

INCREASING THE UNDERSTANDING OF THE GREEN TURTLE POPULATION IN  
PORT CURTIS, 2016-2019: FINAL REPORT  
GPC ERMP CONTRACT No. CA14000241



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Cover photographs:

Scenes from the population monitoring of Green turtles, *Chelonia mydas*, at Port Curtis, April - October 2019.

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#### Executive summary

On 15 October 2015 the Queensland Department of Environment and Heritage Protection (EHP) signed a Consultancy Agreement with Gladstone Ports Corporation (GPC) to undertake a four-year study to *Increase the Understanding of the Green Turtle Population in Port Curtis*.

In the absence of existing data regarding the size of the Green turtle foraging population in Port Curtis, it was planned to have an annual capture of a large number of Green turtles for inclusion in this capture-mark-recapture (tagging, CMR) study with a focus on the Pelican Banks, off the Boyne River estuary and in the Western Basin and Narrows. The number of turtles to be captured annually was to be adjusted by the end of the first year of the study based on the reality of the accessibility of the turtles within the Port:

- The repetitive sampling of the study sites with recaptures of previously tagged turtles to provide the core data required for description of the population structure within the Port;
- CMR analysis to provide quantified estimates of population size, recruitment and survivorship with trends by sex and maturity;
- Growth study analysis;
- Quantified adult breeding rates;
- A gross assessment of health to provide an analysis of the incidence of fractures, Green turtle fibropapilloma tumours and a body condition index.

An established tagged population of green turtles within the Port would provide a 'platform' for more collaborative collaboration with university post-graduate studies to enhance our understanding of the ecology and health of Green turtles within Port Curtis:

- Providing a more focussed selection of turtles for satellite telemetry tracking of movements and habitat use in collaboration with James Cook University, expanding on the telemetry studies commissioned by GPC with inclusion of tracking data from previous and continuing telemetry studies by EHP.
- Defining the genetic stock structure for Green turtles foraging in Port Curtis, in collaboration with Dr Nancy FitzSimmons, Griffith University.
- Turtle health studies:
  - Annual health assessment via blood sampling for haematology and blood chemistry in collaboration with University of Queensland (UQ) School of Veterinary Science (UQSVS).
  - Bioaccumulation of metal toxins by turtles foraging in Port Curtis in collaboration with UQ School of Toxicology.
  - A novel investigation of the toxicology of inorganic contaminants in marine turtles using cell-based bioassays in collaboration with Griffith University.



- Improving the capacity for Stable Isotope (C-N) analysis for interpretation of the diet and habitat use by Green turtles via a collaborative Green turtle diet study with UQ.

This four-year study has built on existing data from the continuing studies of Green turtles in Port Curtis and elsewhere in Queensland by EHP to improve the rigour of analyses as required.

The Green turtle, *Chelonia mydas*, foraging population in Port Curtis

- The Green turtle was the most abundant turtle in intertidal and shallow subtidal waters of Port Curtis.
- The study was completed satisfactorily with 1576 captures of 1232 separate turtles out of a total of 3423 recorded sightings of green turtles during 2016-2019. Captures occurred consistently at multiple study sites within the Port.
- Most turtles were captured via the turtle rodeo method. However, the use of a 300 m blocking net remained the only effective means for capture of turtles in turbid waters.
- Foraging Green turtles aggregated primarily in five areas within Port Curtis that were characterised by being adjacent to outflows from rivers and creeks or, in the case of the Pelican Banks, with outflow from the Port where there is regular reversal of strong tidal currents and associated settlement of sediments to form wide shallow flats supporting seagrass and algal pastures.
- The majority of the Green turtles within Port Curtis forage over the intertidal and subtidal flats adjacent to outside and inside of the outflow areas of the estuaries of Colosseum Creek, Boyne River, South Trees Inlet, Calliope River and the entrance to the Port between Curtis and Facing Islands. This latter area includes the Pelican Banks.
- The team was unable to locate any area with a concentration of foraging Green turtles within the turbid waters of the Western Basin or at the southern end of The Narrows except in the vicinity of Wiggins Island.
- Most juvenile turtles were caught in the shallow intertidal areas and around mangroves or rocky reef during the higher tide levels. Most larger turtles were caught in deeper intertidal and subtidal waters at the Pelican Banks, South Trees, and off southern Wild Cattle Island.
- Based on the flipper tag recovery data, Green turtles foraging within Port Curtis have displayed high fidelity to their respective localised foraging sites, except when adults make breeding migrations.
- Green turtles that forage within Port Curtis are derived primarily from sGBR genetic stock that breeds at the southern Great Barrier Reef courtship areas and nesting beaches.
- Port Curtis is not a significant area for Green turtle aggregation for courtship and mating.
- The sex ratio of Green turtles sampled during 2011-2019 from all study sites collectively varied across the age classes within Port Curtis:
  - adults had approximately equal proportions of females and males (51% females; 1.03:1 ratio)

