-nesting migrations of green turtles (Chelonia mydas) at Wan-An Island, Penghu Archipelago, Taiwan. *Marine Biology,* 137**,** 747-754.
CLUKEY, K. E., LEPCZYK, C. A., BALAZS, G. H., WORK, T. M., LI, Q. X., BACHMAN, M. J. & LYNCH, J. M. 2018. Persistent organic pollutants in fat of three species of Pacific pelagic longline caught sea turtles: Accumulation in relation to ingested plastic marine debris. *Science of The Total Environment,* 610-611**,** 402-411.
COBERLEY, S., S, HERBST, L., H, EHRHART, L., M, BAGLEY, D., A, HIRAMA, S., JACOBSON, E., R & KLEIN, P., A 2001. Survey of Florida green turtles for exposure to a disease-associated herpesvirus. *Diseases of Aquatic Organisms,* 47**,** 159–167.
COICO, R. & SUNSHINE, G. 2015. *Immunology: a short course,* West Sussex, UK, John Wiley & Sons.
COLES, R. & LONG, L. 2000. Stratification of seagrasses in the Great Barrier Reef world heritage area, northeastern Australia, and the implications for management. *Biologia Marina Mediterranea,* 7**,** 345-348.
CONNELL, D. W. 1989. Biomagnification by aquatic organisms — A proposal. *Chemosphere,* 19**,** 1573-1584.
CONTI, B., TABAREAN, I., ANDREI, C. & BARTFAI, T. 2004. Cytokines and fever. *Front Biosci,* 9**,** 1433-1449.
COOPER, T., TRUNKFIELD, H., ZANELLA, A. & BOOTH, W. 1989. An enzyme-linked immunosorbent assay for cortisol in the saliva of man and domestic farm animals. *journal of Endocrinology,* 123**,** R13-R16.
CORTÉS-GÓMEZ, A. A., FUENTES-MASCORRO, G. & ROMERO, D. 2014. Metals and metalloids in whole blood and tissues of Olive Ridley turtles (Lepidochelys olivacea) from La Escobilla Beach (Oaxaca, Mexico). *Marine Pollution Bulletin,* 89**,** 367-375.
CORTÉS-GÓMEZ, A. A., ROMERO, D. & GIRONDOT, M. 2017. The current situation of inorganic elements in marine turtles: A general review and meta-analysis. *Environmental Pollution,* 229**,** 567-585.

CRAY, C., VARELLA, R., BOSSART, G. D. & LUTZ, P. 2001. Altered in vitro immune responses in green turtles (*Chelonia mydas*) with fibropapillomatosis. *Journal of Zoo and Wildlife Medicine,* 32**,** 436-440.

CURRY, S. S., BROWN, D. R., GASKIN, J. M., JACOBSON, E. R., EHRHART, L. M., BLAHAK, S., HERBST, L. H. & KLEIN, P. A. 2000. Persistent Infectivity of a Disease-Associated Herpesvirus in Green Turtles after Exposure to Seawater. *Journal of Wildlife Diseases,* 36**,** 792-797.

DA SILVA, C. C., KLEIN, R. D., BARCAROLLI, I. F. & BIANCHINI, A. 2016. Metal contamination as a possible etiology of fibropapillomatosis in juvenile female green sea turtles *Chelonia mydas* from the southern Atlantic Ocean. *Aquatic Toxicology,* 170**,** 42–51.

DA SILVA, C. C., VARELA, A. S., BARCAROLLI, I. F. & BIANCHINI, A. 2014. Concentrations and distributions of metals in tissues of stranded green sea turtles (Chelonia mydas) from the southern Atlantic coast of Brazil. *Science of The Total Environment,* 466-467**,** 109-118.

DAILEY, M. D. & MORRIS, R. 1995. Relationship of parasites (Trematoda: Spirorchidae) and their eggs to the occurrence of fibropapillomas in the green turtle (Chelonia mydas). *Canadian Journal of Fisheries and Aquatic Sciences,* 52**,** 84-89.

DAVISON, A. J. 2002. Evolution of the herpesviruses. *Veterinary Microbiology,* 86**,** 69-88.

DAY, R. D., CHRISTOPHER, S. J., BECKER, P. R. & WHITAKER, D. W. 2005. Monitoring Mercury in the Loggerhead Sea Turtle, Caretta caretta. *Environmental Science & Technology,* 39**,** 437-446.

DAY, R. D., KELLER, J. M., HARMS, C. A., SEGARS, A. L., CLUSE, W. M., GODFREY, M. H., LEE, A. M., PEDEN-ADAMS, M., THORVALSON, K., DODD, M. & NORTON, T. 2010. Comparison of Mercury burden in chronically debilitated and healthy Loggerhead sea turtles (*Caretta caretta*). *Journal of Wildlife Diseases,* 46**,** 111-117.

DAY, R. D., SEGARS, A. L., ARENDT, M. D., LEE, M. A. & PEDEN-ADAMS, M. A. 2007. Relationship of Blood Mercury Levels to Health Parameters in the Loggerhead Sea Turtle (Caretta caretta). *Environmental Health Perspectives,* 115**,** 1421-1428.

DELANDMETER, P., LEWIS, S. E., LAMBRECHTS, J., DELEERSNIJDER, E., LEGAT, V. & WOLANSKI, E. 2015. The transport and fate of riverine fine sediment exported to a semi-open system. *Estuarine, Coastal and Shelf Science,* 167**,** 336-346.

DEVANTIER, L. M., DE'ATH, G., DONE, T. J. & TURAK, E. 1998. Ecological assessment of a complex natural system: a case study from the great barrier reef. *Ecological Applications,* 8**,** 480-496.

DEZA, F. G., ESPINEL, C. S. & MOMPÓ, S. M. 2009. The immunoglobulin heavy chain locus in the reptile Anolis carolinensis. *Molecular Immunology,* 46**,** 1679-1687.

DIEGO, F. A. & RICHARD, D. R. 2007. Feeding ecology of the East Pacific green sea turtle Chelonia mydas agassizii at Gorgona National Park, Colombia. *Endangered Species Research,* 3**,** 43-51.

DUARTE, A., FAÍSCA, P., LOUREIRO, N. S., ROSADO, R., GIL, S., PEREIRA, N. & TAVARES, L. 2012. First histological and virological report of fibropapilloma associated with herpesvirus in *Chelonia mydas* at Príncipe Island, West Africa. *Archives of Virology,* 157**,** 1155–1159.

DUFFY, D. J., SCHNITZLER, C., KARPINSKI, L., THOMAS, R., WHILDE, J., EASTMAN, C., YANG, C., KRSTIC, A., ROLLINSON, D., ZIRKELBACH, B., YETSKO, K., BURKHALTER, B. & MARTINDALE, M. Q. 2018. Sea turtle

fibropapilloma tumors share genomic drivers and therapeutic vulnerabilities with human cancers. *Communications Biology,* 1**,** 63.

EHRHART, L. 1991. Fibropapillomas in green turtles of the Indian River Lagoon, Florida: distribution over time and area. *Research Plan for Marine Turtle Fibropapilloma (Balazs GH, Pooley SG, eds). Report No. NOAA-TM-NMFS-SWFSC-156. Honolulu: US Department of Commerce, National Oceanographic and Atmospheric Administration, National Marine Fisheries Service***,** 59-61.

ENE, A., SU, M., LEMAIRE, S., ROSE, C., SCHAFF, S., MORETTI, R., LENZ, J. & HERBST, L. H. 2005. Distribution of chelonid fibropapillomatosis-associated herpesvirus variants in Florida: molecular genetic evidence for infection of turtles following recruitment to neritic developmental habitats. *J Wildl Dis,* 41**,** 489–495.

ENERGY, D. O. T. E. A. 2017. Recovery Plan for Marine Turtles in Australia, Commonwealth of Australia 2017.

ENGVALL, E. & PERLMANN, P. 1972. Enzyme-linked immunosorbent assay, ELISA: III. Quantitation of specific antibodies by enzyme-labeled anti-immunoglobulin in antigen-coated tubes. *The Journal of Immunology,* 109**,** 129-135.

ESCHER, B. I., BRAMAZ, N., MUELLER, J. F., QUAYLE, P., RUTISHAUSER, S. & VERMEIRSSEN, E. L. M. 2008. Toxic equivalent concentrations (TEQs) for baseline toxicity and specific modes of action as a tool to improve interpretation of ecotoxicity testing of environmental samples. *Journal of Environmental Monitoring,* 10**,** 612-621.

FABRICIUS, K. & DE'ATH, G. 2000. Biodiversity on the Great Barrier Reef: large-scale patterns and turbidity-related local loss of soft coral taxa. *In:* WOLANSKI, E. (ed.) *Oceanographic Processes of Coral Reefs: Physical and Biological Links in the Great Barrier Reef.* CRC Press.

FAUST, D. R., HOOPER, M. J., COBB, G. P., BARNES, M., SHAVER, D., ERTOLACCI, S. & SMITH, P. N. 2014. Inorganic elements in green sea turtles (*Chelonia mydas*): Relationships among external and internal tissues. *Environmental Toxicology and Chemistry,* 33**,** 2020-2027.

FINLAYSON, K. A., LEUSCH, F. D. L., LIMPUS, C. J. & VAN DE MERWE, J. P. 2019. Towards the development of standardised sea turtle primary cell cultures for toxicity testing. *Ecotoxicology and Environmental Safety,* 173**,** 63-70.

FINLAYSON, K. A., MADDEN HOF, C. A. & VAN DE MERWE, J. P. 2020. Development and application of species-specific cell-based bioassays to assess toxicity in green sea turtles. *Science of The Total Environment,* 747**,** 142095.

FLINT, M., BRAND, A.-F., BELL, I. P. & MADDEN HOF, C. A. 2019. Monitoring the health of green turtles in northern Queensland post catastrophic events. *Science of The Total Environment,* 660**,** 586-592.

FLINT, M., PATTERSON-KANE, J. C., LIMPUS, C. J. & MILLS, P. C. 2010. Health Surveillance of Stranded Green Turtles in Southern Queensland, Australia (2006–2009): An Epidemiological Analysis of Causes of Disease and Mortality. *EcoHealth,* 7**,** 135–145.

FOLEY, A. M., SCHROEDER, B. A., REDLOW, A. E., FICK-CHILD, K. J. & TEAS, W. G. 2005. Fibropapillomatosis in stranded green turtles (*Chelonia mydas*) from the eastern United States (1980–98): trends and associations with environmental factors. *J Wildl Dis,* 41**,** 29–41.

FORBES, G. A. 1996. *The diet and feeding ecology of the green sea turtle (Chelonia mydas) in an algal-based coral reef community.* Ph.D., James Cook University.

FOULKES, E. C. 1996. Metals and biological membranes. *In:* CHANG, L. W., MAGOS, L. & SUZUKI, T. (eds.) *Toxicology of metals.* Boca Raton: CRC publishers.
FRAZIER, J. G. 2005. Marine turtles: the role of flagship species in interactions between people and the sea. *Mast*.
FRITIOFF, Å. & GREGER, M. 2006. Uptake and distribution of Zn, Cu, Cd, and Pb in an aquatic plant Potamogeton natans. *Chemosphere,* 63**,** 220-227.
GAMBÓN-DEZA, F., SÁNCHEZ-ESPINEL, C., MIRETE-BACHILLER, S. & MAGADÁN-MOMPÓ, S. 2012. Snakes antibodies. *Developmental & Comparative Immunology,* 38**,** 1-9.
GARDNER, S. C., FITZGERALD, S. L., VARGAS, B. A. & RODRÍGUEZ, L. M. 2006. Heavy Metal Accumulation in Four Species of Sea Turtles from the Baja California Peninsula, Mexico. *Biometals,* 19**,** 91-99.
GARNETT, S., PRICE, I. & SCOTT, F. 1985. The Diet of the Green Turtle, *Chelonia Mydas* (L.), In Torres Strait. *Wildlife Research,* 12**,** 103–112.
GAUDRY, A., ZEROUAL, S., GAIE-LEVREL, F., MOSKURA, M., BOUJRHAL, F.-Z., EL MOURSLI, R. C., GUESSOUS, A., MOURADI, A., GIVERNAUD, T. & DELMAS, R. 2007. Heavy Metals Pollution of the Atlantic Marine Environment by the Moroccan Phosphate Industry, as Observed through their Bioaccumulation in Ulva Lactuca. *Water, Air, and Soil Pollution,* 178**,** 267-285.
GAUS, C., GRANT, S., JIN, N. L., GOOT, K., CHEN, L., VILLA, A., NEUGEBAUER, F., QI, L. & LIMPUS, C. 2012. Investigations of contaminant levels in green turtles from Gladstone. *National Research Centre for Environmental Toxicology*.
GAUS, C., VILLA, C. A., DOGRUER, G., HEFFERNAN, A., VIJAYASARATHY, S., LIN, C.-Y., FLINT, M., HOF, C. M. & BELL, I. 2019. Evaluating internal exposure of sea turtles as model species for identifying regional chemical threats in nearshore habitats of the Great Barrier Reef. *Science of The Total Environment,* 658**,** 732-743.
GBRMPA 2014. *A vulnerability assessment for the Great Barrier Reef: Marine turtles,* Townsville.
GEORGE, R. H. 2017. Health Problems and Diseases of Sea Turtles. *In:* LUTZ, P. L. & MUSICK, J. A. (eds.) *The Biology of Sea Turtles, Volume I.* Florida, USA: CRC Press.
GONZÁLEZ CARMAN, V., BOTTO, F., GAITÁN, E., ALBAREDA, D., CAMPAGNA, C. & MIANZAN, H. 2014. A jellyfish diet for the herbivorous green turtle Chelonia mydas in the temperate SW Atlantic. *Marine Biology,* 161**,** 339-349.
GRAMENTZ, D. 1988. Involvement of loggerhead turtle with the plastic, metal, and hydrocarbon pollution in the central Mediterranean. *Marine Pollution Bulletin,* 19**,** 11-13.
GRAY, J. S. 2002. Biomagnification in marine systems: the perspective of an ecologist. *Marine Pollution Bulletin,* 45**,** 46-52.
GREENBLATT, R. J., WORK, T. M., BALAZS, G. H., SUTTON, C. A., CASEY, R. N. & CASEY, J. W. 2004. The Ozobranchus leech is a candidate mechanical vector for the fibropapilloma-associated turtle herpesvirus found latently infecting skin tumors on Hawaiian green turtles (Chelonia mydas). *Virology,* 321**,** 101-110.
GREY, H. M. 1963. Phylogeny of the Immune Response. *The Journal of Immunology,* 91**,** 819.
GRILLITSCH, B. & SCHIESARI, L. 2010. The ecotoxicology of metals in reptiles. *In:* SPARLING, D. W., LINDER, G., BISHOP, C. A. & KREST, S. K. (eds.) *Ecotoxicology of amphibians and reptiles.* 2nd ed.: CRC Press.

GUILIZZONI, P. 1991. The role of heavy metals and toxic amterials in the physiological ecology of submersed macrophytes. *Aquatic Botany,* 41**,** 87-109.
GUILLETTE, L. J., GROSS, T. S., MASSON, G. R., MATTER, J. M., PERCIVAL, H. F. & WOODWARD, A. R. 1994. Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. *Environmental Health Perspectives,* 102**,** 680-688.
HALLIWELL, B. & GUTTERIDGE, J. M. 2015. *Free radicals in biology and medicine*, Oxford University Press, USA.
HAMANN, M., GODFREY, M., SEMINOFF, J., ARTHUR, K., BARATA, P., BJORNDAL, K., BOLTEN, A., BRODERICK, A., CAMPBELL, L. & CARRERAS, C. 2010. Global research priorities for sea turtles: informing management and conservation in the 21st century. *Endangered Species Research,* 11**,** 245-269.
HAMANN, M., LIMPUS, C. & OWENS, D. 2002. Reproductive Cycles of Males and Females. *In:* LUTZ, P. L., MUSICK, J. A. & WYNEKEN, J. (eds.) *The Biology of Sea Turtles.* CRC press.
HANCOCK, J. M., FURTADO, S., MERINO, S., GODLEY, B. J. & NUNO, A. 2017. Exploring drivers and deterrents of the illegal consumption and trade of marine turtle products in Cape Verde, and implications for conservation planning. *Oryx,* 51**,** 428-436.
HAWKES, L. A., BRODERICK, A. C., GODFREY, M. H. & GODLEY, B. J. 2009. Climate change and marine turtles. *Endangered Species Research,* 7**,** 137-154.
HAYNES, D. B. 2001. *Pesticide and heavy metal concentrations in Great Barrier Reef sediment, seagrass and dugongs (Dugong dugon).* Ph.D., University of Queensland.
HAZEL, J., HAMANN, M. & LAWLER, I. R. 2013. Home range of immature green turtles tracked at an offshore tropical reef using automated passive acoustic technology. *Marine Biology,* 160**,** 617–627.
HEDGE, L. H., KNOTT, N. A. & JOHNSTON, E. L. 2009. Dredging related metal bioaccumulation in oysters. *Marine Pollution Bulletin,* 58**,** 832-840.
HERBST, L. H. 1994. Fibropapillomatosis of marine turtles. *Ann Rev Fish Dis,* 4**,** 389–425.
HERBST, L. H., ENE, A., SU, M., DESALLE, R. & LENZ, J. 2004. Tumor outbreaks in marine turtles are not due to recent herpesvirus mutations. *Curr Biol,* 14**,** 697–699.
HERBST, L. H. & JACOBSON, E. R. 2002. Practical Approaches for Studying Sea Turtle Health and Disease. *The biology of sea turtles,* 2**,** 385.
HERBST, L. H., JACOBSON, E. R., KLEIN, P. A., BALAZS, G. H., MORETTI, R., BROWN, T. & SUNDBERG, J. P. 1999. Comparative pathology and pathogenesis of spontaneous and experimentally induced fibropapillomas of green turtles (*Chelonia mydas*). *Vet Pathol,* 36**,** 551–564.
HERBST, L. H., JACOBSON, E. R., MORETTI, R., BROWN, T., SUNDBERG, J. P. & KLEIN, P. A. 1995. Experimental transmission of green turtle fibropapillomatosis using cell-free tumor extracts. *Dis Aquat Organ,* 22**,** 1–12.
HERBST, L. H. & KLEIN, P. A. 1995. Green turtle fibropapillomatosis: challenges to assessing the role of environmental cofactors. *Environmental Health Perspectives* 103**,** 27–30.
HERMANUSSEN, S., LIMPUS, C., PAPKE, O., CONNELL, D. & GAUS, C. 2006a. Foraging habitat contamination influences green sea turtle PCDD/F exposure.
HERMANUSSEN, S., LIMPUS, C., PAPKE, O., CONNELL, D. & GAUS, C. Foraging habitat contamination influences green sea turtle PCDD/F exposure. *In:* THOMSEN,

C. & BECHER, G., eds. International Symposium on Halogenated Persistent Organic Pollutants, 2006b Oslo, Norway. Norwegian Institute of Public Health, 592-595.
HIRAMA, S. & EHRHART, L. M. 2007. Description, prevalence and severity of green turtle fibropapillomatosis in three developmental habitats on the east coast of Florida. *Florida Scientist,* 70**,** 435-448.
HIRAMA, S., EHRHART, M., REA, D. & KILTIE, A. 2014. Relating fibropapilloma tumor severity to blood parameters in green turtles Chelonia mydas. *Diseases of Aquatic Organisms,* 111**,** 61-68.
JACOBSON, E. R., BUERGELT, C., WILLIAMS, B. & HARRIS, R. K. 1991. Herpesvirus in cutaneous fibropapillomas of the green turtle, Chelonia mydas. *Dis Aquat Organ,* 12.
JAKOB-HOFF, R. M., MACDIARMID, S. C., LEES, C., MILLER, P. S., TRAVIS, D. & KOCK, R. 2014. *Manual of procedures for wildlife disease risk analysis,* Paris, OIE (World Organisation for Animal Health).
JENSEN, M. P. 2010. *Assessing the composition of green turtle (Chelonia mydas) foraging grounds in Australasia using mixed stock analyses.* University of Canberra Australia.
JENSEN, M. P., BELL, I., LIMPUS, C. J., HAMANN, M., AMBAR, S., WHAP, T., DAVID, C. & FITZSIMMONS, N. N. 2016. Spatial and temporal genetic variation among size classes of green turtles (Chelonia mydas) provides information on oceanic dispersal and population dynamics. *Marine Ecology Progress Series,* 543**,** 241-256.
JENSEN, M. P., FITZSIMMONS, N. N., BOURJEA, J., HAMABATA, T., REECE, J. & DUTTON, P. H. 2019. The evolutionary history and global phylogeography of the green turtle (Chelonia mydas). *Journal of Biogeography,* 46**,** 860-870.
JONES, K. 2019. *Environmental influences on the epidemiology of fibropapillomatosis in green turtles (Chelonia mydas) and consequences for management of inshore areas of the Great Barrier Reef.* Ph.D., James Cook University.
JONES, K., ARIEL, E., BURGESS, G. & READ, M. 2016. A review of fibropapillomatosis in Green turtles (*Chelonia mydas*). *The Veterinary Journal,* 212**,** 48-57.
JONES, K., BURGESS, G., BUDD, A. M., HUERLIMANN, R., MASHKOUR, N. & ARIEL, E. 2020. Molecular evidence for horizontal transmission of chelonid alphaherpesvirus 5 at green turtle (*Chelonia mydas*) foraging grounds in Queensland, Australia. *PLOS ONE,* 15**,** e0227268.
JONES, K., JENSEN, M., BURGESS, G., LEONHARDT, J., VAN HERWERDEN, L., HAZEL, J., HAMANN, M., BELL, I. & ARIEL, E. 2018. Closing the gap: mixed stock analysis of three foraging populations of green turtles (Chelonia mydas) on the Great Barrier Reef. *PeerJ,* 6**,** e5651.
KASKA, Y., CELIK, A., BAG, H., AUREGGI, M., ÖZEL, K., ELÇI, A., KASKA, A. & ELÇI, L. 2004. Heavy metal monitoring in stranded sea turtles along the Mediterranean coast of Turkey. *Fresenius Environmental Bulletin,* 13**,** 769-776.
KELLER, J. M., BALAZS, G. H., NILSEN, F., RICE, M., WORK, T. M. & JENSEN, B. A. 2014. Investigating the Potential Role of Persistent Organic Pollutants in Hawaiian Green Sea Turtle Fibropapillomatosis. *Environmental Science & Technology,* 48**,** 7807-7816.
KENYON, L. O., LANDRY JR, A. M. & GILL, G. A. 2001. Trace Metal Concentrations in Blood of the Kemp's Ridley Sea Turtle(Lepidochelys kempii). *Chelonian Conservation and Biology,* 4**,** 128-135.

KOMOROSKE, L. M., LEWISON, R. L., SEMINOFF, J. A., DEUSTCHMAN, D. D. & DEHEYN, D. D. 2012. Trace metals in an urbanized estuarine sea turtle food web in San Diego Bay, CA. *Science of The Total Environment,* 417-418**,** 108-116.

KORNACKA, A., CYBULSKA, A., BIEŃ, J., GOŹDZIK, K. & MOSKWA, B. 2016. The usefulness of direct agglutination test, enzyme-linked immunosorbent assay and polymerase chain reaction for the detection of Toxoplasma gondii in wild animals. *Veterinary Parasitology,* 228**,** 85-89.

LABRADA-MARTAGÓN, V., TENORIO RODRÍGUEZ, P. A., MÉNDEZ-RODRÍGUEZ, L. C. & ZENTENO-SAVÍN, T. 2011. Oxidative stress indicators and chemical contaminants in East Pacific green turtles (Chelonia mydas) inhabiting two foraging coastal lagoons in the Baja California peninsula. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,* 154**,** 65-75.

LACKOVICH, J. K., BROWN, D. R., HOMER, B. L., GARBER, R. L., MADER, D. R., MORETTI, R. H., PATTERSON, A. D., HERBST, L. H., OROS, J., JACOBSON, E. R., CURRY, S. S. & KLEIN, P. A. 1999. Association of herpesvirus with fibropapillomatosis of the green turtle *Chelonia mydas* and the loggerhead turtle *Caretta caretta* in Florida. *Dis Aquat Organ,* 37**,** 89–97.

LAFFERTY, G. D., PORTER, J. W. & FORD, S. E. 2004. Are Diseases Increasing in the Ocean? *Annual Review of Ecology, Evolution, and Systematics,* 35**,** 31-54.

LAHAYE, V., BUSTAMANTE, P., DABIN, W., VAN CANNEYT, O., DHERMAIN, F., CESARINI, C., PIERCE, G. J. & CAURANT, F. 2006. New insights from age determination on toxic element accumulation in striped and bottlenose dolphins from Atlantic and Mediterranean waters. *Marine Pollution Bulletin,* 52**,** 1219-1230.

LALLAS, P. L. 2001. The Stockholm Convention on Persistent Organic Pollutants. *The American Journal of International Law,* 95**,** 692-708.

LAM, J. C. W., TANABE, S., CHAN, S. K. F., YUEN, E. K. W., LAM, M. H. W. & LAM, P. K. S. 2004. Trace element residues in tissues of green turtles (Chelonia mydas) from South China Waters. *Marine pollution bulletin,* 48**,** 174-182.

LAMBRECHT, B., STEENSELS, M., BORM, S. V., MEULEMANS, G. & BERG, T. V. D. 2007. Development of an M2e-Specific Enzyme-Linked Immunosorbent Assay for Differentiating Infected from Vaccinated Animals. *Avian Diseases,* 51**,** 221-226.

LANDSBERG, J. H., BALAZS, G. H., STEIDINGER, K. A., BADEN, D. G., WORK, T. M. & RUSSELL, D. J. 1999. The Potential Role of Natural Tumor Promoters in Marine Turtle Fibropapillomatosis. *Journal of Aquatic Animal Health,* 11**,** 199-210.

LEE LONG, W., MELLORS, J. & COLES, R. 1993. Seagrasses between Cape York and Hervey Bay, Queensland, Australia. *Marine and Freshwater Research,* 44**,** 19-31.

LEMONS, G., LEWISON, R., KOMOROSKE, L., GAOS, A., LAI, C.-T., DUTTON, P., EGUCHI, T., LEROUX, R. & SEMINOFF, J. A. 2011. Trophic ecology of green sea turtles in a highly urbanized bay: Insights from stable isotopes and mixing models. *Journal of Experimental Marine Biology and Ecology,* 405**,** 25-32.

LESSER, M. P. 2006. Oxidative Stress in Marine Environments: Biochemistry and Physiological Ecology. *Annual Review of Physiology,* 68**,** 253-278.

LEWIS, M. A. & DEVEREUX, R. 2009. Nonnutrient anthropogenic chemicals in seagrass ecosystems: Fate and effects. *Environmental Toxicology and Chemistry,* 28**,** 644-661.

LEWIS, S. S., BRODIE, J. J., ENDO, G. G., LOUGH, J. J. & BAINBRIDGE, Z. Z. 2014. Synthesizing Historical Land Use Change, Fertiliser and Pesticide Usage and Pollutant Load Data in the Regulated Catchments to Quantify Baseline and Changing

Pollutant Loads Exported to the Great Barrier Reef. *Centre of Tropical Water and Aquatic Ecosystem Research (TropWATER), James Cook University*.

LEY-QUIÑÓNEZ, C. P., ZAVALA-NORZAGARAY, A. A., RÉNDON-MALDONADO, J. G., ESPINOSA-CARREÓN, T. L., CANIZALES-ROMÁN, A., ESCOBEDO-URÍAS, D. C., LEAL-ACOSTA, M. L., HART, C. E. & AGUIRRE, A. A. 2013. Selected Heavy Metals and Selenium in the Blood of Black Sea Turtle (Chelonia mydas agasiizzi) from Sonora, Mexico. *Bulletin of Environmental Contamination and Toxicology,* 91**,** 645-651.

LIMPUS, C., JONES, K., ARIEL, E., BURGESS, G., READ, M. & JENSEN, M. The Direction of Research on Fibropapillomatosis on the Great Barrier Reef. *In:* HARGROVE, S., WORK, T., BRUNSON, S., FOLEY, A. M. & BALAZS, G. H., eds. International Summit on Fibropapillomatosis: Global Status, Trends, and Population Impacts, 2015 Hawaii, USA. NOAA Technical Memorandum NMFS-PIFSC-54 36-43.

LIMPUS, C. & MILLER, J. The occurrence of cutaneous fibropapillomas in marine turtles in Queensland. Proceedings of the Australian Marine Turtle Conservation Workshop, 1994. 186-188.

LIMPUS, C. J., LIMPUS, D. J., ARTHUR, K. E. & PARMENTER, C. J. 2005. Monitoring green turtle population dynamics in Shoalwater Bay 2000–2004. *In:* AUTHORITY, G. B. R. M. P. (ed.). Townsville, Australia.

LIMPUS, C. J. & MILLER, J. 1990. The occurrence of cutaneous fibropapillomas in marine turtles in Queensland. *Proceedings of the Australian Marine Turtle Conservation Workshop***,** 186-188.

LU, Y., NERURKAR, V. R., AGUIRRE, A. A., WORK, T. M., BALAZS, G. H. & YANAGIHARA, R. 1999. Establishment and characterization of 13 cell lines from a green turtle (*Chelonia mydas*) with fibropapillomas. *In Vitro Cellular & Developmental Biology - Animal,* 35**,** 389–393.

LU, Y., WANG, Y., YU, Q., AGUIRRE, A. A., BALAZS, G. H., NERURKAR, V. R. & YANAGIHARA, R. 2000. Detection of herpesviral sequences in tissues of green turtles with fibropapilloma by polymerase chain reaction. *Arch Virol,* 145.

LUTCAVAGE, M. E., PLOTKIN, P., WITHERINGTON, B. & LUTZ, P. L. 1997. Human impacts on sea turtle survival. *The biology of sea turtles,* 1**,** 387-409.

MAFFUCCI, F., CAURANT, F., BUSTAMANTE, P. & BENTIVEGNA, F. 2005. Trace element (Cd, Cu, Hg, Se, Zn) accumulation and tissue distribution in loggerhead turtles (Caretta caretta) from the Western Mediterranean Sea (southern Italy). *Chemosphere,* 58**,** 535-542.

MAGADÁN-MOMPÓ, S., SÁNCHEZ-ESPINEL, C. & GAMBÓN-DEZA, F. 2013. Immunoglobulin genes of the turtles. *Immunogenetics,* 65**,** 227-237.

MAGNUSSON, M., HEIMANN, K., QUAYLE, P. & NEGRI, A. P. 2010. Additive toxicity of herbicide mixtures and comparative sensitivity of tropical benthic microalgae. *Marine Pollution Bulletin,* 60**,** 1978-1987.

MALEA, P. & HARITONIDIS, S. 1999. Cymodocea nodosa (Ucria) aschers. as a bioindicator of metals in Thermaikos Gulf, Greece, during monthly samplings. *Botanica marina,* 42**,** 419-430.

MARCHALONIS, J., EALEY, E. & DIENER, E. 1969. Immune response of the Tuatara, *Sphenodon punctatum*. *Australian Journal of Experimental Biology and Medical Science,* 47**,** 367-380.

MARSDEN, I. D. & RAINBOW, P. S. 2004. Does the accumulation of trace metals in crustaceans affect their ecology—the amphipod example? *Journal of Experimental Marine Biology and Ecology,* 300**,** 373-408.

MASCARENHAS, R. & IVERSON, P. J. 2008. Fibropapillomatosis in stranded green turtles (*Chelonia mydas*) in Paraiba State, Northeastern Brazil: evidence of a Brazilian epizootic. *Marine Turtle Newsletter,* 120**,** 3–6.

MASHKOUR, N., JONES, K., HIPOLITO, T. V., KOPHAMEL, S., AHASAN, S., WALKER, G., JAKOB-HOFF, R., WHITTAKER, M., HAMANN, M., BELL, I., ELLIMAN, J., OWENS, L., SALADIN, C., CRESPO-PICAZO, J. L., GARDNER, B., LOGANATHAN, A. L., BOWATER, R., YOUNG, E., BARNES, A., ROBINSON, D., BAVERSTOCK, W., BLYDE, D., MARCH, D., EGHBALI, M., MOHAMMADI, M., FREGGI, D., GILIAM, J., HALE, M., NICOLLE, N., SPIBY, K., WROBEL, D., PARGA, M., MOBARAKI, A., RAJAKARUNA, R., HYLAND, K. P., READ, M. & ARIEL, E. 2020. Disease Risk Analysis in sea turtles: A baseline study to inform conservation efforts. *bioRxiv***,** 2020.03.10.985432.

MASHKOUR, N., MACLAINE, A., BURGESS, G. W. & ARIEL, E. 2018. Discovery of an Australian *Chelonia mydas* papillomavirus via green turtle primary cell culture and qPCR. *Journal of Virological Methods,* 258**,** 13–23.

MEDDINGS, J. I., OWENS, L., BURGESS, G. & ARIEL, E. 2014. Revelations in reptilian and avian immunology: a proposed evolutionary selection pressure for truncated immunoglobulin–Y. *International Journal of Immunological Studies,* 2**,** 29-41.

MOMČILOVIĆ, B. 1988. The Epistemology of Trace Element Balance and Interaction. *In:* HURLEY, L. S., KEEN, C. L., LÖNNERDAL, B. & RUCKER, R. B. (eds.) *Trace Elements in Man and Animals 6.* Boston, MA: Springer US.

MONSERRAT, J. M., MARTÍNEZ, P. E., GERACITANO, L. A., LUND AMADO, L., MARTINEZ GASPAR MARTINS, C., LOPES LEÃES PINHO, G., SOARES CHAVES, I., FERREIRA-CRAVO, M., VENTURA-LIMA, J. & BIANCHINI, A. 2007. Pollution biomarkers in estuarine animals: Critical review and new perspectives. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,* 146**,** 221-234.

MORAIS, R. A., DOS SANTOS, R. G., LONGO, G. O., YOSHIDA, E. T. E., STAHELIN, G. D. & HORTA, P. A. 2014. Direct Evidence for Gradual Ontogenetic Dietary Shift in the Green Turtle, Chelonia mydas. *Chelonian Conservation and Biology,* 13**,** 260-266.

MORTIMER, J. 1982. Feeding ecology of sea turtles. *In:* BJORNDAL, K. A. (ed.) *Biology and conservation of sea turtles. Smithsonian Institute Press, Washington, DC.* Washington, D.C, USA: Smithsonian Institution Press.

MUNKSGAARD, N. C. & LIVINGSTONE PARRY, D. 2002. Metals, arsenic and lead isotopes in near-pristine estuarine and marine coastal sediments from northern Australia. *Marine and Freshwater Research,* 53**,** 719-729.

MURALIKRISHNA, I. V. & MANICKAM, V. 2017. Introduction. *In:* MURALIKRISHNA, I. V. & MANICKAM, V. (eds.) *Environmental Management.* Oxford, UK: Butterworth-Heinemann.

MUSICK, J. A. & LIMPUS, C. J. 1997. Habitat utilization and migration in juvenile sea turtles. *The biology of sea turtles,* 1**,** 137-163.

NATARAJAN, K. & MUTHUKKARUPPAN, V. R. 1985. Distribution and ontogeny of B cells in the garden lizard, Calotesversicolor. *Developmental & Comparative Immunology,* 9**,** 301-310.

NICOLAU, L., MONTEIRO, S. S., PEREIRA, A. T., MARÇALO, A., FERREIRA, M., TORRES, J., VINGADA, J. & EIRA, C. 2017. Trace elements in loggerhead turtles (*Caretta caretta*) stranded in mainland Portugal: Bioaccumulation and tissue distribution. *Chemosphere,* 179**,** 120-126.

NIEBOER, E. & FLETCHER, G. G. 1996. Determinants of reactivity in metal toxicology. *In:* CHANG, L. W., MAGOS, L. & SUZUKI, T. (eds.) *Toxicology of metals.* Taylor & Francis US.

ORLANDO-BONACA, M., FRANCÉ, J., MAVRIČ, B., GREGO, M., LIPEJ, L., FLANDER-PUTRLE, V., ŠIŠKO, M. & FALACE, A. 2015. A new index (MediSkew) for the assessment of the Cymodocea nodosa (Ucria) Ascherson meadow's status. *Marine Environmental Research,* 110**,** 132-141.

ORTH, R. J., CARRUTHERS, T. J. B., DENNISON, W. C., DUARTE, C. M., FOURQUREAN, J. W., HECK, K. L., HUGHES, A. R., KENDRICK, G. A., KENWORTHY, W. J., OLYARNIK, S., SHORT, F. T., WAYCOTT, M. & WILLIAMS, S. L. 2006. A Global Crisis for Seagrass Ecosystems. *BioScience,* 56**,** 987-996.

OWENS, D. W. & RUIZ, G. J. 1980. New Methods of Obtaining Blood and Cerebrospinal Fluid from Marine Turtles. *Herpetologica,* 36**,** 17-20.

PÁEZ-OSUNA, F., CALDERÓN-CAMPUZANO, M. F., SOTO-JIMÉNEZ, M. F. & RUELAS-INZUNZA, J. R. 2010. Lead in blood and eggs of the sea turtle, Lepidochelys olivacea, from the Eastern Pacific: Concentration, isotopic composition and maternal transfer. *Marine Pollution Bulletin,* 60**,** 433-439.

PAGE-KARJIAN, A., GOTTDENKER, N. L., WHITFIELD, J., HERBST, L., NORTON, T. M. & RITCHIE, B. 2017. Potential Noncutaneous Sites of Chelonid Herpesvirus 5 Persistence and Shedding in Green Sea Turtles *Chelonia mydas*. *Journal of Aquatic Animal Health,* 29**,** 136–142.

PAGE-KARJIAN, A., NORTON, T. M., RITCHIE, B., BROWN, C., MANCIA, C., JACKWOOD, M. & GOTTDENKER, N. L. 2015. Quantifying chelonid herpesvirus 5 in symptomatic and asymptomatic rehabilitating green sea turtles. *Endangered Species Research,* 28**,** 135–146.

PAGE-KARJIAN, A., SERRANO, M. E., CARTZENDAFNER, J., MORGAN, A., RITCHIE, B. W., GREGORY, C. R., MCNEILL, J. B., PERRAULT, J. R., CHRISTIANSEN, E. F. & HARMS, C. A. 2020. Molecular Assessment of Chelonid Alphaherpesvirus 5 Infection in Tumor-Free Green (*Chelonia mydas*) and Loggerhead (*Caretta caretta*) Sea Turtles in North Carolina, USA, 2015–2019. *Animals,* 10**,** 1964.

PAGE-KARJIAN, A., TORRES, F., ZHANG, J., RIVERA, S., DIEZ, C., MOORE, P. A., MOORE, D. & BROWN, C. 2012. Presence of chelonid fibropapilloma-associated herpesvirus in tumored and non-tumored green turtles, as detected by polymerase chain reaction, in endemic and non-endemic aggregations, Puerto Rico. *SpringerPlus,* 1**,** 35.

PATRÍCIO, A. R., HERBST, L. H., DUARTE, A., VÉLEZ-ZUAZO, X., SANTOS LOUREIRO, N., PEREIRA, N., TAVARES, L. & TORANZOS, G. A. 2012. Global phylogeography and evolution of chelonid fibropapilloma-associated herpesvirus. *Journal of General Virology,* 93**,** 1035-1045.

PERGENT-MARTINI, C. & PERGENT, G. 2000. Marine phanerogams as a tool in the evaluation of marine trace-metal contamination: an example from the Mediterranean. *International Journal of Environment and Pollution,* 13**,** 126-147.

PERRAULT, J. R. 2014. Mercury and selenium ingestion rates of Atlantic leatherback sea turtles (Dermochelys coriacea): A cause for concern in this species? *Marine Environmental Research,* 99**,** 160-169.

PERRAULT, J. R., LEHNER, A. F., BUCHWEITZ, J. P. & PAGE-KARJIAN, A. 2019. Evidence of accumulation and elimination of inorganic contaminants from the lachrymal salt glands of leatherback sea turtles (Dermochelys coriacea). *Chemosphere,* 217**,** 59-67.

PIKE, D. A. 2013. Climate influences the global distribution of sea turtle nesting. *Global Ecology and Biogeography,* 22**,** 555-566.

PILCHER, N. J., RODRIGUEZ-ZARATE, C. J., ANTONOPOULOU, M. A., MATEOS-MOLINA, D., DAS, H. S. & BUGLA, I. A. 2020. Combining laparoscopy and satellite tracking: Successful round-trip tracking of female green turtles from feeding areas to nesting grounds and back. *Global Ecology and Conservation,* 23**,** e01169.

PITCHAPPAN, R. M. & MUTHUKKARUPPAN, V. R. 1977. Role of the thymus in the immune response to sheep erythrocytes in the lizardCalotes versicolor. *Proceedings of the Indian Academy of Sciences - Section B,* 85**,** 25-33.

POLOCZANSKA, E. S., LIMPUS, C. J. & HAYS, G. C. 2009. Vulnerability of Marine Turtles to Climate Change. *Advances in Marine Biology,* Volume 56**,** 151-211.

POPLE, A., GORDON, A. & NG, J. 1998. Trace metal concentrations in livers and kidneys of sea turtles from south-eastern Queensland, Australia. *Marine and Freshwater Research,* 49**,** 409-414.

PRITCHARD, P., LUTZ, P. & MUSICK, J. 1997. Evolution, phylogeny, and current status. *In:* LUTZ, P. L. & MUSICK, J. A. (eds.) *The biology of sea turtles.* London, England: CRC Press.

QUACKENBUSH, S. L., CASEY, R. N., MURCEK, R. J., PAUL, T. A., WORK, T. M., LIMPUS, C. J., CHAVES, A., DU TOIT, L., PEREZ, J. V., AGUIRRE, A. A., SPRAKER, T. R., HORROCKS, J. A., VERMEER, L. A., BALAZS, G. H. & CASEY, J. W. 2001. Quantitative analysis of herpesvirus sequences from normal tissue and fibropapillomas of marine turtles with real-time PCR. *Virology,* 287**,** 105–111.

QUACKENBUSH, S. L., WORK, T. M., BALAZS, G. H., CASEY, R. N., ROVNAK, J., CHAVES, A., DUTOIT, L., BAINES, J. D., PARRISH, C. R., BOWSER, P. R. & CASEY, J. W. 1998. Three closely related herpesviruses are associated with fibropapillomatosis in marine turtles. *Virol,* 246**,** 392–399.

RAINBOW, P. S. 2002. Trace metal concentrations in aquatic invertebrates: why and so what? *Environmental Pollution,* 120**,** 497-507.

RAINBOW, P. S. 2007. Trace metal bioaccumulation: Models, metabolic availability and toxicity. *Environment International,* 33**,** 576-582.

RAO, B., SUDHAN, C., KUMARI, R. & BEDEKAR, M. 2020. Herpesvirus: An emerging threat to marine turtles. *NISCAIR-CSIR, India,* 4**,** 517-526.

REBOREDA, R. & CAÇADOR, I. 2007. Halophyte vegetation influences in salt marsh retention capacity for heavy metals. *Environmental Pollution,* 146**,** 147-154.

REICH, K. J., BJORNDAL, K. A. & BOLTEN, A. B. 2007. The 'lost years' of green turtles: using stable isotopes to study cryptic lifestages. *Biology letters,* 3**,** 712–714.

REISSER, J., PROIETTI, M., SAZIMA, I., KINAS, P., HORTA, P. & SECCHI, E. 2013. Feeding ecology of the green turtle (Chelonia mydas) at rocky reefs in western South Atlantic. *Marine Biology,* 160**,** 3169-3179.

RESÉNDIZ, E. & LARA-UC, M. M. 2018. Health Assessments in Free-Ranging Sea Turtles: Perspective of Animal Welfare in Wildlife. *Animal Welfare,* 29.

RODENBUSCH, C. R., BAPTISTOTTE, C., WERNECK, M. R., PIRES, T. T., MELO, M. T. D., DE ATAÍDE, M. W., DOS REIS, K., TESTA, P., ALIEVE, M. M. & CANAL, C. W. 2014. Fibropapillomatosis in green turtles Chelonia mydas in Brazil: characteristics of tumors and virus. *Diseases of Aquatic Organisms,* 111**,** 207-217.

ROSS, D. A., GUZMÁN, H. M., VAN HINSBERG, V. J. & POTVIN, C. 2016. Metal contents of marine turtle eggs (Chelonia mydas; Lepidochelys olivacea) from the tropical eastern pacific and the implications for human health. *Journal of Environmental Science and Health, Part B,* 51**,** 675-687.

ROSSI, S., SÁNCHEZ-SARMIENTO, A. M., VANSTREELS, R. E. T., DOS SANTOS, R. G., PRIOSTE, F. E. S., GATTAMORTA, M. A., GRISI-FILHO, J. H. H. & MATUSHIMA, E. R. 2016. Challenges in Evaluating the Severity of Fibropapillomatosis: A Proposal for Objective Index and Score System for Green Sea Turtles (Chelonia mydas) in Brazil. *PLOS ONE,* 11**,** e0167632.

SAKAI, H., ICHIHASHI, H., SUGANUMA, H. & TATSUKAWA, R. 1995. Heavy metal monitoring in sea turtles using eggs. *Marine Pollution Bulletin,* 30**,** 347-353.

SAKAI, H., SAEKI, K., ICHIHASHI, H., KAMEZAKI, N., TANABE, S. & TATSUKAWA, R. 2000a. Growth-Related Changes in Heavy Metal Accumulation in Green Turtle (Chelonia mydas) from Yaeyama Islands, Okinawa, Japan. *Archives of Environmental Contamination and Toxicology,* 39**,** 378-385.

SAKAI, H., SAEKI, K., ICHIHASHI, H., SUGANUMA, H., TANABE, S. & TATSUKAWA, R. 2000b. Species-Specific Distribution of Heavy Metals in Tissues and Organs of Loggerhead Turtle (Caretta caretta) and Green Turtle (Chelonia mydas) from Japanese Coastal Waters. *Marine Pollution Bulletin,* 40**,** 701-709.

SANTOS, M. R. D. D., JÚNIOR, P. D. F., NÓBREGA, Y. C., MERÇON, J., PEREIRA, T. M. & GOMES, L. C. 2017. Stress Response of Juvenile Green Sea Turtles (Chelonia mydas) with Different Fibropapillomatosis Scores. *Journal of Wildlife Diseases,* 53**,** 653-656.

SANTOS, R. G., MARTINS, A. S., BATISTA, M. B. & HORTA, P. A. 2015. Regional and local factors determining green turtle Chelonia mydas foraging relationships with the environment. *Marine Ecology Progress Series,* 529**,** 265-277.

SANTOS, R. G., MARTINS, A. S., TOREZANI, E., BAPTISTOTTE, C., FARIAS, J. N., HORTA, P. A., WORK, T. M. & BALAZS, G. H. 2010. Relationship between fibropapillomatosis and environmental quality: a case study with Chelonia mydas off Brazil. *Diseases of Aquatic Organisms,* 89**,** 87-95.

SCHLACHER-HOENLINGER, M. A. & SCHLACHER, T. A. 1998. Accumulation, contamination, and seasonal variability of trace metals in the coastal zone – patterns in a seagrass meadow from the Mediterranean. *Marine Biology,* 131**,** 401-410.

SCHOFIELD, G., HOBSON, V. J., FOSSETTE, S., LILLEY, M. K. S., KATSELIDIS, K. A. & HAYS, G. C. 2010. BIODIVERSITY RESEARCH: Fidelity to foraging sites, consistency of migration routes and habitat modulation of home range by sea turtles. *Diversity and Distributions,* 16**,** 840-853.

SCHULTZE, B., LIND, P. M., LARSSON, A. & LIND, L. 2014. Whole blood and serum concentrations of metals in a Swedish population-based sample. *Scandinavian Journal of Clinical and Laboratory Investigation,* 74**,** 143-148.

SCHWARZ, K. B. 1996. Oxidative stress during viral infection: A review. *Free Radical Biology and Medicine,* 21**,** 641-649.