- large immature, small immature and recently recruited very small immature turtles showed an overall higher proportion of females in the younger age classes (up to 64% female; 1.77:1 ratio).
- A comparison of sex ratio within multiple foraging areas dominated by Green turtles from the sGBR stock identified that:
  - Lower female biased adult sex ratios are associated with sampling sites in close proximity to the focal courtship and nesting region for the sGBR genetic stock
  - Female biased adult sex ratio is highest at foraging areas 3° or more in latitude away from the core breeding area for the sGBR stock (on the Capricorn-Bunker Group cays).
- The sex ratio of small immature Green turtles from sGBR stock dominated foraging areas has fluctuated mostly within the range of 0.6 to 0.8 females across recent decades and with no obvious tendency towards increased feminisation within the stock.
- Very small immature Green turtles recruited to residency in Port Curtis with a mean CCL = 43.2 cm, with no detectable differences in the size at which they recruited with respect to gender or year of recruitment.
- Very small immature Green turtles recruited into the Port Curtis foraging population at a mean annual proportional rate = 0.14 of the small pre-pubescent Green turtle resident population present in the Port.
- Adult female Green turtles in Port Curtis were larger on average than adult males
- There was no detectable difference in size of either the adult females or males across the seven years of study.
- Adult females within Port Curtis commenced breeding at a relatively small mean CCL = 99.2 cm.
- A low annual recruitment rate of first-time breeders into the female breeding population was recorded (rate = 0.10).
- External examination of foraging turtles in Port Curtis identified only low incidence of compromised health among the turtles captured:
  - Partly or extremely emaciated turtles = 7.9%
  - Turtles fractured from vessel interactions = 3.2%
  - Entangled in fishing gear and marine debris = 0.7%
  - Fibropapillomatosis = 3.6%.
- Fractured turtles:
  - Observed fracture injuries to turtles were consistent with damage caused primarily by medium to large outboard powered vessels moving at speed, not from the larger commercial vessels such as tugs and freighters using the Port infrastructures.

- The majority of fractured turtles (69%) were captured on the Pelican Banks.
- The introduction of a go-slow zone over the Pelican Banks within vessel management policy for Port Curtis has the potential for substantial reduction of vessel related injuries and mortality within the Port.
- Fibropapillomatosis
  - The low incidence of Fibropapillomatosis as recorded by the presence and severity of external tumours in Port Curtis (3.6%) is at the lower range of the incidence of tumoured turtles in coastal bays in Queensland.
  - The incidence of Fibropapillomatosis tumoured turtles in Port Curtis was slightly higher than that recorded at Heron-Wistari Reefs on the outer margin of the GBR.
  - As recorded for Moreton Bay and Shoalwater Bay, Green turtles in Port Curtis displayed a capacity for recovery from Fibropapillomatosis.
  - Fibropapillomatosis is not considered to represent a significant threat to the foraging Green turtle population in Port Curtis.

Population genetics of the Green turtle, *Chelonia mydas*, population foraging in Port Curtis

In summary, genetic analyses of foraging juvenile Green turtles residing in Port Curtis indicate that:

- There were no significant differences in haplotype frequencies between juvenile male and female Green turtles.
- The genetic diversity of juvenile turtles was greater in Port Curtis than observed at foraging grounds dominated by the sGBR genetic stock at Edgecombe Bay, Shoalwater Bay and Moreton Bay due to the presence of several haplotypes observed at low frequencies.
- Although genetic differences were not significant between the Port Curtis sample and the Edgecombe Bay, Shoalwater Bay and Moreton Bay foraging samples, results from the mixed stock analysis were notably different.
- Mixed stock analyses estimated that juvenile turtles originate primarily from the combined sGBR/Coral Sea stock (72.2% - 73.5%), with contributions from New Caledonia (20.8% - 21.6%), and a combination of other (4.4% - 6.9%) genetic stocks.
- In comparison to previous mixed stock analyses of small immature Green turtles during the 1990s and early 2000s at the Edgecombe Bay, Shoalwater Bay and Moreton Bay foraging grounds, the results were unexpected: indicating an average 16.8% decrease in the relative contribution of the sGBR/Coral Sea stock, and average increased contributions of 11.0% from the New Caledonia stock and 3.0% increase in 'other' stocks.

These results highlight the value of assessing the genetic stock composition of other Green turtle foraging populations in eastern Australia, encompassing all age classes,

when endeavouring to understand the shift in haplotype frequencies and estimated contributions of the regional genetic stocks to the foraging turtles in Port Curtis.

#### Green turtle habitat use and site fidelity in Port Curtis

- This satellite telemetry study of the Green turtle population in Port Curtis aimed specifically to increase understanding of habitat use and short-term site fidelity.
- Telemetry data are available from 72 of 73 turtles that were released in Port Curtis with satellite tags between 2010 and 2019.
- Tags transmitted FastGPS and ARGOS PTT locations, and in addition, water temperature and depth from 2014-2019.
- Habitat use was examined within 95% utilisation distributions (UDs) for each tracked individual, delineating the area in which the individual spent 95% of the tracking period, defining the individual's foraging "home range". Sufficient data to generate UD's was received for 72 individuals. The utilisation distribution does not necessarily represent a total home range, as the study only documents habitats utilised in the short-term (over a period of up to 13 months of tracking).
- Average home range area for all tracked individuals was 19.9 km<sup>2</sup> (1-115 km<sup>2</sup>, median=11.4 km<sup>2</sup>).
- These are of a similar scale to home ranges calculated at other Queensland foraging sites. Home range areas were highest for adults and turtles captured on the Pelican Banks and were lowest in 2018 and 2019 compared to other years (all tracked turtles in 2019 were captured close to Wiggins Island).
- Of the 72 individuals for which a 95% UD was generated:
  - 18 had their entire home range within the Port Curtis boundary
  - 53 had at least 2% of their home range area outside Port Curtis
  - The average percentage of home range area outside Port Curtis was 23.7±2.9%.
  - 17 turtles used areas outside the EMRP boundary as part of their 95% UD. These were mostly making periodic trips to offshore reef habitat outside the Port boundary from the Pelican Banks via the northeast channel between Curtis and Facing Islands (n=14). The others were either using estuarine habitats upstream of river outflows (n=2) or making large movements away from their capture site along the coast (n=1).
  - Home ranges of 28 individuals overlapped with shipping channels, though by less than 5% of the home range for 21 of these individuals.
- Satellite-tracked individuals showed a high degree of short-term site fidelity. Of 72 tracked individuals, 60 resettled at their capture site shortly after release.
  - Of these, 45 remained for the duration of the tracked period
  - 7 performed brief trips to other areas and returned