SEMINOFF, J. A. 2004. *Chelonia mydas* [Online]. [Accessed].
SHIMADA, T., LIMPUS, C., JONES, R., HAZEL, J., GROOM, R. & HAMANN, M. 2016. Sea turtles return home after intentional displacement from coastal foraging areas. *Marine Biology,* 163**,** 8.
SHIMADA, T., LIMPUS, C. J., HAMANN, M., BELL, I., ESTEBAN, N., GROOM, R. & HAYS, G. C. 2020. Fidelity to foraging sites after long migrations. *Journal of Animal Ecology,* 89**,** 1008-1016.
SIES, H. 1985. Oxidative stress: introductory remarks. *Oxidative stress.* New York, NY, USA: Academic Press.
SINDERMANN, C. J. 2005. *Coastal pollution: effects on living resources and humans*, CRC Press.
SMITH, G. & COATES, C. 1938. Fibro-epithelial growths of the skin in large marine turtles, *Chelonia mydas* (Linnaeus). *Zoologica,* 23**,** 93–98.
SNOEIJS, T., VAN DEN STEEN, E., EENS, M. & PINXTEN, R. 2007. Kinetics of primary antibody responses to sheep red blood cells in birds: a literature review and new data from great tits and European starlings. *Animal Biology,* 57**,** 79-95.
STORELLI, M. M., STORELLI, A., D'ADDABBO, R., MARANO, C., BRUNO, R. & MARCOTRIGIANO, G. O. 2005. Trace elements in loggerhead turtles (*Caretta caretta*) from the eastern Mediterranean Sea: overview and evaluation. *Environmental Pollution,* 135**,** 163-170.
SULKANEN, S., HALTTUNEN, T., LAURILA, K., KOLHO, K.-L., KORPONAY-SZABÓ, I. R., SARNESTO, A., SAVILAHTI, E., COLLIN, P. & MÄKI, M. 1998. Tissue transglutaminase autoantibody enzyme-linked immunosorbent assay in detecting celiac disease. *Gastroenterology,* 115**,** 1322-1328.
SUZUKI, K. T. & SUZUKI, T. 1996. An introduction to clinical aspects of metal toxicity. *In:* CHANG, L. W., MAGOS, L. & SUZUKI, T. (eds.) *Toxicology of metals.* Taylor & Francis US.
SWIMMER, J. Y. 2000. BIOCHEMICAL RESPONSES TO FIBROPAPILLOMA AND CAPTIVITY IN THE GREEN TURTLE. *Journal of Wildlife Diseases,* 36**,** 102-110.
THOMAS, C., BENNETT, W., GARCIA, C., SIMMONDS, A., HONCHIN, C., TURNER, R., HOF, C. & BELL, I. 2020. Coastal bays and coral cays: Multi-element study of *Chelonia mydas* forage in the Great Barrier Reef (2015–2017). *Science of The Total Environment***,** 140042.
VALDIVIA, P. A., ZENTENO-SAVÍN, T., GARDNER, S. C. & ALONSO AGUIRRE, A. 2007. Basic oxidative stress metabolites in eastern Pacific green turtles (Chelonia mydas agassizii). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,* 146**,** 111-117.
VALYI-NAGY, T. & DERMODY, T. S. 2005. Role of oxidative damage in the pathogenesis of viral infections of the nervous system. *Histology and histopathology,* 20**,** 957-967.
VAN DE MERWE, J. P. 2008. *Persistent organic pollutants and heavy metals in the green sea turtle, Chelonia mydas.* Ph.D., Griffith University, Queensland, Australia.
VAN DE MERWE, J. P., HODGE, M., OLSZOWY, H. A., WHITTIER, J. M. & LEE, S. Y. 2010. Using blood samples to estimate persistent organic pollutants and metals in green sea turtles (Chelonia mydas). *Marine Pollution Bulletin,* 60**,** 579-588.
VAN HOUTAN, K., SMITH, C., DAILER, M. & KAWACHI, M. 2014. Eutrophication and the dietary promotion of sea turtle tumors. *PeerJ,* 2.
VAN HOUTAN, K. S., HARGROVE, S. K. & BALAZS, G. H. 2010. Land Use, Macroalgae, and a Tumor-Forming Disease in Marine Turtles. *PLoS One,* 5.

VÉLEZ-RUBIO, G. M., CARDONA, L., LÓPEZ-MENDILAHARSU, M., MARTÍNEZ SOUZA, G., CARRANZA, A., GONZÁLEZ-PAREDES, D. & TOMÁS, J. 2016. Ontogenetic dietary changes of green turtles (Chelonia mydas) in the temperate southwestern Atlantic. *Marine Biology,* 163**,** 57.

VIJAYASARATHY, S., BADUEL, C., HOF, C., BELL, I., DEL MAR GÓMEZ RAMOS, M., RAMOS, M. J. G., KOCK, M. & GAUS, C. 2019. Multi-residue screening of non-polar hazardous chemicals in green turtle blood from different foraging regions of the Great Barrier Reef. *Science of The Total Environment,* 652**,** 862-868.

VILLA, C., BELL, I., HOF, C. M., LIMPUS, C. & GAUS, C. 2019. Elucidating temporal trends in trace element exposure of green turtles (*Chelonia mydas*) using the toxicokinetic differences of blood and scute samples. *Science of the Total Environment,* 651**,** 2450-2459.

VILLA, C. A., FINLAYSON, S., LIMPUS, C. & GAUS, C. 2015. A multi-element screening method to identify metal targets for blood biomonitoring in green sea turtles (*Chelonia mydas*). *Science of The Total Environment***,** 613-621.

VILLA, C. A., FLINT, M., BELL, I., HOF, C., LIMPUS, C. J. & GAUS, C. 2017. Trace element reference intervals in the blood of healthy green sea turtles to evaluate exposure of coastal populations. *Environmental Pollution,* 220, Part B**,** 1465–1476.

WANG, H.-C. 2005. *Trace metal uptake and accumulation pathways in Kemp's ridley sea turtles (Lepidochelys kempii).* Texas A&M University.

WEBER, M., LOTT, C. & FABRICIUS, K. E. 2006. Sedimentation stress in a scleractinian coral exposed to terrestrial and marine sediments with contrasting physical, organic and geochemical properties. *Journal of Experimental Marine Biology and Ecology,* 336**,** 18-32.

WEI, Z., WU, Q., REN, L., HU, X., GUO, Y., WARR, G. W., HAMMARSTRÖM, L., LI, N. & ZHAO, Y. 2009. Expression of IgM, IgD, and IgY in a Reptile, Anolis carolinensis. *The Journal of Immunology,* 183**,** 3858.

WILLIAMS, E. H., BUNKLEY-WILLIAMS, L., PETERS, E. C., PINTO-RODRIGUEZ, B., MATOS-MORALES, R., MIGNUCCI-GIANNONI, A. A., HALL, K. V., RUEDA-ALMONACID, J. V., SYBESMA, J., DE CALVENTI, I. B. & BOULON, R. H. 1994. An Epizootic of Cutaneous Fibropapillomas in Green Turtles Chelonia mydas of the Caribbean: Part of a Panzootic? *Journal of Aquatic Animal Health,* 6**,** 70-78.

WILLIS, J. M., GAMBRELL, R. P. & HESTER, M. W. 2010. Growth Response and Tissue Accumulation Trends of Herbaceous Wetland Plant Species Exposed to Elevated Aqueous Mercury Levels. *International Journal of Phytoremediation,* 12**,** 586-598.

WORK, T. M., ACKERMANN, M., CASEY, J. W., CHALOUPKA, M., HERBST, L., LYNCH, J. M. & STACY, B. A. 2014. The story of invasive algae, arginine, and turtle tumors does not make sense. *PeerJ PrePrints,* 2**,** e539v1.

WORK, T. M. & BALAZS, G. H. 1999. Relating Tumor Score to Hematology in Green Turtles with Fibropapillomatosis in Hawaii. *Journal of Wildlife Diseases,* 35**,** 804-807.

WORK, T. M., DAGENAIS, J., BALAZS, G. H., SCHUMACHER, J., LEWIS, T. D., LEONG, J.-A. C., CASEY, R. N. & CASEY, J. W. 2009. In vitro biology of fibropapilloma-associated turtle herpesvirus and host cells in Hawaiian green turtles (*Chelonia mydas*). *J Gen Virol,* 90.

WORK, T. M., DAGENAIS, J., BREEDEN, R., SCHNEEMANN, A., SUNG, J., HEW, B., BALAZS, G. H. & BERESTECKY, J. M. 2015. Green turtles (Chelonia mydas) have novel asymmetrical antibodies. *The Journal of Immunology,* 195**,** 5452-5460.

WORK, T. M., DAGENAIS, J., WEATHERBY, T. M., BALAZS, G. H. & ACKERMANN, M. 2017. In-vitro replication of Chelonid herpesvirus 5 in organotypic skin cultures from Hawaiian green turtles (*Chelonia mydas*). *Journal of Virology***,** e00404-17.

WORK, T. M., DAGENAIS, J., WILLIMANN, A., BALAZS, G., MANSFIELD, K. & ACKERMANN, M. 2020. Differences in Antibody Responses against Chelonid Alphaherpesvirus 5 (ChHV5) Suggest Differences in Virus Biology in ChHV5-Seropositive Green Turtles from Hawaii and ChHV5-Seropositive Green Turtles from Florida. *Journal of Virology,* 94**,** e01658-19.

WORK, T. M., RAMEYER, R. A., BALAZS, G. H., CRAY, C. & CHANG, S. P. 2001. Immune status of free-ranging green turtles with fibropapillomatosis from Hawaii. *Journal of Wildlife Diseases,* 37**,** 574–581.

ZAVALA-NORZAGARAY, A. A., LEY-QUIÑÓNEZ, C. P., ESPINOSA-CARREÓN, T. L., CANIZALEZ-ROMÁN, A., HART, C. E. & AGUIRRE, A. A. 2014. Trace Elements in Blood of Sea Turtles Lepidochelys olivacea in the Gulf of California, Mexico. *Bulletin of Environmental Contamination and Toxicology,* 93**,** 536-541.

ZIMMERMAN, L. M., VOGEL, L. A. & BOWDEN, R. M. 2010a. Understanding the vertebrate immune system: insights from the reptilian perspective. *The Journal of Experimental Biology,* 213**,** 661-671.

ZIMMERMAN, L. M., VOGEL, L. A., EDWARDS, K. A. & BOWDEN, R. M. 2010b. Phagocytic B cells in a reptile. *Biology Letters,* 6**,** 270-273.

CHAPTER 3 - Trace element concentrations in forage seagrass species of *Chelonia mydas* along the GBR

3.1. Chapter prelude

3.1.1. Aim:

To investigate and describe concentrations of a suite of ecologically relevant metals in preferred seagrass forage of *C. mydas* at three coastal turtle foraging sites on the Great Barrier Reef (GBR).

3.1.2. Introduction to chapter

Chelonia mydas populations of the Great Barrier Reef tend to primarily forage on seagrass species (Thomas et al., 2020). Some species of seagrass have been found to accumulate trace metal concentrations at levels far greater than the surrounding environment (Bonanno and Di Martino, 2016). To investigate metal exposure in local *C. mydas* populations this chapter focuses on investigating metal concentrations within specific seagrass species known to be common forage material for local populations, as feeding is likely the primary exposure pathway for toxic metal concentrations in marine turtles (Villa et al., 2015). This chapter first identified commonly foraged seagrass species through gastric lavage and taxonomic identification of samples. Samples of all represented seagrass species were then collected from three foraging grounds where turtles were captured: 1) Cockle Bay, adjacent to Townsville and in proximity to intensive land practices (including farming and metal refining), which serve as potential sources of land-based contaminants, 2) Upstart Bay and 3) Edgecumbe Bay, both located south of Townsville where local metal profiles are influenced by agricultural activity, river discharge from the Burdekin river and high erosion rates. Seagrass samples were collected from the same sites before and after the 2018/19 wet season. Seagrass sampling was not conducted by the author at HWK due to financial constraints and no samples were collected at TLK as there is no known seagrass meadows within this site. By analysing each sample with sensitive Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), the concentrations of a suite of ecologically relevant metals (Al, Cd, Co, Cu, Fe, Mg, Mn, Ni, Pb and Zn) were measured. Comparisons with previously published concentrations from the HWK (offshore site) were made to provide some insight as to whether current seagrass metal concentrations were considered elevated relative to the near-pristine offshore site. Additionally, comparisons were made between the three coastal site concentrations. This study found that seagrass metal concentrations were similar at each of the three coastal sites, and

most metals were elevated when compared to data from the offshore site. An increase in some metal concentrations was described following the wet season, particularly in UB.

3.2. Introduction

Marine turtles are air-breathing reptiles. Like mammals, turtles have lungs and must return to the surface to breathe (Wyneken, 2002). Due to this, the primary source of metal element exposure for green turtles (*Chelonia mydas*) is through their diet (Villa et al., 2015). *C. mydas* has a complex life history that includes a diverse diet, dependent on life phase (Bolten, 2002). Juveniles migrate to the coastal zone, inhabiting foraging grounds, where individuals undergo an ontogenetic dietary shift from a carnivorous to an herbivorous diet (Bolten, 2002). Once herbivorous, *C. mydas* forage on a range of material, dependent on region and what species are most predominant (Burkholder et al., 2011). Whilst macroalgal species are common as primary forage material in *C. mydas* diets in some regions (Burkholder et al., 2011), at coastal sites along the Great Barrier Reef (GBR), *C. mydas* feed primarily on seagrass species (Thomas et al., 2020).

In addition to providing forage for *C. mydas*, seagrass fulfil several integral ecological functions, such as sediment stabilisation (Kirkman, 1997, Short et al., 2011), nutrient cycling (Marbà et al., 2007, Touchette, 2007), the sequestration of carbon (Fourqurean et al., 2012), as nursery grounds and foraging material for a wide range of marine organisms (Kirkman, 1997). Most seagrass species grow in the coastal zone, often near anthropogenic activity and potential marine contamination sources (Lewis et al., 2013, Ambo-Rappe, 2014). Declines in seagrass distribution has been widespread in recent decades (Orth et al., 2006, Waycott et al., 2009, Unsworth and Cullen, 2010).

Several seagrass species are considered reliable bioindicators for the health of an ecosystem, as an early warning of any elevated contaminants, or decline in water quality parameters (Bonanno and Orlando-Bonaca, 2018, Malea et al., 2019). Seagrass are efficient at accumulating metals at concentrations higher than that detected in the surrounding water (Bonanno and Di Martino, 2016). Potential bioaccumulation of metals in seagrass may be a significant exposure pathway for turtles to elements at toxic concentrations (Thomas et al., 2020). *C. mydas* display strict site fidelity within a small local foraging range (2 - 3 km^2) and likely inhabit the same seagrass meadow for long periods, regardless of environmental condition and water quality, even if environmental health deteriorates (Hazel et al., 2013).

Metal contamination of marine ecosystems has increased significantly in recent years, with new chemicals frequently being introduced through industrial and agricultural processes and sequestered metal loads remobilised and redistributed due to dredging events and environmental disturbances such as flooding and cyclone activity (Storelli et al., 2005, Thomas et al., 2020). Some metals occur naturally in the marine environment, many of which are deemed essential elements for numerous biochemical and physiological processes (such as iron, Fe; copper, Cu and magnesium, Mg) (da Silva et al., 2016). However, essential elements may have toxic effects if concentrations exceeding optimal thresholds are experienced (as well as extremely low concentrations), particularly over long periods of time. For example, Fe, Cu and Zn may cause reduced immune function in marine organisms when elevated (da Silva et al., 2016). Conversely, numerous metal elements are non-essential for life and are often toxic at very low concentrations (Aggett et al., 2015, de Souza Machado et al., 2016). Non-essential elements commonly detected in the marine environment include, cadmium (Cd) and lead (Pb), with elements such as cobalt (Co) being understudied, but potentially toxic to sea turtles (Thomas et al., 2020). Metals of most concern are those elements (essential and non-essential,) which cause known toxic effects to immune function and biochemical processes such as Cd and Pb (Moszczyński, 1996, da Silva et al., 2016).

Metal contamination (essential and non-essential) of coastal zones and local foraging grounds occurs via several exposure pathways. Freshwater runoff of sediments, during periods of heavy rainfall and atmospheric deposition of metal particles, are the main transportation pathways of metals to the marine environment (Pacyna et al., 1995, Pacyna and Pacyna, 2001, Strzelec et al., 2020). Furthermore, anthropogenic pressures and processes such as mining, metal refining, agricultural chemical application, dredging and drainage of industrial waste, are able to change the distribution and composition of any geoavailable and bioavailable metal concentrations within the coastal zone (Johnston et al., 2010).

This study aimed to compare seagrass trace metal concentrations at several sites along the Great Barrier Reef (GBR), which are important foraging grounds for *C. mydas* and other macro grazers (such as dugongs) (Larkum et al., 2006). Such investigation is significant as it provides data on the prevalence of ecologically relevant metals (deemed toxic or are commonly measured in studies with similar scope) in the region. Metals of focus in this study included non-essential elements, cadmium (Cd), cobalt (Co), nickel (Ni) and lead (Pb), and several

essential elements (e.g. Fe, Cu and Zn), which are thought to be potentially toxic at concentrations exceeding natural ranges (da Silva et al., 2016).

3.3. Materials and methods

3.3.1. Study sites

Three geographically distinct study sites were sampled along the east coast of Australia, adjacent to the GBR (Figure 3.1). Firstly, Cockle Bay (CB) (19 º 10 ' 26.7 "S 146 º 49 ' 32.1"E) is a westerly facing bay of Magnetic Island, eight km off the coast of Townsville, Queensland and forms a part of Cleveland Bay. Industry practices such as, metal processing (including Zn, Cu and Ni), urban runoff from the city of Townsville (population about 200,000) and major sea port practices (including regular channel dredging) take place within the area (Esslemont, 2000). Secondly, Upstart Bay (UB) (19°44 ' 44.4"S 147°36 ' 03.8 "E) is a north facing bay, receiving river discharges from the major catchment of the Burdekin River (which also influences CB to a lesser extent), dominated by agricultural and grazing practices and with a prominent mining background, located 150 km from Townsville. The Burdekin catchment is one of the two largest GBR catchments (the other being the Fitzroy) with an area of 140,000km^2. Finally, Edgecumbe Bay (EB) (20° 6′ 49″ S, 148° 23′ 25″ E) is located south of Bowen, Queensland, approximately 200 km south of Townsville. Within the catchment draining into EB there are a number of point and non-point sources of potential contaminants, including a wastewater treatment plant (for the town of Bowen – population of approximately 10,000), cokeworks and sugarcane farms (mostly on the catchment of the Gregory River in the south of the bay), and rarely, from discharge plumes from the Burdekin River (Clark et al., 2016). Seagrass metal data Thomas et al. (2020) from a fourth site, the Howick island group (HWK), a mid-shelf group of remote reefs found in the northern region of the GBR (14 º 30 ' 11"S 144 º 58 ' 26 "E), was also included here for comparisons. HWK is considered a control site, with limited exposure to land-sourced contamination, located over 130 km from the nearest human settlement (Cooktown) and at least 15 km from the nearest coastal zone catchment. Sampling of seagrass was not conducted by the author at HWK during the current study due to financial and permitting limitations.

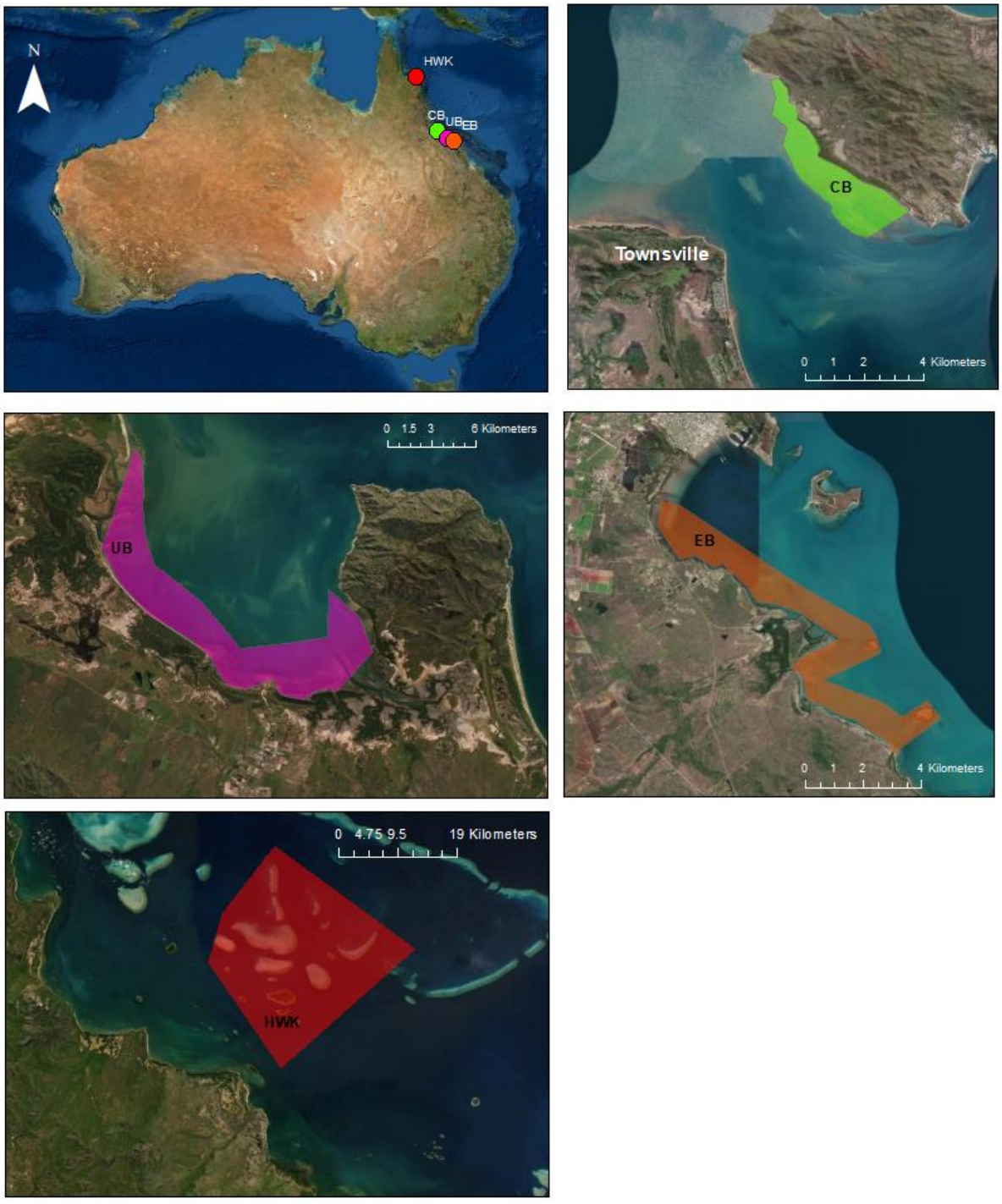


Figure 3.1. Map of three study areas (Cockle Bay (CB), Upstart Bay (UB) and Edgecumbe Bay (EB)) where preferred green turtle seagrass forage was sampled, and the offshore control site from which data was compared (Howick island group, HWK). Each site is colour coordinated between the national overview and localised maps.

3.3.2. Turtle capture and morphometric sampling

In total, 46 turtles were captured using turtle rodeo techniques described in Limpus and Reed (1985). Individually numbered titanium flipper tags (Department of Environmental Sciences, Queensland Government) were applied, as described by Eckert et al. (1999). Curved carapace

length (CCL), from the notch of the supracaudial scute to the line where skin joins the anterior edge of the carapace, along the midline ridge of the carapace, was measured using a flexible tape measure (cm), to the accuracy of ± 0.1 cm (Limpus and Miller, 1994, Limpus and Chaloupka, 1997). Large barnacles were removed from the carapace with long nose plyers if their position obstructed accurate CCL measurements. Animal weight was measured using digital scales (kg) and a custom harness that allowed for balanced weight distribution. The CCL and weight ranges of turtles sampled at each site are detailed in Table 3.1, with most animals captured classed as juveniles (up to 65 cm).

Table 3.1. Sample sizes (n= X) of *C. mydas* captured at each coastal site for gastric lavage sampling. Curved carapace length (CCL, cm) and weight (kg) of *C. mydas* included in this study are included. Weights were not recorded for individuals captured at CB as the weight system malfunctioned, though all individuals looked healthy (not malnourished). All data arranged by study site.

Study Location	Samples	CCL Range (cm)	Weight range (kg)
Cockle Bay (CB)	n=14	42.2 – 61.8	-
Upstart Bay (UB)	n=20	42.0 – 76.4	8.2 – 36.0
Edgecumbe Bay (EB)	n=12	43.2 – 65.0	7.2 – 29.2

3.3.3. Gastric lavage

Throughout the study period a total of 46 *C. mydas* stomach contents were collected, across all sites (CB= 14, UB= 20 and EB= 12), by modifying the protocol outlined in (Forbes and Limpus, 1993). Briefly, a custom-made water pumping mechanism was designed, assembled, and tested by experienced personnel prior to use in the field. A foot pump (Whale babyfoot pump, Whale Marine, Northern Ireland) was connected to a water intake hose and an outtake hose, made from polyurethane tubing (8 mm diameter, with the ends melted and rounded to prevent injury to the turtle's digestive tract). Firstly, captured turtles were elevated, with their head at the lowest point and secured in a fixed position by trained personnel. Turtles were encouraged to open their mouth by applying gentle pressure between the jaws, and once open, a wooden bit was inserted across the mouth to prevent closing. A 15-20 cm length of polyvinyl chloride tubing (10 mm diameter) was inserted into the mouth to offer stability for the insertion of the water tube (150 cm long and 8 mm diameter, marked at every 10 cm for the first 100 cm), which was lubricated with olive oil and slowly inserted down the digestive tract. The tube was slowly rotated during insertion to aid in the breaking up of any food bolus (obstruction of pre-digested material) in the throat. Once the marking, indicating insertion to 50–70 cm was achieved (determined on a case-by-case basis, dependant on turtle size), a constant flow of untreated sea water (1 L per minute) was initiated by regular use of the foot pump. Sea water

was brought into the system by the intake tube, from a clean bucket filled with local sea water. As the water drained from the turtle all forage material was collected using a sieve (310 µm mesh size) positioned below the mouth. The procedure was conducted for no longer than five minutes and the turtle was maintained in the head down position until all water was drained. All equipment was sterilised using hospital grade detergent (Benzalkonium chloride), and thoroughly rinsed between turtles. Samples were then placed on ice until return to the lab, where they were stored at -20 °C until identification and analysis. Due to ethical reasons gastric lavage was kept to a minimum. Furthermore, lavage samples were only collected once from each population as the reason for collection was to identify the primary forage material of each *C. mydas* population. While there may be some fluctuation and variability in diet species between seasons, it is unlikely that the primary foraged species will significantly change in composition.

3.3.4. Forage sample species ID

Forage material was thawed and separated. To identify the seagrass species that were being consumed by *C. mydas* at the study sites, forage species were visually identified by experienced personnel using taxonomical identification guides, where necessary. Forty out of the total 46 (87%) gastric lavage samples collected contained the seagrass species, *Halodule uninervis* (*Halodule*), and the remaining six samples (from CB only, equivalent to 42.3% of CB samples) predominantly contained *Cymodocea serrulata* (*Cymodocea*). Either one of these seagrass species commonly made up 100% of the biomass of an individual lavage sample. Occasionally, in samples collected from CB included small proportions (< 10% of overall biomass) of red algae, however taxonomic identification to the species level was not conducted here as low abundance did not warrant further study. The identification of forage species informed the collection of target seagrass species for the metal concentration analysis.

3.3.5. Seagrass collection

Rather than directly analysing the gastric lavage samples for metals, a total of 82 seagrass samples were collected by hand, either during dedicated field work, or during other sampling efforts (turtle capture and sampling). The reasoning for collecting fresh seagrass samples as opposed to analysing the gastric samples, was to ensure minimum contamination, and to allow for a wider area of each foraging ground to be sampled. Additionally, sampling known forage species meant that analysis could be completed prior to, and following, the wet season, without having to recapture individual green turtles. Seagrass sampling at all sites was conducted before (July – October 2018) and after (February – June 2019) the 2018/19 wet season. Samples per

study site were as follows: n = 16 pre- and n = 12 post-wet season in CB, n = 15 pre- and n = 15 post-wet season in UB and n = 11 pre- and n = 13 post-wet season in EB. As Cs was only present in lavage samples from CB green turtles, this seagrass species was only collected from CB. Preliminary findings found no significant differences between seagrass species, and thus all samples were pooled and referred to collectively. Samples were collected from the intertidal and subtidal zones within known turtle foraging grounds (identified through turtle sampling events). Personnel wore nitrile gloves when handling samples to minimise cross-contamination. Samples were collected at least 100 meters (max 300 m) apart, parallel to the shoreline, either on foot during low tide, or using snorkel techniques (max depth of approximately 2.5 m). Approximately 60 grams of above ground material (leaves and rhizomes) were collected and placed in food grade zip lock bags and stored on ice until return to the laboratory whereby samples were stored at -20 °C, until processing and analysis. Eighteen samples from HWK made up the reference value data provided by Thomas et al. (2020) for comparison. These samples were collected in a similar way to the current study. Briefly, aboveground biomass (leaves) was collected 250 m apart, using gloved hands and samples were frozen until analysis was undertaken. Gastric lavage was not conducted prior to seagrass sampling, in this instance but a range of species was targeted (including, *H. uninervis, C. serrulata*).

3.3.6. Seagrass sample preparation

Prior to analysis, all seagrass leaf samples were thawed and separated from and any below ground material (roots). Small epiphytes growing on the leaf surface were included in the sample, as turtles foraging on seagrass would ingest such epibionts along with the intended forage species. All large debris (shells, shale, sand, etc.) was rinsed off each sample prior to drying, using fresh water. Wet weights were recorded for each sample prior to being oven dried for 48 hr at 60 °C. Dry weights of each sample were then recorded before homogenising into a fine powder using a pestle and mortar (thoroughly cleaned with freshwater between samples to minimise cross-contamination). A minimum of 200 mg of homogenised material (per sample) was submitted to the Advanced Analytical Centre (AAC, James Cook University) for acid digestion and ICP-OES analysis.

3.3.7. ICP-OES analysis

A suite of 10 metals (Al, Cd, Co, Cu, Fe, Mg, Mn, Ni, Pb and Zn) were analysed in each sample. Seagrass samples were digested using a microwave assisted digestion oven (Bergof SW-4). A total of 100 mg of each sample was placed into the digestion vessel. Next, 4 mL SupraPure

(Merk Germany) double distilled HNO_3 and 1 mL AR Grade H_2O_2 were added into the vessel. The sample solution was kept in the fume hood for 2 h until the reaction was complete. Vessels were loaded into the microwave oven and heated to 185°C for 10 minutes. Once cooled, 150 mL of the digested samples were transferred to volumetric flask and diluted 50-fold, with Milli-Q water. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) was conducted using the Agilent 5100-ICP (Agilent Technologies, USA). External calibration strategy was used by applying a series of multi-element standard solutions containing all the elements of interest. HNO_3 and H_2O_2 (acids used in sample digestion, minus the sample) were included as procedure blanks for all elements and used to calculate the limit of detection values (LOD), which was defined as three times the standard deviation of each element's blanks. Three samples were randomly selected and duplicated to check for consistency. To assure instrument calibration quality, independent standards (1 ppm) were included, with reported recoveries ranging from 87% (Al) to 103% (Mg). Two Certified Reference Materials (CRM) (GBW07605 Tea Leaves and NIST 1566 Oyster Tissues) were analysed to validate the analytical method and % recoveries ranged from 92% (Cu) to 118% (Cd). All metal concentrations are reported here as mg/kg of sample dry weight (dw). These recoveries were similar to those reported elsewhere (da Silva et al., 2014, Thomas et al., 2020), and within the range generally considered acceptable (80-120%).

This study and the reference study analysed seagrass samples using similar but distinct analytical techniques. Thomas et al (Thomas et al., 2020) applied inductively coupled plasma mas-spectrometry (ICP-MS) rather than ICP-OES though both are suitable options for the detection of environmental metal loads in organic material such as seagrass. In ICP-OES, digested samples are nebulised and converted to plasma whereby electrons become excited. During the de-excitation phase, light is emitted from atoms and ions at different wavelengths dependant on metal. Wavelengths are then separated, detected, and quantified. In ICP-MS, ions present in the plasma are divided using mass spectrometry which separates and categorises each element by mass/charge ratio. ICP-MS can detect ultra-trace concentrations but both techniques are ideal for measuring the same suit of metals at ppm (mg/kg). Furthermore ICP-MS can differentiate between isotopes of the same element, but this was not applicable in the current study as focus was placed on total metal concentrations only.

3.3.8. Data analysis

Metal concentrations for all samples collected in this study (pre- and post-wet season data sets), and reference value data obtained from Thomas et al. (2020), were reported as mg/kg and any concentrations found to be below limits of detection (LOD) were considered half of the LOD. Pb was removed from further analysis due to having >40% samples <LOD. In addition, Mg and Zn concentrations were removed when comparing current data to the control data from HWK, as these elements were not reported by Thomas et al. (2020).

To reduce the dimensions of the data set we applied Principal Component Analysis (PCA). Spatio-temporal variation between study locations and sampling events (pre- and post-wet season) was conducted for all sites sampled within this study. Additionally, PCA was conducted to measure variation between metal profiles from each coastal site and HWK. The HWK data was collected prior to the wet season, in July / August of 2015-16 and thus for comparison pre-wet season data for coastal sites, CB, UB and EB were used. To determine the most important dimensions in the data two dimension-reduction protocols, scree plots and quality of representation measurements (cos2), were employed. Statistical analysis and plotting of PCA was conducted in the R statistical program (R Core Team, 2019), using the R packages 'Tidyverse'(data exploration (Husson et al., 2017)), 'FactoShiny'(multivariate analysis and plotting) (Husson et al., 2017) and 'FactoMineR' (Factor analysis) (Husson et al., 2013).

3.4. Results

3.4.1 Spatial patterns in metal profiles of preferred green turtle forage

When comparing metal mean concentrations from each site (pre-wet season) against that of the HWK, concentrations were often higher in all three coastal sites, for non-essential elements (Cd, Co and Ni) and essential metals (Cu, Fe and Mn), though difference was observed across all coastal sites for Cd, Co, Fe and Mn only (Table 3.2). Al was the only element where concentrations in seagrass collected from HWK were greater than any coastal sites, and concentrations were lower in HWK relative to CB concentrations. Mn was higher in all coastal sites when compared to HWK, by up to as much as 9-fold in CB.

Table 3.2. Mean concentration (mg/kg dw, rounded to two significant figures) and SD of metal elements at each coastal site (CB, UB and EB), prior to the wet season, and data from a foraging ground at the control site HWK (also collected pre-wet season), provided by Thomas et al. (2020).

	CB		UB		EB		HWK	
Element	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Al	3289	2629	1726	887	1846	753	3020	1600
Cd	0.37	0.12	0.27	0.10	0.33	0.11	0.20	0.07
Co	1.75	0.51	1.87	0.45	1.42	0.66	0.52	0.21
Cu	5.64	2.23	5.05	0.50	2.96	0.62	2.47	3.30
Fe	3382	1533	1954	1147	3123	1088	1696	827
Mn	355	73	246	114	208	111	35	7.67
Ni	3.66	1.27	4.25	1.53	3.87	1.68	3.04	0.90

To investigate whether metal profiles differed spatially, between the three coastal foraging sites and the offshore natural baseline site, HWK, PCA was conducted on pre-wet season data for each site. The scree plot indicated that the first two data dimensions adequately represented most of the variation in the data. Therefore, the suite of eight metals (variables or dimensions) were reduced to two principal components (Dim 1 and 2), which together represented 67.02% of the total variation of the data, presented in Figure 3.2. Data were represented by one cluster, including all analyses metals (variables) indicative of associations between concentrations, with loadings near one another.

Squared cosine (cos^2) indicates the importance of a metal element to a particular principal component (or the quality of representation) and supported the reduction to two principal components. A $cos^2 > 0.65$ true for all metals in the analysis suit, excluding Al and Ni, indicated that most of the data variation was indeed accounted for by the first two dimensions. Rather than removing Al and Ni from the analysis because of low representation, the elements were maintained for consistency. Further confirmation was observed in the scree plot whereby the elbow was at dimension 3 with only variation accounted for by dimension 1 and 2 being present below the line. The Al cluster (top right) aligns closely to Dimension 2 and the others (Cd-Fe-Ni and Co-Cu-Mn clusters), top right and bottom right) aligns closer to Dimension 1 and thus influence the respective components and therefore the entire data set when analysing the results in a reduced space.

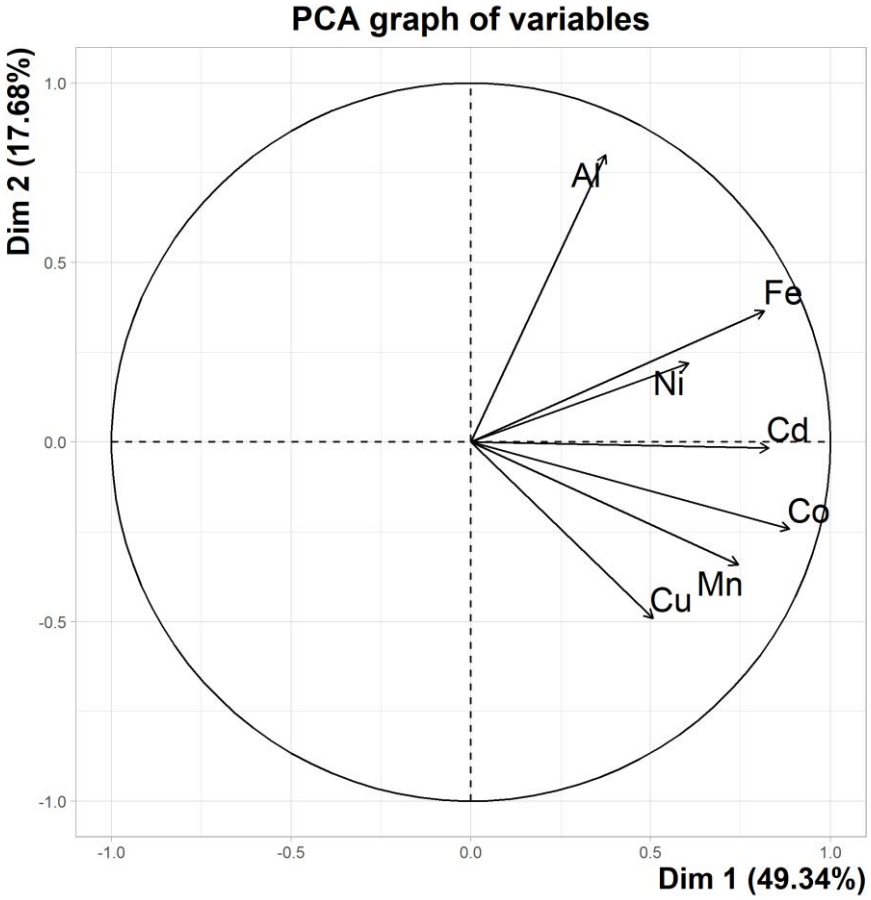


Figure 3.2. Variable loading plot of the PCA findings of all metal concentration data from seagrass collected from coastal sites of this study and baseline data obtained from HWK (Thomas et al., 2020). Dimension 1 (Dim 1) represents 49.34% of total variations and Dim 2 represents 17.68%.

Figure 3.3 represents the same data as Figure 3.2 but visualises individual seagrass samples rather than variable loadings. All points were relatively evenly spread across the plot for each coastal site and no separation between these locations was observed. EB variation was greatest of all sites as demonstrated by largest confidence ellipse and data point spread. All coastal seagrass data was distinctly separate from the HWK data (green), with individual sample loadings being clustered tightly and minimum overlap with other site data occurring. Confidence ellipses for each of the three coastal sites overlapped, indicative of similarities in each site metal profiles. CB (black) overlapped with both UB (blue) and EB (red), suggesting more similarity between CB and the other sites and little to none between UB and EB. HWK ellipse was distant from all coast sites while coastal data was more closely congregated suggesting associations between offshore and coastal metal profiles were less likely than between coastal sites.

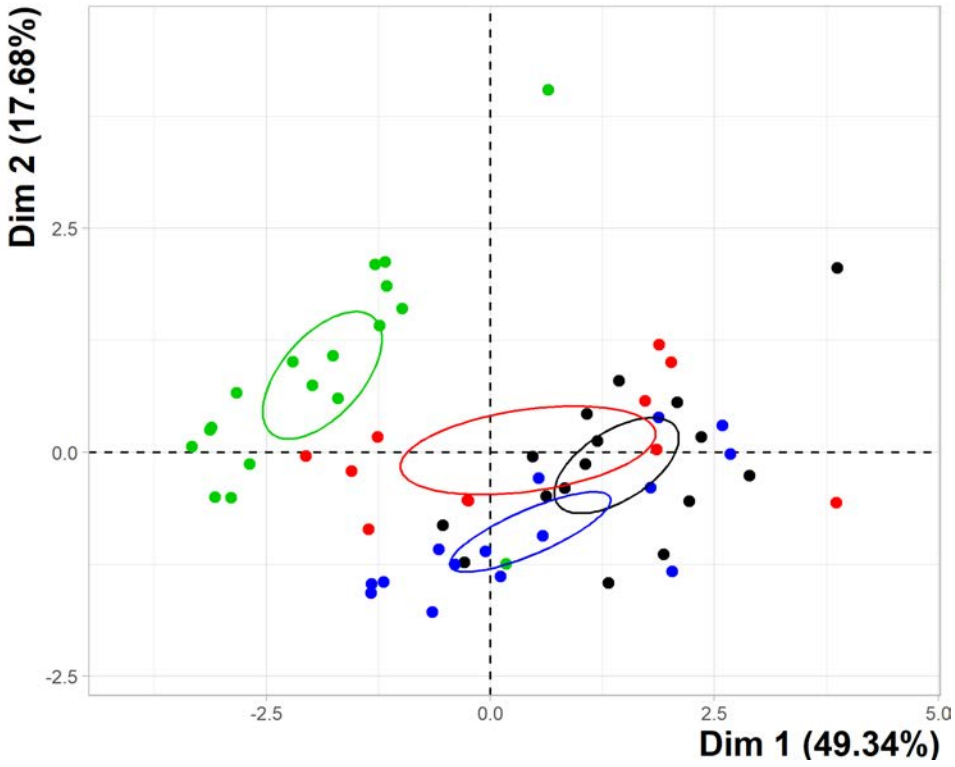


Figure 3.3. PCA individual plot depicting all seagrass metal data reduced to two principal dimensions (Dim 1 and 2). Each point indicates an individual seagrass sample and are categorised by location and represented as different colours (Howick island group, HWK = green, Cockle Bay, CB = black, Upstart Bay, UB = blue and Edgecumbe Bay, EB = red). Confidence ellipses are colour coordinated with the individual sample points. Dimension 1 (Dim 1) represents 49.34% of total variations and Dim 2 represents 17.68%. HWK data obtained from Thomas et al. (2020).

3.4.2 Temporal analysis of seagrass metal profiles between sampling events

To measure any differences in metal concentrations at each coastal site between samples collected prior to and following the wet season, means were compared and variation in means was observed between sampling events at all coastal sites. At CB, most element concentrations decreased over the wet season. Co and Cu were lower post-wet season at CB but higher at UB. Similarly, Zn was greatest post-wet season in UB but lower in EB.

A PCA was conducted to investigate any differences in total metal profile between seagrass samples collected from coastal sites before and after the wet season, and to identify the metals that most influenced the differences between sampling events (Figure 3.4). The scree plot accompanying the analysis determined that most of the data was adequately represented by the first two dimensions (principal components), which represented 56.56% of the variation. Individual samples from both events were evenly distributed across the entire plot (Figure 3.5). The confidence ellipses demonstrate that data from both sampling events show some

association with one another. While ellipses do not intercept or overlap, little space separates them and individual samples from both sets were located within both ellipses.

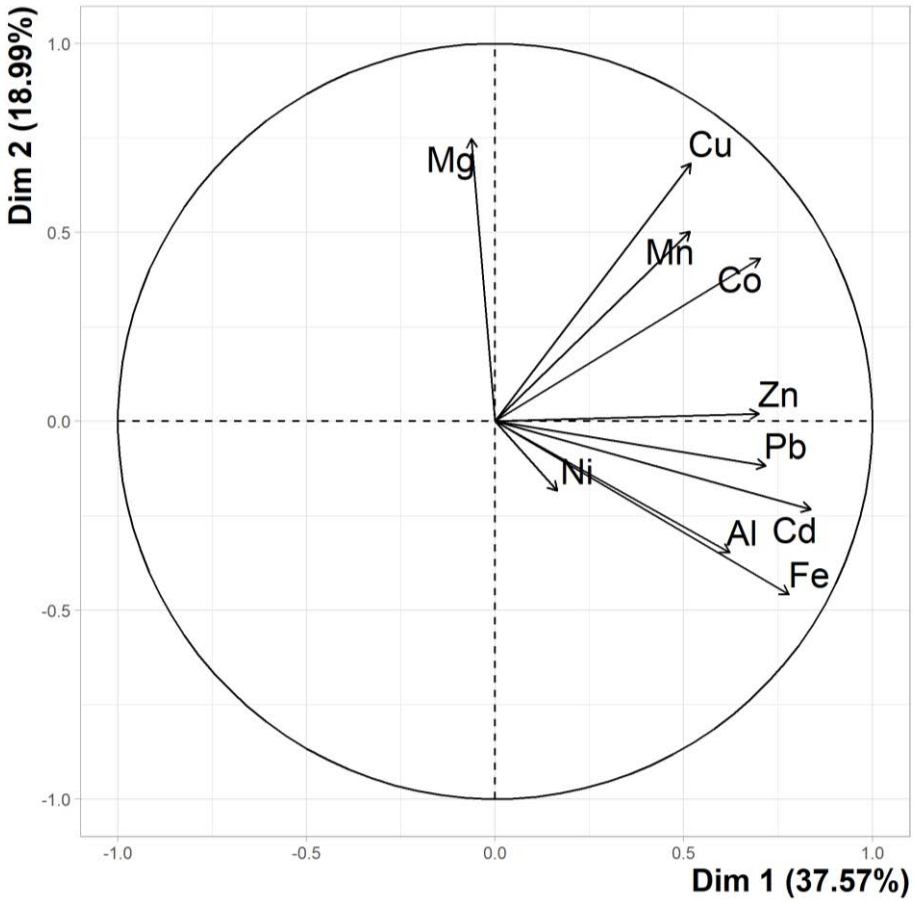


Figure 3.4. Variable loading plot of the PCA findings of all metal concentration data from seagrass collected from coastal sites of this study pre- post-wet season. Dimension 1 (Dim 1) represents 37.57% of total variations and Dim 2 represents 18.99%.

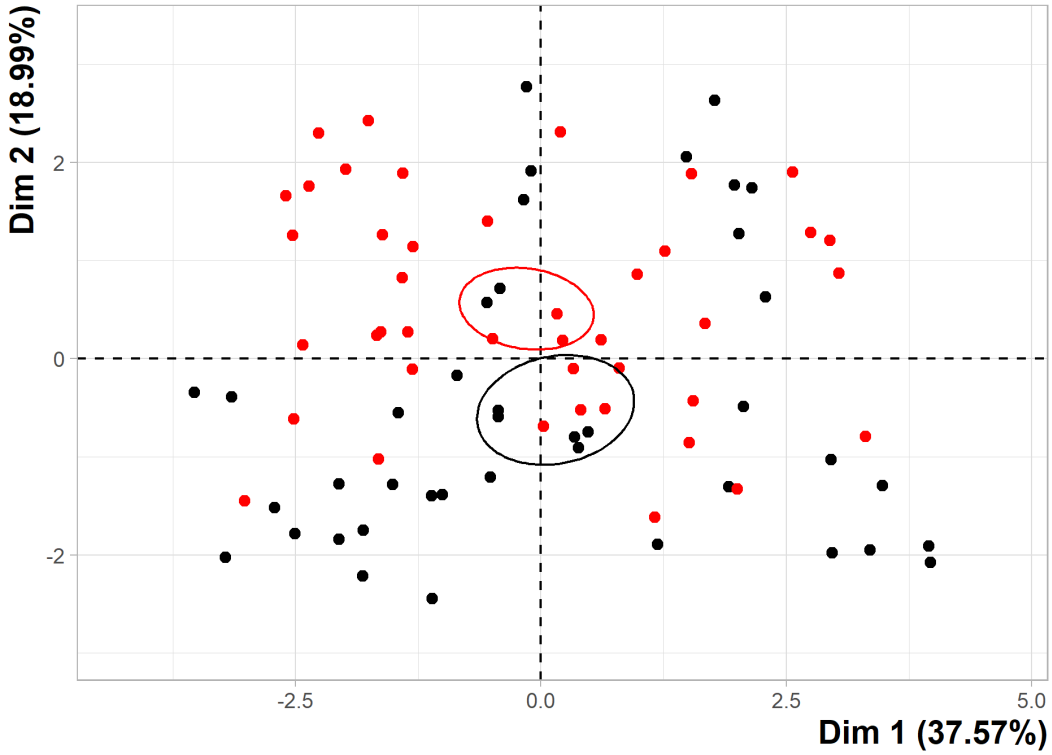


Figure 3.5. PCA individual plot depicting all coastal metal data, collected in this study, reduced to two principal dimensions (Dim 1 and 2). Each point indicates an individual seagrass sample and are categorised by sampling event and categorised as different colours (before wet season = red and after wet season = black. Confidence Ellipses are colour coordinated with the individual sample points. Dimension 1 (Dim 1) represents 37.57% of total variations and Dim 2 represents 18.99%.