- 6 later departed and settled to other areas
  - adult female QA66526 departed the Pelican Banks in November 2016 and travelled over 150 km north along the coast over 6 days towards Shoalwater, where she remained for the remainder of tracking (Table 4, Figure 4.6H).
- 2 departed to courtship areas.
- A further 10 individuals resettled in the capture area after several days at other sites post-release, and six of these remained there for the remaining tracking duration.
- Only two individuals did not return to the original capture site:
  - adult male QA45689 captured on the Pelican Banks in 2014 who travelled south ~20 km to Tannum Sands.
  - adult male QA58291 tracked in 2015 from the Pelican Banks travelled approximately 37 km south to Rodds Bay.
- In 2013, 10 individuals were deployed by CSIRO with satellite transmitters at Pelican Banks and Wiggins Island (Babcock *et al.* 2015).
  - Three individuals tagged on the Pelican Banks departed the capture area shortly after release, travelling distances exceeding any departure track recorded by other satellite tags deployed in Port Curtis 2010-2019.
  - Home ranges reported for CSIRO turtles that remained near the capture area were similar to those from DES deployments in the same year.
  - CSIRO acoustic tracking also showed a higher proportion of turtles moving between the Pelican Banks and Wiggins Island than was recorded by other satellite tracks 2010-2019.
  - The atypical behaviour of these CSIRO turtles may be attributed to habitat damage resulting from extensive flooding that occurred in 2013, the largest occurring in Port Curtis in 100 years, or from any additional disturbance that might be attributed to attachment of both acoustic and satellite transmitters on the same individual. The flooding was followed by very patchy distribution and low biomass of seagrass throughout the port in 2013 (Babcock *et al.* 2015), which may have prompted turtles to expand their foraging ranges.
- From the 72 tracked individuals discussed in this report, the proportion of turtles that used multiple non-contiguous areas as part of their home range, rather than remaining in one area, appeared somewhat higher in years in which Port Curtis experienced major flooding events from the Fitzroy and adjacent outflows (2013 and 2017), though the difference was not statistically significant.
- Individual turtles tended to adhere to spatially confined areas, with few switching between sites as part of their short-term foraging range. Inferences from diet and

ecotoxicology studies may therefore be indicative of microhabitats, rather than the wider Port.

- Tracks of turtles sampled at one study site are likewise unlikely to represent habitat use of turtles in the wider port, and sampling should be targeted directly in areas of interest for monitoring and management with regards to turtles.
- This is a challenging issue to address, as many of these areas (such as dredged channels) are characterised by deep or turbid water and are not suitable for capture by turtle rodeo or blocking nets.
- There was little overlap detected between Green turtle foraging ranges and dredged channels and therefore the movement of large industrial vessels and tugs. In contrast, collision with high-speed recreational vessels may pose a threat to turtles foraging in shallow intertidal waters such as on the Pelican Banks.
- While adult turtles will temporarily leave their foraging home range during breeding migrations, there is some evidence that turtles also are capable of adjusting their space-use in response to disturbance or resource availability.

#### Green turtle population dynamics in Port Curtis

##### Trends in body condition:

- For all foraging sites examined within Port Curtis, there was a broad range in the body condition index (BCI) within size classes:
  - There was no difference in BCI between sexes for the juvenile and subadult turtles
  - Adult females had a greater BCI compared to adult males
- Spatially, BCI was significantly higher for Green turtles sampled in the Western Basin than elsewhere in the Port followed by turtles at South Trees.
- While there was only limited temporal variation in estimated BCI over the 4 yr period, there appeared to be a lower mean BCI in 2018 than in the other years within Port Curtis. Whether the lower BCI for Port Curtis in 2018 was a consequence of the localised high flood level in Gladstone in early 2017 was not investigated.
- When compared with other Green turtle foraging populations dominated by the sGBR genetic stock, Port Curtis displayed an intermediate BCI across all three age classes relative to Moreton Bay (highest age class-specific BCI) and Shoalwater Bay (lowest).

##### Somatic Growth:

- The Green turtles resident in foraging habitats of Port Curtis grew more slowly at any given size or age compared with turtles in other foraging areas dominated by the sGBR stock in Moreton Bay, Heron-Wistari Reefs and Shoalwater Bay.
  - Green turtles living in Port Curtis and Shoalwater Bay commence breeding at a smaller size than those living in Heron-Wistari Reefs and Moreton Bay.

Since Green turtles commence breeding at a larger mean size in habitats that support rapid growth while they commence breeding at a smaller size in habitats which support slow growth, poor growth performance and associated small size at commencement of breeding might reflect suboptimal foraging habitats in Port Curtis.

#### Population size:

- Moderate to high water turbidity which is a long term, pervasive feature of Port Curtis and the temporal variability in availability of turtles within the study sites resulted in sub-optimal numbers of turtles being captured and tagged at most study areas within Port Curtis.
- It was only possible to estimate population size for Green turtles using the foraging grounds at Pelican Banks and the Boyne Island area.
- There were significant numbers of Green turtles resident within these areas of Port Curtis and the site-specific abundance trends were relatively constant over the 4-year period from 2016 onwards.
- The overall 4-year mean population size combined for the combined Pelican Banks-Boyne Island area sites is estimated at 1170 Green turtles (95% credible interval: 1154-1186).
- There are also large numbers of turtles using especially the South Trees and Wild Cattle sites. It is evident that the total resident foraging population within Port Curtis will number in the many thousands of Green turtles.
- In contrast with the other sGBR Green turtle foraging populations in south and central Queensland that are increasing in population size, the foraging population at Pelican Banks and off Boyne Island is stable at best.

#### Breeding biology:

- The annual fluctuations in adult male breeding rates recorded in Port Curtis show comparable synchrony with the previous records of male Green turtles foraging in Shoalwater Bay and Moreton Bay and the female breeding rate recorded at Heron Island.
- There has been a marked lack of synchrony of fluctuations in adult female breeding rate within Port Curtis during the three year period 2017-2019 and the approximately synchronous fluctuations in annual breeding rates previously recorded for females foraging at Shoalwater Bay and Moreton Bay and nesting at Heron Island.
  - The adult female foraging population in Port Curtis has displayed anomalous breeding rates during 2017-2019 relative to other monitored populations within the sGBR stock. No such anomalous breeding rate is evident for the adult male population within Port Curtis during the same period.

#### Dietary ecology of the Green turtles in Port Curtis

- Dietary samples were collected and analysed from 329 Green turtles captured while foraging at nine sites within Port Curtis during 2015-2019.

- Ingested items included one species of mangrove, four species of seagrass, 14 species of red algae (Phylum Rhodophyta), three species of green algae (Phylum Chlorophyta), one species of brown algae (Phylum Ochrophyta), animals in four phyla (Porifera, Mollusca, Cnidaria, and Crustacea) that appeared to be intentionally eaten, and unintentionally eaten items categorised as 'other' (detritus, amphipods and Ozobranchid leeches and plastic debris).
- Food items with a high (>50%) frequency of occurrence at more than one site were the seagrasses *Zostera muelleri*, *Halodule pinnifolia*, *Halophila ovalis*, the red algae *Catenella nipae* and *Bostrychia tenella* and the red mangrove (*Rhizophora stylosa*).
- The diet of the Green turtles varied strongly across the sampling sites within Port Curtis, with grouping of food items into higher-level taxonomic forage categories indicated:
  - A strong predominance of seagrass in turtle diets at Pelican Banks, South Trees, and off Wild Cattle Island.
  - Red algae were the dominant food items at Quoin Island
  - Green algae were the primary food ingested at Wiggins Island.
  - Turtles at the mouth of the Boyne River primarily ate red algae and seagrass and
  - Turtles at Facing Island ate a diet of red algae and mangroves.
- Fifteen Green turtles were sampled for diet at multiple times during the study:
  - All were recaptured at the same sites as their original capture
  - Interval between successive sampling events ranged from 6 wk to 23 mth
  - Based on IRI analyses, 8 of the 15 turtles had different predominant food items across the two sampling periods.
    - Of those, five turtles switched between eating seagrass and algae and the other three changed between two species of seagrass
- Ingested plastic debris generally occurred at a low incidence, with a frequency of occurrence ranging from 0% at multiple sites to 12.5% at Quoin Island.
  - Types of ingested debris included plastic fibres, fishing line, and flat, hard, and soft plastic fragments.
  - All ingested plastic was small, less than 0.5 cm in length and occurring at an insignificant volume.

#### Toxicology of Port Curtis Green turtles

- This study assessed the temporal and spatial accumulation of metals in Green turtles foraging in Port Curtis and provided an assessment on the impact this may be having on Green turtle health, using reference intervals, supported by new toxicological information generated from cell-based bioassays.
- This study presents the longest known temporal analysis of element concentrations in recaptured sea turtles and provides information for managers on the temporal and spatial trends in element concentrations in Port Curtis Green turtles.
- A total of 77 blood samples were collected and analysed, from 37 individual Green turtles captured in at least two different years throughout the study period, 2011-2018.
- All turtles were recaptured at the same site where each was originally captured.



- Sixteen elements (As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Ti, V and Zn) were regularly detected, and measured at concentrations consistently above the reference intervals (RIs).
  - Six elements (Ca, K, Mg, Na, Th, U) had very few blood concentrations above the detection limit, and/or did not have published RIs, and were not included in further analysis and interpretation.
- No unidirectional trend in metal concentrations across the study period was detected (combining Pelican Banks and Boyne River estuary turtles), the temporal trends in blood trace element concentrations in Port Curtis Green turtles followed two broad patterns:
  - Co, Cr, Mn, Mo, Ni - concentrations were generally low in 2011, followed by a spike in the concentrations in 2013/14, a return to low concentrations in 2016, and another spike (except Cr) in 2017/18, although there are signs of concentrations decreasing from 2017 to 2018 in some individuals.
  - As, Ba, Cd, Cu, Fe, Pb, Sb, Se, Ti, V, Zn - concentrations were generally low in 2011, and remained low until a spike in 2017/18, although, again, signs of concentrations decreasing from 2017 to 2018 in some individuals.
- These general trends indicate that the exposure of Port Curtis Green turtles to trace elements has changed over this eight-year sampling period.
  - It is not known what has caused these changes to exposure, although extreme rainfall events (with associated high level flooding) and port activity suggest that climatic and/or anthropogenic activities could be involved.
- In interpreting these data, it is necessary to consider that essential elements, in general, are taken up and regulated more efficiently than non-essential elements. In addition, the residence time of any element in blood is expected to be greatly influenced by species-specific metabolic needs, and detoxification strategies, as well as overall health and individual variation.
  - To date there is a paucity of data for the toxicokinetics and toxicodynamics of trace elements in reptiles.
  - Thus, an observed change in elemental blood concentration from above RI limits back to within RI limits (as observed for some elements) does not necessarily indicate that the risk to turtle health has been reduced, as blood is only a snapshot in time of an active metabolic pathway.
  - Low concentrations of many elements were observed between 2011 and 2016, with signs of additional elements decreasing in 2018, following the 2017 rainfall event. It is likely that, although blood concentrations have reduced in these periods of suspected low exposure conditions, these elements are bioaccumulating in other tissues not sampled in the present study (liver, kidney, brain, etc), where they can elicit toxic effects, particularly chronic effects.