3.5. Discussion

3.5.1. Comparison of metal means to control site concentrations

By comparing seagrass metals in coastal study sites to an ecologically relevant natural baseline population, minimally impacted by anthropogenic activity (in this instance HWK), insight can be gleaned into whether target elements are considered elevated at sites near human influence. Region-specific baseline data is crucial as reference for determining if element concentrations are of concern to local ecosystems or animals. In this study, a total of six out of seven reported metals (Cd, Co, Cu, Fe, Mn, and Ni) were detected at higher concentrations at all coastal sites relative to the HWK data. Al was higher at CB relative to HWK but was lower in both other coastal sites than HWK. When compared to concentrations reported in Thomas et al. (2020) it is worth noting that concentrations were reported to be consistently higher in the reference study for all metals, though many factors play a role in metal concentration reporting (including but not limited to, sampling timings and procedures, prevailing weather conditions prior to and during sampling and relative concentrations of metals in the plant and surrounding environment) (Kirkby, 2012). It is beyond the scope of this study to provide inference as to

why such differences were observed between studies. Metals which form part of the inorganic sediment matrix which is associated with clay based terrigenous sediments (such as Al, Fe and Mn) are expected to be elevated at coastal sites when compared to offshore sites such as HWK (Thomas et al., 2020). Here Cd concentrations were greater at all three coastal sites than those reported in seagrass from HWK (Table 3.1; Thomas et al., 2020), possibly indicating exposure to higher concentrations at all coastal sites monitored. Additionally, coastal Cd concentrations exceeded or were close to control site seagrass data, (0.36 mg/kg), reported by Conti *et al.* (Conti et al., 2015) in temperate seagrass species found in the Mediterranean Sea. One potential source of Cd in the region may be due to soil erosion of old Cd-rich coastal sugar cane paddocks (from long-term application of phosphatic fertiliser (Rayment, 2005)), over time (Wilkinson et al., 2013, Bartley et al., 2015). This exposure pathway is particularly significant during freshwater runoff events and through increased erosion seen in the Burdekin catchment (Kroon et al., 2012), which encompasses UB. Cd is considered a xenobiotic element (not produced or used within the organism) and it is possible that exposure to very low concentrations may cause some toxic effects (da Silva et al., 2016). Due to the low excretion rate and its ability to bioaccumulate in tissues, Cd is considered one metal element that is most likely toxic to organisms (Hueza and Palermo-Neto, 2008), including *C. mydas* (Fraga et al., 2018).

Like Cd, seagrass Co concentrations were elevated at the coastal sites relative to HWK (0.52 mg/kg, Table 3.2), most of all at UB (1.87 mg/kg.), where concentrations were nearly four times greater than at HWK. Co is deemed beneficial to plants as micronutrients and is reported to actively accumulate in seagrass leaves and roots (Schroeder and Thorhaug, 1980, Nicolaidou and Nott, 1998). High concentrations of Co (relative to natural base lines) are of some concern as immunosuppression can be linked to Co exposure (Gaus et al., 2012, Villa et al., 2017, Thomas et al., 2020). Over recent years, consistently high concentrations have been reported in both *C. mydas* blood (Villa et al., 2017) (Chapter 4), and preferred forage samples (Thomas et al., 2020) within the study region, when compared to data obtained from HWK samples. Villa et al. (2017) reported Co blood concentrations in UB exceeded reference intervals (RIs) by up to an order of magnitude. These RIs were calculated to determine if any elements were considered elevated when compared to a base line cohort of clinically healthy turtles that are minimally impacted by anthropogenic chemical influence, in this case from the *C. mydas* population of HWK. This elevation in Co in the local region is supported in the present findings, and by *C. mydas* concentrations reported in Wilkinson *et al.* (unpublished), where Co

was found to be highest in UB followed by CB and EB. UB receives river discharge from the Burdekin River, where historical land use in this region includes Ni and Co mining (Greenvale). Co co-occurs with Ni in natural ore deposits (Wang, 2006). Erosion rates in this region have significantly increased (by up to eight times) since the 1850s and is associated with rangeland beef grazing (Lewis et al., 2007, Wilkinson et al., 2013). Given that Co can be transported in particulate form associated with soil particles and marine sediments, it is plausible that changes in the supply of Co to the coastal region may be associated with increased erosion and transport of fine fractions of sediment (Kroon et al., 2012). Fine sediments (muds and silts) contain higher concentrations of certain metals relative to coarser types (sands and gravel) (Buyang et al., 2019).The increase in fine sediment discharge within the Burdekin may be associated with increased metal loads (particularly if concentrations were higher than previous discharge events), which bind to fine sediments (Thomas et al., 2020). The majority (up to 67%) of sediment discharged from the Burdekin River has been found to deposit in UB with long-distance sediment transport also likely to CB somewhat (Lewis et al., 2014). This pattern is reflected in the current study, whereby forage Co concentrations are greatest at UB and decline (but are still high) in CB.

While the multivariate approach and PCA conducted was able to reduce the dimensions of the data from 9 variables (metal elements) down to two, the results did not indicate any element or interaction of elements that influenced the differences in metal profiles between coastal sites. All variables were loaded evenly and influenced the data set to similar extents. The PCA indicated that metal profiles were distinctly different between HWK and all coastal sites, with strong association between coastal profiles observed in the data. While coastal profiles (particularly CB) were like one another, differences were still found between locations, likely explained by region-specific geoavailability differences, whereby degree of anthropogenic influence and local environmental conditions and processes play important roles in the bioavailability and distribution of persistent chemical contaminants in local environments. Greatest mixing of individual sample data was observed between data from CB and UB, indicative of two study sites with metal profiles most like one another. These sites are located close to one another and are influenced by the Burdekin River out flow, and thus share sediment sources (de Caritat and Grunsky, 2013, Lewis et al., 2014). Furthermore, HWK data was most like EB (eclipse proximity). Out of all three coastal sites EB is likely the least influenced by

anthropogenic impact as interpreted here (consecutively lower metal concentrations, when compared to CB and UB.

3.5.2. Impacts of elevated or toxic metals on seagrass survivability

Metals such as Cu, Cd and Zn are thought to be toxic to seagrass species. Zheng et al. (2018) observed leaf necrosis in *Thalassia hemprichii* after a 5 day treatment to 1 mg/L Cu^{2+}, likely a symptom of malnutrition, as competition for micronutrient uptake binding sites could induce inhibition of transport and function of Ca^{2+}, Mg^{2+}, K^{2+}, which are micronutrients required for numerous metabolic and photosynthetic processes (Wang et al., 2009, Zheng et al., 2018). Furthermore, photosynthetic efficiency may be hindered by phytotoxic effects of some metals, including changes in redox states in leaf cells due to inhibition of antioxidant enzymes, such as superoxide dismutase and peroxidase, which leads to increased production of radical oxygenating species (ROS) that damage photosynthetic apparatus and chlorophyll (Zheng et al., 2018). Necrosis and antioxidant inhibition may accompanied by a significant decline in photosynthetic efficiency (effective quantum yield) (Zheng et al., 2018), which is likely caused by disturbance to photosynthetic electron transport observed in a range of contaminants including photosystem-II herbicides (Wilkinson et al., 2015), also commonly applied in agricultural practices throughout the study region. Reduced photosynthetic function often leads to inhibited growth, survival, and community fitness of exposed meadows (Negri et al., 2015), leading to declines in distribution and inevitably habitat loss for the plethora of species reliant on seagrass ecosystems for a range of ecological functions.

3.5.3. What elevated metal loads mean for C. mydas

One method of attempting to study uptake of environmental concentrations in *C. mydas* is to calculate bioaccumulation factors (BAFs) (Flint et al., 2019), which is the ratio of chemical concentration in blood to that of seagrass (in this instance). While crude, using mean metal concentration values to calculate BAFs for elements in green turtle blood (derived from Chapter 4) from seagrass concentrations (Table 3.2 means) provided some insight into which metals were absorbed more readily (or retained longer) from above ground seagrass forage material (leaves and rhizomes) into the green turtles, in the populations and sites included in this study. For example, in CB, Co BAF was 14 compared to only 4.8 and 1.1 at UB and EB, respectively, indicative of a greater risk of bioaccumulation of Co in turtles foraging in CB, for reasons unknown here. Similarly, Cu BAF was greatest in UB (10.6) when compared to CB (6.8) and EB (1.9). In contrast, Zn BAF remained constant between all coastal sites (2.0-3.0). It is recommended that future research focus on better investigating BAF and bioaccumulation

coefficients for relevant metals, and other influential chemical contaminants in *C. mydas,* to better understand the toxicokinetic potential of such chemicals and the influence environmental concentrations may have on animals.

3.5.4. Variation in metal concentrations following the wet season

While comparisons between sites can be informative, it is difficult to directly compare between sites due to differences in local conditions and metal geoavailability. Geochemistry and bioavailability of metals differs between region, zone and proximity to contaminant sources and are mediated by complex physical, chemical and biological interactions, which are largely poorly-understood (Thomas et al., 2020). Sediment type, texture and mineralogy all play a role in determining availability (Thomas et al., 2020), with particles of fine clay and silt, found in estuarine environments (CB and UB), often carrying significantly greater terrigenous metal loads than coarser carbonate based sediments found offshore (where marine metals tend to be higher than in coastal samples), in areas such as HWK (Thomas et al., 2020). Region-specific variations in sediment profile make it difficult, and often unreliable, to compare metal concentrations directly between sites, as definitive explanation for differences is often unknown. While such comparisons give insight into relative concentrations, they provide little information as to whether such concentrations are at normal loads for the region or if that site is in fact contaminated (Thomas et al., 2020).

In UB for most trace elements, increases in concentrations were reported in samples collected post-wet season, whereas in CB, where the opposite was true (Figure 2). PCA on both location and sampling event (not included here) suggested no variation between metal profiles at either UB or CB and either pre- or post-wet season. This is interesting as during January and February 2019 an extreme flood event caused significant rainfall (an average of 1260 mm over ten days) in the Townsville region, which exceeded historical records (926 mm over ten days in 1953) (Bureau of Meteorology, 2019). This event was initiated by a slow-moving tropical low weather system and associated monsoon trough and impacted numerous areas along the coast, adjacent to the study region (except HWK). Large flood plumes entered the coastal zone from the Ross River (Townsville) and the Burdekin River.

3.6. Conclusion

Across the 2018/19 wet season, trace metal concentrations were measured in preferred *C. mydas* seagrass forage species. Some insight has been gleaned into the profiles of metal exposure in local foraging grounds. However, to build off of this research, a significant increase

in funding and investigation into the ecotoxicological study of environmentally relevant metals and the potential sources of such chemical contaminants is crucial before any insight can be gleaned regarding what the current metal loads likely mean for local seagrass meadows and the macorgrazers which rely on them as forage.

3.7. Permits

Research was carried out under all necessary permits from James Cook University Animals Ethics Committee (A2396), Department of Environment and Science (WISP18586417 and WISP18596817) and Great Barrier Reef Marine Parks Authority (G17/39429.1).

3.8. Summary of contributions

The aim of this chapter was met in the following way:

Aim:

To investigate and describe concentrations of a suite of ecologically relevant metals in preferred seagrass forage of *C. mydas* at three coastal turtle foraging sites on the Great Barrier Reef.

How the aims were achieved

- I designed and developed the study in collaboration with my primary advisor.
- I prepared all permits required to conduct field work and sample collection (Animal ethics, GBRMPA and DES).
- I completed all necessary training to meet safety and proficiency requirements to work efficiently in the field (QLD recreational boat licence, 4x4 driving, advanced and rescue SCUBA and turtle capture and sampling training).
- With assistance from my primary advisor, I planned all aspects of field trips (Riskware risk analysis, volunteer insurance and training, logistics, administration).
- With assistance from my primary advisor, other field collaborators, fellow PhD students and a long list of volunteers, I conducted a series of field trips where I managed volunteer assistance, multiple capture teams and oversaw all aspects of animal handling and welfare during capturing and sampling.
- Identification was made of the primary seagrass species ingested by local *C. mydas* populations and samples of those species were collected from established *C. mydas* foraging grounds at three coastal sites. Seagrass samples were collected from each

coastal site prior to and following the wet season to provide insight into metal exposure fluctuations between events.

- I inputted all data collected and appropriately stored all samples collected ready for sample analysis.
- With assistance from volunteers, I prepared and dried all seagrass samples ready for analysis.
- All seagrass samples were analysed by the Advanced Analytical Centre, JCU as per the protocols selected by the AAC department.
- Detection of a suite of ecologically relevant metal elements was conducted for all samples (ICP-OES) and compared with previously published metal data from a minimally influenced offshore control site.
- Funding was attained through collaboration with Dr. Jon Brodie and awarded grant funds from WWF.
- With assistance from my advisors and Rhondda Jones I conducted all statistical analysis.
- I drafted all aspects of this chapter and with assistance of my advisors, other PhD students and various volunteers I edited and finalised the chapter for inclusion in the thesis.

Summary of outcomes

This study found that target metal concentrations were similar between coastal sites and were often elevated when compared to data from the offshore control site. Some increase in several elements was observed following the wet season within the region.

3.9. References

AGGETT, P., NORDBERG, G. F. & NORDBERG, M. 2015. Essential Metals: Assessing Risks from Deficiency and Toxicity. *In:* NORDBERG, G. F., FOWLER, B. A. & NORDBERG, M. (eds.) *Handbook on the Toxicology of Metals (Fourth Edition).* San Diego: Academic Press.

AMBO-RAPPE, R. 2014. Developing a methodology of bioindication of human-induced effects using seagrass morphological variation in Spermonde Archipelago, South Sulawesi, Indonesia. *Marine Pollution Bulletin,* 86**,** 298-303.

BARTLEY, R., CROKE, J., BAINBRIDGE, Z. T., AUSTIN, J. M. & KUHNERT, P. M. 2015. Combining contemporary and long-term erosion rates to target erosion hot-spots in the Great Barrier Reef, Australia. *Anthropocene,* 10**,** 1-12.

BOLTEN, A. B. 2002. Variation in Sea Turtle Life History Patterns: Neritic vs. Oceanic Developmental Stages. *In:* LUTZ, P. L., MUSICK, J. A. & WYNEKEN, J. (eds.) *The biology of sea turtles.* Boca Raton, FL, USA: CRC press.

BONANNO, G. & DI MARTINO, V. 2016. Seagrass *Cymodocea nodosa* as a trace element biomonitor: Bioaccumulation patterns and biomonitoring uses. *Journal of Geochemical Exploration,* 169**,** 43-49.

BONANNO, G. & ORLANDO-BONACA, M. 2018. Trace elements in Mediterranean seagrasses and macroalgae. A review. *Science of The Total Environment,* 618**,** 1152-1159.

BURKHOLDER, D., HEITHAUS, M., THOMSON, J. & FOURQUREAN, J. 2011. Diversity in trophic interactions of green sea turtles *Chelonia mydas* on a relatively pristine coastal foraging ground. *Marine Ecology Progress Series,* 439**,** 277-293.

BUYANG, S., YI, Q., CUI, H., WAN, K. & ZHANG, S. 2019. Distribution and adsorption of metals on different particle size fractions of sediments in a hydrodynamically disturbed canal. *Science of The Total Environment,* 670**,** 654-661.

CLARK, T. R., LEONARD, N. D., ZHAO, J.-X., BRODIE, J., MCCOOK, L. J., WACHENFELD, D. R., DUC NGUYEN, A., MARKHAM, H. L. & PANDOLFI, J. M. 2016. Historical photographs revisited: A case study for dating and characterizing recent loss of coral cover on the inshore Great Barrier Reef. *Scientific Reports,* 6**,** 19285.

CONTI, M. E., MECOZZI, M. & FINOIA, M. G. 2015. Determination of trace metal baseline values in *Posidonia oceanica, Cystoseira* sp., and other marine environmental biomonitors: a quality control method for a study in South Tyrrhenian coastal areas. *Environmental Science and Pollution Research,* 22**,** 3640-3651.

DA SILVA, C. C., KLEIN, R. D., BARCAROLLI, I. F. & BIANCHINI, A. 2016. Metal contamination as a possible etiology of fibropapillomatosis in juvenile female green sea turtles *Chelonia mydas* from the southern Atlantic Ocean. *Aquatic Toxicology,* 170**,** 42–51.

DA SILVA, C. C., VARELA, A. S., BARCAROLLI, I. F. & BIANCHINI, A. 2014. Concentrations and distributions of metals in tissues of stranded green sea turtles (Chelonia mydas) from the southern Atlantic coast of Brazil. *Science of The Total Environment,* 466-467**,** 109-118.

DE CARITAT, P. & GRUNSKY, E. C. 2013. Defining element associations and inferring geological processes from total element concentrations in Australian catchment outlet sediments: Multivariate analysis of continental-scale geochemical data. *Applied Geochemistry,* 33**,** 104-126.

DE SOUZA MACHADO, A. A., SPENCER, K., KLOAS, W., TOFFOLON, M. & ZARFL, C. 2016. Metal fate and effects in estuaries: a review and conceptual model for better understanding of toxicity. *Science of the Total Environment,* 541**,** 268-281.

ECKERT, K., BJORNDAL, K. A., ABREU-GROBOIS, F. A. & DONNELLY, M. 1999. Research and management techniques for the conservation of sea turtles. Washington, DC: IUCN/SSC Marine Turtle Specialist Group.

ESSLEMONT, G. 2000. Heavy metals in seawater, marine sediments and corals from the Townsville section, Great Barrier Reef Marine Park, Queensland. *Marine Chemistry,* 71**,** 215-231.

FLINT, M., BRAND, A.-F., BELL, I. P. & MADDEN HOF, C. A. 2019. Monitoring the health of green turtles in northern Queensland post catastrophic events. *Science of The Total Environment,* 660**,** 586-592.

FORBES, G. & LIMPUS, C. 1993. A non-lethal method for retrieving stomach contents from sea turtles. *Wildlife Research,* 20**,** 339-343.

FOURQUREAN, J. W., DUARTE, C. M., KENNEDY, H., MARBÀ, N., HOLMER, M., MATEO, M. A., APOSTOLAKI, E. T., KENDRICK, G. A., KRAUSE-JENSEN, D.

& MCGLATHERY, K. J. 2012. Seagrass ecosystems as a globally significant carbon stock. *Nature geoscience,* 5**,** 505-509.

FRAGA, N. S., MARTINS, A. S., FAUST, D. R., SAKAI, H., BIANCHINI, A., DA SILVA, C. C. & AGUIRRE, A. A. 2018. Cadmium in tissues of green turtles (*Chelonia mydas*): A global perspective for marine biota. *Science of The Total Environment,* 637-638**,** 389-397.

GAUS, C., GRANT, S., JIN, N. L., GOOT, K., CHEN, L., VILLA, A., NEUGEBAUER, F., QI, L. & LIMPUS, C. 2012. Investigations of contaminant levels in green turtles from Gladstone. *National Research Centre for Environmental Toxicology*.

HAZEL, J., HAMANN, M. & LAWLER, I. R. 2013. Home range of immature green turtles tracked at an offshore tropical reef using automated passive acoustic technology. *Marine Biology,* 160**,** 617–627.

HUEZA, I. M. & PALERMO-NETO, J. 2008. Toxicologia do chumbo, mercúrio, arsênio e de outros metais. *Toxicologia aplicada à medicina veterinária***,** 641-662.

HUSSON, F., JOSSE, J., LE, S. & MAZET, J. 2013. FactoMineR: multivariate exploratory data analysis and data mining with R. *R package version,* 1.

HUSSON, F., LÊ, S. & PAGÈS, J. 2017. *Exploratory multivariate analysis by example using R*, CRC press.

JOHNSTON, S. G., BURTON, E. D., BUSH, R. T., KEENE, A. F., SULLIVAN, L. A., SMITH, D., MCELNEA, A. E., AHERN, C. R. & POWELL, B. 2010. Abundance and fractionation of Al, Fe and trace metals following tidal inundation of a tropical acid sulfate soil. *Applied Geochemistry,* 25**,** 323-335.

KIRKBY, E. 2012. Chapter 1 - Introduction, Definition and Classification of Nutrients. *In:* MARSCHNER, P. (ed.) *Marschner's Mineral Nutrition of Higher Plants (Third Edition).* San Diego: Academic Press.

KIRKMAN, H. 1997. Seagrasses of Australia. *Canberra: Department of the Environment***,** 36 p.

KROON, F. J., KUHNERT, P. M., HENDERSON, B. L., WILKINSON, S. N., KINSEY-HENDERSON, A., ABBOTT, B., BRODIE, J. E. & TURNER, R. D. R. 2012. River loads of suspended solids, nitrogen, phosphorus and herbicides delivered to the Great Barrier Reef lagoon. *Marine Pollution Bulletin,* 65**,** 167-181.

LARKUM, A., ORTH, R. J. & DUARTE, C. M. 2006. *Seagrasses: Biology, Ecology and Conservation,* Dordrecht, Netherlands, Springer.

LEWIS, S., SMITH, R., O'BRIEN, D., WARNE, M., NEGRI, A., PETUS, C., DA SILVA, E., ZEH, D., TURNER, R. & DAVIS, A. 2013. Assessing the Risk of Additive Pesticide Exposure in Great Barrier Reef ecosystems. *Assessment of the relative risk of water quality to ecosystems of the Great Barrier Reef. Department of the Environment and Heritage Protection, Queensland Government, Brisbane*.

LEWIS, S. E., OLLEY, J., FURUICHI, T., SHARMA, A. & BURTON, J. 2014. Complex sediment deposition history on a wide continental shelf: Implications for the calculation of accumulation rates on the Great Barrier Reef. *Earth and Planetary Science Letters,* 393**,** 146-158.

LEWIS, S. E., SHIELDS, G. A., KAMBER, B. S. & LOUGH, J. M. 2007. A multi-trace element coral record of land-use changes in the Burdekin River catchment, NE Australia. *Palaeogeography, Palaeoclimatology, Palaeoecology,* 246**,** 471-487.

LIMPUS, C. & CHALOUPKA, M. 1997. Nonparametric regression modelling of green sea turtle growth rates (southern Great Barrier Reef). *Marine Ecology Progress Series,* 149**,** 23-34.

LIMPUS, C. & MILLER, J. The occurrence of cutaneous fibropapillomas in marine turtles in Queensland. Proceedings of the Australian Marine Turtle Conservation Workshop, 1994. 186-188.

LIMPUS, C. & REED, P. 1985. The green turtle, *Chelonia mydas*, in Queensland: a preliminary description of the population structure in a coral reef feeding groud. *Biology of Australasian Frogs and Reptiles***,** 47–52.

MALEA, P., MYLONA, Z. & KEVREKIDIS, T. 2019. Trace elements in the seagrass *Posidonia oceanica*: Compartmentation and relationships with seawater and sediment concentrations. *Science of The Total Environment,* 686**,** 63-74.

MARBÀ, N., HOLMER, M., GACIA, E. & BARRON, C. 2007. Seagrass beds and coastal biogeochemistry. *In:* LARKUM, A., ORTH, R. J. & DUARTE, C. M. (eds.) *Seagrasses: biology, ecology and conservation.* Dordrecht, Netherlands: Springer.

METEOROLOGY, B. O. 2019. Special Climate Statement 69—an extended period of heavy rainfall and flooding in tropical Queensland. Bureau of Meteorology.

MOSZCZYŃSKI, P. 1996. Mercury compounds and the immune system: a review. *International journal of occupational medicine and environmental health,* 10**,** 247-258.

NEGRI, A. P., FLORES, F., MERCURIO, P., MUELLER, J. F. & COLLIER, C. J. 2015. Lethal and sub-lethal chronic effects of the herbicide diuron on seagrass. *Aquatic Toxicology,* 165**,** 73-83.

NICOLAIDOU, A. & NOTT, J. A. 1998. Metals in sediment, seagrass and gastropods near a nickel smelter in Greece: Possible interactions. *Marine Pollution Bulletin,* 36**,** 360-365.

ORTH, R. J., CARRUTHERS, T. J. B., DENNISON, W. C., DUARTE, C. M., FOURQUREAN, J. W., HECK, K. L., HUGHES, A. R., KENDRICK, G. A., KENWORTHY, W. J., OLYARNIK, S., SHORT, F. T., WAYCOTT, M. & WILLIAMS, S. L. 2006. A Global Crisis for Seagrass Ecosystems. *BioScience,* 56**,** 987-996.

PACYNA, J. M. & PACYNA, E. G. 2001. An assessment of global and regional emissions of trace metals to the atmosphere from anthropogenic sources worldwide. *Environmental Reviews,* 9**,** 269-298.

PACYNA, J. M., SCHOLTZ, M. T. & LI, Y. F. 1995. Global budget of trace metal sources. *Environmental Reviews,* 3**,** 145-159.

RAYMENT, G. E. 2005. Northeast Australian Experience in Minimizing Environmental Harm From Waste Recycling and Potential Pollutants of Soil and Water. *Communications in Soil Science and Plant Analysis,* 36**,** 121-131.

SCHROEDER, P. B. & THORHAUG, A. 1980. Trace metal cycling in tropical-subtropical estuaries dominated by the seagrass, *Thalassia testudinum*. *American Journal of Botany,* 67**,** 1075-1088.

SHORT, F. T., POLIDORO, B., LIVINGSTONE, S. R., CARPENTER, K. E., BANDEIRA, S., BUJANG, J. S., CALUMPONG, H. P., CARRUTHERS, T. J. B., COLES, R. G., DENNISON, W. C., ERFTEMEIJER, P. L. A., FORTES, M. D., FREEMAN, A. S., JAGTAP, T. G., KAMAL, A. H. M., KENDRICK, G. A., JUDSON KENWORTHY, W., LA NAFIE, Y. A., NASUTION, I. M., ORTH, R. J., PRATHEP, A., SANCIANGCO, J. C., TUSSENBROEK, B. V., VERGARA, S. G., WAYCOTT, M. & ZIEMAN, J. C. 2011. Extinction risk assessment of the world's seagrass species. *Biological Conservation,* 144**,** 1961-1971.

STORELLI, M. M., STORELLI, A., D'ADDABBO, R., MARANO, C., BRUNO, R. & MARCOTRIGIANO, G. O. 2005. Trace elements in loggerhead turtles (*Caretta*

caretta) from the eastern Mediterranean Sea: overview and evaluation. *Environmental Pollution,* 135**,** 163-170.

STRZELEC, M., PROEMSE, B. C., GAULT-RINGOLD, M., BOYD, P. W., PERRON, M. M., SCHOFIELD, R., RYAN, R. G., RISTOVSKI, Z. D., ALROE, J. & HUMPHRIES, R. S. 2020. Atmospheric trace metal deposition near the Great Barrier Reef, Australia. *Atmosphere,* 11**,** 390.

THOMAS, C., BENNETT, W., GARCIA, C., SIMMONDS, A., HONCHIN, C., TURNER, R., HOF, C. & BELL, I. 2020. Coastal bays and coral cays: Multi-element study of *Chelonia mydas* forage in the Great Barrier Reef (2015–2017). *Science of The Total Environment***,** 140042.

TOUCHETTE, B. W. 2007. Seagrass-salinity interactions: Physiological mechanisms used by submersed marine angiosperms for a life at sea. *Journal of Experimental Marine Biology and Ecology,* 350**,** 194-215.

UNSWORTH, R. K. F. & CULLEN, L. C. 2010. Recognising the necessity for Indo-Pacific seagrass conservation. *Conservation Letters,* 3**,** 63-73.

VILLA, C. A., FINLAYSON, S., LIMPUS, C. & GAUS, C. 2015. A multi-element screening method to identify metal targets for blood biomonitoring in green sea turtles (*Chelonia mydas*). *Science of The Total Environment***,** 613-621.

VILLA, C. A., FLINT, M., BELL, I., HOF, C., LIMPUS, C. J. & GAUS, C. 2017. Trace element reference intervals in the blood of healthy green sea turtles to evaluate exposure of coastal populations. *Environmental Pollution,* 220, Part B**,** 1465–1476.

WANG, C., ZHANG, S. H., WANG, P. F., HOU, J., ZHANG, W. J., LI, W. & LIN, Z. P. 2009. The effect of excess Zn on mineral nutrition and antioxidative response in rapeseed seedlings. *Chemosphere,* 75**,** 1468-1476.

WANG, S. 2006. Cobalt—Its recovery, recycling, and application. *JOM,* 58**,** 47-50.

WAYCOTT, M., DUARTE, C. M., CARRUTHERS, T. J. B., ORTH, R. J., DENNISON, W. C., OLYARNIK, S., CALLADINE, A., FOURQUREAN, J. W., HECK, K. L., HUGHES, A. R., KENDRICK, G. A., KENWORTHY, W. J., SHORT, F. T. & WILLIAMS, S. L. 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences,* 106**,** 12377-12381.

WILKINSON, A. D., COLLIER, C. J., FLORES, F. & NEGRI, A. P. 2015. Acute and additive toxicity of ten photosystem-II herbicides to seagrass. *Scientific Reports,* 5**,** 17443.

WILKINSON, S. N., HANCOCK, G. J., BARTLEY, R., HAWDON, A. A. & KEEN, R. J. 2013. Using sediment tracing to assess processes and spatial patterns of erosion in grazed rangelands, Burdekin River basin, Australia. *Agriculture, Ecosystems & Environment,* 180**,** 90-102.

WYNEKEN, J. 2002. The External Morphology, Musculoskeletal System, and Neuro-Anatomy of Sea Turtles. *In:* LUTZ, P. L., MUSICK, J. A. & WYNEKEN, J. (eds.) *The biology of sea turtles.* CRC press.

ZHENG, J., GU, X.-Q., ZHANG, T.-J., LIU, H.-H., OU, Q.-J. & PENG, C.-L. 2018. Phytotoxic effects of Cu, Cd and Zn on the seagrass *Thalassia hemprichii* and metal accumulation in plants growing in Xincun Bay, Hainan, China. *Ecotoxicology,* 27**,** 517-526.

CHAPTER 4 - Green turtle (*Chelonia mydas*) blood and scute metal element loads of the Northern Great Barrier Reef

4.1. Chapter prelude

4.1.1. Aim:

To investigate and describe *C. mydas* blood and scute (carapace) metal profiles in coastal and offshore populations of the GBR.

4.1.2. Introduction to the chapter

While chapter 3 was informative regarding seagrass metal concentrations and potential dietary exposure, metal bioaccumulation and toxicokinetic activity within *C. mydas* is a complex field and an underrepresented area of study, but essential when investigating metal exposure as a threat to local populations. To better understand local marine turtle metal ecotoxicology, this chapter applied similar analysis as conducted in Chapter 3 to quantify blood and scute metal profiles of five *C. mydas* foraging populations: 1) The Howick Group of Islands (HWK), a remote offshore control site (referenced in the previous chapter) that is located in the northern region of the GBR, 15 km from the nearest catchment area, 2) Toolakea Beach (TLK), located 30 km north of Townsville, 3) Cockle Bay (CB), adjacent to Townsville and in proximity to intensive land practices (including farming and metal refining), which serve as potential sources of land-based contaminants, 4) Upstart Bay (UB) and 5) Edgecumbe Bay (EB), both located south of Townsville where local metal profiles are influenced by agricultural activity, river discharge from the Burdekin River and high erosion rates. Blood was analysed as an indicator of short-term (weeks) metal exposure, while scute samples were more indicative of long-term (years) metal storage (Villa et al., 2019). Collection and comparison of these sample types allowed insight into recent and more historical metal exposure. Comparison was made between coastal sites and the offshore population (HWK), as in other chapters. Additionally, published blood reference intervals for a range of target metals were included to provide information as to whether recent metal concentrations were elevated relative to the control site (HWK). It was found that coastal *C. mydas* blood metal profiles were similar between coastal sites, but elevated relative to the control (HWK) population, though most blood metal concentrations were within region-specific reference intervals (Villa et al., 2017) for most elements at most sites. Scute metal were also similar between coastal sites, with overlap between metal profiles of some sites. Some elements were reported at similar concentrations in blood samples relative to scutes (Co, Cu and Fe), though most elements were detected at

greater concentrations in scutes, possibly indicative of higher past exposure or bioaccumulation over time.

4.2. Introduction

Marine turtles are reptiles that are distributed globally throughout tropical and sub-tropical regions (Bowen et al., 1992, Hamann et al., 2002), with six of the seven extant species inhabiting the Great Barrier Reef (GBR). The global status of marine turtles, determined by the International Union for Conservation of Nature (IUCN) red list, varies between vulnerable (such as loggerhead, *Caretta caretta*) (Casale and Tucker, 2017) and critically endangered (including hawksbill, *Eretmochelys imbricata*) (Mortimer and Donnelly, 2008). Such variation in status is likely influenced by diverse threats (often localised) and complex life histories demonstrated by certain species. For example, green turtles (*Chelonia mydas*) are long-lived, slow-growing macrograzers that demonstrate strict foraging site fidelity (only a few square kilometres area), as juveniles and adults (Hazel et al., 2013). Strict fidelity only occurs upon recruitment to coastal foraging grounds, after spending several years in the pelagic zone as post-hatchlings (Hamann et al., 2002). Life-history strategies, such as long life and narrow home ranges, increase the susceptibly of *C. mydas* to stressors, including exposure to and bioaccumulation of ecotoxic contaminants (Gaus et al., 2012, Faust et al., 2014, da Silva et al., 2016). These factors likely contribute to the globally endangered status of *C. mydas* (Seminoff, 2004). Anthropogenic contamination is considered a priority risk to marine turtle health, as a factor in the global decline of *C. mydas* populations (Hamann et al., 2010). Due to the proximity of nearshore environments to anthropogenic land activities (agricultural, industrial and urban practices), inhabiting turtles are regularly exposed to harmful contaminants, including xenobiotic organic chemicals such as herbicides and trace metal loads (Lewis and Devereux, 2009, Kroon et al., 2015).

Acute and long-term exposure to toxic concentrations of some metals likely incurs detrimental health impacts in *C. mydas* (Camacho et al., 2013, Faust et al., 2014, Flint et al., 2015, da Silva et al., 2016). For instance, previous research has reported that concentrations of certain metals could be associated with aetiological component causes (causative factors) of the enzootic neoplastic and debilitating disease, fibropapillomatosis (FP), a marine turtle specific disease that is most prevalent in *C. mydas* (da Silva et al., 2016). FP is characterised by visible tumours that develop on soft tissue areas (such as the neck and flippers) (Jacobson et al., 1989, Balazs, 1991, Herbst, 1994, Mascarenhas and Iverson, 2008). Furthermore, *C.mydas* with FP are often found to be immunosuppressed, though it remains unclear as to whether immunosuppression

causes or is caused by FP expression (Work et al., 2000, Cray et al., 2001, Work et al., 2001). Exposure to several metals has been found to induce immunosuppression, increasing susceptibility to other infections (da Silva et al., 2016), like Chelonid alphaherpesvirus-5 (ChHV5), another possible causative co-factor in the expression of FP clinical signs (Chapter 5, Herbst et al., 1999, Work et al., 2000). Elevated metal loads are transported to the coastal zone and enter the marine environment via several pathways and point sources, including use in industrial and agricultural land practices and from several processes associated with urban settlement (Pacyna et al., 1995, Pacyna and Pacyna, 2001, Strzelec et al., 2020). Naturally geoavailable metal loads also influence the metal profile of a local area. These elements can often be essential, as micronutrients, while others are non-essential, and likely toxic at very low concentrations (Aggett et al., 2015, da Silva et al., 2016, de Souza Machado et al., 2016).

It is important to monitor local metal loads, not only in *C. mydas* populations, but also the wider environment (Chapter 3), to efficiently detect when contaminant levels may be elevated and potentially toxic. In the absence of ecotoxicologically defined endpoints or thresholds for the protection of *C. mydas* populations, one way to investigate levels of metal contamination is to compare data from target sites against pre-defined natural baseline reference ranges obtained from a region that is near-pristine and minimally impacted by anthropogenic pollution. Such values are available for local *C. mydas* blood metal concentrations (Villa et al., 2017). Blood collection is a non-lethal and minimally invasive method of measuring metal loads, and is representative of recent exposure (2-3 weeks), dependant on metal elimination rates (Villa et al., 2017).

While blood sampling and analysis for metals is informative in indicating whether a foraging population of turtles is exposed to elevated metal concentrations, it is somewhat limited when investigating long-term metal concentrations. Previously, internal organs such as the liver and kidney were opportunistically sampled from stranded or moribund turtles, as such tissues are known to be long-term stores of excess metal loads (Grillitsch and Schiesari, 2010, Gaus et al., 2012). This approach is limited as it relies on sporadic access to deceased animals and is not likely representative of average metal loads in free-ranging populations. A better approach, using scute, has been recently implemented and validated (Villa et al., 2019). Similarly, to blood collection, *C. mydas* scute sampling is minimally invasive, and as such is potentially a suitable alternative to sampling internal organs as an indication of past metal exposure (up to

1.4-2.8 years prior to capture) (Vander Zanden et al., 2013, Villa et al., 2019). Scutes are made of keratin (as is the case of human hair and nails), and can be sampled with little risk of harm to the animal (Villa et al., 2019). Each layer provides data on metal concentrations at the time of formation, though no known method is available to pinpoint exposure events. Calculating the accumulated metal loads of scute samples is still informative, as it indicates whether current levels are elevated at a given study site. However, reference values are not currently available for scute metal concentrations (Villa et al., 2019), whereas they are available for blood samples. Further work, therefore, is necessary to understand long-term exposure to elevated trace metals.

The aim of this study was to measure metal concentrations in blood and scute samples of *C. mydas* from five study sites within the GBR, to determine whether exposure to a suite of ecologically relevant metals exceeded locally defined reference intervals for blood concentrations, and whether the scute samples represented a more time integrated indication of past metal exposure. It was hypothesised that turtles sampled from coastal sites would be influenced by land–sourced anthropogenic contamination, and therefore have greater blood metal concentrations than turtles sampled from the reference study site. Additionally, it was hypothesised that metal concentrations would be higher in scute samples than in blood, and that mean concentrations would vary between coastal sites, dependant on local land uses and exposure to anthropogenic contamination.

4.3. Method

4.3.1. Study locations

Five geographically distinct study sites were sampled along the east coast of Queensland, Australia, adjacent to the GBR catchment area (Figure 4.1). Firstly, Howick island group (HWK) is a mid-shelf group of remote reefs found in the Northern region of the GBR (14 º 30 ' 11"S 144 º 58 ' 26 "E). Located over 130 km from the nearest human populace (Cooktown), and at least 15 km from the nearest coastal catchment, HWK was considered relatively removed from land-sourced anthropogenic disturbance. Secondly, Toolakea, TLK (19° 08' 36" S, 146° 34' 56" E), is located 30 km north of Townsville and is predominently inhabited by juvenile *C. mydas*, recently recruited from the pelagic zone. Thirdly, Cockle Bay (CB) (19 º 10 ' 26.7 "S 146 º 49 ' 32.1"E) is a westerly facing bay of Magnetic Island, eight miles off the coast of Townsville, and forms a part of Cleveland Bay. Industry practices such as metal processing (including Zn, Cu, Ni and Co), urban runoff from the city of Townsville (population about 200,000), and major sea port practices (including regular channel dredging) take place within

the region (Esslemont, 2000), and potentially influence both CB and TLK. Fourthly, Upstart Bay (UB) (19°44 ' 44.4"S 147°36 ' 03.8 "E) is a north facing bay located 150 km from Townsville, and receives river discharges from a major catchment, the Burdekin. It is dominated by agricultural and grazing practices, with a legacy of mining activity. The Burdekin catchment is one of the two largest catchments making up the GBR catchment area (the other being the Fitzroy) with an area of 140,000 km^2. Finally, Edgecumbe Bay (EB) (20° 6′ 49″ S, 148° 23′ 25″ E) is located south of Bowen, approximately 200 km south of Townsville. Within the catchment draining into EB, there are a number of sources of potential contaminents, including a wastewater treatment plant (for the town of Bowen, which has a population of approximately 10,000), Cokeworks and sugarcane farms (mostly on the catchment of the Gregory River in the south of the Bay), and occasionally, discharge plumes from the Burdekin River (Clark et al., 2016).

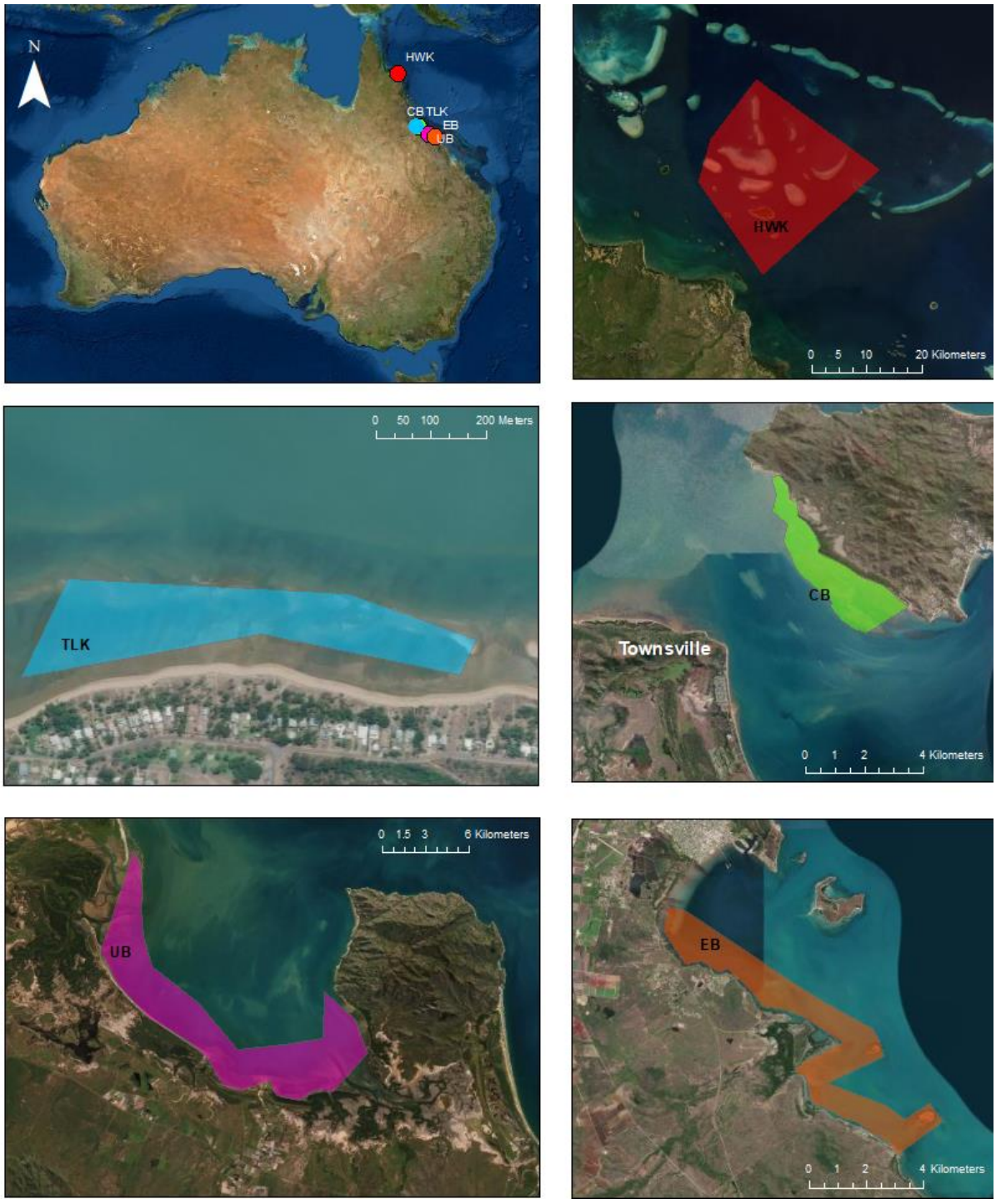


Figure 4.1. Map of five study areas where *C. mydas* were captured and sampled in this study. Abbreviations are defined as follows: HWK = Howick island group, TLK = Toolakea Beach, CB = Cockle Bay, UB = Upstart Bay and EB = Edgecumbe Bay. Each site is colour coordinated between the national overview and localised maps. TLK and CB appear overlapped in the national overview but are geographically distinct on the local scale.

4.3.2. C. mydas capture and morphometric sampling

A total of 122 green turtles were captured from across the five study sites (Table 1). All turtles were captured using turtle rodeo techniques as per Limpus and Read (1985). Individually numbered titanium flipper tags (Department of Environmental Sciences, Queensland Government) were applied as per Eckert et al. (1999). Curved carapace length (CCL) was measured for all turtles captured, using a plastic tape measure (cm) calibrated against metal callipers. Measurements were taken along the midline ridge of the carapace, from the notch of the supracaudial scutes to the posterior edge of the carapace where it joins the skin of the neck, to the accuracy of ± 0.1 cm. Large barnacles were removed with long nose plyers if their location obstructed accurate CCL measurements. Animal weight was measured using digital scales (kg) and a custom harness that allowed for balanced weight distribution. The CCL and weight ranges of turtles sampled at each site are detailed in Table 4.1, with most animals captured classed as juveniles (up to 65 cm). Each turtle captured received a visual health check for fibropapillomatosis tumour expression, of which no sampled individual was positive for here.

Table 4.1. Sample sizes (n= X) for both blood and scute analysis and curved carapace length (CCL, cm) and weight (kg) of all *C. mydas* included in this study. All data are arranged by study site. A weight of >100 (kg) was assigned when the turtle was too large for the available weight system (maximum weight capacity of 100 kg).

Study Location	Blood	Scute	CCL Range (cm)	Weight range (kg)
Howick's (HWK)	n=30	-	42.5 – 72.0	7.8 – 45.0
Toolakea Beach (TLK)	n=9	n=9	36.8 – 49.2	6.3 – 10.8
Cockle Bay (CB)	n=35	n=17	40.1 – 61.8	7.2 – 32.0
Upstart Bay (UB)	n=24	n=24	42.0 – 111.5	8.2 – 29.0
Edgecumbe Bay (EB)	n=24	n=15	43.2 – 108.5	7.2 - >100

4.3.3. Blood sample collection

Non-lethal blood samples were collected from the jugular artery located in the dorsal cervical sinus of juvenile and sub-adult *C. mydas*. Up to 10 mL blood was collected per individual turtle sampled (less than 1% of the turtle's body weight). The venepuncture site was sterilised with single-use alcohol wipes before and after sampling. Blood was sampled using 10 mL syringe fitted with 21-20 gauge needles, as per methods used by Owens and Ruiz (1980). Whole blood (5 mL) was transferred directly to sodium heparin coated evacuated vacutainer tubes (BD Vacutainer, Becton, Dickinson and Co., NJ, USA). All blood samples were stored under chilled

conditions (4-10 °C) until return to the laboratory. Immediately upon return, all samples were refrigerated at 4 °C until analysis.

4.3.4. Scute sample collection

A total of 65 scute samples (approximately 0.5 – 1.0 g each) were collected from the radial edge of the posterior marginal scutes of *C. mydas* individuals captured. The sampling area was first prepared by cleaning with 70% alcohol swabs to remove any epibionts and other fouling. All keratin samples were collected using a sterile diamond tipped hollow drill bit (12 mm diameter). The surrounding area of carapace was again swabbed with isopropanol and pressure was applied, though bleeding was minimal in that area. Samples were placed in sterile 1.5 mL microfuge tubes and stored on ice until return from the field. Once at the laboratory, keratin samples were soaked in 70% isopropanol to remove any remaining epibionts. To minimise environmental contamination of each sample, the outermost scute layer (visible as carapace) was removed prior to storage and was not included in analysis. All keratin samples were then stored at -20 °C until analysis. All turtle and environmental samples collected from HWK was done so on behalf of the author by collaborators and colleagues simultaneously whilst other field work was conducted. These personnel did not have permission nor experience with collecting scute samples correctly. Therefore, scute samples were not collected from turtles captured at HWK but samples were obtained from each of the coastal sites. Furthermore, permits to collect scute samples in the current study were granted after those for blood collection, therefore more turtles were captured and sampled for blood than for scutes at CB and EB, accounting for the disparity in sample numbers for each sample type.

4.3.5. Trace metal analysis

A fully quantitative, multi-element approach was applied to the analysis of a suite of potential trace metal concentrations (Co, Cu, Fe, Mg, Mn, Zn (essential elements) and Al, Cd, Ni and Pb (non-essential elements)) (Table 2). 0.5 g blood samples (blood was too thick to accurately measure volume so were weighed to a 0.1 g accuracy) were digested using a microwave oven (Burghof Speedwave, Germany). Briefly, all samples were weighed and 4 mL double distilled HNO_3 and 0.5 mL AR Grade H_2O_2 were added. Next, samples were heated to 180 $^{\circ}$C for 10 minutes in a microwave oven. Once cooled, the digested samples were quantitatively transferred into 25 mL volumetric flasks and diluted to the mark using Milli-Q water. No further dilution was carried out before ICP-MS analysis. 0.5-1 g of dry and clean scute material was digested similarly to the blood by adding 4 mL double distilled HNO_3 and 0.5 mL AR Grade H_2O_2. Once the reaction was complete, samples were heated to 185 $^{\circ}$C for 10 minutes in

a microwave oven. Once cooled, the digested samples were quantitatively transferred into 25 mL volumetric flasks and diluted to the mark using Milli-Q water. No further dilution was carried out before ICP-MS analysis.

Trace elements analysis was carried out by a Varian 820-MS Inductively Coupled Plasma Mass Spectrometer (Melbourne, Australia). Indium acted as the internal standard to correct for the matrix effects and instrument drift, and for quantification, a series of multi-element standard solutions were used to calibrate the instrument. A series of multi-element standard solutions containing all elements of interest were included to ensure external calibration was met. HNO_3 and H_2O_2 (acids used in sample digestion, minus the sample) were used as procedure blanks and analysis of blanks was conducted for all elements (blood and scutes) and used to calculate the limit of detection values (LOD), three times the standard deviation of the blanks, calculated separately for each element. Randomly selected samples were duplicated (three in total) and included to check for consistency throughout the analysis. To ensure instrument calibration, numerous independent standards (1 ppm) were tested, with reported recoveries ranging from 83% (Co) to 120% (Al). Two Certified Reference Materials (CRM) (GBW07605 Tea Leaves and NIST 1566 Oyster Tissues) were analysed to validate the analytical method and % recoveries ranged from 92% (Cu) to 118% (Cd).