CHAPTER 1  
INTRODUCTION TO THE GREEN TURTLE POPULATION IN PORT CURTIS  
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Port Curtis, a major port in central Queensland, receives outflow from the Calliope and Boyne Rivers. The Port also receives some outflow from the Fitzroy Catchment via The Narrows. Port infrastructure supports coal, LNG, and grain export, bauxite import and alumina export and an alumina smelter and other industry including a power station, tourism to the Great Barrier Reef, vessel transport between Gladstone and the numerous islands of Port Curtis and diverse light industry including cement production and chemical processing. Port Curtis also supports commercial and recreational fishing. Servicing the needs for large vessel movements within Port Curtis has escalated since the 1880s and particularly since the 1960s. Sections of intertidal habitat in the western and southern perimeter of the Port have been converted to infilled land behind rock walls with associated reduction in intertidal habitat (Duke *et al.* 2003; Harris, 2009). Channels and turning basins have been dredged to facilitate access for large vessels.

The turtle population foraging in this modified coastal embayment of Port Curtis has been the focus of increased studies by the Department of Environment and Science (DES) and collaborating university partners since the extreme weather events of the 2010-2011 summer. The two major flooding events of that summer resulted in an abnormal elevation of turtle and dugong mortality and strandings along the Queensland Coast including Port Curtis (Meager and Limpus, 2012; Limpus *et al.* 2012; Gaus *et al.* 2012; Flint *et al.* 2014; Flint *et al.* 2017).

In 2012-2014, CSIRO undertook a multi-year “Integrated study of the Gladstone marine system” (Babcock *et al.* 2015) funded by two of the LNG projects within the Port (Australia Pacific LNG and Shell’s QGC Business). DES Aquatic Threatened Species Program (ATS) partnered with CSIRO for the capture of turtles in 2013 while DES recommenced a continued assessment of the status and population dynamics of Green turtles in Port Curtis. This study included an investigation of Green turtle diet and health (Flint, 2015; Prior *et al.* 2016). Also in 2013, as a consequence of the Magistrates Court of Queensland at Gladstone when considering complaints no. MAG-104829/12(1), 107008/12(8) and 106907/12(9) ruled for funding support to Department of Environment and Heritage Protection (EHP) to undertake a satellite telemetry study of Green turtles foraging in Port Curtis. Thirteen GPS satellite tags were deployed in 2013.

As part of the approval for dredging operations associated with the construction of three LNG plants on south west Curtis Island, the Gladstone Ports Corporation (GPC) was conditioned to implement a range of studies monitoring the ecology and wildlife of Port Curtis under the auspices of an Ecosystem Research and Monitoring Program (ERMP). GPC’s ERMP previously contracted the James Cook University (JCU) in partnership with Queensland Department of Environment and Heritage Protection (EHP), now restructured within the Department of Environment and Science (DES) to deploy GPS satellite tags on Green turtles foraging in Port Curtis over three consecutive years, 2014-2016. The intent of these studies has been to define the behaviour and habitat utilisation of Green turtles within Port Curtis. The

results of that satellite telemetry study have been reported by JCU (Hamann *et al.* 2017).

GPC's ERMP prioritised a focus on marine turtle studies following a Gap Analysis review of marine turtle occurrence and biology within the Port Curtis region (Limpus *et al.* 2013a, b, c, d, e, f). GPC's ERMP contracted EHP to undertake a four year tagging-recapture population study of Green turtles resident in Port Curtis and an associated assessment of their health that commenced in 2016 (GPC ERMP CONTRACT No. CA14000241). The study required the sampling of turtles from a range of sites within the Port including Pelican Banks, Facing Island, Boyne Estuary, and the Western Basin.

As part of the approval for the construction of three LNG plants (Shell's QGC Business, Australia Pacific LNG, Santos GLNG) on south west Curtis Island, the LNG projects also were conditioned to implement a range of studies monitoring the ecology and wildlife of Port Curtis. The combined LNG projects were approved to implement a Long Term Turtle Monitoring Program (LTTMP). Eco Logical Australia was contracted to implement the LTTMP. Eco Logical Australia (ELA) subsequently in 2016 contracted the EHP to provide access to a representative annual sample of foraging Green turtles within Port Curtis for in depth health studies by contracted investigators at University of Queensland (UQ) School of Veterinary Science and Griffith University School of Environment. These health studies encompass the assessment of Green turtle haematology, blood chemistry, toxicology, and disease.

The EHP-DES led study builds on knowledge gained during previous studies of Green turtles in the region (reviewed by Limpus, 2007; Limpus *et al.* 2013 b) and, in particular, studies within the Port with respect to Green turtles foraging across a range of habitats. This report summarises the results of the four year GPC ERMP funded study and the third year of the ELA funded study.

## Methods

### Study Sites

Five long-term standard study sites within Port Curtis were sampled during 2016-2019 (Figure 1.1):

- Pelican Banks intertidal and subtidal habitats in north-eastern Port Curtis and abutting Curtis Island.
- Facing Island intertidal rocky reef and mangrove habitats along the western side of the island.
- Intertidal flats adjacent to the Boyne River estuary and Boyne Island.
- Western Basin, with emphasis on Wiggins Island intertidal flats adjacent to the outflow of the Calliope River into the Port and adjacent to port infrastructure adjacent to RG Tanna and Wiggins Island coal loading terminals.
- Quoin Island intertidal rocky reef and mangrove habitats.