4.3.6. Data analysis

For all blood and scute samples, metal concentrations were reported as mg/L for blood and mg/kg (dw) for scutes, and all concentrations found to be below Limits of detection (<LOD) were assigned a value half of the LOD (Wendelberger and Campbell, 1994, Verbovšek, 2011, Villa et al., 2019, Thomas et al., 2020). While there is some dispute as to what the most suitable method of substituting non-detect data for analysis this method is still commonly used for normally distributed data as it is simple to apply and causes less error than other options, though this method may also provide greater underestimation of mean concentrations than other methods, such as LOD/√2 (Verbovšek, 2011). Ni and Pb blood concentrations were not included in analysis as >40% of samples were <LOD. Blood metal concentrations at five sites were compared to the blood metal (Co, Cu, Fe, Mg, Mn, Zn only) reference intervals reported in Villa et al. (2017). Mean metal concentrations were compared between blood samples collected in 2014 (Villa et al., 2017) and 2017 – 2019 (current study) for the three sites included in both studies (HWK, CB and UB). EB and TLK was not included in this analysis as investigation was not conducted within those sites during the reference study.

To reduce the complexity of potential associations between variables (metal elements) and to best analyse whether similarities exist between metals in either blood or scute samples, multivariate analysis was conducted. Dimension reduction of all data was achieved by applying a multivariate principal component analysis (PCA). By applying PCA, spatial and temporal variation between study locations and sampling events were investigated. To determine the most important dimensions in the data, two dimension-reduction protocols, scree plots and quality of representation measurements (cos2) were used. As no scutes were collected from HWK this site is not represented in the scute PCA findings. Statistical analysis and plotting of PCA was conducted in the R statistical program (R Core Team, 2019), using the R packages 'Tidyverse' (data exploration), 'Factoshiny'(multivariate analysis and plotting)(Husson et al., 2017) and 'FactoMineR' (Factor analysis) (Husson et al., 2013).

4.4. Results

4.4.1. C. mydas blood and scute metal concentrations

When comparing coastal *C. mydas* blood metal concentrations to baseline levels from the control site it was found that concentrations were similar between HWK and coastal sites for most elements analysed (Table 4.2). This was particularly true for the essential elements Cu, Fe and Zn, where concentrations were comparable between data from HWK and the majority, if not all, of coastal sites concentrations. Al and Mg were detected at greater concentrations at EB when compared to HWK but were at similar concentrations to HWK at the other sites. Co concentrations were observed to be greatest in UB (0.4 ± 0.3 mg/L) and decreased the further north sites were located. A higher mean Co concentration (compared to HWK, TLK and CB) was also detected in samples from EB (0.3 ± 0.2 mg/L), although not quite as high as in UB.

Table 4.2. The average blood concentration (mg/L) and standard deviation for each metal, rounded to two significant figures. All data are arranged by site, from north to south.

Element	HWK	TLK	CB	UB	EB
Al	0.7 ± 0.5	0.2 ± 0.1	0.5 ± 0.6	0.6 ± 0.5	1.2 ± 2.4
Co	0.02 ± 0.02	0.1 ± 0.2	0.1 ± 0.2	0.4 ± 0.3	0.3 ± 0.2
Cu	0.6 ± 0.1	0.5 ± 0.1	0.7 ± 0.5	0.6 ± 0.2	0.6 ± 0.2
Fe	267 ± 55	193 ± 88	199 ± 104	267 ± 100	303 ± 204
Mg	73 ± 8.5	94 ± 9.9	84 ± 11	88 ± 14	111 ± 57

Mn	0.1 ± 0.1	0.03 ± 0.02	0.1 ± 0.1	0.1 ± 0.04	0.1 ± 0.2
Zn	8.8 ± 2.2	6.5 ± 2.6	7.8 ± 4.1	8.8 ± 2.9	11 ± 6.7

4.4.2. Temporal changes in blood metal concentrations

The mean metal concentrations in turtle blood reported here for HWK, CB and UB (collected 2017-2019) were compared with those analysed in Villa et al. (2017), collected in 2014 (Table 4.3). For HWK, all elements, except Mn were within standard deviation ranges between studies. Mn in HWK blood samples was detected at a mean concentration of up to five times greater in the current study when compared against the reference study (0.1 ± 0.05). For CB (CLV in reference study), five out of six elements (Cu, Fe, Mg, Mn and Zn) were within standard deviation range between studies, with Co detected at a higher mean concentration in 2014 (0.2 ± 0.1), at up to double the concentration compared to current findings (0.1 ± 0.2). For UB, all six elements were similar and within standard deviation range between studies. For each of the compared sites, as well as EB and TLK, most metal elements included were within or below RI values derived from HWK blood metal data in Villa et al. (2017). Only Mn concentrations were greater than Mn RIs at HWK and exceeded RIs in blood collected from CB, UB and EB. Mg exceeded RIs at UB EB and TLK but were within range at CB. Cu did not exceed RIs at any of the coastal sites. Co concentrations at CB, UB, EB and TLK exceeded RIs by up to 14-fold. Fe and Zn concentrations were within range at all sites.

Table 4.3. Mean metal concentrations (mg/L) and SD in addition to previously recorded metal concentrations (collected in 2014) and established reference intervals (RI), calculated by (Villa et al., 2017), for *C. mydas* blood collected from HWK (Howick island group), CB (Cockle Bay), UB (Upstart Bay) and EB (Edgecumbe Bay). Asterix (*) depicts metal elements which concentrations were detected, in the current study, above that of the reference intervals included. EB and TLK were not sampled in the reference study and therefore values are not available for comparison.

HWK

Element	Present average	Villa average	Villa calculated RI
Co	0.02 ± 0.02	0.02 ± 0.001	0.007 – 0.03
Cu	0.6 ± 0.1	0.5 ± 0.1	0.29 – 0.7
Fe	267 ± 55	290 ± 49	210 – 410
Mg	73 ± 9	69 ± 7	55.0 – 85
Mn*	0.1 ± 0.05	0.02 ± 0.01	0.008 – 0.04
Zn	8.8 ± 2.2	10 ± 1.7	7.3 – 14

CB

Element	Present average	Villa average	Villa calculated RI
Co*	0.1 ± 0.2	0.2 ± 0.1	0.007 – 0.03
Cu	0.7 ± 0.5	0.7 ± 0.2	0.3 – 0.7
Fe	200 ± 104	260 ± 67	210 – 410
Mg	84 ± 11	91 ± 11	55 – 85
Mn*	0.1 ± 0.1	0.05 ± 0.02	0.008 – 0.04
Zn	7.7 ± 4.1	9.6 ± 2.7	7.3 – 14

UB

Element	Present average	Villa average	Villa calculated RI
Co*	0.4 ± 0.3	0.5 ± 0.2	0.007 – 0.03
Cu	0.6 ± 0.2	0.6 ± 0.2	0.3 – 0.7
Fe	267 ± 100	300 ± 85	210 – 410
Mg*	88 ± 14	99 ± 12	55 – 85
Mn*	0.09 ± 0.04	0.07 ± 0.04	0.008 – 0.04
Zn	8.9 ± 2.9	11 ± 2.5	7.3 – 14

EB

Element	Present average	Villa average	Villa calculated RI
Co*	0.3 ± 0.2	-	0.007 – 0.03
Cu	0.6 ± 0.2	-	0.3 – 0.7
Fe	300 ± 205	-	210 – 410
Mg*	110 ± 60	-	55 – 85
Mn*	0.1 ± 0.2	-	0.008 – 0.04
Zn	11 ± 6.7	-	7.3 – 14

TLK

Element	Present average	Villa average	Villa calculated RI
Co*	0.1 ± 0.2	-	0.007 – 0.03
Cu	0.5 ± 0.1	-	0.3 – 0.7
Fe	193 ± 88	-	210 – 410
Mg*	94 ± 9.9	-	55 – 85

Mn	0.03 ± 0.02	-	0.008 – 0.04
Zn	6.5 ± 2.6	-	7.3 – 14

4.4.3. C. mydas scute metal concentrations

Like metal concentrations analysed in blood samples (Table 4.2), most elements in scute samples were detected at comparable concentrations between coastal study sites (Table 4.4), with TLK as an exception as metal concentrations were largely different in TLK when compared to other sites, particularly CB and UB. For instance, Co was lower in TLK than UB and the same as CB and EB, as Co concentrations were not different in scutes sampled from *C. mydas* in UB, CB and EB. This finding differs to that for Co measured in blood, where concentrations were higher in UB than all sites. Scute Mg and Zn concentrations were higher in TLK than all other sites Concentrations of Fe were higher in CB compared to all other sites. Unlike blood, elements Cd, Ni and Pb were detected in scutes from all sites. Cd was marginally higher in concentration at TLK but was comparative between all sites. Ni was highest at CB (3.3 ± 7.3 mg/kg), by as much as 3 times, when compared to EB (0.9 ± 1.0 mg/kg) and TLK (1.1 ± 1.0 mg/kg). Pb was detected at consistent concentrations in scute samples from all sites.

When observational comparison was made between blood and scute metal concentrations most elements (Al, Mg, Mn, and Zn) were detected at concentrations far greater in scute than blood. For Mg, scute concentrations (6030 mg/kg mean scute concentration) were on average 64-times higher than blood concentrations (94 mg/L mean blood concentration). Similarly, Al, Mn and Zn were 220-times, 11-times, and 24-times higher in scute than blood, respectively. Co concentrations were similar between blood and scutes. Cu and Fe were similar, if not lower, in scute samples than blood.

Table 4.4. The mean scute element concentrations (mg/kg dw) and standard deviation for each coastal site (Toolakea Beach (TLK), Cockle Bay (CB), Upstart Bay (UB) and Edgecumbe Bay (EB)), rounded to two significant figures. All data are arranged by site, from north to south. Average concentration presented as mg/kg (dw). Scutes were not collected from HWK and thus are not represented here.

Element	TLK	CB	UB	EB
Al	48 ± 36	262 ± 230	138 ± 140	60 ± 92
Cd	0.1 ± 0.03	0.07 ± 0.03	0.05 ± 0.04	0.07 ± 0.03
Co	0.1 ± 0.02	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Cu	0.1 ± 0.03	0.3 ± 0.4	0.3 ± 0.6	0.3 ± 0.3
Fe	48 ± 30	171 ± 148	82 ± 94	52 ± 65
Mg	7400 ± 700	5500 ± 1490	5810 ± 1260	5400 ± 1500
Mn	6 ± 2	8 ± 4	13 ± 9	10 ± 6
Ni	1.1 ± 1.0	3.3 ± 7.3	1.3 ± 1.6	0.9 ± 1.0
Pb	0.29 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.07
Zn	302 ± 104	181 ± 58	165 ± 67	148 ± 80

4.4.4. Multivariate dimension reduction of blood metal data

PCA was conducted to investigate differences in total metal profile in all blood samples between locations, and to assess which metals most influenced differences between sites (Figure 4.2). The scree plot indicated that the first two data dimensions adequately represented most of the variation in the data. Therefore, the suite of seven metals (variables or dimensions) were reduced to two principal components (Dim 1 and 2), which together represented 71.86% of the total variation of the data. Data were represented by two distinct clusters of metals (variables), Fe-Zn-Co-Cu (Fe cluster) and Al-Mg-Mn (Mg cluster), indicative of associations between elements, with loadings near one another (Figure 4.2). Squared cosine (Cos2) indicated the importance of a metal element to a particular principal component (or the quality of representation) and supported the reduction to two principal components. A Cos2 >0.60 was true for all metals in the analysis suite, excluding Co and Cu, which were represented by the first four dimensions, indicating that most of the data variation was indeed accounted for by the first two dimensions. The Fe cluster (top right) aligned closely to Dim 2, while the Mg-cluster (bottom right) aligned closer to Dim 1. Thus, each cluster influences the respective components, and therefore the entire data set, when analysing the results in a reduced space.

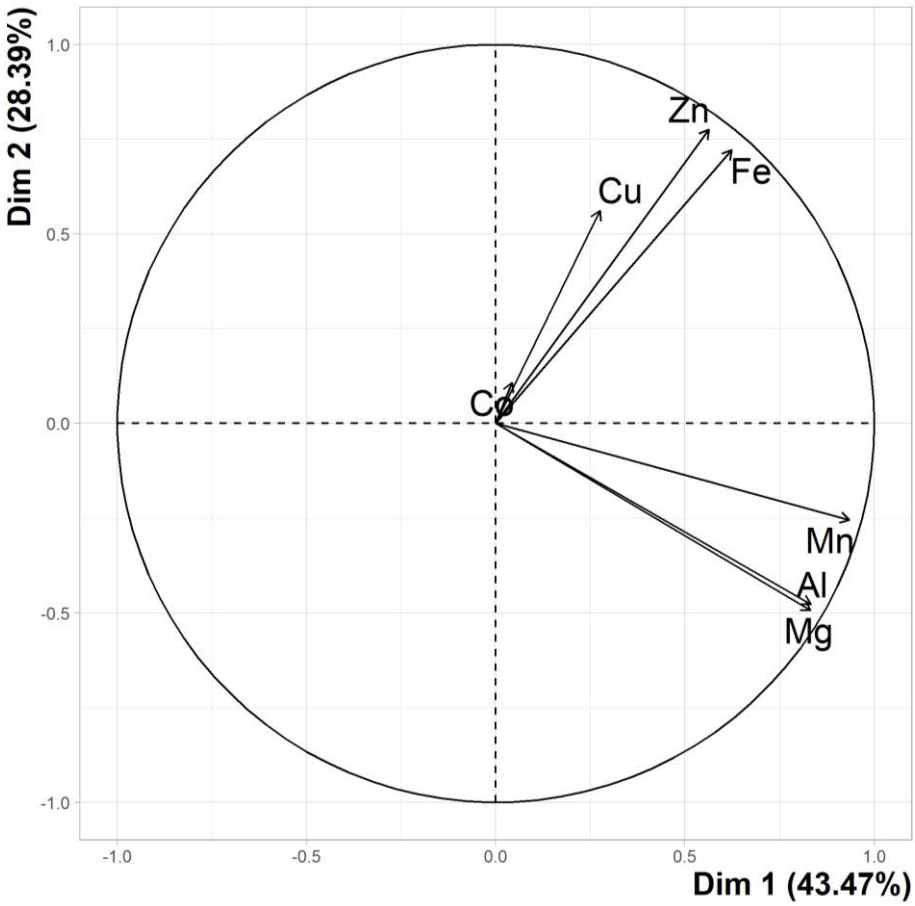


Figure 4.2. Variable loading plot of the Principal Component Analysis findings of all metal concentration data from blood collected from *C. mydas* at all five study sites (Howick's island group (HWK), Toolakea beach (TLK), Cockle Bay (CB), Upstart Bay (UB), Edgecumbe Bay, (EB)). Dimension 1 (Dim 1) represents 43.47% of total variations and Dim 2 represents 28.39%.

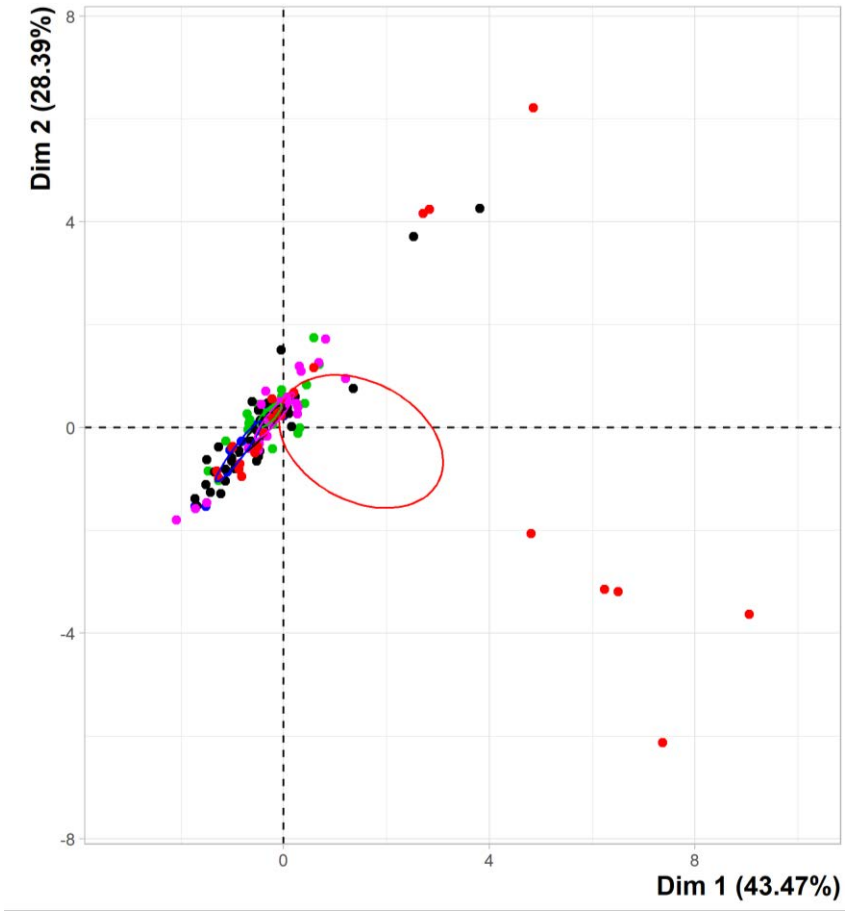


Figure 4.3. PCA individual plot depicting all blood metal data reduced to two principal dimensions (Dim 1 and 2). Points indicate individual turtles, and are categorised by study site, represented as different colours: HWK (Howick island group) = green, TLK (Toolakea Beach) = blue, CB (Cockle Bay) = black, UB (Upstart Bay) = pink and EB (Edgecumbe Bay) = red. Confidence ellipses are colour coordinated with the individual sample points. Dim 1 represents 43.47% of total variations and Dim 2 represents 28.39%.

When looking at individual sample PCA data per study location (Figure 4.3), it was apparent that variation within each site (confidence ellipses) was closely associated between all sites, with ellipses overlapping and individual points converging, in the centre of the plot, indicative of similar blood metal profiles between sites. Variation is comparatively low in all sites other than EB, with a wider spread in the data and larger elliptical area. These findings support the data in Table 4.2, whereby EB metal concentrations were noticeably more variable than other sites.

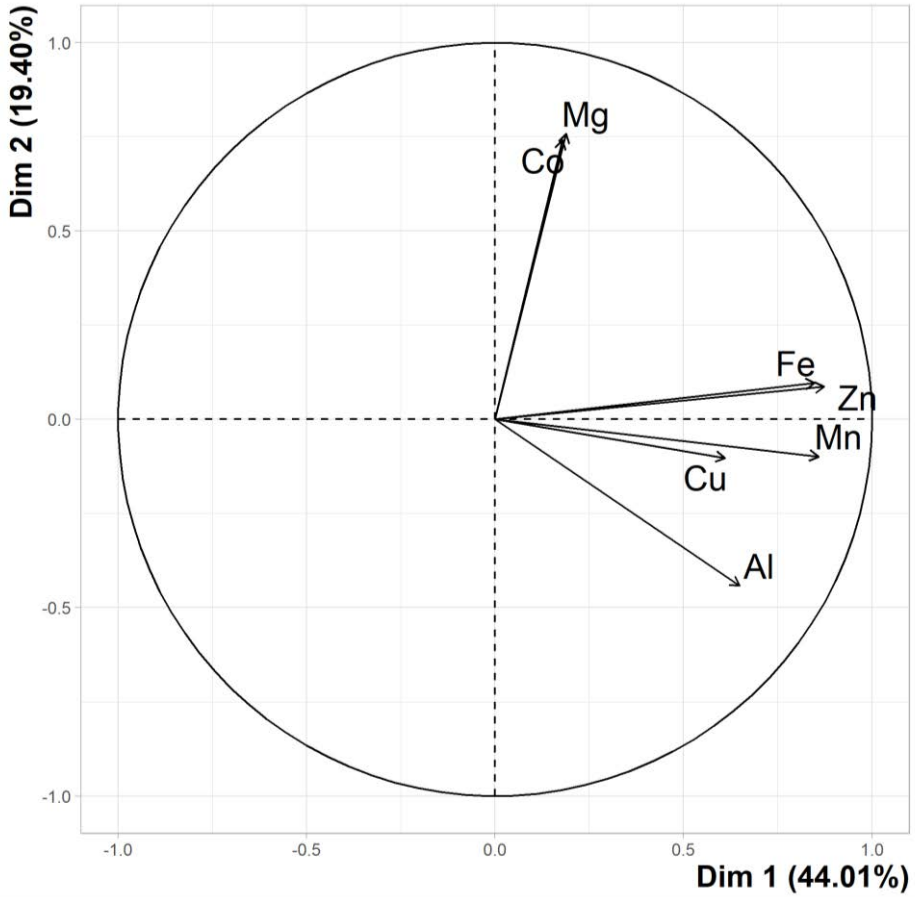


Figure 4.4. Variable loading plot of the Principal Component Analysis findings of all metal concentration data from blood collected from *C. mydas* at four study sites (Howick's island group (HWK), Toolakea Beach (TLK), Cockle Bay (CB) and Upstart Bay (UB)). Data presented here is the same as in Figure 2 and 3 excluding data from Edgecumbe Bay (EB). Dimension 1 (Dim 1) represents 44.01% of total variations and Dim 2 represents 19.40%.

In Figure 4.3 EB data dominates the plot, with the widest spread of individual points, causing a loss in resolution regarding the distribution of data from each of the other included sites. In Figure 4.4 and 4.5 the same PCA data is represented as in Figure 4.3 but with EB data excluded. As previously seen in figure 4.2, the variables (metals) influenced the data in two distinct clusters, though differing between analysis (Mg-Co and Al-Cu-Fe-Mn-Zn). Strong associations between metals in each cluster is suggested by proximity of each loading and influence was equally shared between all variables (length of the loadings).

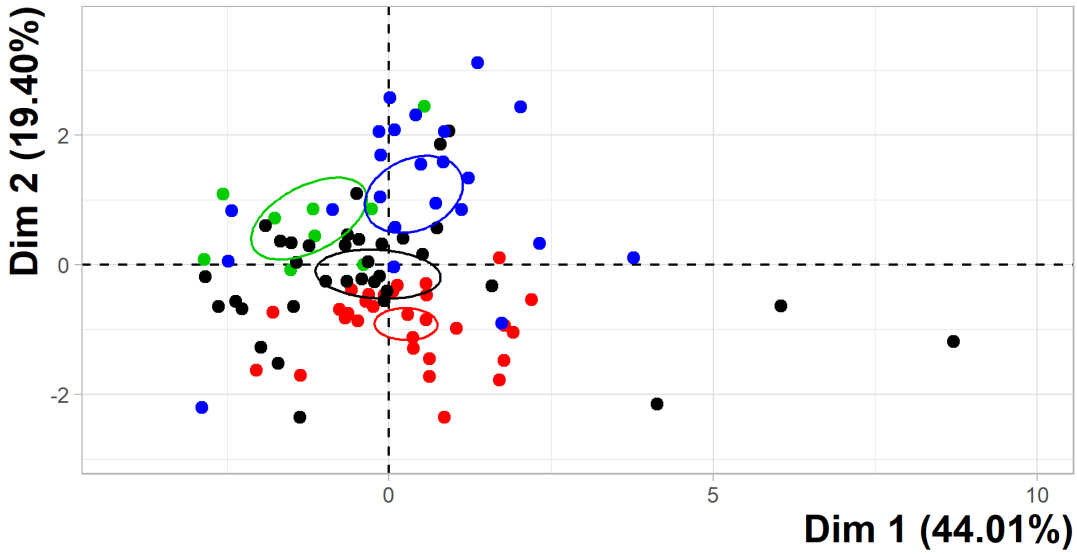


Figure 4.5. PCA individual plot depicting all blood metal data reduced to two principal dimensions (Dim 1 and 2). Points indicate individual turtles, and are categorised by study site, represented as different colours: HWK (Howick island group) = red, TLK (Toolakea Beach) = Green, CB (Cockle Bay) = black, UB (Upstart Bay) = blue. Data presented here is the same as in Figure 2 and 3 excluding data from Edgecumbe Bay (EB). Confidence ellipses are colour coordinated with the individual sample points. Dim 1 represents 44.01% of total variations and Dim 2 represents 19.40%.

Figure 4.5 represents the same data as Figure 4.4 and thus the position of loadings in Figure 4.4 correspond to the position of individual points in Figure 4.5. Thus, it was found that the metal profile of blood samples collected in UB were most strongly influenced by elements Co and Mg, while CB and HWK were primarily influenced by the Al-cluster. Overlap of confidence ellipses (and thus close associations in metal profiles) were observed between TLK and UB only and all other coastal sites variability was distinct from one another but some overlap between individual sample profiles occurred between sites (mixing of points).

4.4.5. Multivariate dimension reduction of scute metal data

PCA was conducted to investigate differences in total metal profile in *C. mydas* scute samples between locations, and to assess which metals most influenced differences between sites (Figure 4.6 and 4.7). The scree plot indicated that the first two data dimensions adequately represented most of the variation in the data. Therefore, the suite of metals (variables) was reduced to two principal components (Dim 1 and 2), which together represented 48.61% of the total variation of the data. As previously observed in blood metal PCA findings, variables (metals) influenced the scute metal data in two distinct clusters, Cd-Mg-Zn and Al-Co-Cu-Mn, with Ni and Fe negatively correlated to one another and influencing the data to a lesser degree (smaller loadings) relative to the other elements. Strong associations between metals in each

cluster was suggested by proximity of each loading and influence was equally shared between all variables (length of the loadings).

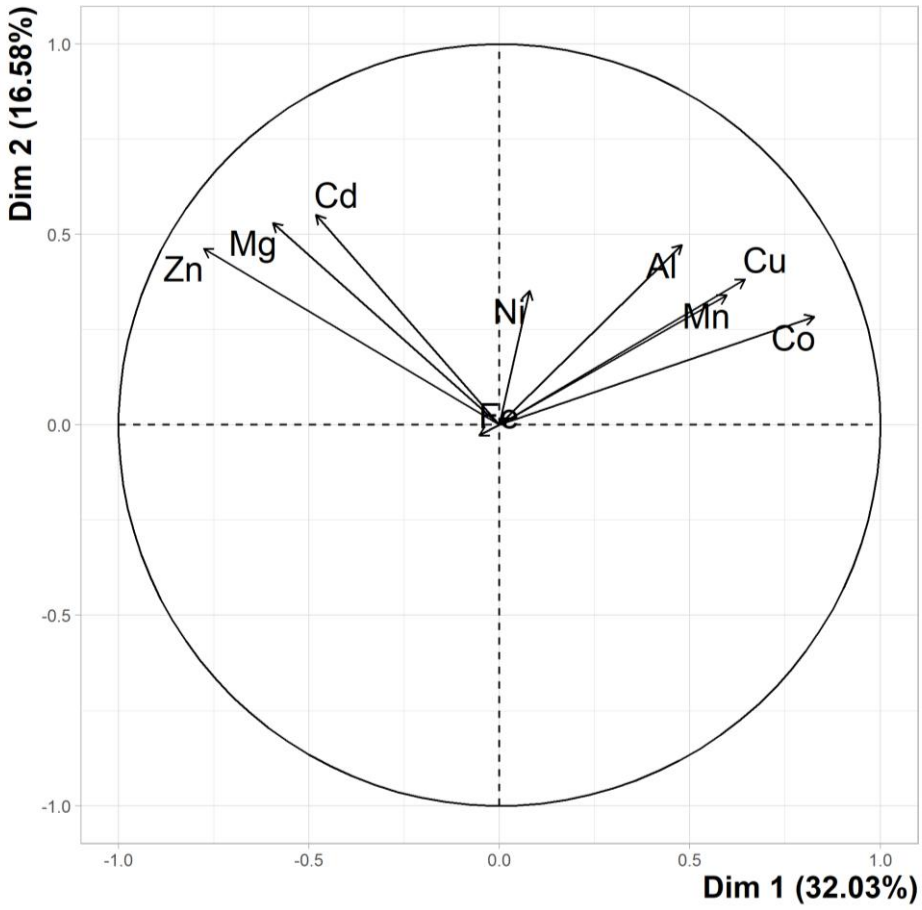


Figure 4.6. Variable loading plot of the Principal Component Analysis findings of all metal concentration data from scutes collected from *C. mydas* four study sites (TLK, CB, UB, EB). Dimension 1 (Dim 1) represents 32.03% of total variations and Dim 2 represents 16.58%.

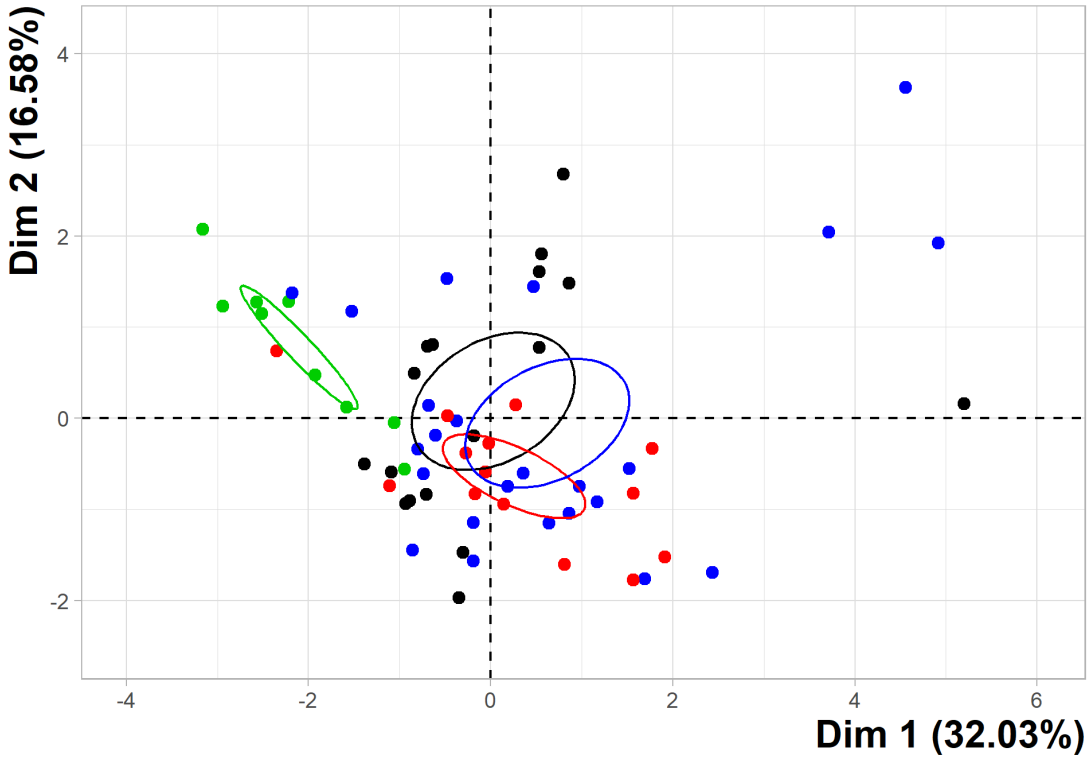


Figure 4.7. PCA individual plot depicting all scute metal data reduced to two principal dimensions (Dim 1 and 2). Points indicate individual turtles, and are categorised by study site, represented as different colours: TLK (Toolakea Beach) = green, CB (Cockle Bay) = black, UB (Upstart Bay) = blue and EB (Edgecumbe Bay) = red. Confidence ellipses are colour coordinated with the individual sample points. Dim 1 represents 32.03% of total variations and Dim 2 represents 16.58%.

When looking at individual scute sample PCA data per study location (Figure 4.7), it was apparent that variation within each site (confidence ellipses) was closely associated between CB, UB, and EB, with ellipses overlapping and individual points converging, in the centre of the plot, indicative of similar scute metal profiles between sites. TLK metal profile was distinct from all other sites as indicated by isolation of confidence ellipse and less mixing of individual points. Variation was similar in all sites though was observably lower in TLK data. These findings support the data in Table 4.4, whereby TLK scute metal concentrations tended to be lower when compared to the other sites. When looking at both 4.6 and 4.7 together it was apparent that TLK profile was most influenced by Cd, Mg and Zn concentrations whereas the loadings of the Al cluster of elements (also Ni and Fe less so) was most representative of the metal profiles of CB, UB and EB.

4.5. Discussion

4.5.1. C. mydas metal concentrations between coastal and reference populations

It was predicted that *C. mydas* blood metal concentrations would be lower, and metal profile most different at the control site (HWK) when compared to each coastal site studied. Previous research conducted by WWF and the Rivers to Reefs to Turtles project showed that metal loads were consistently higher in coastal *C. mydas* populations (Villa et al., 2017) and forage material (seagrass) (Thomas et al., 2020) compared to the natural baseline metal concentrations in an area minimally influenced by land based contaminants and industrial activity, HWK (Villa et al., 2017). These trends were supported by work conducted in *C. mydas* seagrass forage (Chapter 3), but not as consistently in the current study. Several elements (Al, Co, Mg and Mn) were occasionally higher in blood collected from one or more coastal site compared to the HWK though most elements were detected at concentrations similar between sites. This was true particularly for essential elements such as Cu and Fe and Mn. Essential elements tend to be maintained within optimal ranges through homeostatic processes (Bury et al., 2003, Aggett et al., 2015) and therefore variability would be small in a population consistently exposed to optimal concentrations of such elements, as expected at HWK.

Reference intervals were calculated by Villa et al. (2017) for all target metals. RIs were derived from HWK baseline blood concentrations and provided insight into whether current metal loads were elevated when compared to the upper and lower 95% prediction intervals in the healthy and minimally exposed population. Data from the current study found that the suite of *C. mydas* blood metal concentrations (all but Mn) at HWK were within the homeostatic ranges previously observed by Villa et al. (2017), suggestive of no recent fluctuations in concentrations between sampling events of both studies. It is unknown as to why Mn may be elevated at this time. In comparison, several coastal blood metal concentrations were detected at concentrations greater than the published RIs. At all four coastal sites Co, Mg and often Mn (at CB, UB and EB) were detected at concentrations which exceeded the RIs and the remaining metals were within the defined reference ranges (Villa et al., 2017). No insight is gleaned from this descriptive study regarding possible sources of elevated metal availability at these sites.

Chemical contaminants are known to interact in complex mixtures, which could have synergistic, antagonistic, or additive influences on one another (Gatidou et al., 2015, Wilkinson et al., 2015). When variable chemical mixture interactions are undefined a univariate statistical approach is considered less than ideal due to multicollinearity in the data (Finlayson et al.,

2021). To account for limitations encountered when comparing single metal elements, multivariate analysis allows for the analysis of metals as co-variables within the overall metal profile, rather than distinct stressors that act in isolation of any others. PCA was implemented and the dimensions of the data were reduced to measure for particularly influential metals or differences between study site profiles that might have dominated the data variability. When applying PCA on all the sites (Figure 4.3), no separation was detected between study sites (overlapping confidence ellipses) and this was likely due to the high variability in data from EB. To improve the resolution of the data set EB was removed from the PCA (Figure 4.5). From this data it was observed that differences between metal profiles at each coastal site were evident. Individual sample profiles of blood collect from UB were particularly distinct from the other sites (lack of data point mixing) and were influenced primarily by Co and Mg, which lends credence to the elevated mean Co and Mg concentrations detected at UB when compared to the other sites. Some association between metal profiles of UB and TLK samples was implied (converging confidence ellipses) though no other sites were determined to be like others.

Coastal zone ecosystems and offshore regions vary in a range of geochemical characteristics, which can influence the bioavailability and geoavailability of certain elements at a particular locality (Rabajczyk, 2010). The proximity to river discharge catchments, sediment type, and grain size are fundamental characteristics that play a role in a site's metal profile. Freshwater runoff of contaminated sediments and water from land-based practices into creeks and river systems is a major transport pathway of elevated land-based, non-essential and potentially harmful metal loads (Pacyna et al., 1995, Pacyna and Pacyna, 2001). Further confounding the risk, fine clay-based sediment types, which dominant in estuarine and intertidal zones (including CB and UB), often carry higher trace metal concentrations than larger and coarser bicarbonate sediments found offshore (Weber et al., 2006). As such, making direct comparison between site type is unreliable, and therefore further emphasis was not placed on comparisons of metal concentrations between coastal sites. The finding that trace metal concentrations were similar between coastal populations and the offshore control population is likely indicative of essential elements at the coastal sites likely being within their respective natural and optimal homeostatic ranges (Bury et al., 2003, Aggett et al., 2015).

Most scute concentrations were similar between coastal sites, particularly between CB, UB and EB (Table 4.4 and Figure 4.6 and 4.7). TLK was slightly different as several essential elements

(Al, Fe and Mn) were lower there than at the other sites. This finding was comparable to the means from the blood analyses, where it was suggested that these elements were likely within natural baselines. Mg and Zn concentrations were significantly greater in *C. mydas* scutes from TLK than any other coastal site, including CB (the study area closest to the Zn refinery), though Zn is also naturally geoavailable in certain substrates (Scott et al., 2013). Like Zn, Mg is an essential element and is associated with carbonate-based sediments found in bays, not dominated by river discharge such as TLK and EB. Variation in sediment composition between sites is known to play a role in local natural metal geoavailability (Thomas et al., 2020), but likely does not lend an explanation for significant fluctuation in metal loads over time. Zn and Mg concentrations did not follow the same trend in blood samples as in scutes, which may be indicative of past exposure to elevated elements (up to 2.8 years prior to sampling) (Villa et al., 2019). No explanation as to historical contamination events or sources that may have influenced this trend are offered here. Unlike blood sampling, which is an established and widely applied method of metal monitoring in turtles, scute metal analysis is still a novel approach not readily adopted as an alternative long-term metal concentration store. Because of this, no published reference intervals are available for scute metal concentrations (Villa et al., 2019), and thus no indication as to whether current coastal concentrations are within expected ranges can be stated. Unlike Villa et al. (2019), the scope of this study did not allow for scute sample collection from a control site such as the HWK. Therefore, reference intervals could not be calculated, and steady-state relationships between blood and scute concentrations in control animals (HWK) could not be defined. If such information was available, deviations from the steady-state in the coastal sites would have been possible and would have further elucidated as to whether elevation in scute metals not seen in blood was indeed due to past exposure to elevated concentrations (Villa et al., 2019).

Individual scute sample data and overall confidence ellipses for most coastal population overlapped consistently, except for TLK which was distinct from all other sites. Association between metal profiles of CB and UB is similar to that reported in Villa et al. (2017), further indicative of the absence of any significant contamination event in recent years (since 2014) at any sites in this study (or that all sites may have been impacted by contamination in similar ways throughout the entire study region). When comparing blood and metal mean concentrations, Cu, Fe and Co concentrations were similar between sampling type, inferring that these elements have remained at steady-state concentrations within the region in recent

years (Villa et al., 2019). In contrast, elements such as Al, Mg and Zn were detected at concentrations far greater in scutes than blood (a 220-fold difference in concentration was observed for Al). No definitive reason for such a disparity between sampling types can be given here and further toxicokinetic investigation is encouraged to better understand the affinity of some metals to different storage tissues in marine turtles, including absorption to keratinous scute tissue. As scute metal concentrations are considered to be indicative of exposure and uptake of metals up to 2.8 years prior to sample, it may be inferred that any elevated concentrations, when compared to blood metal concentrations (indicative of short term exposure of between 2 and 3 weeks), is a result of exposure to past concentrations that have since declined (Villa et al., 2019). In contrast, the higher concentrations of these elements detected in scutes is likely indicative of bioaccumulation over time (Grillitsch and Schiesari, 2010). Due to the persistent nature of metal elements in the marine environment and the long-lived life history strategy and strict fidelity to narrow foraging ranges (Hazel et al., 2013), the accumulation of elements to levels greater than the surrounding environment or blood (and other short-term metal stores) is common and somewhat anticipated (Caurant et al., 1999, Nicolau et al., 2017, Ross et al., 2017, Mondragón et al., 2020).

4.5.2. Temporal variation in C. mydas blood metals

Blood samples are considered a short-term store for metal concentrations and are representative of recent exposure on or close to (2-3 weeks) the point of capture. Because no recaptures of animals occurred during this study, temporal differences within sites could not be made. To address this limitation, the study design ensured that similar methodologies to that conducted by Villa et al. (2017) were applied for the collection, storage and analysis of bloods and data. Such a universal study design allowed for direct comparison between data collected at different times, but from the same locations. The study areas that were measured in both sites and compared here were HWK, CB and UB. Blood metal concentrations detected in *C. mydas* from HWK were similar between studies, with most mean concentrations being within range of one another. Mn was the only element to exceed the range of blood concentrations previously detected at HWK though knowledge as to why this may be the case is currently lacking. Similarly, when comparing mean concentrations at both CB and UB most elements were similar or within range between studies. Co was the sole element to be detected at higher concentrations between studies, with it being detected at concentrations up to double that of current levels in 2014 in CB samples. This finding suggests that concentrations are currently lower than previously and that recent exposure to Co at CB is reduced in the local *C. mydas*

population. The scope of this study does not allow for inference to be made as to why this may be the case.

4.5.3. Consistently elevated Co concentrations

Cobalt was consistently higher in blood and scutes at all coastal *C. mydas* populations relative to concentrations recorded in HWK, but particularly high in UB turtles. Such findings are true, not only for the blood and scute analysis conducted here, but also in previous analysis of *C.mydas* blood (Villa et al., 2017) and scutes (Villa et al., 2019), and also in primary seagrass species within the study region (Chapter 3 and (Thomas et al., 2020)). The consistently elevated Co profile observed in UB is likely influenced by natural deposits of Ni and Co that saw the establishment of industrial practices such as intensive mining and refining industries to process the available ore. Furthermore, the Burdekin River (which outlets into UB) and adjacent catchment area has seen significant increases in erosion rate since the mid-1800s, associated with agricultural land practices such as beef grazing and the cultivation of range land for cattle (Lewis et al., 2007, Wilkinson et al., 2013), likely leading to the distribution and transport of enriched sediment into waterways. As previously discussed, estuarine and river-borne sediment tend to be fine in grain size and metals have a high affinity for such surfaces. Therefore it is feasible that elevated Co and Ni concentrations have likely been consistently transported over time to the coastal environment (Kroon et al., 2012).

Co concentrations in the current study may be at levels which could present negative health impacts in exposed marine species, such as *C. mydas*. The biochemical implications of elevated Co concentrations are poorly understood, though recent research has found that Co may induce liver dysfunction (Villa et al., 2017), inflammatory responses (Villa et al., 2017, Gaus et al., 2019) and oxidative stress (Pulido and Parrish, 2003, Permenter et al., 2014, Finlayson et al., 2019a, Finlayson et al., 2019c). The blood concentrations reported at UB in the current study exceeded the upper limit of the reported reference interval (0.0071 – 0.033) by nearly 13-fold. While such concentrations were reported, inference as to whether such concentrations are toxic to the local *C. mydas* cannot be made without first conducting a comprehensive hazardous risk assessment, of which was beyond the scope of this study. Definitive relationships cannot be made between the Co concentrations reported here and toxic impacts but metal-induced oxidative stress via the production of toxic reactive oxygen species (ROS) is of particular interest in the investigation of fibropapillomatosis (FP) aetiology. Chronic damage caused by oxidative stress has the potential to induce immunosuppression (da Silva et al., 2016, Gaus et

al., 2019), which is believed to increase susceptibility to further infection (such as ChHV5) (Gaus et al., 2019), possibly associated with the expression of FP (da Silva et al., 2016, Jones et al., 2016, Page-Karjian, 2019). Unfortunately, it was not possible to determine whether Co, or any other metals in the current study, were associated with FP, as most elements were at concentrations that presented little toxicological risk to local *C. mydas* populations. Furthermore, no turtles captured in this study presented with visible FP tumours, and thus comparisons could not be made between clinically healthy and diseased individuals to investigate differences in metal loads. While causation could not be drawn between FP and metal exposure, it is highly recommended that Co should be considered a priority element in future investigation. Further investigation should be conducted into the ecotoxicological implications of elevated exposure to metal loads on *C. mydas* and to research how metal-induced immunosuppression may play a role in the development of FP clinical signs and other toxicity-induced health complications in susceptible *C. mydas* populations. While *In vivo* investigation of metals on marine turtles is not possible for obvious ethical concerns, an *in vitro* cytotoxic assay has been recently validated to measure the toxic effects of metal concentrations on *C. mydas* primary skin fibroblasts (Finlayson et al., 2019a, Finlayson et al., 2019b). It is strongly suggested that future effort should be placed on applying this and other such sensitive ecotoxicological assays to investigate the potential toxic effects which environmentally relevant metals may be of most concern.

4.6. Conclusions

A suite of ecologically relevant metal elements was measured in blood and scute samples from resident *C. mydas* at four coastal sites and compared with a reference site within the northern GBR. Metal profiles were similar between all coastal study sites, and profiles of some coastal sites were more like that of the control site than others coastal populations. Blood metal concentrations at all sites (coastal and control) were generally within published reference intervals (except Co), suggesting that metal loads at the time of sampling were not at concentrations deemed of major concern. In contrast, scute concentrations of some elements were greater than those in blood within the same sites, which may be indicative of previous exposure of local *C. mydas*, to certain elements at elevated concentrations (Mg, Mn, and Zn). Furthermore, some blood metal concentrations differed temporally when compared with previous research. Particularly, concentrations of Co were found to be magnitudes greater than baseline values and may be of concern as a possible aetiological agent of disease expression and other health conditions in *C. mydas*. Further study investigating overall risk and

susceptibility of *C. mydas* to elevated metal elements, such as Co, is required to better manage and protect populations inhabiting at-risk coastal areas. As more sensitive methods are validated and implemented in marine turtle ecotoxicology, future study design should strive to promote comparison between multiple study findings where possible. Such an approach will greatly improve baseline data and thus the sensitivity of the analysis. Further effort should be applied to develop the use of scute material as a long-term indication of metal exposure in individuals. Development of region-specific natural baseline reference intervals is an essential step that would aid comparison between regions and will allow the development of a holistic view over areas of particular concern.

4.7. Permits

All research was carried out under permits from James Cook University Animals Ethics Committee (A2396), Department of Environment and Science (WISP18586417 and WISP18596817) and Great Barrier Reef Marine Parks Authority (G17/39429.1).

4.8. Summary of contributions

The aim of this chapter was met in the following way:

Aim:

To investigate and describe *C. mydas* blood and scute (carapace) metal profiles in coastal and offshore populations of the GBR.

How the aims were achieved

- I designed and developed the study in collaboration with my primary advisor.
- I prepared all permits required to conduct field work and sample collection (Animal ethics, GBRMPA and DES).
- I completed all necessary training to meet safety and proficiency requirements to work efficiently in the field (QLD recreational boat licence, 4x4 driving and turtle capture and sampling training).
- With assistance from my primary advisor, I planned all aspects of field trips (Riskware risk analysis, volunteer insurance and training, logistics, administration).
- With assistance from my primary advisor, other field collaborators, fellow PhD students and a long list of volunteers, I conducted a series of field trips where I

managed volunteer assistance, multiple capture teams and oversaw all aspects of animal handling and welfare during capturing and sampling.

- Over 120 blood and 60 scute samples were collected from local *C. mydas* populations and analysed with ICP-MS for a suite of ecologically relevant metal elements.
- Comparison of concentrations was made between coastal and control populations, with published reference intervals and between blood and scute sample types to provide insight into past and present metal profiles.
- I inputted all data collected and appropriately stored all samples collected ready for sample analysis.
- Sara Kophamel and Dr. Ian Bell collected *C. mydas* blood samples and relevant morphometric data from the Howick Island group on my behalf and following my provided protocol.
- All blood and scute samples were analysed by the Advanced Analytical Centre, JCU as per the protocols selected by the AAC department.
- Detection of a suite of ecologically relevant metal elements was conducted for all samples (ICP-MS) and compared with previously published metal data from the minimally influenced offshore control site, HWK.
- Funding of this analysis was provided by a successful CPHMVS HDRE grant application and use of student research funds (SSA).
- With assistance from my advisors and Rhondda Jones I conducted all statistical analysis.
- I drafted all aspects of this chapter and with assistance of my advisors, other PhD students and various volunteers I edited and finalised the chapter for inclusion in the thesis.

Summary of outcomes

Coastal blood metal concentrations were typically elevated when compared with concentrations recorded in the offshore control population (HWK), though most elements were within reference intervals derived from previously reported HWK concentrations. Some elements were at similar concentrations in blood and scutes, but most were higher in scute samples.

4.9. References

AGGETT, P., NORDBERG, G. F. & NORDBERG, M. 2015. Essential Metals: Assessing Risks from Deficiency and Toxicity. *In:* NORDBERG, G. F., FOWLER, B. A. & NORDBERG, M. (eds.) *Handbook on the Toxicology of Metals (Fourth Edition).* San Diego: Academic Press.

BALAZS, G. H. 1991. Current status of fibropapillomas in the Hawaiian green turtle,*Chelonia mydas*. *Research Plan for Marine Turtle Fibropapilloma (Balazs GH, Pooley SG, eds). Report No. NOAA-TM-NMFS-SWFSC-156. Honolulu: US Department of Commerce, National Oceanographic and Atmospheric Administration, National Marine Fisheries Service***,** 47-57.

BOWEN, B. W., MEYLAN, A. B., ROSS, J. P., LIMPUS, C. J., BALAZS, G. H. & AVISE, J. C. 1992. Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. *Evolution,* 46**,** 865-881.

BURY, N. R., WALKER, P. A. & GLOVER, C. N. 2003. Nutritive metal uptake in teleost fish. *Journal of Experimental Biology,* 206**,** 11.

CAMACHO, M., LUZARDO, O. P., BOADA, L. D., LÓPEZ JURADO, L. F., MEDINA, M., ZUMBADO, M. & ORÓS, J. 2013. Potential adverse health effects of persistent organic pollutants on sea turtles: Evidences from a cross-sectional study on Cape Verde loggerhead sea turtles. *Science of The Total Environment,* 458–460**,** 283-289.

CASALE, P. & TUCKER, A. D. 2017. *Caretta caretta (amended version of 2015 assessment)* [Online]. [Accessed Downloaded on 13 October 2020].

CAURANT, F., BUSTAMANTE, P., BORDES, M. & MIRAMAND, P. 1999. Bioaccumulation of Cadmium, Copper and Zinc in some Tissues of Three Species of Marine Turtles Stranded Along the French Atlantic Coasts. *Marine Pollution Bulletin,* 38**,** 1085-1091.

CLARK, T. R., LEONARD, N. D., ZHAO, J.-X., BRODIE, J., MCCOOK, L. J., WACHENFELD, D. R., NGUYEN, A. D., MARKHAM, H. L. & PANDOLFI, J. M. 2016. Historical photographs revisited: A case study for dating and characterizing recent loss of coral cover on the inshore Great Barrier Reef. *Scientific reports,* 6**,** 19285.

CRAY, C., VARELLA, R., BOSSART, G. D. & LUTZ, P. 2001. Altered in vitro immune responses in green turtles (*Chelonia mydas*) with fibropapillomatosis. *Journal of Zoo and Wildlife Medicine,* 32**,** 436-440.

DA SILVA, C. C., KLEIN, R. D., BARCAROLLI, I. F. & BIANCHINI, A. 2016. Metal contamination as a possible etiology of fibropapillomatosis in juvenile female green sea turtles *Chelonia mydas* from the southern Atlantic Ocean. *Aquatic Toxicology,* 170**,** 42–51.

DE SOUZA MACHADO, A. A., SPENCER, K., KLOAS, W., TOFFOLON, M. & ZARFL, C. 2016. Metal fate and effects in estuaries: a review and conceptual model for better understanding of toxicity. *Science of the Total Environment,* 541**,** 268-281.

ECKERT, K., BJORNDAL, K. A., ABREU-GROBOIS, F. A. & DONNELLY, M. 1999. Research and management techniques for the conservation of sea turtles. Washington, DC: IUCN/SSC Marine Turtle Specialist Group.

ESSLEMONT, G. 2000. Heavy metals in seawater, marine sediments and corals from the Townsville section, Great Barrier Reef Marine Park, Queensland. *Marine Chemistry,* 71**,** 215-231.

FAUST, D. R., HOOPER, M. J., COBB, G. P., BARNES, M., SHAVER, D., ERTOLACCI, S. & SMITH, P. N. 2014. Inorganic elements in green sea turtles (*Chelonia mydas*): Relationships among external and internal tissues. *Environmental Toxicology and Chemistry,* 33**,** 2020-2027.

FINLAYSON, K. A., LEUSCH, F. D. & VAN DE MERWE, J. P. 2019a. Primary green turtle (*Chelonia mydas*) skin fibroblasts as an in vitro model for assessing genotoxicity and oxidative stress. *Aquatic Toxicology,* 207**,** 13-18.

FINLAYSON, K. A., LEUSCH, F. D. L., LIMPUS, C. J. & VAN DE MERWE, J. P. 2019b. Towards the development of standardised sea turtle primary cell cultures for toxicity testing. *Ecotoxicology and Environmental Safety,* 173**,** 63-70.

FINLAYSON, K. A., LEUSCH, F. D. L. & VAN DE MERWE, J. P. 2019c. Cytotoxicity of organic and inorganic compounds to primary cell cultures established from internal tissues of *Chelonia mydas*. *Science of The Total Environment,* 664**,** 958-967.

FINLAYSON, K. A., LEUSCH, F. D. L., VILLA, C. A., LIMPUS, C. J. & VAN DE MERWE, J. P. 2021. Combining analytical and in vitro techniques for comprehensive assessments of chemical exposure and effect in green sea turtles (Chelonia mydas). *Chemosphere,* 274**,** 129752.

FLINT, M., EDEN, P. A., LIMPUS, C. J., OWEN, H., GAUS, C. & MILLS, P. C. 2015. Clinical and Pathological Findings in Green Turtles (*Chelonia mydas*) from Gladstone, Queensland: Investigations of a Stranding Epidemic. *EcoHealth,* 12**,** 298-309.

GATIDOU, G., STASINAKIS, A. S. & IATROU, E. I. 2015. Assessing single and joint toxicity of three phenylurea herbicides using Lemna minor and Vibrio fischeri bioassays. *Chemosphere,* 119**,** S69-S74.

GAUS, C., GRANT, S., JIN, N. L., GOOT, K., CHEN, L., VILLA, A., NEUGEBAUER, F., QI, L. & LIMPUS, C. 2012. Investigations of contaminant levels in green turtles from Gladstone. *National Research Centre for Environmental Toxicology*.

GAUS, C., VILLA, C. A., DOGRUER, G., HEFFERNAN, A., VIJAYASARATHY, S., LIN, C.-Y., FLINT, M., HOF, C. M. & BELL, I. 2019. Evaluating internal exposure of sea turtles as model species for identifying regional chemical threats in nearshore habitats of the Great Barrier Reef. *Science of The Total Environment,* 658**,** 732-743.

GRILLITSCH, B. & SCHIESARI, L. 2010. The ecotoxicology of metals in reptiles. *In:* SPARLING, D. W., LINDER, G., BISHOP, C. A. & KREST, S. K. (eds.) *Ecotoxicology of amphibians and reptiles.* 2nd ed.: CRC Press.

HAMANN, M., GODFREY, M., SEMINOFF, J., ARTHUR, K., BARATA, P., BJORNDAL, K., BOLTEN, A., BRODERICK, A., CAMPBELL, L. & CARRERAS, C. 2010. Global research priorities for sea turtles: informing management and conservation in the 21st century. *Endangered Species Research,* 11**,** 245-269.

HAMANN, M., LIMPUS, C. & OWENS, D. 2002. Reproductive Cycles of Males and Females. *In:* LUTZ, P. L., MUSICK, J. A. & WYNEKEN, J. (eds.) *The Biology of Sea Turtles.* CRC press.

HAZEL, J., HAMANN, M. & LAWLER, I. R. 2013. Home range of immature green turtles tracked at an offshore tropical reef using automated passive acoustic technology. *Marine Biology,* 160**,** 617–627.

HERBST, L. H. 1994. Fibropapillomatosis of marine turtles. *Ann Rev Fish Dis,* 4**,** 389–425.

HERBST, L. H., JACOBSON, E. R., KLEIN, P. A., BALAZS, G. H., MORETTI, R., BROWN, T. & SUNDBERG, J. P. 1999. Comparative pathology and pathogenesis of spontaneous and experimentally induced fibropapillomas of green turtles (*Chelonia mydas*). *Vet Pathol,* 36**,** 551–564.

HUSSON, F., JOSSE, J., LE, S. & MAZET, J. 2013. FactoMineR: multivariate exploratory data analysis and data mining with R. *R package version,* 1.

HUSSON, F., LÊ, S. & PAGÈS, J. 2017. *Exploratory multivariate analysis by example using R*, CRC press.

JACOBSON, E. R., MANSELL, J. L., SUNDBERG, J. P., JAJJAR, L., REICHMANN, M. E., EHRHART, L. M., WALSH, M. & MURRU, F. 1989. Cutaneous fibropapillomas of green turtles (*Chelonia mydas*). *J Comp Pathol,* 101**,** 39–52.

JONES, K., ARIEL, E., BURGESS, G. & READ, M. 2016. A review of fibropapillomatosis in Green turtles (*Chelonia mydas*). *The Veterinary Journal,* 212**,** 48-57.

KROON, F., BERRY, K., BRINKMAN, D., DAVIS, A., KING, O., KOOKANA, R., LEWIS, S., LEUSCH, F., MAKARYNSKYY, O. & MELVIN, S. 2015. Identification, impacts, and prioritisation of emerging contaminants present in the GBR and Torres Strait marine environments. *Report to the National Environmental Science Programme. Reef and Rainforest Research Centre Limited, Cairns (138 pp.)*.

KROON, F. J., KUHNERT, P. M., HENDERSON, B. L., WILKINSON, S. N., KINSEY-HENDERSON, A., ABBOTT, B., BRODIE, J. E. & TURNER, R. D. R. 2012. River loads of suspended solids, nitrogen, phosphorus and herbicides delivered to the Great Barrier Reef lagoon. *Marine Pollution Bulletin,* 65**,** 167-181.

LEWIS, M. A. & DEVEREUX, R. 2009. Nonnutrient anthropogenic chemicals in seagrass ecosystems: Fate and effects. *Environmental Toxicology and Chemistry,* 28**,** 644-661.

LEWIS, S. E., SHIELDS, G. A., KAMBER, B. S. & LOUGH, J. M. 2007. A multi-trace element coral record of land-use changes in the Burdekin River catchment, NE Australia. *Palaeogeography, Palaeoclimatology, Palaeoecology,* 246**,** 471-487.

LIMPUS, C. & READ, M. 1985. The Green sea turtle, *Chelonia mydas,* in Queensland: a preliminary description of the population structure of a coral reef feeding ground. *In:* GRIGG, G. C., SHINE, R. & EHMANN, H. (eds.) *Biology of Austrlian Frogs and Reptiles.* Sydney, NSW: Royal Zoological Society

MASCARENHAS, R. & IVERSON, P. J. 2008. Fibropapillomatosis in stranded green turtles (*Chelonia mydas*) in Paraiba State, Northeastern Brazil: evidence of a Brazilian epizootic. *Marine Turtle Newsletter,* 120**,** 3–6.

MONDRAGÓN, M. E., LUZARDO, O. P., ZUMBADO, M., RODRÍGUEZ-HERNÁNDEZ, Á., BERRIEL, C. R., RAMÍREZ-GOMEZ, H. V., ISLAS, C. G.-R., FISHER, R. F. A. & MARTÍNEZ, J. R. R. 2020. Incidence of 49 elements in the blood and scute tissues of nesting hawksbill turtles (*Eretmochelys imbricata*) in Holbox Island. *Regional Studies in Marine Science***,** 101566.

MORTIMER, J. A. & DONNELLY, M. 2008. *Eretmochelys imbricata.* [Online]. [Accessed Downloaded on 13 October 2020].

NICOLAU, L., MONTEIRO, S. S., PEREIRA, A. T., MARÇALO, A., FERREIRA, M., TORRES, J., VINGADA, J. & EIRA, C. 2017. Trace elements in loggerhead turtles (*Caretta caretta*) stranded in mainland Portugal: Bioaccumulation and tissue distribution. *Chemosphere,* 179**,** 120-126.

OWENS, D. W. & RUIZ, G. J. 1980. New Methods of Obtaining Blood and Cerebrospinal Fluid from Marine Turtles. *Herpetologica,* 36**,** 17-20.

PACYNA, J. M. & PACYNA, E. G. 2001. An assessment of global and regional emissions of trace metals to the atmosphere from anthropogenic sources worldwide. *Environmental Reviews,* 9**,** 269-298.

PACYNA, J. M., SCHOLTZ, M. T. & LI, Y. F. 1995. Global budget of trace metal sources. *Environmental Reviews,* 3**,** 145-159.

PAGE-KARJIAN, A. 2019. Fibropapillomatosis in Marine Turtles. *In:* MILLER, E. R., LAMBERSKI, N. & CALLE, P. P. (eds.) *Fowler's Zoo and Wild Animal Medicine Current Therapy.* St. Louis, Missouri, USA: WB Saunders.

PERMENTER, M. G., DENNIS, W. E., SUTTO, T. E., JACKSON, D. A., LEWIS, J. A. & STALLINGS, J. D. 2014. Exposure to Cobalt Causes Transcriptomic and Proteomic Changes in Two Rat Liver Derived Cell Lines. *PLOS ONE,* 8**,** e83751.

PULIDO, M. D. & PARRISH, A. R. 2003. Metal-induced apoptosis: mechanisms. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,* 533**,** 227-241.

RABAJCZYK, A. 2010. The speciation and physico-chemical forms of metals in surface waters and sediments AU - Namieśnik, Jacek. *Chemical Speciation & Bioavailability,* 22**,** 1-24.

ROSS, D. A. N., GUZMÁN, H. M., POTVIN, C. & VAN HINSBERG, V. J. 2017. A review of toxic metal contamination in marine turtle tissues and its implications for human health. *Regional Studies in Marine Science,* 15**,** 1-9.

SCOTT, C., PLANAVSKY, N. J., DUPONT, C. L., KENDALL, B., GILL, B. C., ROBBINS, L. J., HUSBAND, K. F., ARNOLD, G. L., WING, B. A., POULTON, S. W., BEKKER, A., ANBAR, A. D., KONHAUSER, K. O. & LYONS, T. W. 2013. Bioavailability of zinc in marine systems through time. *Nature Geoscience,* 6**,** 125-128.

SEMINOFF, J. A. 2004. *Chelonia mydas* [Online]. [Accessed].

STRZELEC, M., PROEMSE, B. C., GAULT-RINGOLD, M., BOYD, P. W., PERRON, M. M., SCHOFIELD, R., RYAN, R. G., RISTOVSKI, Z. D., ALROE, J. & HUMPHRIES, R. S. 2020. Atmospheric trace metal deposition near the Great Barrier Reef, Australia. *Atmosphere,* 11**,** 390.

THOMAS, C., BENNETT, W., GARCIA, C., SIMMONDS, A., HONCHIN, C., TURNER, R., HOF, C. & BELL, I. 2020. Coastal bays and coral cays: Multi-element study of *Chelonia mydas* forage in the Great Barrier Reef (2015–2017). *Science of The Total Environment***,** 140042.

VANDER ZANDEN, H. B., BJORNDAL, K. A. & BOLTEN, A. B. 2013. Temporal consistency and individual specialization in resource use by green turtles in successive life stages. *Oecologia,* 173**,** 767-777.

VERBOVŠEK, T. 2011. A comparison of parameters below the limit of detection in geochemical analyses by substitution methods Primerjava ocenitev parametrov pod mejo določljivosti pri geokemičnih analizah z metodo nadomeščanja. *RMZ Mater Geoenvironment,* 58**,** 393-404.

VILLA, C., BELL, I., HOF, C. M., LIMPUS, C. & GAUS, C. 2019. Elucidating temporal trends in trace element exposure of green turtles (*Chelonia mydas*) using the toxicokinetic differences of blood and scute samples. *Science of the Total Environment,* 651**,** 2450-2459.

VILLA, C. A., FLINT, M., BELL, I., HOF, C., LIMPUS, C. J. & GAUS, C. 2017. Trace element reference intervals in the blood of healthy green sea turtles to evaluate exposure of coastal populations. *Environmental Pollution,* 220, Part B**,** 1465–1476.

WEBER, M., LOTT, C. & FABRICIUS, K. E. 2006. Sedimentation stress in a scleractinian coral exposed to terrestrial and marine sediments with contrasting physical, organic and geochemical properties. *Journal of Experimental Marine Biology and Ecology,* 336**,** 18-32.

WENDELBERGER, J. & CAMPBELL, K. 1994. Non-detect data in environmental investigations. *American Statistical Association*.

WILKINSON, A. D., COLLIER, C. J., FLORES, F. & NEGRI, A. P. 2015. Acute and additive toxicity of ten photosystem-II herbicides to seagrass. *Scientific Reports,* 5**,** 17443.

WILKINSON, S. N., HANCOCK, G. J., BARTLEY, R., HAWDON, A. A. & KEEN, R. J. 2013. Using sediment tracing to assess processes and spatial patterns of erosion in grazed rangelands, Burdekin River basin, Australia. *Agriculture, Ecosystems & Environment,* 180**,** 90-102.

WORK, T. M., BALAZS, G. H., RAMEYER, R. A., CHANG, S. P. & BERESTECKY, J. 2000. Assessing humoral and cell-mediated immune response in Hawaiian green turtles, *Chelonia mydas*. *Veterinary Immunology and Immunopathology,* 74**,** 179-194.

WORK, T. M., RAMEYER, R. A., BALAZS, G. H., CRAY, C. & CHANG, S. P. 2001. Immune status of free-ranging green turtles with fibropapillomatosis from Hawaii. *Journal of Wildlife Diseases,* 37**,** 574–581.

CHAPTER 5 - Seroprevalence of ChHV5 in Green turtle (*Chelonia mydas*) populations of the Great Barrier Reef.

5.1. Chapter prelude

5.1.1. Aim:

To survey and describe the seroprevalence of ChHV5-specific antibodies in *C. mydas* from coastal and offshore populations of the northern and central regions of the GBR.

5.1.2. Introduction to chapter

To investigate trace metal exposure of local *C.* mydas populations, Chapter 3 first described *C. mydas* seagrass forage metal concentrations, and Chapter 4 investigated *C. mydas* blood and scute metal profiles for each sample location of this study. This was done to describe local metal concentrations and, in an attempt, to better understand aspects of metal-induced toxicological impacts and the risk such elements pose to local turtle populations and their habitats. In this chapter focus is shifted from ecotoxicology to microbiology, where a recently validated species-specific Enzyme-Linked Immunosorbent Assay (ELISA) was applied to identify specific antibodies against Chelonid-alphaherpesvirus 5 (ChHV5), the infectious agent potentially associated with the disease fibropapillomatosis (FP). This study surveyed GBR *C. mydas* populations, for the first time, to better understand local viral exposure, and whether certain populations are more susceptible to contracting the virus. The same five study sites sampled in Chapter 4 were sampled here: The Howick Group of Islands (HWK), a remote offshore control site located in the northern region of the GBR, 15 km from the nearest catchment area and minimally influenced by anthropogenic interaction, 2) Toolakea Beach (TLK), located 30 km north of Townsville, 3) Cockle Bay (CB), adjacent to Townsville and in proximity to intensive land practices (including farming and metal refining), which serve as potential sources of land-based contaminants, 4) Upstart Bay (UB) and 5) Edgecumbe Bay (EB), both located south of Townsville where local metal profiles are influenced by agricultural activity, river discharge from the Burdekin River and high erosion rates. FP is enzootic to CB and UB. It appeared that seroprevalence of ChHV5-specific antibodies were similar between sites, and little difference was observed spatially, temporally or between sites with and without history of FP cases. This chapter formed an oral presentation given at the Australia Marine Turtle Symposium 2018.

5.2. Introduction

The green turtle (*Chelonia mydas*) is a predominantly herbivorous marine reptile found in tropical and subtropical regions of the world (Garnett et al., 1985, Pritchard et al., 1997). The complex life history of green turtles means that they inhabit a range of environments, including the oceanic zone and the neritic zone as well as the natal region from where they hatched (Aguirre and Lutz, 2004, Reich et al., 2007, Bell, 2013). As a result, managing and conserving this species is challenging. Due to a long-life span and strict foraging site fidelity, *C. mydas* face a relatively large array of anthropogenic and natural threats including, but not limited to, hunting, fishery bycatch incidents and disease (Bjorndal, 1995, Herbst and Klein, 1995, Wyneken, 2002, Van Houtan et al., 2010). A disease that holds significant concern for *C. mydas* populations is fibropapillomatosis (FP). FP is a transmissible neoplastic disease (Work et al., 2001) characterised by external papilloma-like lesions in soft tissue regions (eyes, neck and flippers) (Mascarenhas and Iverson, 2008) and, in severe cases, tumorous lesions, surrounding internal organs (Jacobson et al., 1989, Balazs and Pooley, 1991, Herbst, 1994, Mascarenhas and Iverson, 2008). Such lesions can be mechanically cumbersome, reducing sight and movement, (Balazs, 1991, Chaloupka and Balazs, 2005, Work et al., 2014) and may lead to organ failure (Smith and Coates, 1938, Balazs, 1986, Herbst, 1994, Quackenbush et al., 1998). FP prevalence in Australian *C. mydas* populations, has been reported to be between 0 and 70% as determined by visual assessment of lesions (Quackenbush et al., 1998, Aguirre et al., 2000, Limpus et al., 2005, Flint et al., 2010). While FP is considered enzootic to several sites (CB and EB) along the Great Barrier Reef (GBR) (Ariel et al., 2017b, Jones et al., 2020), little is currently known about the epizootiology of the disease.

Chelonid-alphaherpesvirus-5 (ChHV5) is considered the primary aetiological agent of FP (Davison and McGeoch, 2010), though a multi-factorial hypothesis is most likely, with environmental co-factors, such as toxic metals (Aguirre et al., 1994, da Silva et al., 2016) and elevated UV radiation (Duffy et al., 2018), potentially playing a significant aetiological role (Herbst et al., 1999, Aguirre and Lutz, 2004, Foley et al., 2005, dos Santos et al., 2010, Van Houtan et al., 2010, Duffy et al., 2018). Culturing of this virus has not been possible, until recently (Work et al., 2017), which has hampered efforts to test Koch's postulates for determining whether ChHV5 is the primary causative agent of FP (Lackovich et al., 1999, Lu et al., 1999, Coberley et al., 2001a, Work et al., 2009, Work et al., 2017). Like other herpesviruses, there is evidence that ChHV5 establishes a latent infection, with episodes of reactivation, particularly when the host becomes stressed or immunocompromised (Herbst et

al., 1995, Work et al., 2014, Ariel et al., 2017b). ChHV5 DNA is consistently detected in FP tumour tissue when applying quantitative PCR techniques (Quackenbush et al., 1998, Lackovich et al., 1999, Work et al., 2009, Page-Karjian et al., 2017). However, evidence of ChHV5 exposure has also been detected in individuals which do not have FP, when testing for DNA (Quackenbush et al., 2001, Page-Karjian et al., 2017, Page-Karjian et al., 2020a, Page-Karjian et al., 2020b) and through applying a serodiagnostic approach (Page-Karjian et al., 2020a, Page-Karjian et al., 2020b, Work et al., 2020).

Enzyme Linked Immunosorbent Assay (ELISA) offers an alternative to viral DNA detection by detecting specific antibody prevalence, which can give a larger window of opportunity to detect past presence of virus in a population. Antibody prevalence studies are a well-established technique in mammals (Yoshii et al., 2003, Inagaki et al., 2016, Yang et al., 2016) and sero-surveys are slowly gaining importance in reptiles as a method of testing for viral exposure (Coberley et al., 2001b, Allender et al., 2006, Graham et al., 2012, Ariel et al., 2017a, Page-Karjian et al., 2020a, Page-Karjian et al., 2020b, Work et al., 2020). An indirect ELISA, detecting ChHV5-specific antibodies in green turtles, has recently been developed by Work et al. (2020). Interestingly, Work et al. (2020) found that Atlantic turtles from Florida were uniformly seropositive to ChHV5, regardless of their lesion status. In contrast, in Pacific turtles from Hawaii, the same authors detected strong antibody reactivity mainly in animals with FP lesions, while only a lesser antibody response was recognized in non-lesioned individuals, independent of whether they lived in enzootic FP areas. Moreover, two different isotypes of turtle antibodies were addressed in the same study, i.e., 7S IgY, supposed to represent recent antigen exposure, and 5.7S IgY, whose emergence is believed to require chronic antigen exposure. Using monoclonal antibodies against these IgY types, Work et al. (2020) found that samples from Hawaiian turtles, with confirmed ChHV5-shedding, invoked a strong 5.7S reaction against ChHV5, while results for samples from non-shedders were often detected at lower antibody titers relative to shedding individuals. Furthermore, Page-Karjian et al. (2020b) conducted a comprehensive study on both wild free-ranging and rehabilitating *C. mydas* and *C. caretta* (Loggerhead) of Carolina, USA, also in the Atlantic ocean (like Florida), whereby qPCR was implemented to detect ChHV5 DNA and ELISA was conducted to measure for antibodies against ChHV5. The study found that none of the wild animals were positive for ChHV5 DNA, whereas a small proportion of the rehabilitating individuals sampled were positive for DNA and over half tested positive for antibodies against ChHV5. Similarly, (Page-

Karjian et al., 2020a) conducted a study investigating ChHV5 DNA and antibodies against the virus in nesting female adult *C. mydas* of another Florida population. It was reported that some wild turtles sampled were positive for DNA and approximately a third were positive for antibodies against ChHV5. These finds suggested that ChHV5 is enzootic and stable within this population.

Based on these previous studies, we hypothesized that the antibody reaction of Australian turtles against ChHV5 might be more like Hawaiian turtles than to Atlantic turtles. However, we also considered that Australian turtles may have their own way of interacting with ChHV5. To address these issues, we applied the same ELISA (Work et al., 2020) to measure the prevalence and extent of antibodies against ChHV5 F-US-4 (Ackermann et al., 2012) in turtles captured from the northern and central region of the GBR. We tested sera from *C. mydas* populations in FP enzootic as well as historically FP-free regions. This study is the first known survey of anti-ChHV5 antibody prevalence in turtles of the GBR, Australia.

5.3. Materials and Methods

5.3.1. Study locations

Five geographically distinct study sites were sampled along the east coast of Australia, adjacent to the GBR catchment area (Figure 5.1). The Howick's group of Islands and reefs (HWK) is a mid-shelf group of remote reefs found in the northern region of the GBR (14º 30 ' 11" S 144º 58 ' 26 " E). The HWK population is considered a relevant base line cohort when comparing health and pollution parameters in coastal populations (Villa et al., 2017), due to its location so far away from anthropogenic influence. Coastal sites included in the study were Toolakea beach (TLK), Cockle Bay (CB), Upstart Bay (UB) and Edgecombe Bay (EB) in north Queensland, Australia. Firstly, TLK (19° 08' 36" S, 146° 34' 56" E) is located 30 km north of Townsville is predominently inhabited by juvenile *C. mydas*, recently recruited from the pelagic zone. CB (19º 10 ' 26.7 " S 146 º 49 ' 32.1" E) is a bay of Magnetic Island, 13 km off the coast of Townsville. Within this area anthropogenic activity includes an international import-export port, metal refining, agricultural land practices and intensive coastal development. UB (19° 44 ' 44.4" S 147° 36 ' 03.8 " E) is located 150 km south of Townsville. While land use is also extensive in this region, human activity is less diverse, with agriculture being the major industry, though mining was previous conducted in the area. EB (20° 6′ 49″ S, 148° 23′ 25″ E) is located south of Bowen, approximately 200 km south of Townsville. Land use is similar to Upstart Bay. FP is historically enzootic to CB and EB (Ariel et al., 2017b,

Jones et al., 2020). Serum samples from *C. mydas* populations in EB were collected in the present study (EBp) and analysed alongside archived samples (data unpublished) collected in 2010 (EBa).

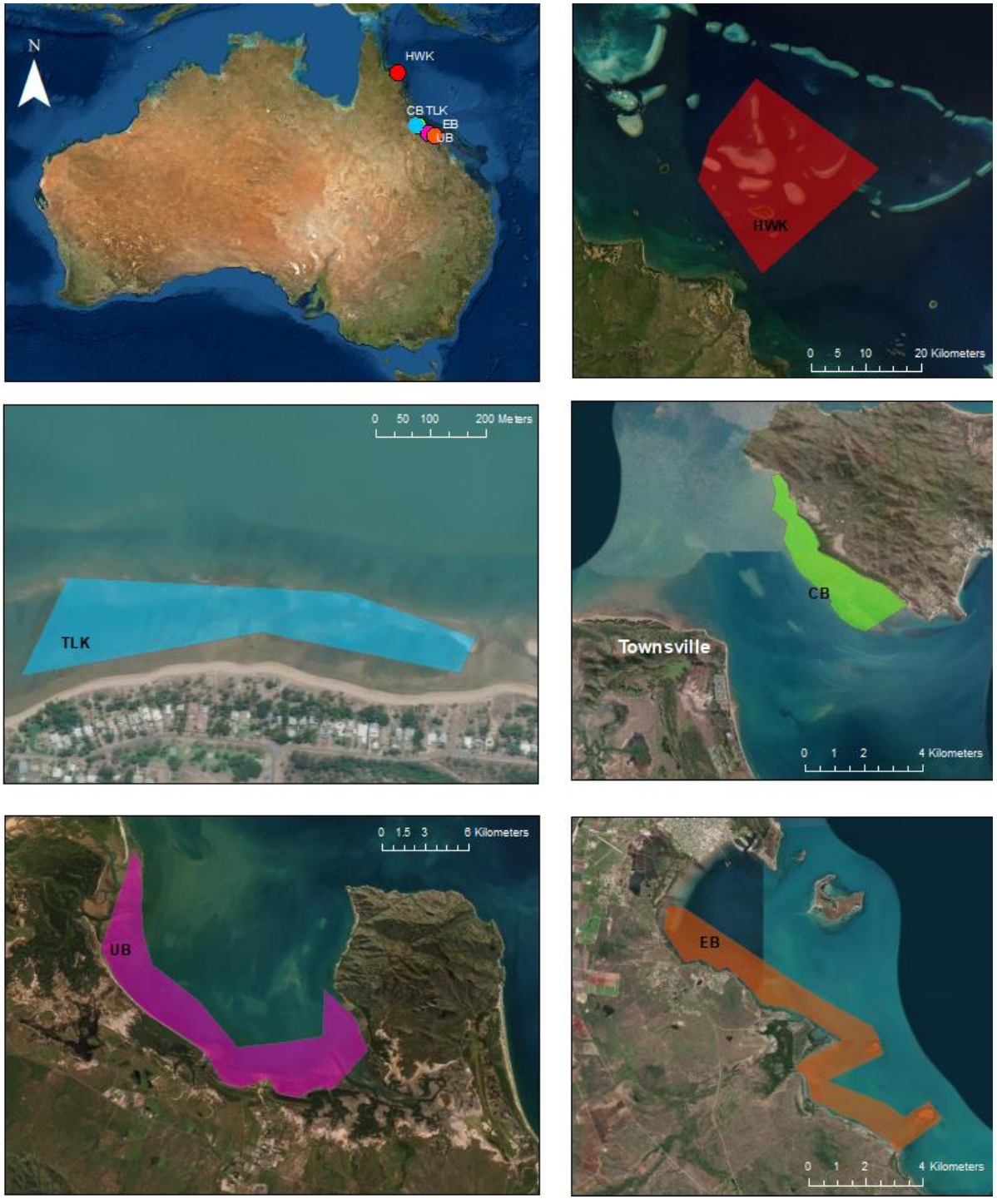


Figure 5.1. Map of five study areas where *C. mydas* were captured and sampled in this study. Abbreviations are defined as follows: HWK = the Howick's Group of Islands and Reefs, TLK = Toolakea Beach, CB = Cockle Bay, UB = Upstart Bay and EB = Edgecumbe Bay. Each site is colour coordinated between the national overview and localised maps. TLK and CB appear overlapped in the national overview but are geographically distinct on the local scale.

All research in the present study was carried out under permits from James Cook University Animals Ethics Committee (A2396), Department of Environment and Science (WISP18586417 and WISP18596817) and Great Barrier Reef Marine Parks Authority (G17/39429.1). The archived samples were collected under permits from James Cook University Animals Ethics Committee (A1501), Department of Environment and Science (WISP06619309) and Great Barrier Reef Marine Parks Authority (G10/33220.1). The negative control serum for the ELISA test was kindly made available from another study of captive *C. mydas* hatchlings which were collected and kept under permits from James Cook University Animals Ethics Committee (A2309) and Department of Environmental Protection (WITK15765815).

5.3.2. C. mydas capture

A total of 131 *C. mydas* were captured (HWK, n= 26; CB, n= 20; TLK, n= 20; UB, n= 25; EBp, n= 20), using turtle rodeo techniques in shallow near shore areas (Limpus and Reed, 1985) and beach capture methods in tidal pools for TLK turtles between 2017 and 2019. Additionally, a sample set of 20 turtles was included from archived serum samples collected in 2011 (EBa) (Limpus and Read, 1985).

5.3.3. Morphometric data

Individually numbered titanium flipper tags (Department of Environmental Sciences, Queensland Government) were applied as described by Eckert et al. (1999). Curved carapace length (CCL) was measured, using a flexible tape measure. Measurements were taken from the notch of the supracaudial scute to the line where skin joins carapace, along the midline ridge of the carapace, to the accuracy of ± 0.1 cm. Large barnacles were removed with long nose plyers if their position obstructed accurate CCL measurements. Most samples included in this study were from juveniles (up to 65 cm CCL) though several samples from sub-adults (65 to 95 cm CCL) and adults (> 90 cm) were also analysed (Table 5.1). A thorough visual inspection, of soft tissue areas, for FP lesion development was made for each turtle. None of the turtles captured during the current study were observed to be positive for FP. Six of the 20 archived serum samples derived from turtles positive for FP.

5.3.4. Serum collection

Up to 5 ml of blood was collected from the jugular vein of each turtle sampled. Blood was placed into microfuge tubes and stored on ice until return to the laboratory on the same day.

Upon return, samples were placed at 4°C overnight. All samples were then centrifuged for 3 minutes at 1610 *g* to separate serum from all other blood constituents and was stored in cryotube vials at -80 °C until analysis.

5.3.5. GST-tagged glycoprotein antigen production

A GST-tagged ChHV5 envelope glycoprotein, namely, F-US-4 exofuse C-myc-GST and the mock antigen, C-myc-GST were constructed by Willimann (2018) as described by Work et al. (2020). Glycoprotein DNA was identified from recombinant bacmid cloned baculovirus vectors and modified to include a C-terminal C-myc-GST tag, allowing efficient binding to the glutathione-conjugated casein (Sehr et al., 2001). Baculovirus DNA was then transfected into SF9 insect cells (Life Technology, USA) and cells were cultured until a MOI of 5 (4 x 10^7 cells per T150 flask + 40 ml SF900-type III Media) was reached. The cell supernatant was then harvested. Protein expression was measured via immunostaining of cells as well as western immunoblotting of cell and cell supernatants.

5.3.6. Purification of anti-turtle primary monoclonal antibodies

Two non-purified hybridoma supernatants containing anti-turtle IgY antibodies, anti-7s and anti-5.7s (or CO2 and D70 respectively), were provided by Thierry Work (United States Geological survey, Honolulu, USA). The protein fraction of the supernatant was purified by elution, with a protein G Hi Trap system (Sigma Aldrich, USA). Protein content of each purified fraction was measured by applying a BCA protein assay (Thermo Fisher Scientific, USA). The most concentrated fractions consistently read as having a protein content of greater than 250 μg/ml.

5.3.7. Indirect Enzyme-Linked immunosorbent Assay to measure ChHV5 IgY response

The GST-tagged glycoproteins and the purified anti-7s and 5.7 monoclonal antibodies (Work et al., 2020) were tested in several serial dilution validation assays. Anti-C-myc monoclonal antibodies were used to measure the binding properties of the antigens. Anti-turtle monoclonal antibodies were tested with selected known negative and positive turtle sera. Serial dilutions were tested for all assay reagents to establish the optimum ELISA conditions (Work et al., 2020).

ChHV5-specific IgY reactivity was measured by applying an indirect ELISA to all serum samples collected from each study site (Work et al., 2020). Briefly, 96 well Nunc Maxisorp immune plates (Thermo Fischer Scientific, USA) were coated with glutathione casein. Antigens (F-US-4 and C-myc-GST), at optimal dilutions (1:40 and 1:80, respectively) were added and plates incubated, for 1 hr at ambient temperature. Serum samples were diluted 1:25 in High salt (1M NaCl) phosphate buffer + 0.3% tween-20 with 2% milk powder. Plates were then incubated with anti-7s and 5.7s monoclonal antibodies (Work et al., 2015) at a dilution of 0.2 µg/ml in PBS-TM. Plates were then incubated with goat anti-mouse polyclonal antibodies (1:1000 dilution in PBS-TM). Colour development was achieved with the chromogen, 3, 3', 5, 5'- Tetramethylbenzidine (TMB, Thermo Scientific). Absorbance was measured at 650 nm after 25 minutes (Tecan Hydroflex Infinite F50 plate reader, Tecan, Switzerland). All reaction was stopped after 30 minutes with 0.5 M Phosphoric acid (H_3PO_4) and absorbance was measured again at 450 nm following a 10-minute incubation.

5.3.8. Controls used for indirect ELISA

For every serum sample analysed against antigen F-US-4 exofuse C-myc-GST, the same sample was analysed for the mock antigen, C-myc-GST control. By calculating the ΔOD (Delta Optical Density, F-US-4 OD minus C-myc-GST OD), any non-specific background was subtracted. The negative control sera were pooled from five captive *C. mydas* hatchlings kept in isolation for nine months post hatching. Negative control samples were included on each ELISA plate alongside the serum samples from free-living turtles.

5.3.9. Low and high responder cut off values

Gold standard cut off parameters were applied to calculate the negative cut off value for this study (Work et al., 2020). The low responder negative cut-off value was calculated for the pool of negative hatchling serum for each plate, as average ΔOD plus three times the standard deviation (3SD) of the average. Cut-off values for each plate were compared and were found to be within 0.1 of each other. All separate corresponding plate controls were then treated as replicates and averages were calculated. From this, a cut off value was calculated, allowing for comparison of all samples across all plates. In addition to this sensitivity-focused cut-off, a second cut-off value of 0.3 ΔOD was applied to enable comparison to previously published cut-off values (Lange et al., 2009, Work et al., 2020).

5.3.10. Data analysis

Prior to analysis, all data was tested for normality and relevant statistical tests were then chosen. All ΔOD data were transformed into categorical data (0 or 1) to best represent whether that sample was positive or negative for a response to ChHV5 antigen exposure, using the low responder negative cut-off value to determine positive or negative prevalence of ChHV5 antibodies. When stratifying the data to compare low and high responder sero-prevalence, data was transformed to three categories (0, 1 and 2) to represent three groups (negative ChHV5 IgY response), low responders (individuals with ΔOD greater than the low responder cut off) and high responders (>0.3), respectively. A Chi-squared of independence test (χ^2) was conducted to measure comparisons between prevalence data. In addition to prevalence comparisons, mean ΔOD values for all samples from each study site were compared using the Kruskal-Wallis test. For all outputs, statistical significance was deemed as a p value < 0.05. All statistical analysis was carried out in SPSS (IBM, USA).

5.4. Results

The mean, standard deviation, and range of CCL of turtles sampled per site is collated in Table 5.1. In all study sites, juvenile turtles (up to 65 cm CCL) were the size class most frequently encountered (between 56.4 and 100% of turtles captured at each site) when using turtle rodeo capture techniques. Briefly, the average CCL of all wild turtles sampled in this study was 50.5 cm, with the smallest individual being 36.8 cm CCL (TLK) and the largest being 116.0 cm CCL (HWK).

Table 5.1. Mean, standard deviation (SD) and min to max range of CCL (cm) of all turtles captured and sampled in this study per study site.

	TLK[1]	CB[2]	UB[3]	HWK[4]	EBp[5]	EBa[5]
Mean	45.1	45.4	52.0	51.6	60.5	48.1
SD	4.2	3.1	7.8	7.4	14.7	7.5
Range	36.8 - 56	40.7 - 54.8	42 - 104.8	40.7 - 116	42.9 - 93	41.2 - 75.1

[1]Toolakea beach; [2]Cockle Bay; [3]Upstart Bay (UB); [4]Howick's Islands; [5]Edgecombe Bay (p=present samples from 2019; a= archived samples from 2010)

5.4.1. Defining the true negative cut off value

A pool of sera from five 9-months old captive hatchlings was used to determine the negative cut-off values for our ELISAs. For this purpose, the F-US4 and, in control wells, the c-myc-GST antigen were bound to casein-glutathione-coated ELISA plates and the reactions were

measured as described in Materials and Methods separately by using monoclonal antibodies against either 7S or 5.7S turtles IgY. The results of three independent experiments are shown in Fig. 1.

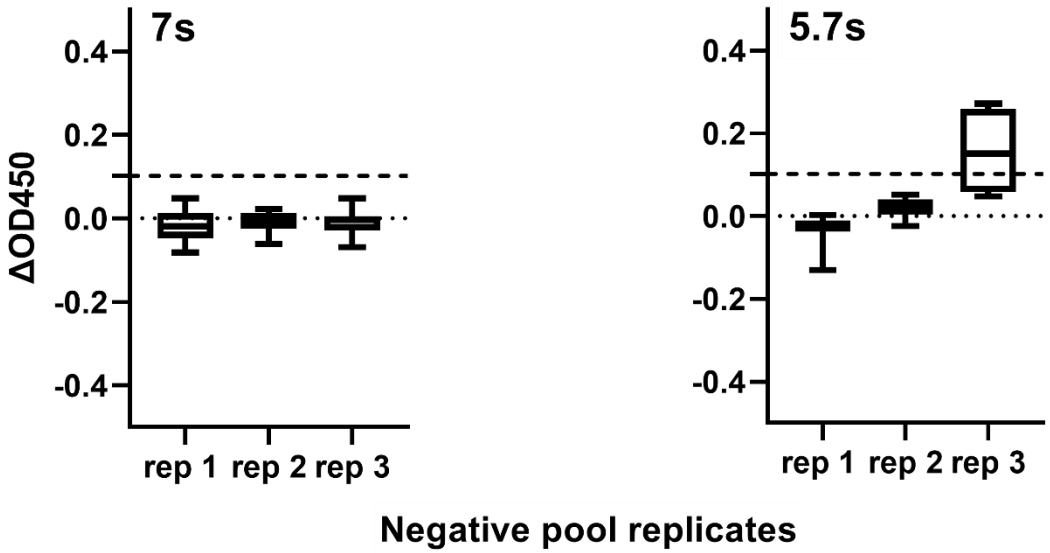


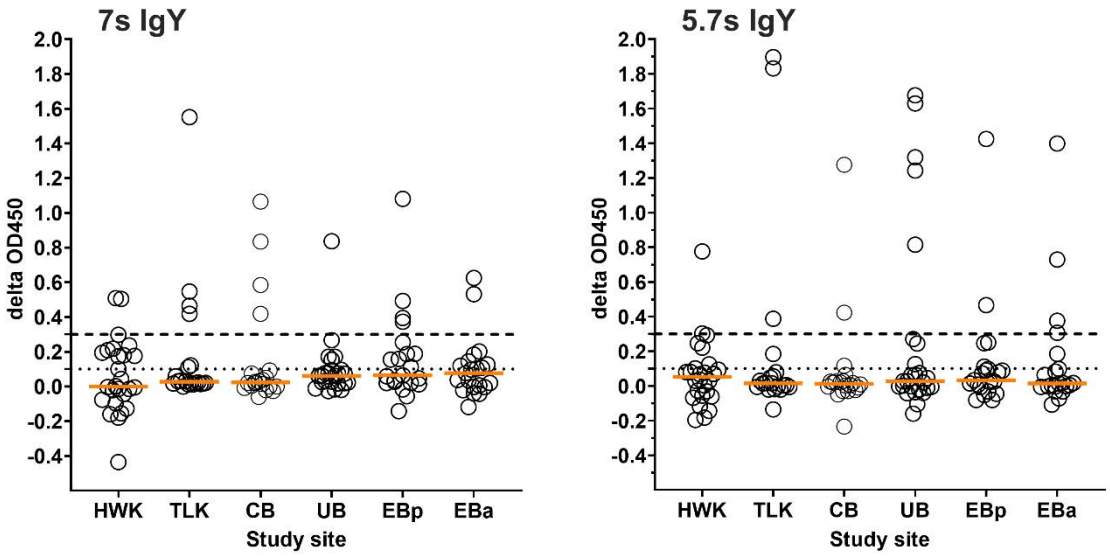
Figure 5.2. Box plots of the replicate (rep 1-3) findings of the negative control sample pool of captive hatchlings ΔOD450. Separate plots represent 7s and 5.7s IgY type, respectively. Box is 10 and 90 percentiles, whiskers are 1.5 interquartile range, and mid-line represents the mean. The dotted line defines 0 and the dashed line shows the negative cut off, 0.1 ΔOD450 (0.101, rounded to one decimal place), calculated from the data.

The majority of replicates of the negative controls in the pool, used to define the negative cut off value, were close to 0 ΔOD450, when assayed for 7s and 5.7s IgY against ChHV5 (Figure 5.2) and therefore were considered suitable to calculate the negative cut off. The mean ΔOD450 of all 7s replicates is consistent and indicative of a stable assay whereas the average ΔOD450 of the 5.7s are diverse. While the means of replicate one and two are close to 0 and whiskers overlap, the mean of replicate three exceeds zero and 0.1 ΔOD450 (the calculated cutoff value). Such variance may suggest issues with this assay. All negative control ΔOD450s were pooled per plate (and treat as replicates) and a box and whisker analysis was applied. The negative cut off value was calculated as the average negative control ΔOD450 average, both 7s and 5.7s IgY datasets combined, plus three standard deviations. In the study the true negative cut off was defined as 0.1 ΔOD450.

5.4.2. Indirect ELISA measuring anti-ChHV5 IgY

An indirect ELISA was conducted to measure the seroprevalence of a sample set of size matched turtles from five sites along the Northern and Central GBR (131 individuals in total,

111 current and 20 archived samples). All samples were tested for antibodies of two types of turtle specific IgY, 5.7s and 7s IgY. The two specified cut-off values (0.1 and 0.3 ΔOD450)



were applied to all data to calculate prevalence and to stratify positive samples into low and high responders.

Figure 5.3. Scatter plots showing all individual turtles (represented by empty circles) per site (HWK = the Howick group of islands and reefs, TLK = Toolakea beach, CB = Cockle Bay, UB = Upstart Bay and EB = Edgecumbe Bay). Sero-reactivity was displayed as ΔOD450 (nm) for both 7s and 5.7s IgY. The dashed line represents the high specificity cut off, 0.3 (high responders) and dotted line indicates the negative control cut off, 0.1 (low responders). The solid orange line, for each site, represents the average ΔOD450, of all turtles sampled within each site. Kruskal Wallace analysis was conducted to compare mean ΔOD450 between sites.

Seropositive individuals (turtles with ΔOD450 greater than the low responder cut off, 0.1) were detected at all study sites (Figure 5.3). When all serum samples were stratified to give three categories (negative, low, and high responders), the number of high responders in HWK was fewer than most other sites and ΔOD450 was lower, when compared to other sites (for both IgY types, Table 5.2). High ΔOD450 was detected in TLK though prevalence of high responders was low. At most sites (other than CB) for both IgY isotypes, the prevalence of low responders was greater than that for high responders to ChHV5 antibodies. In contrast, few low responders were detected in CB and most turtles positive for anti-ChHV5 antibodies at that site were high responders. When examining the total number of positive anti-ChHV5 7s IgY Ab individuals at all sites, the order of seroprevalence (from highest to lowest) was EBp (45%), HWK (38.5%), EBa (35%), TLK (25%), UB (20%) and CB (20%) (Table 5.2). In contrast, when looking at 5.7s IgY prevalence the order was as follows: UB (28%), EBp (25%), EBa (25%), HWK (19.2%), TLK (15%) and CB (10%) (Table 5.2). However, no statistical

significance was observed between seroprevalence at all FP sites for both IgY responses (χ^2, p= 0.163 for 7s and p= 0.876 for 5.7s). Furthermore, when the averages of each site ΔOD450 (after the 3SD cut off, 0.1, was subtracted) were compared, no significant difference was observed between any sites for 7s and 5.7s IgY responses (Kruskal-Wallis, p= 0.278 for 7s and p= 0.763 for 5.7s, respectively).

Table 5.2. Total and high responder (HR) seroprevalence of anti-ChHV5 antibodies at all sites (north to south; HWK = the Howick group of islands and reefs, TLK = Toolakea beach, CB = Cockle Bay, UB = Upstart Bay and EB = Edgecumbe Bay), in the current study. Separate percentages were calculated for anti-ChHV5 7s IgY and 5.7s IgY. HR seroprevalence is the percentage of the total number of samples for each site.

Site	7s seroprevalence (%)	7s HR (%)	5.7 seroprevalence (%)	5.7s HR (%)
HWK	38.5	17.7	19.2	7.7
TLK	25.0	20.0	15.0	15.0
CB	20.0	15.0	10.0	10.0
UB	20.0	4.0	28.0	16.0
EBp	45.0	20.0	25.0	0.0
EBa	35.0	10.0	25.0	15.0

5.4.3. Coastal turtle Anti-ChHV5 IgY seroprevalence compared with an offshore population

To best investigate whether ChHV5 prevalence differed between coastal and offshore *C. mydas* populations, mean seroprevalence (%) of all coastal sites with no history of FP (TLK and UB) and coastal sites with history of FP cases (CB and EBp) were calculated and compared against that of HWK (Figure 5.4). When the coastal sites (without FP history), TLK and UB were combined and surveyed for anti-ChHV5 7s IgY, an average ChHV5 seroprevalence of 22.5% was calculated. Coastal sites with FP case history (CB and EBp) had a slightly higher seroprevalence (32.5%) than other coastal sites but was lower than seroprevalence reported in the offshore control site, HWK (38.5%). For anti-ChHV5 5.7s IgY antibodies, the average seroprevalence of all coastal sites without FP history was 21.5%, which was higher than coastal sites with FP history (17.5%) and the offshore site (19.2%). No significant difference in prevalence was observed between all sites for both 7s and 5.7s responses (χ^2, p= 0.385 for 7s IgY and p= 0.837 for 5.7s IgY, respectively).