Based on advice from the JCU Seagrass Ecology Group, two new study sites were included for sampling during 2018 and sampling continued during 2019:

- South Trees intertidal and subtidal habitats adjacent to the main shipping channel and the Queensland Alumina Limited wharves.
- Subtidal flats off the southern end of Wild Cattle Island adjacent to Colosseum Creek estuary.

During 2019, previously un-sampled sites supporting foraging Green turtle were searched for evidence of turtles moving from standard sampling sites to alternate sites:

- Intertidal flats along the western margin of Chinaman Island

A selection of these study sites were searched daily for turtles during each of four 8-12 day duration study trips to the Port during each of the four study years, 2016-2019.

Water turbidity varied widely across the spatial scale of the Port and temporally in response to the twice daily tidal cycle, the changing tidal range across the lunar cycle, wind speed and direction and river runoff following recent rains. The elevated turbidity of Port Curtis waters was first reported in 1800 by Matthew Flinders (1814) and in our experience remains a characteristic of the Port in present times.

The lowest turbidity water was encountered outside the immediate Port area off Wild Cattle Island. Low turbidity water occurred on the Pelican Banks, at South Trees, and at the mouth of the Boyne River, particularly for the first few hours of incoming tides. Capture of turtles by the turtle rodeo method is restricted to the shallower waters where the bottom is visible and hence foraging turtles can be seen at the bottom. Sites for attempted capture of turtles were selected on a daily basis with respect to the tidal cycle, wind direction and speed for the day.

The Narrows were identified in the ERMP conditions as a site for specific study of Green turtles within the Port.

- Several days of boat-based searches for areas within the southern third of The Narrows and Grahams Creek during the 2016 field trips failed to find any areas with a concentration of turtles. This area was searched extensively again during 2018 field studies and again no areas with concentrated Green turtles were located.
- A vessel based assessment of the northern end of The Narrows was conducted during 31 July-1 August and 9-10 November 2017 to identify suitable areas for the capture of marine turtles. No areas of intertidal habitat with a concentration of foraging marine turtles were identified. Isolated Green turtles were observed but all were within turbid channels with a water depth exceeding 3.5 m.
- On 6 July 2019, a search for turtles was conducted using three vessels operating independently along approximately 13km of The Narrows and adjacent side branches of the stream from -23.62971°S, 151.04688°E to -23.67160°S, 151.12207°E (north and south of Ramsay's Crossing) during 11:30 – 13:30hr. Only two juvenile Green turtles were observed during this survey. None were captured by the rodeo capture method.

No site was found within The Narrows where the water was clear enough to consistently capture turtles by the turtle rodeo method and the habitat was not conducive to effective capture using blocking nests. As a consequence, no systematic mark-recapture study was initiated within The Narrows.

Similarly, there was difficulty in locating accessible turtles within the western Basin, other than at Wiggins Island. For example:

- Several days of boat-based searches of the perimeter of the Western Basin during the 2016 field trips failed to find any areas with a concentration of turtles to the west of Wiggins Island.
- On one to three days during each field trip during 2017, generalised transects were conducted through potential habitats where netting could be applied for the capture of turtles within the Western Basin. The team was unable to locate any area with a concentration of foraging Green turtles within the Western Basin that would have been suitable for use of the blocking net.
- Three searches of the entire perimeter of the Western Basin were conducted during 2019 in search of suitable study areas for turtles.
  - 21 May: During a boat-based search for turtles around the perimeter of western Basin, turtles were recorded aggregated only at the algal/seagrass flats at Wiggins Island.
  - 14 & 16 September: A boat-based search for turtles around the perimeter of Western Basin from the RG Tanna wharf to Fisherman's landing in the south and the LNG wharves on the Curtis Island shore only recorded turtles aggregated at the algal/seagrass flats at Wiggins Island.

The capture of turtles on the Wiggins Island intertidal flats was restricted mostly to netting which had variable success across the years.

#### Turtle capture

The standard methods of the DES Queensland Turtle Conservation (QTC) Project developed for assessing the population dynamics of foraging marine turtles (Limpus *et al.* 2005) were used in the present study.

Most turtles were captured by the turtle rodeo method (Limpus, 1978) of jumping from a catch boat to catch and restrain the turtle. Because of the turbidity of waters within the Port, the rodeo method was restricted in use to shallow waters typically less than 2 m deep, i.e., at depths where the turtle could be seen at the bottom (Figure 1.2). Two purpose built turtle catch boats (Figure 1.2A) were routinely deployed during daily sampling of turtles within the Port during each study trip. With the elevated turbidity of waters within much of the Port, alternate methods were employed as conditions permitted. A small proportion of turtles were captured by beach-jumping whereby catchers walked in shallow water usually less than 0.7 m deep to jump on foraging or basking turtles. Turtles were also captured using in a 100 m to 300 m long blocking net set across the drainage flow off the flats on a falling tide. The net was deployed at approximately one hour after high tide in water of no more than two metres depth. Catchers walked along the inside of the net to capture turtles as they swam into the net (Figure 1.3). Catch boats remained in attendance at the net to provide oversight of safe operations on the net and to take on board turtles as they were captured. The net was removed when the water depth at the net dropped to approximately 0.7 m. This normally gave approximately a two hour time window for capture of turtles from the adjacent flats as they moved back to deeper water with the falling tide. Netting was used on the intertidal flats of Pelican Banks, adjacent to Wiggins Island and at South Trees.