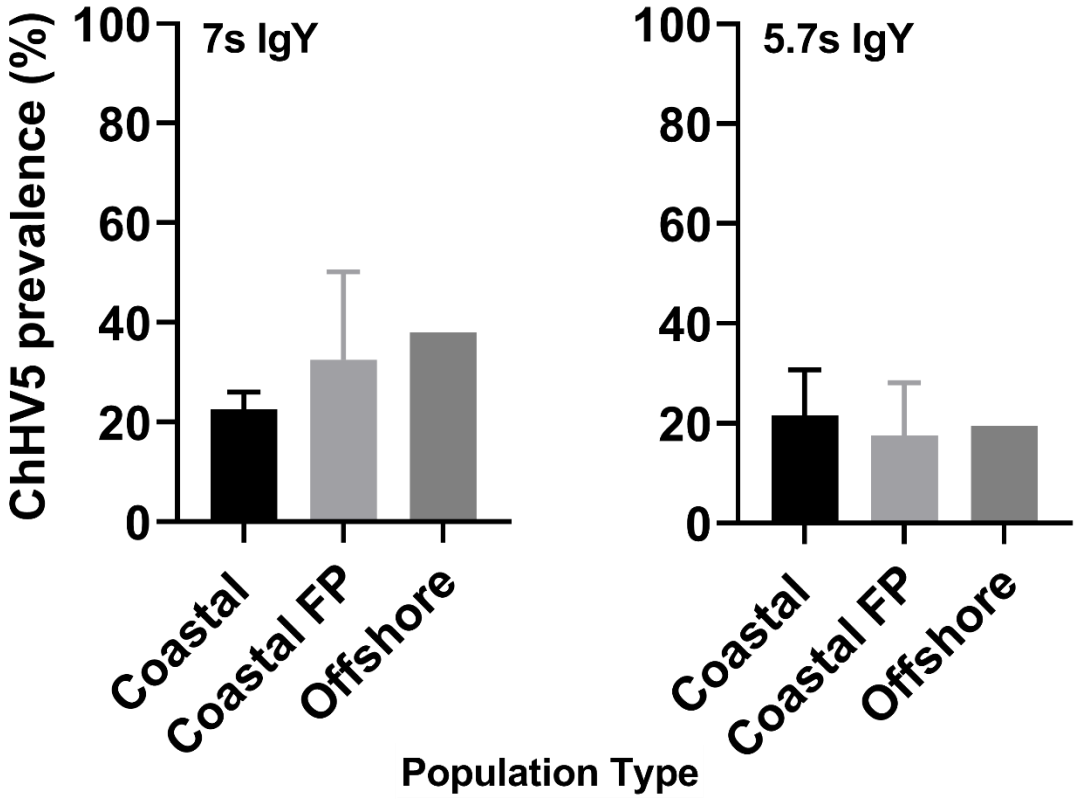


Figure 5.4 Average seroprevalence (%) of all coastal study sites, without FP history (TLK and UB), coastal sites which FP is enzootic (CB and EBp) compared to that of HWK (offshore). Seroprevalence of 7s and 5.7s IgY responses to ChHV5 presented separately. Error bars represent SD.

5.4.4. Variation in ChHV5 IgY seroprevalence between FP enzootic and non-enzootic study sites

Anti-ChHV5 7s IgY seroprevalence was slightly higher in FP enzootic sites (32.5%) for 7s compared to FP non-enzootic sites (27.8%, Figure 5.5). Anti-ChHV5 5.7s IgY seroprevalence was 19.0% in FP enzootic sites and 20.73% in FP non-enzootic study sites. These differences were not statistically significant (χ^2, p= 0.555 for 7s and p= 0.429 for 5.7s, respectively).

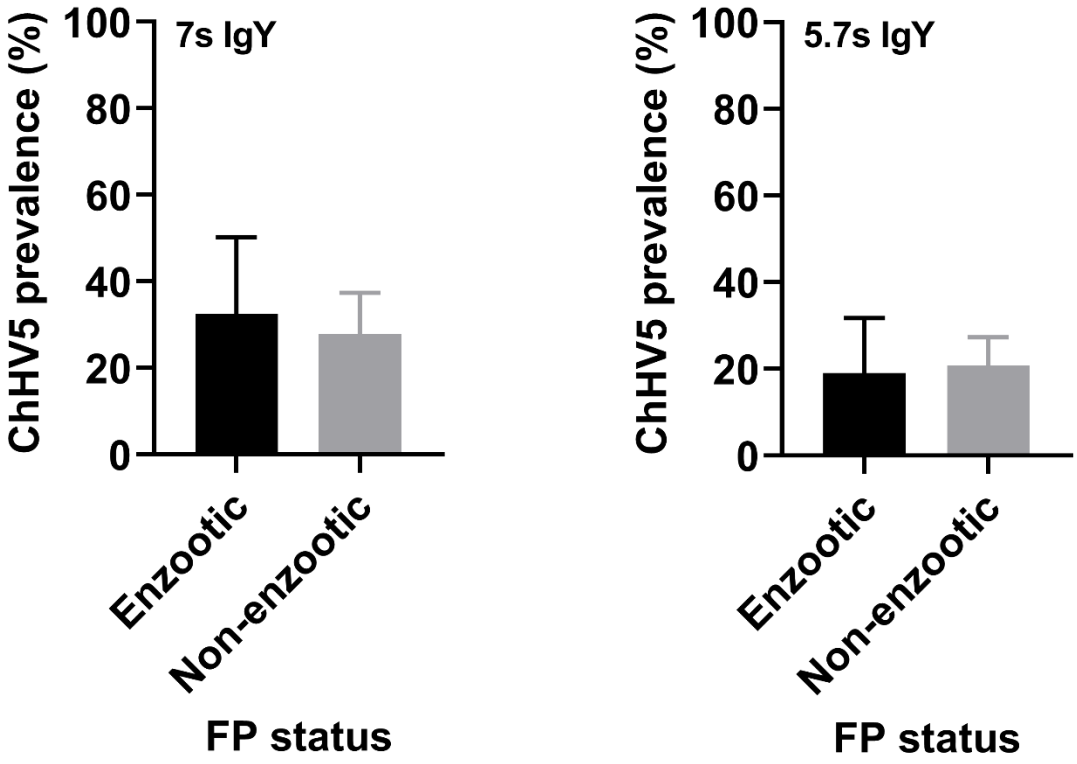


Figure 5.5. Bar charts representing the overall ChHV5 prevalence for sites where FP is known to be enzootic (CB and EBp) and for sites where FP lesions are yet to be reported (HWK, TLK and UB). Error bars represent SD. Prevalence for 7s and 5.7s IgYs against ChHV5 were measured for all sites.

5.4.5. Temporal variation in anti -ChHV5 IgY Seroprevalence

A marginally higher anti-ChHV5 7s IgY seroprevalence was reported between EB present (EBp, 45%) and archived (EBa, 35%, Figure 5.6). On the other hand, the prevalence of 5.7s response to ChHV5 was identical between data sets (25%). No significant difference in prevalence was determined, for either 7s and 5.7s IgY types (χ^2, p= 0.752 for 7s and p= 0.705 for 5.7s, respectively).

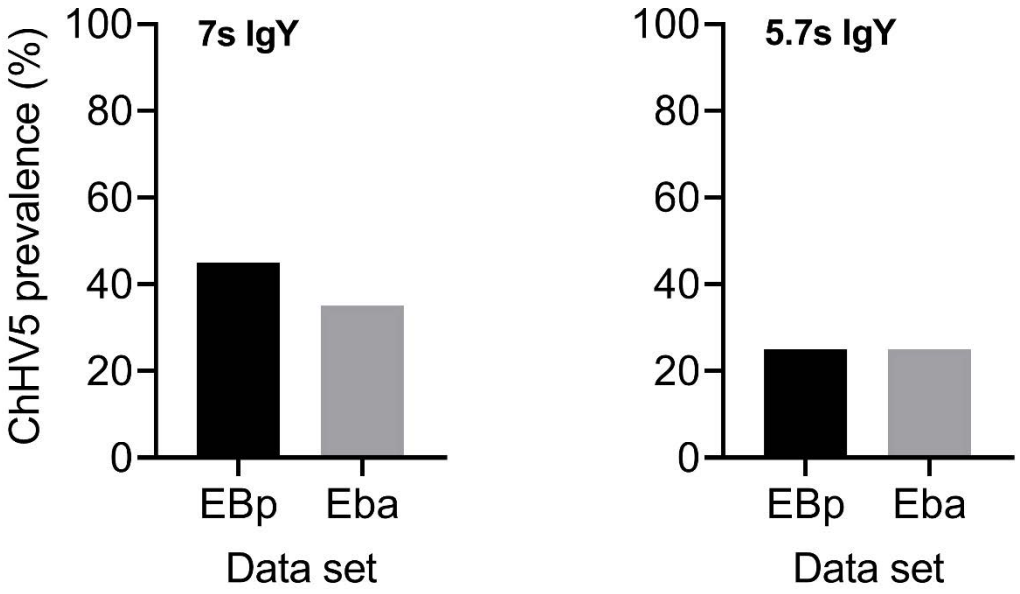


Figure 5.6. Bar charts representing the overall ChHV5 prevalence observed in EBp (serum samples collected from EB (Edgecumbe Bay) as part of this study) data and in EBa (samples collected from EB in 2011 and archived) Prevalence for 7s and 5.7s IgY against ChHV5 were measured for both time periods.

5.4.6. ChHV5 seroprevalence in turtles with and without FP lesions

None of the turtles included in this study (collected between 2017 and 2019) had visible FP lesions, at the time of sampling. A total of six (30%) green turtles sampled in 2010, from EB (EBa), were reported as positive for FP lesions at the time of capture.

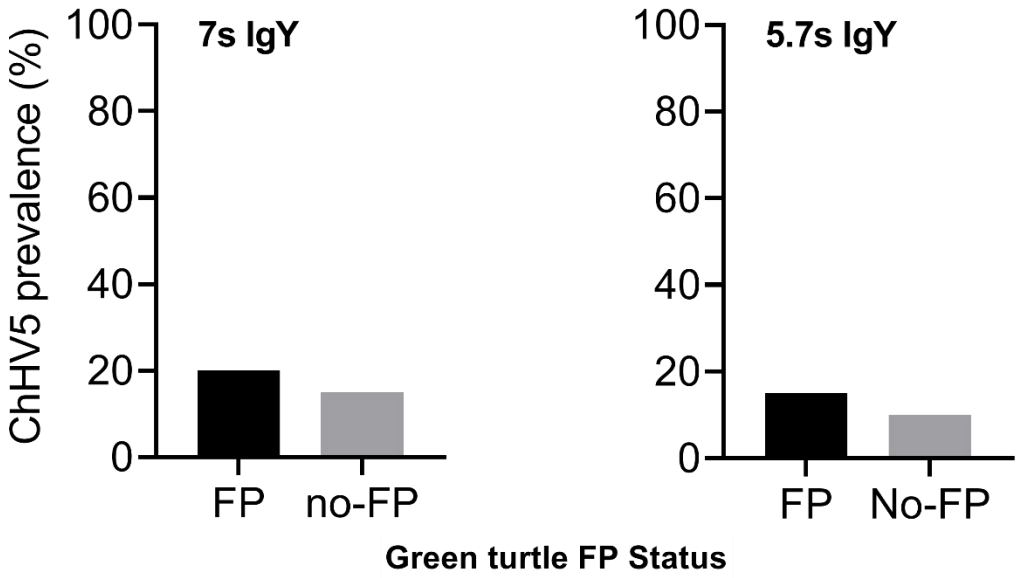


Figure 5.7. Bar charts representing the seroprevalence (%) of anti-ChHV5 antibodies observed in turtles with (n= 6) and without FP lesions (n=14), captured in 2010 (EBa data set). Seroprevalence data for 7s and 5.7s IgY against ChHV5 were reported.

Of the six turtles to be reported to have FP, 20 % tested positive for 7s IgYs and 15% were positive for 5.7s IgYs against ChHV5 (Figure 5.7.). In comparison, 15% and 10% of turtles without FP tested positive for 7s and 5.7s IgYs, respectively. As for all previous comparisons, no significant difference was determined between FP positive and FP negative green turtles, when investigating for both IgY subtypes (χ^2, p= 0.38 for 7s and p= 0.74 for 5.7s, respectively).

5.5. Discussion

Evidence points to ChHV5 as the likely primary aetiological agent of FP with a close association between tumour growth and the presence of ChHV5 DNA being observed (Lu et al., 2000, Quackenbush et al., 2001, Herbst et al., 2004, Ene et al., 2005, Duarte et al., 2012, Patricio et al., 2012). However, experimental data on ChHV5 pathogenesis and transmission is lacking, due to culturing of the virus only being recently achieved (Work et al., 2017). Additionally, the life history of *C. mydas* is complex, with an individual inhabiting a range of environments over time, making the study of ChHV5 epizootiology challenging (Jones et al., 2020). Mounting evidence supports the hypothesis that ChHV5 is horizontally transmitted upon juvenile turtles recruiting to the neritic zone (nearshore) foraging regions (Ene et al., 2005, Patricio et al., 2012, Jones et al., 2020). Serological detection of ChHV5 exposure

(through measuring for ChHV5-specific IgY responses) in wild turtles is, therefore, an efficient method for surveying the distribution and prevalence of the virus.

5.5.1. Comparing ChHV5 seroprevalence between global populations

Work et al. (2020) recently established a standard protocol for analysing ChHV5 IgY seroprevalence and this method was applied to Australian samples. By measuring ΔOD values using the specific ELISA protocol implemented in this study, comparisons can be made between sample sets within the region as well as between other research findings. For instance, by applying two distinct cut-offs: three times standard deviation (3SD) above the average ΔOD of the negative control pool (0.102 ΔOD) and an ΔOD greater than 0.3 as reported in Work *et al.* (Work et al., 2020), samples were stratified into high, low and negative responder groups. The ΔOD in this study represents either 7s or 5.7s turtle IgY binding to a specific ChHV5 antigen (surface glycoprotein, F-US-4 exofuse C-myc-GST).

This study revealed that 7s IgY seroprevalence ranged from 20–45% of turtles assayed whereas 5.7s IgY was only detected in 10–28% of individuals (Table 2). These findings suggest potential similarities between turtles of the local study area and results for Hawaiian green turtles. Work et al. (2020) reported that ChHV5 seroprevalence in Hawaii was 10-40% and 40–60% in Florida, for turtles without FP. Work et al. (2020), found that antibody titre positively correlated with FP lesion severity in Hawaii green turtles but not in Florida populations. Furthermore, Work et al. (2020) found that the number of Hawaiian anti-ChHV5 IgY high responders increased with increased average FP tumour score (severity of lesion growth) for each population investigated. In other words, turtles with the high tumour scores were likely to be high responders to ChHV5. This may explain why ChHV5 seroprevalence was not significantly different between sites in this study, since none of the turtles captured between 2017 and 2019 had visible FP lesions. Like the current study, Page-Karjian et al. (2020b) measured ChHV5 antibody prevalence in free ranging and rehabilitating marine turtles captured in a Carolina, USA population, all of which were observed to be negative for FP. This study recorded a ChHV5 antibody prevalence of 57% in the rehabilitating turtles, which was higher than the current study. Interestingly, ChHV5 antibody prevalence was greatest in *C. caretta* when compared to the *C. mydas* sampled. This study is the first known study to investigate ChHV5 antibodies in Loggerhead turtles. Additionally, (Page-Karjian et al., 2020a) also conducted ELISA to analyse ChHV5 antibody prevalence in nesting female *C. mydas* in Florida. The prevalence of ChHV5 antibodies detected in this study were more like those

reported in the current study, where 29% of animals tested positive for ChHV5 antibodies. No animals with FP were included in these studies and therefore little inference can be made as to whether ChHV5 antibody prevalence was uniform between turtles with and without FP tumours.

In the current study, turtle seropositive for IgYs (either 7s or 5.7s isotypes) to ChHV5 were predominantly classified as low responders (below 0.3 ΔOD cut off), though a small proportion of individuals were high responders within each study site (above 0.3 ΔOD). In Hawaii, 5.7s IgY high responders were rare in turtles without visible FP lesions. Both IgY isotypes studied here are indicative of different phases of the immune response to ChHV5. A detectable 7s IgY response likely infers recent exposure and possibly initial infection (similar to IgM in humans), whereas an elevated 5.7s IgY response suggests extended or latent ChHV5 viral exposure (similar to IgG function) (Work et al., 2020). It is implied then, that the absence of FP in high 5.7s IgY responding individuals, although the turtle has been exposed to the virus and may harbour a chronic infection, other environmental co-factors are crucial for the development of lesions (Herbst et al., 2008). For instance, a deterioration in conditions, such as an elevation in trace element pollution (Villa et al., 2017) and UV intensity (Duffy et al., 2018)), may cause FP development in the high 5.7s IgY responders (Work et al., 2014). As ChHV5 DNA is typically found in FP lesions, but rarely observed in other tissues, it is possible that viral shedding occurs predominantly when lesions first form and viral replication is highest, before ChHV5 becomes latent (Work et al., 2014).

Further confounding our knowledge, anti-ChHV5 5.7s IgY titres (ΔOD) may decline over time, during the latency phase. ChHV5, like other alphaherpesvirus may maintain viral replication in nerve ganglia cells of the host, at rates low enough to avoid detection by the host immune system (Rock, 1993, Koyuncu et al., 2017, Cohen, 2020). While it is unknown which cell types are targeted by the ChHV5 latent phase (Page-Karjian et al., 2015, Page-Karjian et al., 2017), a seronegative response may indicate that the turtle had either never been exposed to ChHV5 or that the IgY response to ChHV5 had fallen below the detectable threshold of the assay. Furthermore, a noticeable decline in FP prevalence has occurred within the study region in recent years and so too may have the number of ChHV5 high responders. Therefore, the prevalence of ChHV5 infected turtles may be higher than reported here. If seropositive 5.7s IgY responders (both high and low) were spatially and temporally mapped, insight into ChHV5

transmission pathways may be possible. This could be achieved by surveying the seroprevalence of IgYs in turtles at all known foraging grounds within an area, during multiple sampling events, including the study of recaptured individuals sampled more than once, over time. Re-capture data would enable the detection of any deterioration in 5.7s IgY response over time.

5.5.2. ChHV5 seroprevalence between coastal and offshore foraging grounds

No significant differences were found when seroprevalence of ChHV5 antibodies in samples from the offshore site, where FP has never been reported (HWK), were compared to samples from turtles inhabiting coastal foraging grounds known to harbour turtles with FP. One possible explanation is that the sample sizes used in this study were not large enough to be representative of each population and therefore further investigation into ChHV5 IgY seroprevalence should be conducted with greater sampling sizes at existing study sites. Furthermore, another explanation is that herpesvirus is ubiquitous, and the real cause of FP disease expression is something completely different from what previous research has considered. For instance, it is possible that another virus (instead of, or as well as ChHV5) is the primary aetiological agent of FP. In fact, Mashkour et al. (2018) detected a novel papillomavirus in FP tumour tissue of green turtles of the GBR. This line of investigation warrants further exploration to better understand FP disease expression.

At each of the GBR study sites, a subset of turtles (10-45%) had a detectable anti-ChHV5 IgY response indicating that serological detection of ChHV5 exposure is possible in turtles without FP. Environmental factors have been suggested to play a role in FP development, including elevated metal concentrations (Villa et al., 2015, da Silva et al., 2016, Villa et al., 2017), elevated UV (Duffy et al., 2018, Duffy and Martindale, 2019) and toxic algal blooms (Arthur et al., 2006). If these factors are below the threshold necessary to invoke a sufficient stress response in the turtles, FP disease expression may not occur, even if a turtle is infected with ChHV5. Investigation into *C. mydas* blood metal concentrations may provide additional information regarding FP lesion development at these study sites. To better understand the development of FP, a multifactorial approach to investigating environmental co-factors is crucial.

5.5.3. ChHV5 seroprevalence within FP enzootic study sites

FP is known to be enzootic to at least two sites of this study region (CB and EB, specifically Brisk bay) (Ariel et al., 2017b, Jones et al., 2020). However, prevalence appears to have declined in recent years and corresponds to mass mortality and stranding events in 2011. Cases were identified as emaciated green turtles from foraging grounds along the Queensland coast, which had been impacted, following a series of natural disasters, including cyclone Yasi (Bell and Ariel, 2011). The loss of seagrass beds likely impacted green turtle populations significantly, as high site fidelity is demonstrated, with the younger population demographic likely succumbing first. Turtles with FP lesions tend to be juveniles and therefore more vulnerable to starvation and the mortality event may have reduced the number of animals with FP and therefore the overall infection pressure on the population for years to come. When ChHV5 seroprevalence data for both FP enzootic sites were pooled and compared against the remaining sites (no previous FP case history), no significant difference was found for either 7s or 5.7s IgY assays. Statistical similarities observed in ChHV5 7s IgY seroprevalence between study sites may be indicative of an equal infection pressure at all sites. Which could infer a ubiquitous presence of ChHV5 throughout the GBR region.

5.5.4. ChHV5 seroprevalence between individuals with and without FP lesions

While FP is enzootic in EB, no significant difference in ChHV5 seroprevalence was observed over time. Between 2011 and 2016 FP prevalence in the entire bay was approximately 11% (Jones et al., 2020), though data recorded in the field for turtles captured in 2010 (EBa) indicated that 6 out of 20 (30% FP prevalence) turtles, had visible external lesion growth. One reason for the greater prevalence of FP reported for this cohort of turtles, when compared to the average FP prevalence for the wider population, is that capture and sampling effort was likely focused predominantly on the area of EB where a FP prevalence convergence zone (hotspot) is located (Jones, 2019). While FP prevalence data is available for these turtles, lesion severity (size and frequency) was not, therefore no assumptions can be made regarding the effect of FP severity on the level of ChHV5 IgY response in these populations. However, analysis was conducted here to compare seroprevalence of ChHV5 IgYs between those animals with and without FP lesions. Low seroprevalence of anti-ChHV5 IgYs was reported in turtles with and without FP for both IgY subtypes. 20% and 15% of individuals with FP were also seropositive for anti-ChHV5 IgYs (7s and 5.7s IgY, respectively). One potential reason for observing some turtles with FP testing negative for ChHV5 IgY could be due to a large amount of time passing between initial infection and the sampling conducted here. This may cause the

titre for each IgY to have previously peaked and since declined below the detection threshold of the assay. Similarly, 15% and 10% of individuals without FP were still positive for anti-ChHV5 IgYs (7s and 5.7s, respectively). This data supports the possibility that sole association to ChHV5 exposure is not adequate for the aetiology of FP and other factors (possibly viral or environmental) likely play significant role in disease expression (Mashkour et al., 2021). Such findings warrant further investigation into this association as well as any additional factors and stressors which may also play a role. Alternatively, as FP cases were only present in the archived sample set (EBa), the sample size may not be an adequate representation of the entire population and therefore a larger data set is necessary to better understand the relationship between FP and ChHV5. It is worth noting that some antibody degradation is possible in the archived samples over time, but the risk was considered to be minimal as the archived samples contained sera positive for ChHV5 IgYs and sera that was negative. Additionally, archived samples were maintained at -80 °C from collection until analysis with no freeze-thaw cycles, which is considered the gold standard for the long term storage of antibodies in mammal serum and plasma samples (Thachil et al., 2015), with -20°C also being suitable for long term storage of reptile samples (Ariel et al., 2017a).

Chelonia mydas are long lived and are thought to remain in local foraging grounds for much of their life (Hazel et al., 2013). If turtle populations from the GBR and Hawaii are exposed to similar viral subtypes and pathogenesis is indeed comparable, turtles with FP in the GBR may have a fatality rate of 70% (Bennett et al., 1999). As severity of FP disease expression was not considered at time of capture, it stands to reason that FP prevalence within this local population may have been higher than 30% with a large proportion potentially having died and therefore removed from the population before sampling. Juvenile turtles are believed to be exposed to ChHV5 upon recruitment to the neritic zone through horizontal transmission (Ene et al., 2005, Jones et al., 2020), when turtles with FP shed infected epidermal cells (Herbst et al., 2008, Page-Karjian et al., 2017, Yetsko et al., 2020). When considering potential for high death rates of infected animals and a consistent primary viral exposure rate, ChHV5 seroprevalence and infectivity of the virus appear to remain constant, even during periods of low FP lesion prevalence. Horizontal transmission of ChHV5 in juvenile *C. mydas* upon recruitment to coastal foraging grounds, where FP is endemic – and not from mother to egg, allows the assumption that the hatchling sera collected for use as the negative control pool were indeed

negative for ChHV5 antibodies. Such an assumption can be confidently made as these turtles had not been in contact with wild turtles or known FP hotspots prior to sample collection.

Future work should be focused on the epizootiological survey of local marine turtles (not just *C. mydas*) for FP lesions and ChHV5 viral exposure (7s and 5.7s IgY responses) simultaneously, with all data synthesised to map out regions which are potentially susceptible to increased FP expression. Additionally, suspected FP hotspots should be studied to better understand processes influencing convergence of individuals with the disease. The implementation of a dual methods approach may prove useful. While the ELISA applied here is a sensitive serological assay for measuring past exposure to ChHV5, the application of other tests, such as PCR (which tests for ChHV5 DNA, indicative of current infection) are also useful in the epizootiological study of viruses. Both techniques provide incomplete pictures of viral exposure and infectivity, thus the two techniques should be used in combination to provide greater diagnostic insight into past and present ChHV5 infection in turtles (Page-Karjian et al., 2020b). Finally, investigation into environmental co-factors and their impact on *C. mydas* health and disease is necessary to determine stressors which play a definitive role in the decline in immune function and local FP prevalence. If an inter-disciplinary and multi-factorial research approach is taken, our ability to predict and manage FP susceptible turtle populations could improve dramatically.

5.6. Conclusions

The chelonid alphaherpesvirus, ChHV5, is thought to be the primary etiological agent of the debilitating disease, FP, but seroprevalence of antibodies to this virus in local *C. mydas* populations of the GBR has not been investigated previously. This study found that a proportion of turtles (10-45%) at each site were seropositive for at least one of two known IgY isotypes (7s and 5.7s IgY) against the virus. No significant difference in seroprevalence was detected between turtle populations at any site irrespective of location being coastal (TLK, CB, UB, EBp) or offshore (HWK) sites, FP enzootic sites (CB and EBp) or sites with no reports of FP (HWK, TLK, UB). Finally, ChHV5 seroprevalence was statistically similar over time at one site (EB) in this study and no differences were found between individuals with and without FP. Findings here support the hypothesis that, stress from environmental co-factors or co-infections, in addition to ChHV5 infection, is necessary for the development of FP lesions in *C. mydas* inhabiting foraging grounds of the GBR.

5.7. Summary of contributions

The aim of this chapter was met in the following way:

Aim:

To survey and describe the seroprevalence of ChHV5-specific antibodies in *C. mydas* from coastal and offshore populations of the northern and central regions of the Great Barrier Reef.

How the aims were achieved

- I designed and developed the study in collaboration with my primary advisor.
- I prepared all permits required to conduct field work and sample collection (Animal ethics, GBRMPA and DES).
- I completed all necessary training to meet safety and proficiency requirements to work efficiently in the field (QLD recreational boat licence, 4x4 driving and turtle capture and sampling training).
- I enrolled and completed a post graduate intensive course in epidemiology and public health to best prepare me to conduct this study.
- With assistance from my primary advisor, I planned all aspects of field trips (Riskware risk analysis, volunteer insurance and training, logistics, administration).
- With assistance from my primary advisor, other field collaborators, fellow PhD students and a long list of volunteers, I conducted a series of field trips where I managed volunteer assistance, multiple capture teams and oversaw all aspects of animal handling and welfare during capturing and sampling.
- I inputted all data collected and appropriately stored all samples collected ready for sample analysis.
- Sara Kophamel and Dr. Ian Bell collected *C. mydas* serum samples and relevant morphometric data from the Howick Island group on my behalf and following my provided protocol.
- My primary advisor provided archived serum samples from EB for the inclusion in this study.
- The testing of all serum samples was done during a study visit to the University of Zurich (UZH), Switzerland. I collaborated with Prof. Mathias Ackermann at the Institute of Virology at UZH to validate and conduct the ELISA protocol.

- Conducted the assay by following previously published protocols to allow for comparison between sites within this study but also with previous findings from Hawaiian *C. mydas* populations.
- Funding of this analysis was provided by a successful CPHMVS HDRE grant application, use of student research funds (SSA) and awarded funds from a WWF grant.
- With assistance from my advisors and Rhondda Jones I conducted all statistical analysis.
- I created the seminar which I presented at the Australian Marine Turtle Symposium 2018 discussing the findings of this study.
- I drafted all aspects of this chapter and with assistance of my advisors, other PhD students and various volunteers I edited and finalised the chapter for inclusion in the thesis.

Summary of outcomes

This study found that seroprevalence of ChHV5 antibodies were comparable between all sites surveyed here. Similarly, average seroprevalence findings were similar to that of *C. mydas* investigated in a Hawaiian population (Work et al., 2020).

5.8. References

ACKERMANN, M., KORIABINE, M., HARTMANN-FRITSCH, F., DE JONG, P. J., LEWIS, T. D., SCHETLE, N., WORK, T. M., DAGENAIS, J., BALAZS, G. H. & LEONG, J.-A. C. 2012. The Genome of Chelonid Herpesvirus 5 Harbors Atypical Genes. *PLOS ONE,* 7**,** e46623.

AGUIRRE, A., LIMPUS, C., SPRAKER, T. & BALAZS, G. Survey of fibropapillomatosis and other potential diseases in marine turtles from Moreton Bay, Queensland, Australia. *In:* KALB, H. & WIBBELS, T., eds. Proceedings of the Nineteenth Annual Symposium on Sea Turtle Conservation and Biology. H. Kalb and T. Wibbels. South Padre Island, Texas, 2000. U.S. Depatment of Commerce, NOAA Technical Memorandum NMFS-SEFSC, 36.

AGUIRRE, A. A., BALAZS, G. H., ZIMMERMAN, B. & GALEY, F. D. 1994. Organic contaminants and trace metals in the tissues of green turtles (*Chelonia mydas*) afflicted with fibropapillomas in the Hawaiian islands. *Marine Pollution Bulletin,* 28**,** 109–114.

AGUIRRE, A. A. & LUTZ, P. L. 2004. Marine Turtles as Sentinels of Ecosystem Health: Is Fibropapillomatosis an Indicator? *EcoHealth,* 1**,** 275–283.

ALLENDER, M. C., MITCHELL, M. A., PHILLIPS, C. A., GRUSZYNSKI, K. & BEASLEY, V. R. 2006. Hematology, plasma biochemistry, and antibodies to select

viruses in wild-caught eastern massasauga rattlesnakes (*Sistrurus catenatus catenatus*) from Illinois. *Journal of Wildlife Diseases,* 42**,** 107–114.

ARIEL, E., ELLIOTT, E., MEDDINGS, J. I., MILLER, J., SANTOS, M. B. & OWENS, L. 2017a. Serological survey of Australian native reptiles for exposure to ranavirus. *Diseases of Aquatic Organisms,* 126**,** 173–183.

ARIEL, E., NAINU, F., JONES, K., JUNTUNEN, K., BELL, I., GASTON, J., SCOTT, J., TROCINI, S. & BURGESS, G. 2017b. Phylogenetic variation of chelonid alphaherpesvirus 5 in green turtle (*Chelonia mydas*) populations along the Queensland Coast, Australia. *Journal of Aquatic Animal Health***,** 150–157.

ARTHUR, K., SHAW, G., LIMPUS, C. & UDY, J. 2006. A review of the potential role of tumour-promoting compounds produced by Lyngbya majuscula in marine turtle fibropapillomatosis. *African Journal of Marine Science,* 28**,** 441–446.

BALAZS, G. & POOLEY, S. Research plan for marine turtle fibropapilloma: results of a December

1990 workshop. . NOAA-TMNMFS-SWFC-156., 1991 Hawaii, USA. Department of Commerce, National Oceanographic and Atmospheric Administration.

BALAZS, G. H. 1986. Fibropapillomas in Hawaiian green turtles. *Marine Turtle Newsletter,* 39**,** 1-3.

BALAZS, G. H. 1991. Current status of fibropapillomas in the Hawaiian green turtle,*Chelonia mydas*. *Research Plan for Marine Turtle Fibropapilloma (Balazs GH, Pooley SG, eds). Report No. NOAA-TM-NMFS-SWFSC-156. Honolulu: US Department of Commerce, National Oceanographic and Atmospheric Administration, National Marine Fisheries Service***,** 47-57.

BELL, I. 2013. Algivory in hawksbill turtles: *Eretmochelys imbricata* food selection within a foraging area on the Northern Great Barrier Reef. *Marine Ecology,* 34**,** 43–55.

BELL, I. & ARIEL, E. 2011. Dietry shifts in green turtles. *Seagrass- Watch.* Queensland, Australia: Seagrass- Watch HQ.

BENNETT, P., KEUPER-BENNETT, U. & BALAZS, G. Photographic evidence for the regression of 447 fibropapillomas afflicting green turtles at Honokawai, Maui, in the Hawaiian islands. Proc 19th Ann Symp SeaTurtle Biol Conservation,, 1999 South Padre Island, TX. 37–39.

BJORNDAL, K. A. Biology and conservation of sea turtles. World conference on sea turtle conservation, 1995 Washington D.C., USA. Smithsonian Institution Press Washington.

CHALOUPKA, M. & BALAZS, G. 2005. Modelling the effect of fibropapilloma disease on the somatic growth dynamics of Hawaiian green sea turtles. *Marine Biology,* 147**,** 1251–1260.

COBERLEY, S., S, HERBST, L., H, EHRHART, L., M, BAGLEY, D., A, HIRAMA, S., JACOBSON, E., R & KLEIN, P., A 2001a. Survey of Florida green turtles for exposure to a disease-associated herpesvirus. *Diseases of Aquatic Organisms,* 47**,** 159–167.

COBERLEY, S. S., HERBST, L. H., BROWN, D. R., EHRHART, L. M., BAGLEY, D. A., SCHAF, S. A., MORETTI, R. H., JACOBSON, E. R. & KLEIN, P. A. 2001b. Detection of Antibodies to a Disease-Associated Herpesvirus of the Green Turtle, <em>Chelonia mydas</em&gt. *Journal of Clinical Microbiology,* 39**,** 3572.

COHEN, J. I. 2020. Herpesvirus latency. *The Journal of Clinical Investigation,* 130**,** 3361–3369.

DA SILVA, C. C., KLEIN, R. D., BARCAROLLI, I. F. & BIANCHINI, A. 2016. Metal contamination as a possible etiology of fibropapillomatosis in juvenile female green

sea turtles *Chelonia mydas* from the southern Atlantic Ocean. *Aquatic Toxicology,* 170**,** 42–51.

DAVISON, A. & MCGEOCH, D. 2010. Create genus Scutavirus (type species: the currently unassigned species Chelonid herpesvirus 5) in subfamily Alphaherpesvirinae, family Herpesviridae. Code.

DOS SANTOS, R. G., MARTINS, A. S., TOREZANI, E., BAPTISTOTTE, C., FARIAS, J. D. N., HORTA, P. A., WORK, T. M. & BALAZS, G. H. 2010. Relationship between fibropapillomatosis and environmental quality: a case study with *Chelonia mydas* off Brazil. *Dis Aquat Organ,* 89**,** 87–95.

DUARTE, A., FAÍSCA, P., LOUREIRO, N. S., ROSADO, R., GIL, S., PEREIRA, N. & TAVARES, L. 2012. First histological and virological report of fibropapilloma associated with herpesvirus in *Chelonia mydas* at Príncipe Island, West Africa. *Archives of Virology,* 157**,** 1155–1159.

DUFFY, D. J. & MARTINDALE, M. Q. 2019. Perspectives on the expansion of human precision oncology and genomic approaches to sea turtle fibropapillomatosis. *Communications Biology,* 2**,** 54.

DUFFY, D. J., SCHNITZLER, C., KARPINSKI, L., THOMAS, R., WHILDE, J., EASTMAN, C., YANG, C., KRSTIC, A., ROLLINSON, D., ZIRKELBACH, B., YETSKO, K., BURKHALTER, B. & MARTINDALE, M. Q. 2018. Sea turtle fibropapilloma tumors share genomic drivers and therapeutic vulnerabilities with human cancers. *Communications Biology,* 1**,** 63.

ECKERT, K., BJORNDAL, K. A., ABREU-GROBOIS, F. A. & DONNELLY, M. 1999. Research and management techniques for the conservation of sea turtles. Washington, DC: IUCN/SSC Marine Turtle Specialist Group.

ENE, A., SU, M., LEMAIRE, S., ROSE, C., SCHAFF, S., MORETTI, R., LENZ, J. & HERBST, L. H. 2005. Distribution of chelonid fibropapillomatosis-associated herpesvirus variants in Florida: molecular genetic evidence for infection of turtles following recruitment to neritic developmental habitats. *J Wildl Dis,* 41**,** 489–495.

FLINT, M., PATTERSON-KANE, J. C., LIMPUS, C. J. & MILLS, P. C. 2010. Health Surveillance of Stranded Green Turtles in Southern Queensland, Australia (2006–2009): An Epidemiological Analysis of Causes of Disease and Mortality. *EcoHealth,* 7**,** 135–145.

FOLEY, A. M., SCHROEDER, B. A., REDLOW, A. E., FICK-CHILD, K. J. & TEAS, W. G. 2005. Fibropapillomatosis in stranded green turtles (*Chelonia mydas*) from the eastern United States (1980–98): trends and associations with environmental factors. *J Wildl Dis,* 41**,** 29–41.

GARNETT, S., PRICE, I. & SCOTT, F. 1985. The Diet of the Green Turtle, *Chelonia Mydas* (L.), In Torres Strait. *Wildlife Research,* 12**,** 103–112.

GRAHAM, S. P., HASSAN, H. K., CHAPMAN, T., WHITE, G., GUYER, C. & UNNASCH, T. R. 2012. Serosurveillance of Eastern Equine Encephalitis Virus in Amphibians and Reptiles from Alabama, USA. *The American Journal of Tropical Medicine and Hygiene,* 86**,** 540–544.

HAZEL, J., HAMANN, M. & LAWLER, I. R. 2013. Home range of immature green turtles tracked at an offshore tropical reef using automated passive acoustic technology. *Marine Biology,* 160**,** 617–627.

HERBST, L. H. 1994. Fibropapillomatosis of marine turtles. *Ann Rev Fish Dis,* 4**,** 389–425.

HERBST, L. H., ENE, A., SU, M., DESALLE, R. & LENZ, J. 2004. Tumor outbreaks in marine turtles are not due to recent herpesvirus mutations. *Curr Biol,* 14**,** 697–699.

HERBST, L. H., JACOBSON, E. R., KLEIN, P. A., BALAZS, G. H., MORETTI, R., BROWN, T. & SUNDBERG, J. P. 1999. Comparative pathology and pathogenesis of

spontaneous and experimentally induced fibropapillomas of green turtles (*Chelonia mydas*). *Vet Pathol,* 36**,** 551–564.

HERBST, L. H., JACOBSON, E. R., MORETTI, R., BROWN, T., SUNDBERG, J. P. & KLEIN, P. A. 1995. Experimental transmission of green turtle fibropapillomatosis using cell-free tumor extracts. *Dis Aquat Organ,* 22**,** 1–12.

HERBST, L. H. & KLEIN, P. A. 1995. Green turtle fibropapillomatosis: challenges to assessing the role of environmental cofactors. *Environmental Health Perspectives* 103**,** 27–30.

HERBST, L. H., LEMAIRE, S., ENE, A. R., HESLIN, D. J., EHRHART, L. M., BAGLEY, D. A., KLEIN, P. A. & LENZ, J. 2008. Use of baculovirus-expressed glycoprotein H in an enzyme-linked immunosorbent assay developed to assess exposure to chelonid fibropapillomatosis-associated herpesvirus and its relationship to the prevalence of fibropapillomatosis in sea turtles. *Clin Vaccine Immunol,* 15**,** 843–851.

INAGAKI, E., SAKAI, M., HIRANO, M., MUTO, M., KOBAYASHI, S., KARIWA, H. & YOSHII, K. 2016. Development of a serodiagnostic multi-species ELISA against tick-borne encephalitis virus using subviral particles. *Ticks and Tick-borne Diseases,* 7**,** 723–729.

JACOBSON, E. R., MANSELL, J. L., SUNDBERG, J. P., JAJJAR, L., REICHMANN, M. E., EHRHART, L. M., WALSH, M. & MURRU, F. 1989. Cutaneous fibropapillomas of green turtles (*Chelonia mydas*). *J Comp Pathol,* 101**,** 39–52.

JONES, K. 2019. *Environmental influences on the epidemiology of fibropapillomatosis in green turtles (Chelonia mydas) and consequences for management of inshore areas of the Great Barrier Reef.* Ph.D., James Cook University.

JONES, K., BURGESS, G., BUDD, A. M., HUERLIMANN, R., MASHKOUR, N. & ARIEL, E. 2020. Molecular evidence for horizontal transmission of chelonid alphaherpesvirus 5 at green turtle (*Chelonia mydas*) foraging grounds in Queensland, Australia. *PLOS ONE,* 15**,** e0227268.

KOYUNCU, O. O., MACGIBENY, M. A., HOGUE, I. B. & ENQUIST, L. W. 2017. Compartmented neuronal cultures reveal two distinct mechanisms for alpha herpesvirus escape from genome silencing. *PLOS Pathogens,* 13**,** e1006608.

LACKOVICH, J. K., BROWN, D. R., HOMER, B. L., GARBER, R. L., MADER, D. R., MORETTI, R. H., PATTERSON, A. D., HERBST, L. H., OROS, J., JACOBSON, E. R., CURRY, S. S. & KLEIN, P. A. 1999. Association of herpesvirus with fibropapillomatosis of the green turtle *Chelonia mydas* and the loggerhead turtle *Caretta caretta* in Florida. *Dis Aquat Organ,* 37**,** 89–97.

LANGE, C. E., TOBLER, K., FAVROT, C., MÜLLER, M., NÖTHLING, J. O. & ACKERMANN, M. 2009. Detection of Antibodies against Epidermodysplasia Verruciformis-Associated Canine Papillomavirus 3 in Sera of Dogs from Europe and Africa by Enzyme-Linked Immunosorbent Assay. *Clinical and Vaccine Immunology,* 16**,** 66.

LIMPUS, C. & READ, M. 1985. The Green sea turtle, *Chelonia mydas,* in Queensland: a preliminary description of the population structure of a coral reef feeding ground. *In:* GRIGG, G. C., SHINE, R. & EHMANN, H. (eds.) *Biology of Austrlian Frogs and Reptiles.* Sydney, NSW: Royal Zoological Society

LIMPUS, C. & REED, P. 1985. The green turtle, *Chelonia mydas*, in Queensland: a preliminary description of the population structure in a coral reef feeding groud. *Biology of Australasian Frogs and Reptiles***,** 47–52.

LIMPUS, C. J., LIMPUS, D. J., ARTHUR, K. E. & PARMENTER, C. J. 2005. Monitoring green turtle population dynamics in Shoalwater Bay 2000–2004. *In:* AUTHORITY, G. B. R. M. P. (ed.). Townsville, Australia.

LU, Y., NERURKAR, V. R., AGUIRRE, A. A., WORK, T. M., BALAZS, G. H. & YANAGIHARA, R. 1999. Establishment and characterization of 13 cell lines from a green turtle (*Chelonia mydas*) with fibropapillomas. *In Vitro Cellular & Developmental Biology - Animal,* 35**,** 389–393.

LU, Y., WANG, Y., YU, Q., AGUIRRE, A. A., BALAZS, G. H., NERURKAR, V. R. & TANAGIHARA, R. 2000. Detection of herpesviral sequences in tissues of green turtles with fibropapilloma by polymerase chain reaction. *Arch Virol,* 145.

MASCARENHAS, R. & IVERSON, P. J. 2008. Fibropapillomatosis in stranded green turtles (*Chelonia mydas*) in Paraiba State, Northeastern Brazil: evidence of a Brazilian epizootic. *Marine Turtle Newsletter,* 120**,** 3–6.

MASHKOUR, N., JONES, K., WIRTH, W., BURGESS, G. & ARIEL, E. 2021. The Concurrent Detection of Chelonid Alphaherpesvirus 5 and Chelonia mydas Papillomavirus 1 in Tumoured and Non-Tumoured Green Turtles. *Animals,* 11**,** 697.

MASHKOUR, N., MACLAINE, A., BURGESS, G. W. & ARIEL, E. 2018. Discovery of an Australian *Chelonia mydas* papillomavirus via green turtle primary cell culture and qPCR. *Journal of Virological Methods,* 258**,** 13–23.

PAGE-KARJIAN, A., CHABOT, R., STACY, N., MORGAN, A. S., VALVERDE, R., STEWART, S., COPPENRATH, C., MANIRE, C. A., HERBST, L. H., GREGORY, C. R., RITCHIE, B. W. & PERRAULT, J. 2020a. Comprehensive health assessment of green turtles Chelonia mydas nesting in southeastern Florida. *Endangered Species Research,* 42.

PAGE-KARJIAN, A., GOTTDENKER, N. L., WHITFIELD, J., HERBST, L., NORTON, T. M. & RITCHIE, B. 2017. Potential Noncutaneous Sites of Chelonid Herpesvirus 5 Persistence and Shedding in Green Sea Turtles *Chelonia mydas*. *Journal of Aquatic Animal Health,* 29**,** 136–142.

PAGE-KARJIAN, A., NORTON, T. M., RITCHIE, B., BROWN, C., MANCIA, C., JACKWOOD, M. & GOTTDENKER, N. L. 2015. Quantifying chelonid herpesvirus 5 in symptomatic and asymptomatic rehabilitating green sea turtles. *Endangered Species Research,* 28**,** 135–146.

PAGE-KARJIAN, A., SERRANO, M. E., CARTZENDAFNER, J., MORGAN, A., RITCHIE, B. W., GREGORY, C. R., MCNEILL, J. B., PERRAULT, J. R., CHRISTIANSEN, E. F. & HARMS, C. A. 2020b. Molecular Assessment of Chelonid Alphaherpesvirus 5 Infection in Tumor-Free Green (*Chelonia mydas*) and Loggerhead (*Caretta caretta*) Sea Turtles in North Carolina, USA, 2015–2019. *Animals,* 10**,** 1964.

PATRICIO, A. R., HERBST, L. H., DUARTE, A., VELEZ-ZUAZO, X., SANTOS LOUREIRO, N., PEREIRA, N., TAVARES, L. & TORANZOS, G. A. 2012. Global phylogeography and evolution of chelonid fibropapilloma-associated herpesvirus. *J Gen Virol,* 93**,** 1035–1045.

PRITCHARD, P., LUTZ, P. & MUSICK, J. 1997. Evolution, phylogeny, and current status. *In:* LUTZ, P. L. & MUSICK, J. A. (eds.) *The biology of sea turtles.* London, England: CRC Press.

QUACKENBUSH, S. L., CASEY, R. N., MURCEK, R. J., PAUL, T. A., WORK, T. M., LIMPUS, C. J., CHAVES, A., DU TOIT, L., PEREZ, J. V., AGUIRRE, A. A., SPRAKER, T. R., HORROCKS, J. A., VERMEER, L. A., BALAZS, G. H. & CASEY, J. W. 2001. Quantitative analysis of herpesvirus sequences from normal tissue and fibropapillomas of marine turtles with real-time PCR. *Virology,* 287**,** 105–111.

QUACKENBUSH, S. L., WORK, T. M., BALAZS, G. H., CASEY, R. N., ROVNAK, J., CHAVES, A., DUTOIT, L., BAINES, J. D., PARRISH, C. R., BOWSER, P. R. &

CASEY, J. W. 1998. Three closely related herpesviruses are associated with fibropapillomatosis in marine turtles. *Virol,* 246**,** 392–399.

REICH, K. J., BJORNDAL, K. A. & BOLTEN, A. B. 2007. The 'lost years' of green turtles: using stable isotopes to study cryptic lifestages. *Biology letters,* 3**,** 712–714.

ROCK, D. L. 1993. The molecular basis of latent infections by alphaherpesviruses. *Seminars in Virology,* 4**,** 157–165.

SEHR, P., ZUMBACH, K. & PAWLITA, M. 2001. A generic capture ELISA for recombinant proteins fused to glutathione S-transferase: validation for HPV serology. *Journal of Immunological Methods,* 253**,** 153-162.

SMITH, G. & COATES, C. 1938. Fibro-epithelial growths of the skin in large marine turtles, *Chelonia mydas* (Linnaeus). *Zoologica,* 23**,** 93–98.

THACHIL, A., GERBER, P. F., XIAO, C.-T., HUANG, Y.-W. & OPRIESSNIG, T. 2015. Development and Application of an ELISA for the Detection of Porcine Deltacoronavirus IgG Antibodies. *PLOS ONE,* 10**,** e0124363.

VAN HOUTAN, K. S., HARGROVE, S. K. & BALAZS, G. H. 2010. Land Use, Macroalgae, and a Tumor-Forming Disease in Marine Turtles. *PLoS One,* 5.

VILLA, C. A., FINLAYSON, S., LIMPUS, C. & GAUS, C. 2015. A multi-element screening method to identify metal targets for blood biomonitoring in green sea turtles (*Chelonia mydas*). *Science of The Total Environment***,** 613-621.

VILLA, C. A., FLINT, M., BELL, I., HOF, C., LIMPUS, C. J. & GAUS, C. 2017. Trace element reference intervals in the blood of healthy green sea turtles to evaluate exposure of coastal populations. *Environmental Pollution,* 220, Part B**,** 1465–1476.

WILLIMANN, A. 2018. *The prevalence of antibodies against envelope proteins of Chelonid herpesvirus 5 is inconsistent with the current understanding of the pathogenesis and epidemiology of fibropapillomatosis in marine turtles.* University of Zurich.

WORK, T. M., DAGENAIS, J., BALAZS, G. H., SCHETTLE, N. & ACKERMANN, M. 2014. Dynamics of Virus Shedding and In Situ Confirmation of Chelonid Herpesvirus 5 in Hawaiian Green Turtles With Fibropapillomatosis. *Veterinary Pathology,* 52**,** 1195–1201.

WORK, T. M., DAGENAIS, J., BALAZS, G. H., SCHUMACHER, J., LEWIS, T. D., LEONG, J.-A. C., CASEY, R. N. & CASEY, J. W. 2009. In vitro biology of fibropapilloma-associated turtle herpesvirus and host cells in Hawaiian green turtles (*Chelonia mydas*). *J Gen Virol,* 90.

WORK, T. M., DAGENAIS, J., BREEDEN, R., SCHNEEMANN, A., SUNG, J., HEW, B., BALAZS, G. H. & BERESTECKY, J. M. 2015. Green Turtles Have Novel Asymmetrical Antibodies. *The Journal of Immunology,* 195**,** 5452.

WORK, T. M., DAGENAIS, J., WEATHERBY, T. M., BALAZS, G. H. & ACKERMANN, M. 2017. In-vitro replication of Chelonid herpesvirus 5 in organotypic skin cultures from Hawaiian green turtles (*Chelonia mydas*). *Journal of Virology***,** e00404-17.

WORK, T. M., DAGENAIS, J., WILLIMANN, A., BALAZS, G., MANSFIELD, K. & ACKERMANN, M. 2020. Differences in Antibody Responses against Chelonid Alphaherpesvirus 5 (ChHV5) Suggest Differences in Virus Biology in ChHV5-Seropositive Green Turtles from Hawaii and ChHV5-Seropositive Green Turtles from Florida. *Journal of Virology,* 94**,** e01658-19.

WORK, T. M., RAMEYER, R. A., BALAZS, G. H., CRAY, C. & CHANG, S. P. 2001. Immune status of free-ranging green turtles with fibropapillomatosis from Hawaii. *Journal of Wildlife Diseases,* 37**,** 574–581.

WYNEKEN, J. 2002. The External Morphology, Musculoskeletal System, and Neuro-Anatomy of Sea Turtles. *In:* LUTZ, P. L., MUSICK, J. A. & WYNEKEN, J. (eds.) *The biology of sea turtles.* CRC press.

YANG, M., XU, W., BITTNER, H., HORSINGTON, J., VOSLOO, W., GOOLIA, M., LUSANSKY, D. & NFON, C. 2016. Generation of mAbs to foot–and–mouth disease virus serotype A and application in a competitive ELISA for serodiagnosis. *Virology Journal,* 13**,** 195.

YETSKO, K., FARRELL, J., STAMMNITZ, M. R., WHITMORE, L., WHILDE, J., EASTMAN, C. B., RAMIA, D. R., THOMAS, R., KRSTIC, A., LINSER, P., CREER, S., CARVALHO, G., BURKHALTER, B., MURCHISON, E. P., SCHNITZLER, C. & DUFFY, D. J. 2020. Mutational, transcriptional and viral shedding dynamics of the marine turtle fibropapillomatosis tumor epizootic. *bioRxiv***,** 2020.02.04.932632.

YOSHII, K., HAYASAKA, D., GOTO, A., OBARA, M., ARAKI, K., YOSHIMATSU, K., ARIKAWA, J., IVANOV, L., MIZUTANI, T., KARIWA, H. & TAKASHIMA, I. 2003. Enzyme-linked immunosorbent assay using recombinant antigens expressed in mammalian cells for serodiagnosis of tick-borne encephalitis. *Journal of Virological Methods,* 108**,** 171–179.

CHAPTER 6 - Thesis discussion

Throughout this thesis the aim was to investigate links between trace metals and FP in local *C. mydas* population at sites within the northern and central GBR. The work conducted included aspects of serology and marine ecotoxicology, and significant insights were gleaned in the furtherment of knowledge on those subjects. Synthesis of the findings and outcomes of each chapter of this thesis will be amalgamated here. Firstly, the main findings from each chapter will be summarised and then discussed in relation to the wider aim of this thesis to better understand the effect of environmental factors on development of FP in *C. mydas*.

6.1. Chelonia mydas preferred forage metal profiles (Chapter 3)

To commence investigation, I began with investigating environmental metal exposure to determine the status of metal contamination in preferred seagrass spp. and to determine elements of most concern. To establish possible influence of the wet season and heavy rainfall I compared seagrass metal concentrations at three coastal sites from before and after the 2018/19 wet season. The aim of this chapter was:

> To investigate and describe concentrations of a suite of ecologically relevant metals in preferred seagrass forage of *C. mydas* at three coastal turtle foraging sites on the Great Barrier Reef (GBR).

This aim was achieved by first identifying specific primarily foraged seagrass species (*H. uninervis primarily)* by analysing *C. mydas* stomach contents for seagrass species and then collecting samples of this species from established *C. mydas* foraging grounds. When seagrass samples were analysed for a suite of ecologically relevant elements (Al, Cd, Co, Cu, Fe, Mg, Mn, Ni, Pb and Zn) metal profiles were similar in all sampled coastal sites, but distinct from the control site situated offshore. Elements that varied in concentration between coastal and offshore sites tended to be elevated in the coastal site, which was expected considering land-based contaminants are higher closest to the source, and within proximity to urban and industrial land uses (Gaudry et al., 2007, Thomas et al., 2020, Vijayasarathy et al., 2019, Brodie et al., 2019, Villa et al., 2017). Following the 2018/19 wet season, increases in some metal concentrations were observed when looking at the overall metal profiles, though variation in metal concentrations meant that some overlap and similarity (before and after WS metal profile confidence ellipses were closely association) was observed.

While the targeted metal concentrations in this study were elevated in coastal sites relative to the HWK, all concentrations (except Mn) were comparable to observed findings within

seagrass of north Australia, at sites in proximity to CB and UB, which were sampled in the current study (Thomas et al., 2020, Haynes, 2001, Prange and Dennison, 2000, Vonk et al., 2017). Site specific geochemical differences in sediments and bioavailability of metals play a significant role in dictating the availability of metals (Thomas et al., 2020, Villa et al., 2017), and thus ecological health and contamination should be considered on a local scale. Total metal profiles and patterns of elevation were expected to differ at the two different study site types (coastal and offshore). In this chapter, metal profiles were deemed to be at concentrations of concern in the local region (elements such as Co were elevated above baseline concentrations), but not high enough to be considered at risk levels, regarding seagrass health (Thomas et al., 2020). Definitive statements on toxicity of ecologically relevant concentrations could not be made as the scope of this study did not allow for *in vivo* ecotoxicological assay of the impact of reported metal loads on relevant end points (such as photosynthetic efficiency and growth) in targeted seagrass species. Furthermore, it was not possible to make assumptions on overall seagrass and environmental health as only one aspect (seagrass metal loads) of contamination and ecotoxicological investigation was conducted. A larger scope would be necessary to comprehensively investigate specific toxicokinetic properties of certain elements on species (such as transport, absorption, detoxification, and excretion), to adequately predict relative risk factors and ecotoxicological implications of elevated and toxic metals on coastal seagrass (and presumably *C. mydas*). As reptiles, marine turtles breathe air and thus the primary metal exposure pathway is through voluntary ingestion of forage material (Grillitsch and Schiesari, 2010). As macrograzers, *C. mydas* inhabit coastal foraging grounds that are often near urban settlement and anthropogenic influence and thus are at risk from prolonged exposure to toxic elements (Villa et al., 2017, Komoroske et al., 2011, van de Merwe et al., 2010, Grillitsch and Schiesari, 2010, Van de Merwe, 2008, Aguirre et al., 1994). To assess the risk encountered by local turtle populations the surveying of population metal profiles was necessary.

6.2. Chelonia mydas blood and scute metal loads (Chapter 4)

To determine direct metal exposure in local *C. mydas* populations, intensive capture and sampling effort was placed on four coastal sites (TLK, CB, UB and EB) and the control site, HWK. The same suite of metals reported in the previous chapter was analysed in blood and scute (keratin). Comparison between coastal sites and HWK was conducted to provide insight into whether metal concentrations were elevated at sites closes to potential sources of contamination. As metal concentrations were elevated in seagrass from sites adjacent to human activity, relative to the control site, it can be inferred here that blood and scute metal

concentrations would also be higher at the coastal sites, when compared to HWK data. Blood and scute samples provide non-invasive methods of examining short-term (blood, 2-3 week) (Villa et al., 2017, van de Merwe et al., 2010, da Silva et al., 2016, Villa et al., 2015, Grillitsch and Schiesari, 2010) and long-term (scute, approximately 1.4-2.8 years, but may fluctuate dependant on metal element and toxicokinetic pressures) exposure (Villa et al., 2019). Comparison between the two may provide evidence as to historical or recent exposures, and whether current profiles are higher or lower than in recent years.

The aim of this chapter was:

To investigate and describe *C. mydas* blood and scute (carapace) metal profiles in coastal and offshore populations of the GBR.

Blood metal concentrations were consistently lower than seagrass concentrations (Chapter 3), and most were similar between all sites, sometimes including the offshore control site, though for most elements, concentrations were elevated (to varying degrees) at coastal sites when compared to HWK. However, most elements were within published reference intervals specific to the study region, derived from HWK data (Villa et al., 2017). These findings support the theory that most of the target metals were not elevated above baseline concentrations within the study region at the time of sampling, though differences were observed in blood concentrations between coastal and offshore populations. Furthermore, scute concentrations for the same targeted metals largely followed the same pattern and concentrations of most elements were again similar between coastal sites. Scute samples were not obtained from the control population in this study (due to lack of necessary permits for collection obtained by those who collected the blood samples on authors behalf), and reference values were not available to measure deviance from a natural baseline data set. It was therefore not possible to define whether current concentrations of target elements were elevated at the coastal sites (Villa et al., 2019). Some elements (Co, Cu and Fe) were detected at similar concentrations between blood (short term indication of metal exposure) and scutes (long term indicator) suggesting that steady-state exposure to these elements was likely in local *C. mydas* populations over recent years (Villa et al., 2019). On the other hand, most elements (Al, Mg, Mn, and Zn) were magnitudes higher in scutes compared to blood. Greater scute concentrations might suggest elevation in previous metal exposure (Villa et al., 2019). Or, alternatively, due to the persistent nature of trace elements, bioaccumulation of concentrations in storage tissues is likely in long-lived species such as *C. mydas* (Grillitsch and Schiesari, 2010). In vertebrates trace elements

often have strong affinity to specific organs and thus organ-specific bioaccumulation of certain elements often takes place (organotropism) (Grillitsch and Schiesari, 2010). Such affinity of some elements to certain storage tissues may lend possible explanation to why concentrations were substantially greater in scutes relative to blood in this study. Bioaccumulation of elements occurs when the uptake rate exceeds that of elimination, and the rate of accumulation is related to the detoxification mechanisms of species and specific tissues (Jakimska et al., 2011, Grillitsch and Schiesari, 2010). Bioaccumulation of elements is dependent on exposure time, with bioaccumulated concentrations increasing with longer exposure to environmental concentrations (Jakimska et al., 2011, Zauke et al., 2006, Kahle and Zauke, 2003, Ritterhoff and Zauke, 1997, Grillitsch and Schiesari, 2010), and with high trophic transfer rates from a lower trophic level (primary producers in the case of *C. mydas*) (Jakimska et al., 2011).

As mentioned, little inference can be made as to the possible toxic outcomes of elevated metal concentrations, or to any evaluation in level of risk posed to local *C. mydas* populations included in the study. To measure the toxic impacts of elevated metals direct ecotoxicological investigation into potential effects of elements on live specimens would be required. While *in vivo* investigations into the impact of toxic metals on marine turtles would be the gold standard for measuring any toxic impacts, such an approach is not possible due to a myriad of ethical concerns. In response to this limitation an *in vitro* cytotoxic assay was recently designed and validated to measure the potential toxic effects of trace metal concentrations on *C. mydas* primary skin fibroblasts (Finlayson et al., 2019b, Finlayson et al., 2019a), providing opportunity for rapid assessment of risk posed by environmentally relevant metal concentrations of the study area of interest. Through advancement in cell culturing of *C. mydas* cell lines (Finlayson et al., 2020, Mashkour et al., 2018) cytotoxic investigation into specific toxicokinetic activity and biochemical implications of toxic metal exposure has been conducted. Finlayson et al. (2020) assayed cultured *C. mydas* skin and liver cells in response to a suite of targeted metals (including Co, Cu and Mn) and found that the effect concentrations (causing mortality of 20 % of cells) for the metals tested in the bioassay were greater than concentrations detected in animals captured. This study successfully demonstrated how such biochemical approaches could be applied in future research to investigate direct toxicity of any chemical contaminants (organic and inorganic) of ecological concern. Further determination of acute and chronic toxic effects of environmentally relevant concentrations for elements of concern is necessary to determine reliable thresholds for local populations, which would allow

for more informed management and policy decisions to better protect local foraging grounds and *C. mydas* populations.

Cobalt (Co) was the sole metal element that was consistently high in all instances of investigation in this thesis (seagrass, blood and scute). Co concentration was unfailingly greatest in UB and other coastal locations (to a lesser extent) compared to the HWK control population, defined local baseline reference values, and previously reported global concentrations in coastal seagrass meadows and *C. mydas* populations. This was to be expected as such findings were previously reported in blood and scute samples collected from the study region (Villa et al., 2019, Villa et al., 2017). The geochemical composition of the Burdekin region is rich in Co (Wilkinson et al., 2013). The outlet of the Burdekin River, which is a significant source of land-based sediments and contaminants (Brodie et al., 2017), flows into UB, and thus it is not surprising that Co concentrations are highest at this site. Additionally, trends within this thesis suggest concentrations dilute the further from the influence of the Burdekin River, suggesting that terrestrial runoff and erosion of the Burdekin region is a significant source of Co, Ni and likely numerous other trace metals that may be available in sediments or readily used in anthropogenic land uses.

6.3. Chelonia mydas seroprevalence of ChHV5 along the GBR (Chapter 5)

The debilitating marine turtle disease, fibropapillomatosis (FP) is a research topic of high priority (Hamann et al., 2010). FP was first recorded over 80 years ago (Smith and Coates, 1938, Lucke, 1938) and has received ongoing academic attention since, with global efforts increasing in recent years to better understand various aspects of transmission and expression of the disease. Whilst FP is an established area of investigation, little is known regarding specific component causes responsible for the development of tumours in certain individuals within *C. mydas* populations. One assumption that has been placed on FP investigation is that ChHV5 is likely the primary aetiological infection agent that is a necessary cause for the development of tumours in individuals. Recently, progress has been made in the sero-surveillance techniques of populations for specific antibodies against the virus in other parts of the world, an aspect that I believe is essential for understanding the association of ChHV5 to FP, and whether it is as important to the aetiology of the disease as previously believed. This chapter of my thesis focused on surveying the prevalence of ChHV5 with the aim:

To survey and describe the seroprevalence of ChHV5-specific antibodies in *C. mydas* from coastal and offshore populations of the northern and central regions of the GBR.

In order to achieve this aim I applied a validated serodiagnostic survey protocol to investigate the prevalence of exposure to ChHV5 by detecting ChHV5 specific antibodies in serum samples from the same five *C. mydas* populations previously sampled in Chapters 3 and 4. Seroprevalence of two ChHV5 (7s and 5.7s IgY) antibodies ranged from 20 to 45 % and 10 to 28 % (for 7s and 5.7s IgY types respectively), and were statistically similar, both spatially (between all sites, HWK, TLK, CB, UB and EB) and temporally (over time between 2010 and 2017/19), in EB specifically. For instance, no difference was detected when comparing seroprevalence at sites with a history of FP cases (CB and EB) and sites with no FP history (HWK, TLK and UB), and similarly when comparing samples from individuals with FP and without, at the time of sampling.

6.4. Thesis findings regarding FP disease expression

When planning this project and designing each study I predicted that some distinction would be apparent between coastal study populations and the offshore control *C. mydas* population for ChHV5 seroprevalence and metal element concentrations. Whilst such distinctions were present between site types for metal exposure, little difference was detected between populations for ChHV5 antibody seroprevalence. Such an outcome is no less interesting when considering the application of novel approaches (such as ELISA and scute analysis) was used to pioneer investigation into aspects of the epidemiology of ChHV5 as an indication of susceptibility and risk of FP to local *C. mydas* populations residing in the north and central regions of the GBR. The finding that suggested homogeneous seroprevalence of ChHV5-specific antibodies at all studied sites is telling in itself as it implies equal risk to the expression of FP, regardless of history of FP cases, if ChHV5 is indeed the primary aetiological agent or necessary cause of FP tumour development. From the findings presented here and other investigations, one thing is certain: cryptic aspects of the factors leading to FP expression still allude investigations. The finding here suggests that viral transmission of ChHV5 is likely equal within all bays and *C. mydas* populations, irrespective of proximity to other populations. Like other herpesviruses (such as Herpes simplex type 1 (HSV-1) and type 2, (HSV-2) in humans) (Soh et al., 2020, Connolly et al., 2020, Fiorini et al., 1986), ChHV5 may be ubiquitous throughout the marine environment and in *C. mydas* individuals of local populations of the GBR, though further surveying of viral distribution would be necessary to better determine the commonality of ChHV5 in marine turtles. The similarity in ChHV5 antibody prevalence between sites with historically high and low prevalence of FP lesions may indicate that ChHV5 is in fact not as crucial in the aetiology of FP as once believed, and the consistent

detection of viral DNA in tumour tissues is merely incidental or less significant as a component cause of FP. *Chelonia mydas* virology is largely an emerging field (Ariel, 2011), with focus placed on herpesviruses in the literature. By implementing technological advancements and increased effort in screening of various sample types would allow for more reliability in the diagnosis and isolation of wildlife viruses (Leland and Ginocchio, 2007). For instance, Mashkour *et al.* (Mashkour et al., 2018) reported evidence of a marine turtle-specific papillomavirus (CmPV) in *C. mydas* with FP from animals captured along the northern GBR. The study applied a combination of diagnostic protocols (viral isolation via cell culture and molecular detection of viral DNA with Polymerase Chain Reaction (qPCR)) and found FP tumour tissue samples from CB and EB were positive for CmPV. (Mashkour et al., 2018) were the first to report consistent detection of a *C. mydas* CmPV in FP lesions and serves as an example for the need to further screen marine turtles for a wide range of viruses. This potential association between CmPV and FP begs the question as to whether assumptions placed on ChHV5 as primary causative agent of FP need to be reconsidered. Extended effort should be placed on screening for other novel viruses, and future diagnostic surveys of FP tumour biopsies should include papillomavirus. CmPV serological analysis was beyond the scope of this thesis but is strongly recommended to further elucidate its role in FP tumour development.

Definitive association between elevated trace metal exposure and FP disease prevalence in *C. mydas* cannot be made, as the suite of metals included for investigation here were deemed to not be elevated at any of the study sites at the time of sampling. Metals were below toxic thresholds and within local reference intervals, and no turtles with FP were captured during the project to allow comparison between metal loads in healthy and diseased individuals. Such realities make ecotoxicological investigation into environmental contamination and wildlife disease difficult but is a positive outcome for the local turtle populations, which are likely healthy and successful, at least with respects to the disease and ecotoxicological aspects researched here. As no FP positive individuals were sampled for blood or scute metal analysis, and comparisons could not be made, answers as to whether toxic metal loads are significant component causes of FP remain inconclusive, and further investigation is necessary. Metals are a complex area of study, as potential sources are numerous, little is understood about the interaction effects and environmental consequences of toxic concentrations of metals and different species of each element. As discussed previously, metal-induced immunosuppression is likely a significant characteristic of toxic metals regarding the development of FP (Cray et al., 2001, da Silva et al., 2016). Any contaminants or mix of chemicals (or other environmental

stressors) that cause a decrease in immune function or increased susceptibility to infection are of interest as potential causes of the disease. Furthermore, FP aetiology is likely not restricted to one form of environmental pressure with evidence suggesting other co-factors (Duffy et al., 2018). The one thing that remains clear is that a multifactorial approach is required to best ascertain the necessary causal factors responsible for FP expression within local and global *C. mydas* populations.

6.5. Thesis limitations

This project was able to achieve fundamental investigation into aspects of *C. mydas* health and exposure to environmental contamination and infection, through application of cutting edge and novel techniques, however some limitations were encountered and are discussed here. The scope of this thesis was somewhat limited regarding the resources available to investigate a wide range of ecologically relevant chemicals. A multifactorial approach to the ecotoxicological risk of chemical contaminants is strongly suggested. As contaminants are not encountered in isolation by green turtles, neither should the analysis of such data when addressing knowledge gaps in a particularly complex area of study such as FP disease expression. With the advent of sensitive and reliable technology a broader approach may become more feasible. As demonstrated here, ICP-MS can be used to measure a wide suite of trace metals in numerous sample types, allowing for paired sample analysis comparable between sites and similar studies. The main limitation of this analytical technique was cost. The financial budget of this project, like many PhDs, was relatively small and thus strategic planning and design was necessary to best address all research questions and aims. Sample size and statistical power was considered, and it was decided that implementing larger sample sizes and fewer ecologically relevant metal elements, rather than a range of chemical analysis techniques inclusive of a diverse suite of contaminants (not just metals), would provide more robust data to build on knowledge, and give insight into any future research directions and approaches. With a larger monetary budget and research team this project would have aimed to survey a significantly wider suite of contaminants and would have included more *C. mydas* populations in a larger study region.

The methods used in this thesis to measure metal concentrations in seagrass, blood and scute samples provided snapshots of metal exposure of an individual turtle or the wider environment at the time of collection. While this is informative and useful in knowledge building, the marine environment is extremely dynamic and complex, with an ever-growing source of chemical contamination and other environmental processes and events, all with the potential to influence

metal distribution and bioavailability. The approach taken in this project intended to investigate a diverse set of sample types to best investigate local *C. mydas* metal exposure with the resources available. With a greater time budget, other more efficient monitoring programs may have been implemented to report concentration fluctuation over a longer time and with higher resolution data, but by the very nature of long-term monitoring the time constraints did not allow such an approach here. The aim of this thesis was to build on global knowledge of the ecotoxicological impacts of metals on *C. mydas* health as potential aetiological co-factors of FP. As a result of this thesis, significant data sets of metal concentrations can be added to global data sets necessary to establish or refine natural baseline values and local reference intervals of environmental metal concentrations in *C. mydas,* and associated habitats in which they reside and forage. Furthermore, the scope of this thesis did not provide opportunity to measure biochemical parameters associated with declined immune function (total white cell counts) and oxidative stress (lipid peroxidation), two main factors considered significant in the development of FP.

6.6. Future research directions

Building on the limitations of this thesis and through the synthesis of my findings, insight into potential direction for future research was gleaned, and some suggestions are discussed here. Firstly, further effort is required to monitor concentrations of a wide and diverse suite of established and emerging marine contaminants, both in the local region, but also at any site where contamination is of concern. Furthermore, effort should be focused on the investigation into relevant ecotoxicological end points for targeted contaminants of concern to calculate toxic thresholds for various indicative health parameters for both coastal seagrass species and for *C. mydas.* Additionally, effort should be made to calculate the bioaccumulation factor of trace metals from blood (principal transport medium) to long term storage tissues and organs, particularly scutes, liver and kidney. Once species-specific susceptibility to such contaminants is better understood, definitive insight into whether certain contaminants are associated with FP development may be obtained.

Regarding aspects of seroprevalence of antibodies to ChHV5, this thesis provides a starting point for surveying of ChHV5 in Australian *C. mydas* populations. It is suggested that future efforts are carried out to apply comparable protocols to measure seroprevalence of ChHV5 antibodies in other populations and in individuals with current or previous FP clinical signs. Furthermore, in light of the findings by Mashkour et al. (2018), it would be prudent to also survey for antibodies against CmPV and other viruses. As more data is reported, a clearer

picture of distribution and further insight into possible transmission pathways will be possible. Such information will allow further study of whether ChHV5 and other viral agents are strongly associated with FP, and whether there is a primary aetiological agent of the disease. Concurrent to universal ChHV5 seroprevalence surveying, it is strongly suggested that further study effort should be placed on locating and mapping the distribution and prevalence of FP positive turtles in ecologically relevant populations. Until a reliable prevalence of FP cases is calculated and regularly updated, association between the disease and associated causes will not be possible, knowledge gaps and questions will persist, and answers will continue to allude. Finally, future virological studies should aim to continue the work of previous studies to further survey *C. mydas*, with and without FP tumours, for viruses that could be associated with disease expression, preferably using a joint approach including serodiagnostic and molecular techniques, as seen with the papillomavirus recently discovered in tumour tissue in Australian turtles (Mashkour et al., 2018).

In this thesis, I investigated possible links between trace-metal concentrations in seagrass and turtle tissues and the prevalence of specific antibodies against ChHV5 in *C. mydas* serum from coastal and offshore populations along the GBR. There was no clear link between investigated environmental contaminants and sites of historical presence of elevated numbers of FP in turtle populations. Furthermore, and contrary to expectations, the seroprevalence of antibodies to ChHV5 remained at comparable levels across all sites. From the findings of this thesis, it is doubtful that exposure to ChHV5 alone can elicit tumour growth as the virus appears to be ubiquitous across the GBR.

6.7. References

AGUIRRE, A. A., BALAZS, G. H., ZIMMERMAN, B. & GALEY, F. D. 1994. Organic contaminants and trace metals in the tissues of green turtles (*Chelonia mydas*) afflicted with fibropapillomas in the Hawaiian islands. *Marine Pollution Bulletin,* 28**,** 109–114.

ARIEL, E. 2011. Viruses in reptiles. *Vet Res,* 42**,** 1-12.

BRODIE, J., GRECH, A., PRESSEY, B., DAY, J., DALE, A. P., MORRISON, T. & WENGER, A. 2019. The Future of the Great Barrier Reef: The Water Quality Imperative. *In:* WOLANSKI, E., DAY, J. W., ELLIOTT, M. & RAMACHANDRAN, R. (eds.) *Coasts and Estuaries.* Elsevier.

BRODIE, J. E., LEWIS, S. E., COLLIER, C. J., WOOLDRIDGE, S., BAINBRIDGE, Z. T., WATERHOUSE, J., RASHEED, M. A., HONCHIN, C., HOLMES, G. & FABRICIUS, K. 2017. Setting ecologically relevant targets for river pollutant loads to meet marine water quality requirements for the Great Barrier Reef, Australia: A preliminary methodology and analysis. *Ocean & Coastal Management,* 143**,** 136-147.

CONNOLLY, S. A., JARDETZKY, T. S. & LONGNECKER, R. 2020. The structural basis of herpesvirus entry. *Nature Reviews Microbiology*.

CRAY, C., VARELLA, R., BOSSART, G. D. & LUTZ, P. 2001. Altered in vitro immune responses in green turtles (*Chelonia mydas*) with fibropapillomatosis. *Journal of Zoo and Wildlife Medicine,* 32**,** 436-440.

DA SILVA, C. C., KLEIN, R. D., BARCAROLLI, I. F. & BIANCHINI, A. 2016. Metal contamination as a possible etiology of fibropapillomatosis in juvenile female green sea turtles *Chelonia mydas* from the southern Atlantic Ocean. *Aquatic Toxicology,* 170**,** 42–51.

DUFFY, D. J., SCHNITZLER, C., KARPINSKI, L., THOMAS, R., WHILDE, J., EASTMAN, C., YANG, C., KRSTIC, A., ROLLINSON, D., ZIRKELBACH, B., YETSKO, K., BURKHALTER, B. & MARTINDALE, M. Q. 2018. Sea turtle fibropapilloma tumors share genomic drivers and therapeutic vulnerabilities with human cancers. *Communications Biology,* 1**,** 63.

FINLAYSON, K. A., LEUSCH, F. D. & VAN DE MERWE, J. P. 2019a. Primary green turtle (*Chelonia mydas*) skin fibroblasts as an in vitro model for assessing genotoxicity and oxidative stress. *Aquatic Toxicology,* 207**,** 13-18.

FINLAYSON, K. A., LEUSCH, F. D. L., LIMPUS, C. J. & VAN DE MERWE, J. P. 2019b. Towards the development of standardised sea turtle primary cell cultures for toxicity testing. *Ecotoxicology and Environmental Safety,* 173**,** 63-70.

FINLAYSON, K. A., MADDEN HOF, C. A. & VAN DE MERWE, J. P. 2020. Development and application of species-specific cell-based bioassays to assess toxicity in green sea turtles. *Science of The Total Environment,* 747**,** 142095.

FIORINI, G., BERNASCONI, P., SINICO, R. A., CHIANESE, R., POZZI, F. & D'AMICO, G. 1986. Increased frequency of antibodies to ubiquitous viruses in essential mixed cryoglobulinaemia. *Clinical and experimental immunology,* 64**,** 65-70.

GAUDRY, A., ZEROUAL, S., GAIE-LEVREL, F., MOSKURA, M., BOUJRHAL, F.-Z., EL MOURSLI, R. C., GUESSOUS, A., MOURADI, A., GIVERNAUD, T. & DELMAS, R. 2007. Heavy Metals Pollution of the Atlantic Marine Environment by the Moroccan Phosphate Industry, as Observed through their Bioaccumulation in Ulva Lactuca. *Water, Air, and Soil Pollution,* 178**,** 267-285.

GRILLITSCH, B. & SCHIESARI, L. 2010. The ecotoxicology of metals in reptiles. *In:* SPARLING, D. W., LINDER, G., BISHOP, C. A. & KREST, S. K. (eds.) *Ecotoxicology of amphibians and reptiles.* 2nd ed.: CRC Press.

HAMANN, M., GODFREY, M., SEMINOFF, J., ARTHUR, K., BARATA, P., BJORNDAL, K., BOLTEN, A., BRODERICK, A., CAMPBELL, L. & CARRERAS, C. 2010. Global research priorities for sea turtles: informing management and conservation in the 21st century. *Endangered Species Research,* 11**,** 245-269.

HAYNES, D. B. 2001. *Pesticide and heavy metal concentrations in Great Barrier Reef sediment, seagrass and dugongs (Dugong dugon).* Ph.D., University of Queensland.

JAKIMSKA, A., KONIECZKA, P., SKÓRA, K. & NAMIEŚNIK, J. 2011. Bioaccumulation of Metals in Tissues of Marine Animals, Part I: the Role and Impact of Heavy Metals on Organisms. *Polish Journal of Environmental Studies,* 20.

KAHLE, J. & ZAUKE, G.-P. 2003. Trace metals in Antarctic copepods from the Weddell Sea (Antarctica). *Chemosphere,* 51**,** 409-417.

KOMOROSKE, L. M., LEWISON, R. L., SEMINOFF, J. A., DEHEYN, D. D. & DUTTON, P. H. 2011. Pollutants and the health of green sea turtles resident to an urbanized estuary in San Diego, CA. *Chemosphere,* 84**,** 544-552.

LELAND, D. S. & GINOCCHIO, C. C. 2007. Role of Cell Culture for Virus Detection in the Age of Technology. *Clinical Microbiology Reviews,* 20**,** 49-78.

LUCKE, B. 1938. Studies on tumors in cold-blooded vertebrates. *Annual Report of the Tortugas Laboratory of the Carnegie Institute.*

MASHKOUR, N., MACLAINE, A., BURGESS, G. W. & ARIEL, E. 2018. Discovery of an Australian *Chelonia mydas* papillomavirus via green turtle primary cell culture and qPCR. *Journal of Virological Methods,* 258**,** 13–23.
PRANGE, J. A. & DENNISON, W. C. 2000. Physiological Responses of Five Seagrass Species to Trace Metals. *Marine Pollution Bulletin,* 41**,** 327-336.
RITTERHOFF, J. & ZAUKE, G.-P. 1997. Trace metals in field samples of zooplankton from the Fram Strait and the Greenland Sea. *Science of the Total Environment,* 199**,** 255-270.
SMITH, G. & COATES, C. 1938. Fibro-epithelial growths of the skin in large marine turtles, *Chelonia mydas* (Linnaeus). *Zoologica,* 23**,** 93–98.
SOH, T., DAVIES, C., MUENZNER, J., HUNTER, L., BARROW, H., CONNOR, V., BOUTON, C., SMITH, C., EMMOTT, E. & ANTROBUS, P. 2020. Whole-cell and plasma membrane proteomics of herpes simplex virus-1 infection reveals cell surface remodelling via pUL56-mediated GOPC degradation. *Cell Reports*.
THOMAS, C., BENNETT, W., GARCIA, C., SIMMONDS, A., HONCHIN, C., TURNER, R., HOF, C. & BELL, I. 2020. Coastal bays and coral cays: Multi-element study of *Chelonia mydas* forage in the Great Barrier Reef (2015–2017). *Science of The Total Environment***,** 140042.
VAN DE MERWE, J. P. 2008. *Persistent organic pollutants and heavy metals in the green sea turtle, Chelonia mydas.* Ph.D., Griffith University, Queensland, Australia.
VAN DE MERWE, J. P., HODGE, M., OLSZOWY, H. A., WHITTIER, J. M. & LEE, S. Y. 2010. Using blood samples to estimate persistent organic pollutants and metals in green sea turtles (Chelonia mydas). *Marine Pollution Bulletin,* 60**,** 579-588.
VIJAYASARATHY, S., BADUEL, C., HOF, C., BELL, I., DEL MAR GÓMEZ RAMOS, M., RAMOS, M. J. G., KOCK, M. & GAUS, C. 2019. Multi-residue screening of non-polar hazardous chemicals in green turtle blood from different foraging regions of the Great Barrier Reef. *Science of The Total Environment,* 652**,** 862-868.
VILLA, C., BELL, I., HOF, C. M., LIMPUS, C. & GAUS, C. 2019. Elucidating temporal trends in trace element exposure of green turtles (*Chelonia mydas*) using the toxicokinetic differences of blood and scute samples. *Science of the Total Environment,* 651**,** 2450-2459.
VILLA, C. A., FINLAYSON, S., LIMPUS, C. & GAUS, C. 2015. A multi-element screening method to identify metal targets for blood biomonitoring in green sea turtles (*Chelonia mydas*). *Science of The Total Environment***,** 613-621.
VILLA, C. A., FLINT, M., BELL, I., HOF, C., LIMPUS, C. J. & GAUS, C. 2017. Trace element reference intervals in the blood of healthy green sea turtles to evaluate exposure of coastal populations. *Environmental Pollution,* 220, Part B**,** 1465–1476.
VONK, J. A., SMULDERS, F. O. H., CHRISTIANEN, M. J. A. & GOVERS, L. L. 2017. Seagrass leaf element content: a global overview. *Marine Pollution Bulletin***,** 123-133.
WILKINSON, S. N., HANCOCK, G. J., BARTLEY, R., HAWDON, A. A. & KEEN, R. J. 2013. Using sediment tracing to assess processes and spatial patterns of erosion in grazed rangelands, Burdekin River basin, Australia. *Agriculture, Ecosystems & Environment,* 180**,** 90-102.
ZAUKE, G.-P., PROWE, F. & KIRF, M. 2006. Heavy metals in crustaceans from the Iberian deep sea plain. *Scientia Marina,* 70**,** 271-279.

Glossary of terms

Term	Definition
Enzootic	A predictably regular temporal pattern of disease affecting animals of a certain population or group.
Epizootiology	The sum of factors controlling the occurrence of a disease or pathogen in animals.
Horizontal viral transmission	The spread of a viral infection from one individual to another usually by contact with bodily secretions and excretions containing the viral agent.
Hypoproteinemia	Lower-than-normal levels of protein in the body.
Lytic	Of, relating to or the cause of lysis.
Natal region	The area of which marine turtles lay eggs and where hatchlings first emerge. Often in the vicinity of the specific beach where life begins for marine turtles.
Neritic zone	The habitable zone between the low water mark and approximately 200 m depth
Pathognomonic	A characteristic of a particular disease.
Reference Intervals	Intervals calculated from a group or population of healthy individuals of a given species for a given test.

Appendices

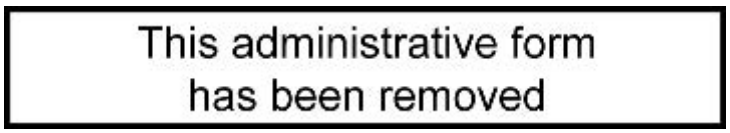
Appendix 1 – Animal Ethics approval for chapter 3, 4 and 5.

This administrative form has been removed

Appendix 2 – GBRMPA scientific research permit approval for chapter 3, 4 and 5.

This administrative form has been removed

Appendix 3 – Queensland Department of Environment and Science (formerly DEHP) scientific Purposes Permit (non-protected areas) approval for chapter 3, 4 and 5.



Appendix 4 – Queensland Department of Environment and Science (formerly DEHP) scientific Purposes Permit (protected areas) approval for chapter 3, 4 and 5.

This administrative form has been removed

Appendix 5 – Tag ID, CCL and weight of all *C. mydas* sampled in chapter 4 and 5.

Tag ID	CCL	Weight	Location
QA69058	55.2	19.1	HWK
QA69019	60.8	22.4	HWK
QA49510	62.9	24.3	HWK
QA49024	58.0	20.2	HWK
QA47557	65.0	29.5	HWK
QA48976	57.3	18.4	HWK
QA49390	53.8	16.8	HWK
QA48723	63.1	28.3	HWK
QA69612	63.7	28.0	HWK
QA69607	42.5	7.8	HWK
QA39526	49.7	12.9	HWK
QA69608	50.5	14.3	HWK
QA69606	64.4	33.0	HWK
QA69066	52.0	13.6	HWK
QA49327	54.7	19.6	HWK
QA51264	67.7	38.0	HWK
QA32005	64.1	30.0	HWK
QA69064	45.2	10.4	HWK
QA40151	56.5	20.4	HWK
K89104	68.0	36.0	HWK
QA69609	41.2	7.8	HWK
QA69010	72.0	45.0	HWK
QA69023	43.0	9.2	HWK
QA69068	69.1	34.0	HWK
QA69024	64.3	29.3	HWK
QA49394	56.4	18.7	HWK
QA49036	56.0	19.4	HWK
QA68860	53.5	18.5	HWK
QA51291	57.0	14.0	HWK
QA39694	67.5	31.5	HWK
QA62467	45.8	10.6	TLK
QA62468	44.7	10.7	TLK
QA62472	36.8	6.4	TLK
QA62471	46.3	10.5	TLK
QA62226	43.8	9.6	TLK
QA62228	42.8	9.3	TLK
QA62227	45.5	10.7	TLK
QA62463	49.2	13.2	TLK
QA62470	46.7	10.8	TLK

QA62125	56.3	21.3	CB
QA62391	42	13.8	CB
NO TAG - SICk	44	8.2	CB
QA62395	45.3	7.3	CB
QA62125	56.3	21.3	CB
QA62110	46.7	9.24	CB
QA62111	40.1	7.15	CB
QA62113	49.6	17.4	CB
QA70100	41.2	8.19	CB
QA70099	54.7	20.03	CB
QA70268	48	11.7	CB
QA70272	49.2	10.5	CB
QA70274	46.6	9.8	CB
QA70337	51.6	15.7	CB
QA70339	64	32	CB
QA70341	45.3	9.75	CB
QA70343	56.7	20.8	CB
QA70345	45.3	8.4	CB
QA70349	43.5	8.05	CB
QA62114	44.2	8.7	CB
QA62117	49.6	131	UB
QA62303	53.6	18.8	UB
QA62317	67.5	18.4	UB
QA62314	64.5	29	UB
QA62315	45.7	9.6	UB
QA62316	42	8.2	UB
QA62143	49.8	12.4	UB
QA62185	42.9	8.7	UB
QA62399	56.9	19.9	UB
QA62398	44.5	8.6	UB
QA62144	44.8	8.4	UB
QA51060	66.2	36	UB
QA62478	61.9	23.5	UB
QA62479	53.9	18.5	UB
QA51466	57.5	20.4	UB
QA62480	53	17.3	UB
QA62481	55.2	18.75	UB
QA62482	43.2	9.5	UB
QA62483	43.6	8.9	UB
QA62484	48.5	11.9	UB

QA62370	44.8	10.1	EB
QA62371	92.8	>100	EB
QA62166	60.8	26.2	EB
QA62170	98.2	>100	EB
QA62318	49.9	13.7	EB
QA62351	93	>100	EB
QA62115	48.2	11.58	EB
QA62353	75.2	49.4	EB
QA62102	83.8	>100	EB
QA62183	61.7	23.14	EB
QA36886	56.8	21.54	EB
K81504	65	29.18	EB
QA62124	46.3	10.59	EB
QA62123	57.2	17.85	EB
QA62104	43.2	7.2	EB
QA62319	48.8	10.9	EB
QA42386	46.2	10	EB
QA62106	56.2	19.1	EB
QA62301	48.8	11.1	EB
QA62321	48.5	9.2	EB

Appendix 6 - Raw data from ICP-MS analysis of seagrass samples reported on in chapter 3

Site -sample #	Al	Cd	Co	Cu	Fe	Mg	Mn	Ni	Pb	Zn	location	event
CB-01	2996.14	0.38	1.31	4.15	2102.41	7403.92	411.84	3.04	1.12	21.67	CB	Before
CB-02	1766.75	0.24	0.9	4.35	1683.57	7238.45	328.87	3.43	0.25	19.89	CB	Before
CB-03	1642.27	0.3	1.5	3.36	1381.93	6830.9	358.3	2.3	0.25	18.14	CB	Before
CB-04	3006.93	0.32	1.48	5.25	2897.78	7511.67	333.15	3.71	1.09	28.86	CB	Before
CB-05	4469.56	0.24	1.89	5.37	3349.59	7499.7	464.63	5.01	1.72	27.42	CB	Before
CB-06	3399.21	0.2	1.31	3.7	2658.56	7040.82	481.37	3.17	0.25	23	CB	Before
CB-07	3746.68	0.36	1.51	3.81	2563.51	7376.53	422.57	3.16	1.75	19.29	CB	Before
CB-08	6786.71	0.45	2.19	5.76	4540.93	7308.35	395.84	4.95	1.79	22.9	CB	Before
CB-09	12920.98	0.77	3.09	6.4	8392.73	7184.22	392.65	7.76	4.36	31.27	CB	Before
CB-10	3962.47	0.34	1.46	4.16	2856.92	7012.9	354.83	3.21	1.26	17.38	CB	Before
CB-12	3124.18	0.43	1.68	9.96	4214.76	8240.29	238.98	2.75	0.99	56.03	CB	Before
CB-13	2264.53	0.33	1.36	5.93	3230.66	8675.79	248.49	2.71	1.86	35.47	CB	Before
CB-14	3298	0.39	1.85	1.85	4046.66	8251.65	286.99	2.98	1.22	42.58	CB	Before
CB-15	3964.55	0.41	1.93	6.67	3916.8	8686.55	338.3	3.22	2.49	39.36	CB	Before
CB-16	3576.44	0.38	2.36	7.03	3916.69	8409.32	411.34	3.98	2.06	30.42	CB	Before
CB-17	2629.39	0.35	1.89	9.61	3295.35	8688.74	321.1	3.39	2.87	33.21	CB	Before
CB-18	1988.56	0.34	2.11	8.61	2437.77	8557.97	247.36	3.41	2.27	30.65	CB	Before
CB-A1	2377.48	0.45	1.02	5.81	1782.49	7735.96	305.79	2.43	0.25	19.34	CB	After
CB-A2	651.36	0.3	0.19	3.65	556.88	7008.57	149.7	1.71	0.25	16.09	CB	After
CB-A4	4018	0.39	1.05	3.97	4603.01	7459.46	121.57	4.04	2.17	24.89	CB	After
CB-A5	2660.69	0.47	1	4.48	2530.46	7127.79	263.4	3.8	1.22	23.61	CB	After
CB-A6	2433.19	0.34	0.85	3.94	1758.59	6998.19	229.15	2.8	0.25	18.53	CB	After
CB-A7	4975.87	0.44	1.76	4.81	3516.77	6835.17	250.9	4.2	1.78	25.32	CB	After
CB-A8	6544.2	0.53	1.8	5.22	4602.96	7209.99	216.88	6.06	2.11	28.35	CB	After
CB-A9	5728.26	0.52	2.1	4.1	4611.08	6544.14	288.1	4.81	1.8	24.88	CB	After
CB-A10	3085.25	0.4	1.01	4.12	2742.83	7056.37	144.81	3.97	0.25	24.82	CB	After
CB-A11	2845.35	0.34	0.74	2.79	2852.53	7176.86	157.45	2.98	0.69	22.14	CB	After

CB-A12	7083.3	0.6	1.93	4.7	5207.82	7238.27	289.04	5.46	2.14	25.52	CB	After
CB-A13	5728.26	0.52	2.1	4.1	4263.6	7494.4	325.46	4.01	1.57	30.29	CB	After
CB-A14	2442.6	0.36	1.16	3.17	2090.84	6916.33	289.34	2.7	0.7	27.94	CB	After
UB-01	1542.61	0.29	1.86	5.52	1313.8	8236.38	206.23	5.78	0.25	14.41	UB	Before
UB-02	1359.83	0.25	1.78	4.94	1334.89	9258.18	194.46	3.31	0.25	10.41	UB	Before
UB-03	1491.95	0.25	1.57	5.17	1520.46	8652.37	166.1	3.04	0.93	12.24	UB	Before
UB-04	1636.38	0.24	2.15	6.26	1376.33	8573.64	196.33	3.68	0.25	13.14	UB	Before
UB-05	818.64	0.23	1.4	4.85	930.84	9001.13	145.73	3.07	0.25	11.95	UB	Before
UB-06	3217.57	0.29	2.54	4.93	2595.87	8215.02	179.64	6.63	0.93	14.8	UB	Before
UB-07	3967.05	0.38	2.71	5.33	2980.22	8898.21	283.22	5.09	1.57	11.5	UB	Before
UB-08	1965.98	0.22	2.01	5.03	2082.28	8380.72	143.77	5.9	0.25	12.81	UB	Before
UB-09	1056.52	0.17	1.35	5.23	1090.83	8769.12	192.49	2.71	0.25	18.02	UB	Before
UB-10	915.69	0.21	1.1	4.35	1017.82	8112.65	276.32	2.33	0.25	13.13	UB	Before
UB-11	2249.65	0.3	2.12	5.35	2215.83	8484.9	593.19	4.72	0.25	16.92	UB	Before
UB-12	1323.38	0.24	1.84	4.23	1846.44	10108.94	256.14	3.56	0.25	11.73	UB	Before
UB-13	688.65	0.16	1.5	4.4	1196.35	8974.6	374.48	3.56	0.25	12.1	UB	Before
UB-14	1891.19	0.29	2.25	5.18	2377.73	8919.31	257.3	7.21	0.25	14.34	UB	Before
UB-15	1768.4	0.56	1.81	5.03	5435.76	6671.73	221.11	3.14	1.07	30.34	UB	Before
UB-A1	1276.01	0.37	1.27	5.32	2683.92	7236.42	153.08	2.92	0.25	23.88	UB	After
UB-A2	1233.48	0.35	1.56	6.61	1754.86	7725.36	417.8	3.67	0.25	26.54	UB	After
UB-A3	1813.13	0.44	2.26	8.51	4035.36	9123.96	320.64	5.4	0.25	26.66	UB	After
UB-A4	2592.42	0.58	2.64	6.12	6372.35	6898.12	208.54	5.66	1.72	26.77	UB	After
UB-A5	1091.92	0.45	0.85	4.93	3743.63	7661.65	151.27	2.77	0.25	27.47	UB	After
UB-A6	1792.98	0.42	2.29	10.32	2601.44	7017.44	397.08	4.43	0.67	35.26	UB	After
UB-A7	2360.2	0.48	2.85	9.46	3246.58	7467.17	359.36	4.17	0.25	28.93	UB	After
UB-A8	1910.52	0.57	2.27	7.34	4378.61	7716.38	344.95	4.48	0.9	24.68	UB	After
UB-A9	1425.15	0.44	2.59	8.44	2288.64	7659.51	576.34	3.32	0.93	23.56	UB	After
UB-A10	1571.39	0.51	2.29	8.56	2914.54	7180.87	388.08	3.94	0.94	29.99	UB	After
UB-A11	1385.49	0.61	2.14	5.32	7149.3	6784.29	150.48	5.04	1.71	49.8	UB	After
UB-A12	664.62	0.49	2.81	4.78	5319.57	6620.6	232.4	2.17	1.27	30.38	UB	After