It had been anticipated that turtles could be captured by tunnel-netting within the turbid waters of the Western Basin and The Narrows. This netting technique involves

placing large lengths of net at a fixed location for the duration of the falling tide to capture turtles as they move off the intertidal flats. However, it was found that with the high velocity tidal currents within these areas, tunnel netting would not be practicable or cost effective for capturing turtles at these study sites.

Captured turtles were lifted into the turtle catch boats (Figure 1.2E) for transport back to the DES Marine Parks Workshop at the Gladstone Marina where they were processed for the required data and tissue samples (Figure 1.4). Unrestrained turtles were held on their backs to prevent them wandering on the concrete and abrading their plastrons. After processing, the turtles were loaded back to the catch boats and released back in the Port that evening or on the following morning.

#### Data collection

The turtles were tagged with standard titanium turtle tags, one on each front flipper (Limpus, 1992, Figure 1.5A). The length of turtles was taken as the midline curved carapace length (CCL,  $\pm 0.2$  cm) measured with a flexible fibreglass tape from the skin-carapace junction at the anterior of the carapace back to the posterior ventral margin of the junction of the post vertebral scales (Limpus, 1985). Large barnacles that would have interfered with the CCL measurement were removed before CCL was measured. Turtles with damage to the posterior carapace measurement point were not measured for CCL. Turtles over 30 kg were weighed while suspended from a Salter dial balance or an electric balance ( $WT \pm 0.1$  kg). Turtles under 30 kg were weighed on a top pan electronic balance ( $\pm 0.01$  kg, Figure 1.5B).

Juvenile Green turtles that had recently recruited to benthic foraging in coastal waters from plankton feeding in pelagic surface waters were identified by characteristics defined by Limpus *et al.* (2005) (Figure 1.6):

- White ventral surfaces, sharp edges to their carapaces and two pronounced longitudinal plastral ridges.
- Within a few months, the white ventral surfaces become discoloured by green algal growth and the plastron colour changes to pale yellow while the sharp edges are abraded (Figure 1.6).
- *Chelonibia* barnacles less than approximately 100 d old (30 mm in length) (Doell, 2017).

For some turtles, the identification of sex, maturity and breeding status was supplied from prior history from nesting beach and courtship area studies or prior gonad examination of turtles previously captured in foraging studies.

Genetic stocks of Green turtles in eastern Australia have been defined by FitzSimmons and Limpus (2014). Previous studies with Green turtle foraging populations dominated by the sGBR genetic stock (Limpus *et al.* 1994) indicate little morphological differentiation between immature male and female Green turtles (Figure 1.7). Based on these Moreton Bay data, tail length can be used for identification of males when carapace length exceeds 80 cm and tail length exceeds 5 cm. In these cases, the immature male has a narrower tail than the female (C. Limpus, unpublished data). In addition, a review of unpublished Green turtle tail length data in the Queensland Turtle Data Base, males can be scored as pubescent immature when the tail exceeds 16 cm and as adult when the tail length exceeds 28 cm.

For turtles with no prior history or without well differentiated tails indicating they were males, the sex, maturity, and breeding status were determined via examination of the gonads and associated ducts of the turtles using laparoscopy and/or ultrasonography (Figure 1.5C, D). The interpretation of the gonad observation followed the standard defined procedures within the DES Queensland Turtle Conservation Project (Limpus *et al.* 1994, Limpus, 1993; Limpus and Limpus, 2003; Limpus *et al.* 2005). Puberty in the context of marine turtles continues to be defined as the life history stage during which the gonads and associated ducts differentiate from the generalised immature structures to those of the adult turtle. Immature turtles for which the sex and maturity had been determined in a previous year were not re-assessed in subsequent years.

Operationally the breeding status of turtles were recognised using the characteristics defined by Limpus *et al.* (2005):

Adult males were scored as:

- preparing for breeding in the coming breeding season if testis had distended seminiferous tubules and epididymis had a distinct enlarged white tube.
- not preparing for breeding in the coming breeding season if the testis did not have distended seminiferous tubules and the epididymus had a non-distended translucent tube.

Adult females were scored as (each of these conditions is mutually exclusive of the others):

- preparing for breeding in the coming breeding season if the ovary had enlarged (> 1 cm diameter) vitellogenic follicles.
- bred in the previous breeding season if the ovary had large healing corpora lutea (> 3 mm diameter) on fluid filled vesicles (Fig. 2b) and enlarged atretic follicles.
- bred in the season before last if the ovary had corpora albicantia ~3 mm in diameter and surrounded by white radiating folds of connective tissue.

The interpretation of the breeding history of individual turtles obtained via laparoscopy could project two years into the past for adult females examined in any one year but the interpretation of male breeding history could only be applied for the year of observation.

Each turtle was examined for evidence of external injuries of anthropogenic origin such as injuries from propeller cuts or blunt force trauma fractures to the carapace or head and entanglement in fishing gear or ropes. In addition, each turtle was examined for external evidence of disease or poor health. Turtles were scored for severity of Fibropapillomatosis tumours following Work and Balazs (1999). Limpus *et al.* (2012) scored turtle health via a gross external body condition index using qualitative external appearance of the turtle based on concavity of the plastron. For the current Port Curtis study this condition index was modified to accommodate scoring for UQ School of Veterinary Science requirements with a five-stage qualitative index of body condition based on a body condition score used by Flint (2009). Turtles in very good body condition were assigned a body condition = 4: plastron well rounded with no concavity along the area of the infra-marginal scutes; no concavity in the anterior midline of the plastron; well-muscled proximal portion of the front flipper; no concavity under the costal scutes adjacent to the marginal scutes. Turtles in extremely poor body condition were assigned a body condition index = 1:

very concave margin of the infra-marginal scutes with the marginal scutes; very concave midline of the plastron behind the ectoplastron bone; pronounced concavity of the carapace under the costal scutes adjacent to the marginal scutes; wastage of muscle over the humerus off the front flippers or, for the most extreme, body condition index = 0 where bones are protruding from the plastron (Figure 1.8).