UB-A13	620.55	0.28	2.35	5.71	2651.1	6482.77	326.31	3.19	0.25	20.05	UB	After
UB-A14	822.14	0.32	1.8	12.13	1545.89	8272.84	204.94	5.02	0.25	24.01	UB	After
UB-A15	1500.35	0.31	2.64	4.73	1860.84	9032.78	339.04	6.95	1.02	11.39	UB	After
EB-01	1309.72	0.57	2.66	2.95	4718.52	6191.88	450.74	5	0.25	14.72	EB	Before
EB-03	1063.05	0.21	0.56	1.96	1917.47	6843.11	40.47	3.05	0.25	18.91	EB	Before
EB-09	2416.88	0.42	2.25	2.89	4212.22	7197.75	290.85	2.79	0.25	24.26	EB	Before
EB-11	3112.23	0.39	1.8	2.46	4592.16	7282.79	207.79	4.73	2.05	19.13	EB	Before
EB-13	2694.04	0.35	1.77	2.6	3439.92	7893.85	244.07	7.54	0.25	23.76	EB	Before
EB-16	2622.77	0.36	1.57	3.87	3851.2	8267.65	260.28	5.51	0.25	28.96	EB	Before
EB-22	1566.8	0.28	1.41	3.3	1999.71	7882.88	218.59	3.72	0.25	16.08	EB	Before
EB-28	1047.3	0.31	1.08	4.09	2993.15	7807.87	160.41	3.14	0.25	23.65	EB	Before
EB-30	960.71	0.22	0.89	3.16	2073.5	7719.73	212.11	2.21	0.25	16.31	EB	Before
EB-32	1731.18	0.24	0.91	2.72	2112.83	6947.39	108.08	1.85	1.11	24.07	EB	Before
EB-34	1777.71	0.24	0.78	2.6	2440.06	7914.88	97.25	2.99	0.25	13.33	EB	Before
EB-A1	2617.15	0.32	0.97	2.6	2912.35	7484.94	152.09	5.64	0.25	12.25	EB	After
EB-A2	1800.77	0.29	0.71	2.53	3072.94	6740.67	78.18	3.36	0.66	10.28	EB	After
EB-A3	2644.11	0.32	0.9	2.04	2929.8	6799.1	126.95	3	0.25	11.82	EB	After
EB-A4	2161.37	0.43	1.65	4.22	4654.35	7444.67	266.84	5.45	0.25	19.33	EB	After
EB-A5	2727.01	0.35	1	2.05	3932.63	7011.42	94.01	8.01	0.25	11.38	EB	After
EB-A6	1901.84	0.33	0.85	2.33	2979.43	6064.93	125.72	4.28	0.25	11.8	EB	After
EB-A7	2478.68	0.41	1.77	3.27	4481.17	7564.2	253.86	6.05	0.25	19.55	EB	After
EB-A8	1268.88	0.19	0.56	1.4	2265.94	6618.66	42.3	5.07	0.25	10.66	EB	After
EB-A9	2295.68	0.31	1.07	2.67	2720.8	7332.39	160.56	7.12	0.95	12.66	EB	After
EB-A10	1885.26	0.28	0.96	2.86	2146.75	7394.8	60.83	6.7	0.25	15.11	EB	After
EB-A11	1438.39	0.19	0.94	2.19	2000.76	6479.89	33.9	5.76	0.25	20.56	EB	After
EB-A12	869.42	0.11	0.38	2.07	1034.35	8021.17	65.85	3.54	0.76	18.3	EB	After
EB-A13	1294.33	0.18	0.8	2.12	2094.98	6811.71	52.09	5.15	0.25	18.98	EB	After

Appendix 7 - Raw data from ICP-MS analysis of blood (B) and Scute (S) samples reported on in chapter 4

Turtle	Al	Co	Cu	Fe	Mg	Mn	Zn	location
69058	0.934	0.035	0.949	331.62	84.43	0.152	12.54	HWK
69019	1.29	0.03	0.691	308.6	82.32	0.161	10.41	HWK
49510	1.41	0.031	0.718	253.51	71.54	0.237	7.76	HWK
49024	0.588	0.019	0.802	267.59	75.44	0.126	8.61	HWK
47557	0.665	0.02	0.695	261.04	69.85	0.109	9.05	HWK
48976	1.66	0.017	0.728	258.77	80.57	0.193	8.54	HWK
49390	0.803	0.011	0.661	277.72	60.02	0.124	9.57	HWK
48723	0.744	0.017	0.584	260.6	77.42	0.097	9.16	HWK
69612	1.4	0.063	0.788	204.01	59.64	0.208	5.39	HWK
69607	0.834	0.012	0.753	295.84	75.82	0.133	9.55	HWK
39526	1.12	0.018	0.65	237.82	70.76	0.123	8.9	HWK
69608	1.49	0.011	0.82	322.32	83.18	0.115	11.24	HWK
69606	0.319	0.021	0.517	254.56	70	0.056	8.33	HWK
69066	0.444	0.016	0.675	262.66	78.92	0.071	8.73	HWK
49327	0.448	0.009	0.775	270.64	83.24	0.098	10.39	HWK
51264	0.402	0.011	0.408	258.43	73.81	0.088	9.35	HWK
32005	0.26	0.026	0.43	205.76	50.51	0.055	7.13	HWK
69064	0.297	0.023	0.569	118.74	75.23	0.073	3.94	HWK
40151	0.429	0.013	0.615	295.91	81.51	0.087	8.53	HWK
89104	0.368	0.02	0.576	311.93	75.22	0.091	11.6	HWK
69609	0.321	0.042	0.838	230.35	80.08	0.074	5.68	HWK
69010	0.329	0.019	0.703	419.71	83.28	0.077	14.87	HWK
69023	0.438	0.131	0.669	260.47	65.04	0.067	6.86	HWK
69068	0.287	0.017	0.536	259.6	69.43	0.072	7.14	HWK
69024	0.18	0.014	0.578	281.69	69.17	0.035	7.92	HWK
49394	0.211	0.012	0.517	304.67	71.39	0.055	8.97	HWK
49036	1.09	0.01	0.481	270.46	69.98	0.06	10.79	HWK

68860	0.175	0.012	0.566	321.48	75.06	0.093	8.82	HWK
51291	0.242	0.02	0.595	271.66	74.06	0.047	9.86	HWK
39694	0.295	0.02	0.383	142.97	54.47	0.046	5.27	HWK
QA62467	0.112	0.06	0.65	296.057	95.917	0.028	9.262	TLK
QA62468	0.187	0.077	0.603	58.856	107.616	0.013	2.331	TLK
QA62472	0.353	0.046	0.396	282.124	82.685	0.051	9.136	TLK
QA62471	0.103	0.044	0.459	58.425	86.97	0.017	2.843	TLK
QA62226	0.117	0.084	0.643	205.53	97.23	0.022	6.594	TLK
QA62228	0.194	0.06	0.529	225.918	90.364	0.025	6.826	TLK
QA62227	0.092	0.024	0.581	173.846	82.054	0.033	6.38	TLK
QA62463	0.05	0.043	0.391	168.473	95.813	0.033	5.757	TLK
QA62315	0.462	0.503	0.62	262.842	109.798	0.087	9.209	TLK
QA70352	0.149	0.016	0.537	157.371	54.539	0.044	6.687	CB
QA70351	0.105	0.016	0.652	214.767	92.993	0.057	8.397	CB
QA62452	0.164	0.022	0.75	246.233	89.767	0.055	10.075	CB
QA62458	0.159	0.066	0.57	166.158	84.236	0.06	5.413	CB
QA62383	0.138	0.019	0.42	176.83	90.034	0.028	7.14	CB
QA62473	0.079	0.014	0.52	70.7	82.969	0.012	2.812	CB
QA62455	1.469	0.005	0.72	92.628	60.761	0.016	4.512	CB
QA62457	0.368	0.124	0.568	270.426	79.974	0.036	9.351	CB
QA62456	0.15	0.013	0.405	124.847	74.585	0.027	4.244	CB
QA62459	0.127	0.029	1.195	61.087	77.232	0.014	1.916	CB
QA62454	0.259	0.101	1.754	225.241	89.872	0.032	8.171	CB
QA62453	0.05	0.005	0.739	225.129	77.038	0.026	10.061	CB
QA70223	0.51	0.005	0.523	126.968	99.391	0.023	4.976	CB
QA62387	0.69	0.056	0.448	203.847	83.81	0.025	7.206	CB
QA70221	0.46	0.009	0.333	125.361	65.95	0.069	4.113	CB
QA62113	0.391	0.055	0.66	209.17	80.56	0.076	9.48	CB
QA62125	0.255	0.023	0.459	236.45	80.04	0.084	9.07	CB
QA62391	1.96	0.038	1.39	637.94	84.28	0.385	24.94	CB

NO TAG - SICK	2.42	0.005	1.15	306.33	82.67	0.294	10.51	CB
QA62395	1.31	0.14	2.96	340.51	96.88	0.294	13.05	CB
QA62125	0.588	0.024	0.467	239.82	81.09	0.086	9.68	CB
QA62110	0.236	0.057	0.716	68.41	101.02	0.064	3.09	CB
QA62111	0.216	0.131	0.641	193.73	86.6	0.085	7.01	CB
QA70100	0.215	0.062	0.452	66.85	73.71	0.05	2.53	CB
QA70099	0.205	0.607	0.562	243.41	95.68	0.201	8.69	CB
QA70268	1.31	0.38	0.56	278.23	73.18	0.13	12.01	CB
QA70272	0.251	0.042	0.924	145.89	111.63	0.1	5.78	CB
QA70274	0.347	0.095	0.554	131.77	72.8	0.077	5.16	CB
QA70337	0.199	0.129	0.543	246.37	90.23	0.159	11.36	CB
QA70339	0.261	0.563	0.567	264.9	92.15	0.139	10.21	CB
QA70341	0.185	0.214	0.529	157.41	82.53	0.098	4.85	CB
QA70343	0.342	0.313	0.516	250.44	77.91	0.113	9.75	CB
QA70345	1.09	0.042	0.839	142.79	90.9	0.11	5.86	CB
QA70349	0.283	0.026	0.742	182.46	85.5	0.13	7.93	CB
QA62114	0.174	0.123	0.634	147.91	91.06	0.155	6.47	CB
QA62148	0.914	0.192	0.197	57.188	43.835	0.027	2.015	UB
QA39698	0.196	0.151	0.313	101.882	95.347	0.021	3.785	UB
QA62317	0.256	0.193	0.681	386.41	89.1	0.08	11.7	UB
QA62307	0.3	0.308	0.528	314.781	65.963	0.058	10.175	UB
QA62310	1.8	0.177	0.829	261.77	84.11	0.138	8.9	UB
QA62117	0.157	0.699	0.808	233.58	80.64	0.072	7.61	UB
QA62144	0.3	0.957	0.611	357.46	92.72	0.103	10.94	UB
QA62143	0.251	0.567	0.55	233.6	96.21	0.093	7.36	UB
QA62476	0.658	0.356	0.267	68.174	75.436	0.018	3.319	UB
QA62314	0.559	0.671	0.79	352.53	100.5	0.127	12.18	UB
QA62315	0.186	0.768	0.481	222.42	85.6	0.147	7.32	UB
QA62398	0.824	0.99	0.462	179.87	92.18	0.102	6.63	UB
QA62483	0.393	0.092	0.917	261.42	126.28	0.094	9.42	UB

QA62303	2.45	0.458	0.83	362.03	96.13	0.184	11.88	UB
QA62478	0.227	0.619	0.522	272.56	96.32	0.098	9.64	UB
QA62399	0.292	0.633	0.526	313.75	82.65	0.142	9.14	UB
QA51466	0.629	0.45	0.52	313.54	92.37	0.137	10.41	UB
QA62480	0.213	0.484	0.531	275.09	88.08	0.101	10.76	UB
QA51060	0.412	0.223	0.495	257.6	87	0.093	9.28	UB
QA62431	0.216	0.115	0.507	197.64	96.84	0.058	7.45	UB
QA62316	0.756	0.202	0.751	465.7	84.66	0.104	13.02	UB
QA62479	0.406	0.212	0.55	307.35	93.42	0.089	11.26	UB
QA62484	0.256	0.193	0.681	386.41	89.1	0.08	11.7	UB
QA62185	0.713	0.426	0.531	211.92	91.26	0.086	7.15	UB
QA62122	0.22	0.158	0.722	122.319	85.214	0.038	6.869	EB
QA62121	0.289	0.357	0.498	298.474	92.628	0.035	10.755	EB
QA62107	0.307	0.327	0.387	126.344	86.416	0.03	5.29	EB
K39869	0.225	0.083	0.645	174.251	83.823	0.029	6.242	EB
QA62123	0.328	0.224	0.527	193.8	95.46	0.085	9.64	EB
QA62124	0.248	0.384	0.477	180.28	96.25	0.102	7.32	EB
QA42166	9.26	0.282	0.502	322.9	349.19	0.763	9.59	EB
QA36886	0.207	0.479	0.386	317.92	92.03	0.114	12.52	EB
QA62115	0.306	0.154	0.659	132.55	93.79	0.119	4.22	EB
QA62386	0.346	0.449	0.5	257.17	101.5	0.074	10.68	EB
QA62106	0.273	0.262	0.496	265.76	94.7	0.084	9.74	EB
QA62104	0.593	0.243	0.625	208.61	96.7	0.081	6.41	EB
QA62370	7.55	0.117	0.682	339.54	293.45	0.555	9.7	EB
QA62371	14.3	0.079	0.315	167.2	441.09	0.876	4.42	EB
QA62170	15.8	0.084	0.624	436.13	430.81	0.858	12.76	EB
QA62318	0.273	0.489	0.441	137.14	69.98	0.056	4.44	EB
QA62351	18.6	0.089	0.52	365.39	263.45	0.523	10.99	EB
QA62353	0.543	0.413	1.3	920.28	118.17	0.327	30.64	EB
QA62102	1.01	0.075	1.01	726.27	96.43	0.163	23.27	EB

QA62183	0.748	0.104	0.958	677.81	91.06	0.179	24.24	EB
K81504	0.84	0.525	0.746	331.9	88.4	0.131	12.68	EB
QA62319	0.547	0.342	0.582	280.71	91.21	0.114	8.92	EB
QA62301	0.244	0.378	0.266	182.64	100.37	0.053	6.66	EB
QA62321	0.228	0.638	0.66	127.45	101.81	0.072	4.8	EB

Appendix 8 – Advanced Analytical Centre, JCU ICP-MS Certified reference materials (CRM) and respective % recoveries. This applies for the ICP-MS analysis conducted in chapter 3 and 4.

Recovery of NIST1566 Oyster & GBW07605 Tea Leaves

Recovery					
Element					
Al	NIST1566		GBW07605		
Cd		86%	N/A		No certified value available
Co		101%		118%	
Cu		95%		92%	
Fe		95%		96%	
Mg		103%		92%	
Mn		98%		92%	
Ni		104%		102%	
Pb		105%		103%	
Zn		100%		103%	
				104%	

Appendix 9 – Chapter 3 seagrass metal concentration Raw data plus the HWK data from Thomas et al 2020 PCA R code

Loaded Packages

```
library(tidyverse)
library(Factoshiny)
library(FactoMineR)
```

Reading and formatting the data (Seagrass metal data from Appendix 5)

```
sgbeforehwk <- read.csv("sgbeforehwk.csv")
sgbeforehwk <- rename(sgbeforehwk,sample=ï..Site..sample..)
view(sgbeforehwk)
```

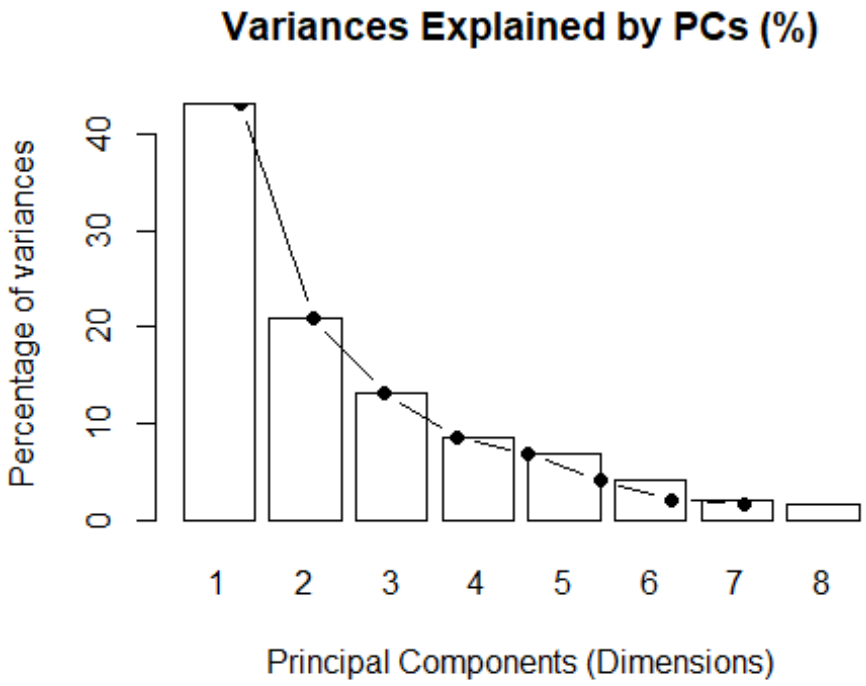
Plotting PCA of my seagrass data and HWK from Thomas et al 2020

```
sgbeforehwk.PCA<-PCA(sgbeforehwk[,-c(1)],quali.sup=c(8,9),graph=FALSE)
plot.PCA(sgbeforehwk.PCA,choix='var',cex=1.65,cex.main=1.65,cex.axis=1.65)
plotellipses(sgbeforehwk.PCA,
keepvar=8,invisible=c('quali','ind.sup'),cex=1.45,cex.main=1.45,cex.axis=1.45,label ='none')
```

Plotting Skree plot

```
eig.val <- sgallhwkres.PCA$eig
barplot(eig.val[, 2],
        names.arg = 1:nrow(eig.val),
        main = "Variances Explained by PCs (%)",
        xlab = "Principal Components (Dimensions)",
        ylab = "Percentage of variances",
        col ="white")
lines(x = 1:nrow(eig.val), eig.val[, 2],
      type = "b", pch = 19, col = "black")
```

Appendix 10 – Skree plot for PCA comparing seagrass metal data with that of HWK reported in Thomas et al 2020 PCA



Appendix 11 - Chapter 3 seagrass metal concentration Raw data before and after the 2018/19 wet season PCA R code

Loaded Packages

```
library(tidyverse)
library(Factoshiny)
library(FactoMineR)
```

Reading and formatting the data (Seagrass metal data from Appendix 5)

```
sgall <- read.csv("Seagrass All.csv")
sgall <- rename(sgall,sample=ï..Site..sample..)
sgall <- sgall[1:84,1:13]
sgall <- na.omit(sgall)
view(sgall)
```

Plotting PCA of my seagrass data

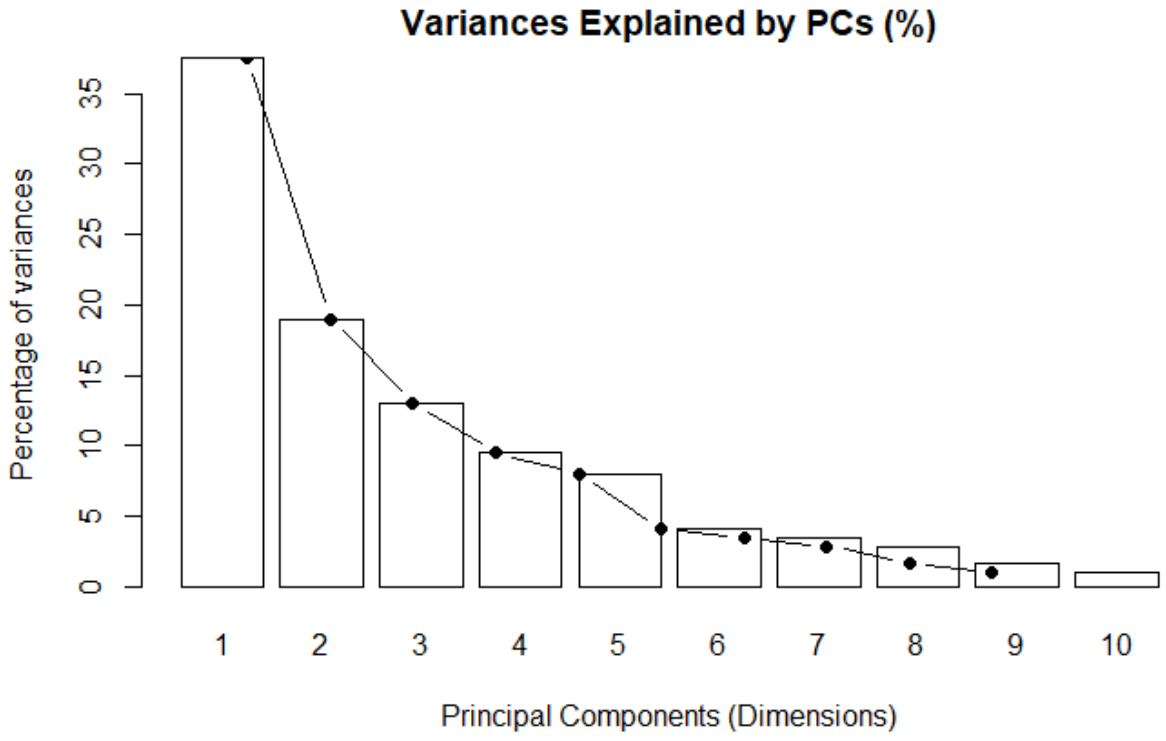
```
sgalleventres.PCA<-PCA(sgall[,-c(1,12)],quali.sup=c(11),graph=FALSE)
```

```
plot.PCA(sgalleventres.PCA,choix='var')

plotellipses(sgalleventres.PCA,
keepvar=11,invisible=c('quali','ind.sup'),cex=1.4,cex.main=1.4,cex.axis=1.4,label ='none')
```

Plotting Skree plot

```
eig.val <- res.PCA$eig

barplot(eig.val[, 2],
        names.arg = 1:nrow(eig.val),
        main = "Variances Explained by PCs (%)",
        xlab = "Principal Components (Dimensions)",
        ylab = "Percentage of variances",
        col ="white")
lines(x = 1:nrow(eig.val), eig.val[, 2],
      type = "b", pch = 19, col = "black")
```

Appendix 12 – Skree plot for PCA comparing seagrass metal data before and after the 2018/19 wet season



Appendix 13 – All blood metal Principal Component Analysis (PCA) R code

Loaded Packages

```
library(tidyverse)
library(Factoshiny)
library(FactoMineR)
```

Reading and formatting the data (all blood metal from Appendix 6)

```
allblood <- read.csv("allblood.csv")
allblood <- rename(allblood,Turtle = ï..Turtle)
```

Plotting PCA of all blood data

```
allbloodres.PCA<-PCA(allblood[,-c(1)],quali.sup=c(8),graph=FALSE)
plot.PCA(allbloodres.PCA,choix='var',cex=1.65,cex.main=1.65,cex.axis=1.65)
plotellipses(allbloodres.PCA,
keepvar=8,invisible=c('quali','ind.sup'),cex=1.4,cex.main=1.4,cex.axis=1.4,label ='none')
```

Display eigen values

```
allbloodres.PCA$eig
```

Display variables, including Cos2

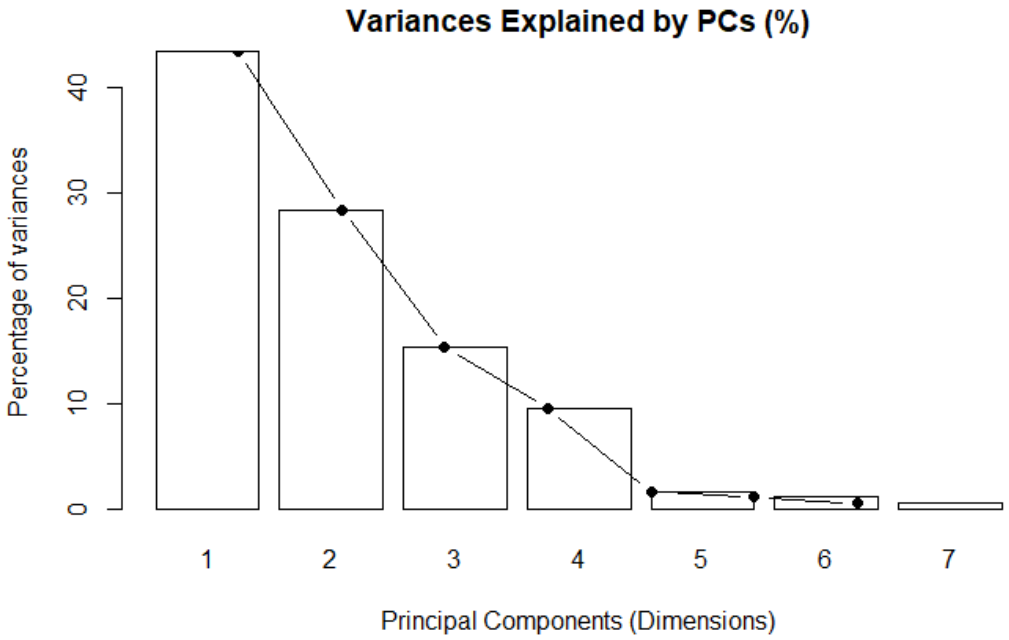
```
allbloodres.PCA$var
```

Plotting Skree plot

```
allbloodeig.val <- allbloodres.PCA$eig
barplot(allbloodeig.val[, 2],
        names.arg = 1:nrow(eig.val),
        main = "Variances Explained by PCs (%)",
        xlab = "Principal Components (Dimensions)",
        ylab = "Percentage of variances",
        col ="white")
lines(x = 1:nrow(eig.val), eig.val[, 2],
```

type = "b", pch = 19, col = "black")

Appendix 14 - Skree plot for PCA analysis of all blood samples in chapter 4



Appendix 15 – PCA R code for analysis of blood metal concentration data minus EB data

Loaded Packages

```
library(tidyverse)
library(Factoshiny)
library(FactoMineR)
```

Reading and formatting the data (all metal from Appendix 6 minus EB)

```
bloodEB <- read.csv("allbloodwoeb.csv")
bloodEB <- rename(bloodEB,Turtle = ï..Turtle)
bloodEB <- na.omit(bloodEB)
```

Plotting PCA of blood (minus EB) data

```
bloodEB.PCA<-PCA(bloodEB[,-c(1)],quali.sup=c(8),graph=FALSE)
plot.PCA(bloodEB.PCA,choix='var',cex=1.6,cex.main=1.6,cex.axis=1.6)
```

```
plotellipses(bloodEB.PCA,
keepvar=8,invisible=c('quali','ind.sup'),cex=1.6,cex.main=1.6,cex.axis=1.6,label ='none')
```

Display eigen values

```
bloodEB.PCA$eig
```

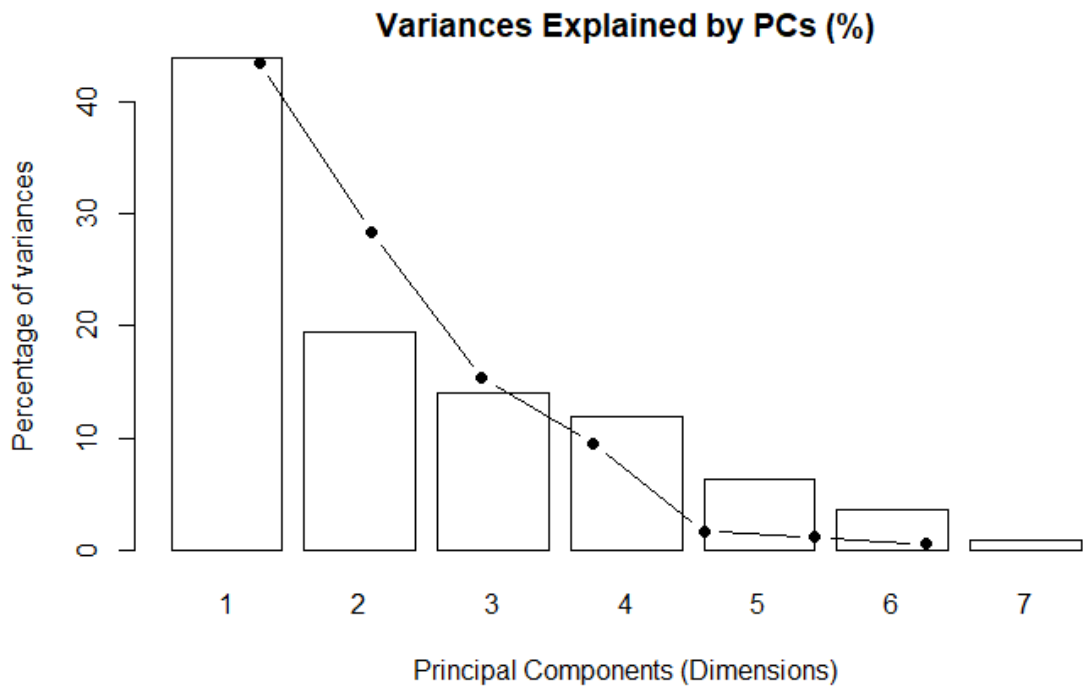
Display variables, including Cos2

```
allbloodres.PCA$var
```

Plotting Skree plot

```
bloodEB.val <- bloodEB.PCA$eig
barplot(bloodEB.val[, 2],
        names.arg = 1:nrow(eig.val),
        main = "Variances Explained by PCs (%)",
        xlab = "Principal Components (Dimensions)",
        ylab = "Percentage of variances",
        col ="white")
lines(x = 1:nrow(eig.val), eig.val[, 2],
      type = "b", pch = 19, col = "black")
```

Appendix 16 – Skree plot for PCA analysis of all blood samples in chapter 4 minus EB data



Appendix 17 – Scute metal Principal Component Analysis (PCA) R code

Loaded Packages

```
library(tidyverse)
library(Factoshiny)
library(FactoMineR)
```

Reading and formatting the data (all scute from Appendix 6)

```
scute <- read.csv("scute.csv")
scute <- rename(scute,Turtle = ï..Turtle)
scute <- na.omit(scute)
```

Plotting PCA of scute data

```
scute.PCA<-PCA(scute[,-c(1,10)],quali.sup=c(10),graph=FALSE)
plot.PCA(scute.PCA,choix='var')
plotellipses(scute.PCA,
keepvar=10,invisible=c('quali','ind.sup'),cex=1.6,cex.main=1.6,cex.axis=1.6,label ='none')
```

Display eigen values

```
scute.PCA$eig
```

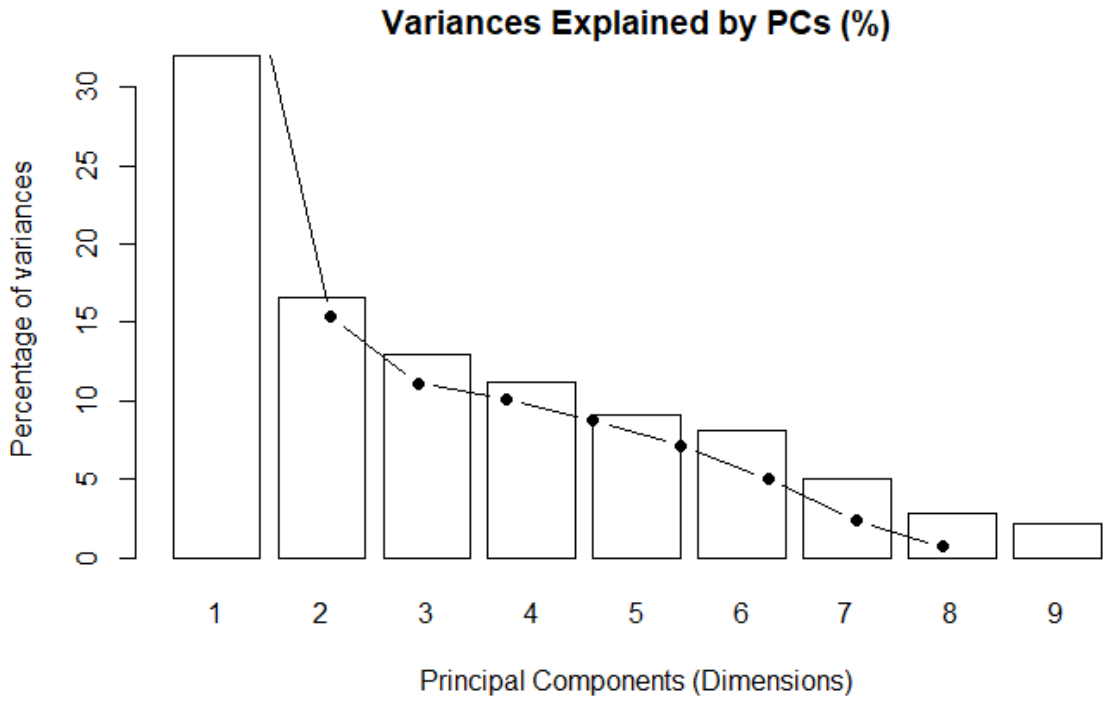
Display variables, including Cos2

```
scute.PCA$var
```

Plotting Skree plot

```
scute.val <- scute.PCA$eig
barplot(scute.val[, 2],
        names.arg = 1:nrow(eig.val),
        main = "Variances Explained by PCs (%)",
        xlab = "Principal Components (Dimensions)",
        ylab = "Percentage of variances",
        col ="white")
lines(x = 1:nrow(eig.val), eig.val[, 2],
      type = "b", pch = 19, col = "black")
```

Appendix 18 – Skree plot for PCA analysis of scute samples in chapter 4



Appendix 19 – Chapter 5 Optical density raw data from ELISA using the CO2 monoclonal protocol from *C.mydas* sampled at each study site and positive (PC) and negative controls (NC) included on each plate.

SITE	ID	Cmyc-GST O.D.	F-US-4 O.D.
TLK	QA62460	1.079	1.497
TLK	QA62461	1.718	1.732
TLK	QA62462	1.787	1.846
TLK	QA62463	0.187	0.298
TLK	QA62465	0.162	0.175
TLK	QA62467	0.226	0.257
TLK	QA62468	0.088	0.107
TLK	QA62469	0.133	0.153
TLK	QA7380	0.558	1.023
TLK	QA62126	0.296	1.848
TLK	QA62470	0.181	0.194
TLK	QA62471	1.408	1.530
TLK	QA62472	0.136	0.135
TLK	QA62226	0.145	0.168
TLK	QA62227	0.147	0.168
TLK	QA62228	0.123	0.140
TLK	QA20330	0.587	1.133
TLK	QA62129	0.100	0.133
TLK	QA62131	0.181	0.212
TLK	QA62136	0.138	0.155
CB	QA70352	0.000	0.122
CB	QA70345	0.146	0.150
CB	QA70274	0.121	0.099
CB	QA70220	0.225	0.164
CB	QA70222	0.253	1.088
CB	QA70205	0.067	0.080
CB	QA70206	0.114	0.207
CB	QA70208	0.140	0.191
CB	QA70207	0.135	0.720
CB	QA70212	0.343	0.342
CB	QA70210	0.421	0.495
CB	QA70211	0.188	0.228
CB	QA70204	0.430	0.460
CB	QA70209	0.127	0.101
CB	QA62456	0.151	0.170
CB	QA70223	0.172	0.172
CB	QA62459	0.147	0.168

CB	QA62458	0.175	1.240
CB	QA62454	1.325	1.352
CB	QA70351	0.201	0.194
NC	F1	0.100	0.445
NC	F4	0.078	0.151
NC	F6	0.090	0.163
NC	G4	0.073	0.114
NC	G5	0.081	0.154
NC	G6	0.349	0.325
PC	H3	0.323	1.547
	anti-cmyc	0.563	0.823

UB	QA62307	0.577	0.672
UB	QA62310	0.367	0.463
UB	QA62148	0.711	0.737
UB	QA62317	0.340	0.513
UB	QA62314	0.471	0.503
UB	QA62476	0.384	0.556
UB	QA51061	0.249	0.310
UB	QA62478	0.356	0.384
UB	QA51466	1.153	1.420
UB	QA39698	0.364	0.353
UB	QA62117	0.275	0.294
UB	QA62303	0.330	0.304
UB	QA62315	0.571	0.631
UB	QA62143	0.381	0.536
UB	QA62399	0.295	0.372
UB	QA62479	0.994	1.832
UB	QA62480	0.793	0.867
UB	QA62481	0.363	0.426
UB	QA62484	0.370	0.447
UB	QA62316	0.624	0.603
UB	QA62185	1.008	1.025
UB	QA62398	0.320	0.305
UB	QA62144	0.888	0.911
UB	QA62482	0.317	0.378
UB	QA62483	0.560	0.636
HWK	QA69745	0.528	0.452
HWK	QA69815	0.531	0.434
HWK	QA48905	0.456	0.301
HWK	QA69019	0.348	0.327

HWK	QA49510	0.468	0.642
HWK	QA49024	0.375	0.593
HWK	QA47557	0.676	0.913
HWK	QA51264	0.417	0.291
HWK	QA32005	1.811	1.651
HWK	QA69010	1.555	1.120
HWK	QA39526	0.413	0.399
HWK	QA69608	0.591	0.593
HWK	QA69066	0.748	0.849
HWK	QA49327	0.292	0.286
HWK	QA69064	0.350	0.307
HWK	QA40151	0.382	0.380
HWK	QA51273	1.082	1.278
HWK	QA51810	0.718	1.227
HWK	QA49394	0.679	0.888
HWK	QA49036	1.326	1.370
HWK	QA69023	0.814	0.994
HWK	QA69607	1.784	1.605
HWK	QA69609	0.493	0.479
HWK	QA69801	0.855	1.032
HWK	QA69805	0.769	1.274
HWK	QA69811	0.902	1.200
UB	F1	0.990	1.123
UB	F4	0.467	1.151
UB	F6	0.426	0.909
UB	G4	0.429	1.158
UB	G5	0.456	0.953
UB	G6	0.571	1.168
UB	H3	0.718	2.042
UB	anti-cmyc	1.963	2.350
HWK	F1 - NC	1.390	1.854
HWK	F4 - NC	0.744	1.587
HWK	F6 - NC	0.540	0.908
HWK	G4 - NC	0.517	1.255
HWK	G5 - NC	0.640	0.934
HWK	G6 - NC	0.677	1.335
HWK	H3 - PC	0.671	2.308
HWK	anti-cmyc	1.102	2.192
UB	C2 31/10/2017	0.283	0.233
UB	C2 14/3/2018	0.248	0.229

UB	C2 5/9/2018	0.270	0.240
UB	C2 19/12/2018	0.254	0.278
UB	C3 31/10/2017	0.224	0.224
UB	C3 14/3/2018	0.323	0.236
UB	C3 5/9/2018	0.255	0.305
UB	C3 19/12/2018	0.255	0.303
UB	D1 31/10/2017	0.311	0.228
UB	D2 31/10/2017	0.259	0.259
UB	E1 31/10/2017	0.273	0.228
HWK	C2 31/10/2017	0.242	0.180
HWK	C2 14/3/2018	0.247	0.233
HWK	C2 5/9/2018	0.284	0.303
HWK	C2 19/12/2018	0.304	0.325
HWK	C3 31/10/2017	0.189	0.160
HWK	C3 14/3/2018	0.264	0.173
HWK	C3 5/9/2018	0.206	0.188
HWK	C3 19/12/2018	0.313	0.311
HWK	D1 31/10/2017	0.397	0.404
HWK	D2 31/10/2017	0.192	0.190
HWK	E1 31/10/2017	0.206	0.350

EB	QA62171	0.273	0.293
EB	QA62169	0.332	0.491
EB	QA62353	0.331	0.704
EB	QA62102	0.345	0.366
EB	QA62351	0.322	0.385
EB	QA62323	0.263	0.289
EB	QA29733	0.266	0.379
EB	QA62181	0.231	0.420
EB	QA62199	0.358	0.405
EB	QA62178	0.318	0.506
EB	QA62183	0.318	0.302
EB	QA42318	0.468	0.326
EB	QA62107	0.367	0.622
EB	QA62301	0.356	0.415
EB	QA62320	0.341	0.354
EB	QA62122	0.366	0.760
EB	QA62121	0.563	1.056
EB	QA62340	0.392	0.548
EB	QA62337	0.313	0.348
EB	QA62338	0.792	1.874

EB Archived	K93037	0.313	0.272
EB Archived	K93030	0.662	0.668
EB Archived	K93098	0.645	0.683
EB Archived	K93080	0.421	0.528
EB Archived	K71744	0.290	0.367
EB Archived	K93032	0.500	0.496
EB Archived	K93028	0.339	0.374
EB Archived	K93031	0.347	0.344
EB Archived	K93036	0.331	0.292
EB Archived	K93038	0.422	0.954
EB Archived	K93039	0.420	0.400
EB Archived	K93051	0.386	0.490
EB Archived	K93052	0.282	0.304
EB Archived	K93074	0.477	0.661
EB Archived	QA9473	0.501	0.646
EB Archived	K97483	0.463	0.583
EB Archived	QA9471	0.376	1.001
EB Archived	QA9465	0.374	0.501
EB Archived	QA9462	0.498	0.700
EB Archived	QA9471	0.916	1.013
NC	C2	0.384	0.355
NC	C2	0.707	0.391
NC	C2	0.360	0.376
NC	C3	0.348	0.566
NC	C3	0.364	0.516
NC	C3	0.405	0.414
PC	H3	0.630	2.003
anti-cmyc		2.069	2.098

Appendix 20 – Chapter 5 Optical density raw data from ELISA using the D70 monoclonal protocol from *C.mydas* sampled at each study site and positive (PC) and negative controls (NC) included on each plate.

SITE	ID	Cmyc-GST O.D.	F-US-4 O.D.
TLK	QA62460	0.241	0.320
TLK	QA62461	0.203	0.216
TLK	QA62462	0.526	0.505
TLK	QA62463	0.164	0.552
TLK	QA62465	0.161	0.209
TLK	QA62467	0.095	0.113
TLK	QA62468	0.137	0.143
TLK	QA62469	0.142	0.120
TLK	QA7380	0.165	1.997
TLK	QA62126	0.122	0.307
TLK	QA62470	0.088	0.070
TLK	QA62471	0.082	0.138
TLK	QA62472	0.174	0.168
TLK	QA62226	0.064	0.080
TLK	QA62227	0.219	0.084
TLK	QA62228	0.094	0.088
TLK	QA20330	0.144	2.042
TLK	QA62129	0.065	0.095
TLK	QA62131	0.073	0.069
TLK	QA62136	0.062	0.065
CB	QA70352	0.188	0.139
CB	QA70345	0.380	0.350
CB	QA70274	0.131	0.127
CB	QA70220	0.088	0.063
CB	QA70222	0.465	0.888
CB	QA70205	0.074	0.065
CB	QA70206	0.192	0.162
CB	QA70208	0.165	0.190
CB	QA70207	0.116	0.233
CB	QA70212	0.158	0.167
CB	QA70210	0.109	0.112
CB	QA70211	0.107	0.112
CB	QA70204	0.071	0.089
CB	QA70209	0.078	0.094
CB	QA62456	0.156	0.220
CB	QA70223	0.105	0.124
CB	QA62459	0.128	0.153
CB	QA62458	0.268	1.544

CB	QA62454	1.519	1.284
CB	QA70351	0.117	0.145
NC	F1	0.050	0.083
NC	F4	0.059	0.054
NC	F6	0.058	0.065
NC	G4	0.056	0.060
NC	G5	0.075	0.060
NC	G6	0.065	0.130
PC	H3	0.072	0.175
	anti-cmyc	0.419	0.614

UB	QA62307	0.509	1.827
UB	QA62310	0.356	1.985
UB	QA62148	0.456	0.477
UB	QA62317	0.182	0.308
UB	QA62314	0.272	0.319
UB	QA62476	0.289	0.316
UB	QA51061	0.219	0.210
UB	QA62478	0.633	0.878
UB	QA51466	0.556	0.827
UB	QA39698	0.251	1.929
UB	QA62117	0.432	0.410
UB	QA62303	0.230	0.293
UB	QA62315	0.282	0.241
UB	QA62143	0.313	0.310
UB	QA62399	0.521	0.361
UB	QA62479	0.270	1.085
UB	QA62480	0.311	0.308
UB	QA62481	0.314	0.341
UB	QA62484	0.483	0.485
UB	QA62316	0.501	0.396
UB	QA62185	0.378	0.373
UB	QA62398	0.341	0.406
UB	QA62144	0.279	0.353
UB	QA62482	0.373	0.333
UB	QA62483	0.307	0.272
HWK	QA69745	0.528	1.304
HWK	QA69815	0.540	0.666
HWK	QA48905	0.427	0.529
HWK	QA69019	0.696	0.764
HWK	QA49510	0.449	0.331

HWK	QA49024	0.540	0.617
HWK	QA47557	0.376	0.308
HWK	QA51264	0.398	0.468
HWK	QA32005	0.513	0.370
HWK	QA69010	0.420	0.500
HWK	QA39526	0.404	0.407
HWK	QA69608	0.478	0.508
HWK	QA69066	0.694	0.498
HWK	QA49327	0.460	0.404
HWK	QA69064	0.386	0.415
HWK	QA40151	0.366	0.339
HWK	QA51273	0.701	0.519
HWK	QA51810	0.350	0.387
HWK	QA49394	0.526	0.466
HWK	QA49036	0.475	0.443
HWK	QA69023	0.317	0.412
HWK	QA69607	0.748	0.827
HWK	QA69609	0.392	0.639
HWK	QA69801	0.570	0.862
HWK	QA69805	0.584	0.806
HWK	QA69811	0.507	0.810
UB	F1 - NC	0.220	0.301
UB	F4 - NC	0.247	0.265
UB	F6 - NC	0.241	0.337
UB	G4 - NC	0.251	0.270
UB	G5 - NC	0.241	0.263
UB	G6 - NC	0.306	0.443
UB	H3 - PC	0.829	1.061
UB	anti-cmyc	1.343	1.534
HWK	F1 - NC	0.406	1.050
HWK	F4 - NC	0.309	1.109
HWK	F6 - NC	0.383	0.682
HWK	G4 - NC	0.554	0.960
HWK	G5 - NC	0.741	1.187
HWK	G6 - NC	0.998	1.249
HWK	H3 - PC	1.038	1.337
HWK	anti-cmyc	1.377	1.822
UB	C2 31/10/2017	0.204	0.174
UB	C2 14/3/2018	0.297	0.284
UB	C2 5/9/2018	0.317	0.187

UB	C2 19/12/2018	0.176	0.132
UB	C3 31/10/2017	0.200	0.176
UB	C3 14/3/2018	0.226	0.230
UB	C3 5/9/2018	0.340	0.344
UB	C3 19/12/2018	0.161	0.152
UB	D1 31/10/2017	1.579	0.191
UB	D2 31/10/2017	0.203	0.189
UB	E1 31/10/2017	0.230	0.202
HWK	C2 31/10/2017	0.247	0.254
HWK	C2 14/3/2018	0.262	0.299
HWK	C2 5/9/2018	0.305	0.397
HWK	C2 19/12/2018	0.191	0.229
HWK	C3 31/10/2017	0.288	0.314
HWK	C3 14/3/2018	0.350	0.293
HWK	C3 5/9/2018	0.377	0.392
HWK	C3 19/12/2018	0.248	0.300
HWK	D1 31/10/2017	0.289	0.265
HWK	D2 31/10/2017	0.269	0.271
HWK	E1 31/10/2017	0.240	0.282
EB	QA62171	0.223	0.247
EB	QA62169	0.182	0.294
EB	QA62353	0.290	0.541
EB	QA62102	0.266	0.274
EB	QA62351	0.406	0.362
EB	QA62323	0.180	0.173
EB	QA29733	0.290	0.756
EB	QA62181	0.298	0.387
EB	QA62199	0.208	0.299
EB	QA62178	0.224	0.297
EB	QA62183	0.433	0.532
EB	QA42318	0.308	0.342
EB	QA62107	0.265	0.512
EB	QA62301	0.294	0.320
EB	QA62320	0.364	0.378
EB	QA62122	0.265	1.690
EB	QA62121	0.378	0.457
EB	QA62340	0.316	0.235
EB	QA62337	0.566	0.485
EB	QA62338	0.311	0.279

EB Archived	K93037	0.337	0.305
EB Archived	K93030	0.489	0.417
EB Archived	K93098	0.414	0.382
EB Archived	K93080	0.242	0.340
EB Archived	K71744	0.141	0.146
EB Archived	K93032	0.491	0.867
EB Archived	K93028	0.325	0.320
EB Archived	K93031	0.274	0.357
EB Archived	K93036	0.271	0.289
EB Archived	K93038	0.417	1.146
EB Archived	K93039	0.378	0.393
EB Archived	K93051	0.265	0.282
EB Archived	K93052	0.233	0.232
EB Archived	K93074	0.420	0.727
EB Archived	QA9473	0.288	0.285
EB Archived	K97483	0.294	0.299
EB Archived	QA9471	0.292	1.691
EB Archived	QA9465	0.224	0.223
EB Archived	QA9462	0.304	0.489
EB Archived	QA9471	0.546	0.609
NC	C2	0.322	0.594
NC	C2	0.244	0.293
NC	C2	0.236	0.294
NC	C3	0.184	0.297
NC	C3	0.157	0.308
NC	C3	0.197	0.420
PC	H3	0.233	0.492