Data recorded for each turtle during this four-year, 2016-2019, GPC ERMP commissioned study are collated into the DES QTC Data Base. This relational data base collated the temporal and spatial distribution of all turtles sighted or captured. The data included tag numbers, date, location, time of sighting, species, sex, age class, carapace length, weight, tail length and coded data summarising the breeding condition of adults, body condition and external evidence of injury or disease, and a summary of experiments conducted on the turtles. Analysis of data from the present study has been supplemented during analysis with additional data within the QTC data base, recorded during prior DES studies and incidental records within Port Curtis.

Throughout this study, the emphasis will be on comparing the performance of the foraging Green turtle population of Port Curtis with the performance of foraging Green turtle populations sampled elsewhere in eastern Australia and particular with foraging populations dominated by the sGBR Green turtle genetic stock.

Additional collaborative specialised studies were undertaken to enhance our understanding of the population dynamics and health of this foraging Green turtle population in Port Curtis and to support other contracted studies by GPC ERMP and LNG LTTMP. This provided opportunities for collaborative studies to be developed between DES and universities with resulting support for numerous post graduate studies. In recognition of the invaluable contribution of diverse academic support within the DES QTC Project in Port Curtis, the final reporting of the study is presented in a series of separate reports:

## **Chapter 2: The Green turtle, *Chelonia mydas*, foraging population in Port Curtis**

Analysis of the sighting and capture data will provide a description of the recorded distribution, relative abundance by study sites within the Port and age class and gender, distribution of breeding stocks that support this foraging population, foraging site fidelity based on flipper tag recoveries, size range of turtles by gender, age class and study sites, annual breeding rates and summary of external evidence of injuries and disease.

The methods are reported in Chapter 1 and analyses and results are reported in Chapter 2.

## **Chapter 3: Population genetics of the Green turtle, *Chelonia mydas*, population foraging Port Curtis**

Small skin samples (~2 mm<sup>3</sup>) were collected from more than 100 foraging Green turtles in Port Curtis. Samples were originally stored in 20%DSMO in saturated NaCl solution, but later were stored into 70-95% ethanol. These samples were banked at the DES Turtle laboratory at Ecosciences Precinct, Dutton Park Brisbane.

Samples from turtles classed as new recruits will be analysed to quantify the distribution of mitochondrial (mt) DNA haplotypes (variants) in males and females. These results will be compared to the distribution of mtDNA haplotypes previously



observed in adult and immature turtles at Shoalwater Bay and Moreton Bay (Jensen *et al.* 2016). This will allow us to determine if there have been any differences in stock composition of juvenile Green turtles recruiting into the benthic foraging population of turtles in the of Port Curtis in recent years. It will also inform us more broadly for the region, which has previously indicated a strong predominance of the sGBR genetic stock, whether there have been any changes since the 1990s.

The specific methods, analyses and results are reported in Chapter 3.

#### **Chapter 4: Green turtle habitat use**

This is an extension of the DES long-term satellite telemetry studies in Port Curtis. The first satellite tags were deployed on Green turtles in 2010 as part of an environmental survey conducted by GHD in relation to planned infrastructure and dredging developments. The tags were deployed in collaboration with DES. DES commenced satellite telemetry studies in Port Curtis in 2013 following the Magistrates Court of Queensland at Gladstone when considering complaints no. MAG-104829/12(1), 107008/12(8) and 106907/12(9) provided funds to EHP to implement a satellite telemetry study in Port Curtis. This was followed by the three-year study, 2014-2016, funded by GPC ERMP to JCU with DES-EHP as a collaborating partner (Hamann *et al.* 2017). Additional GPS satellite tags were deployed in collaboration with DES QTC Project on Green turtle captured while foraging on intertidal flats at:

- 2017: Pelican Banks (n = 3).
- 2018: Colosseum Creek estuary (n = 2) and One Tree (n = 2) funded by JCU Centre for Tropical Water & Aquatic Ecosystem Research (Tropwater) and at One Tree (n = 1) and Pelican Banks (n = 1) funded by Eco Logical Australia (ELA) as part of the LNG LTTMP.
- 2019: Wiggins Island (n = 10) funded by ELA as part of the LNG LTTMP.

A total of 71 GPS satellite tags were deployed on Green turtles of both genders and across all age classes and five study sites within Port Curtis during this 10 year period. The data received via the ARGOS satellite system were accessed via the Wildlife Computers Portal (Wildlife Computers, 2015). A further 10 GPS satellite tags were deployed on Green turtles at the Pelican Banks and Wiggins Island by CSIRO in 2013 (Babcock *et al.* 2013). Data from these tags was not available for analysis in the present study but findings are discussed in Chapter 4.

The specific methods, analyses and results of the telemetry study are reported in Chapter 4.

#### **Chapter 5: Green turtle population dynamics in Port Curtis**

Statistical analysis of the tagging-recapture data will be applied to define a range of demographic data for the foraging Green turtle population within Port Curtis, including population size, recruitment rates and trends, growth rates, annual breeding rates and body condition.

The specific methods, analyses and results are reported in Chapter 5.

#### **Chapter 6: Dietary ecology of the Green turtles in Port Curtis**

This is an extension of two recent diet studies of Green turtles foraging within Port Curtis in collaboration with our DES QTC Project:

- 2013: BScHon study by B. Prior, UQ.
- 2015-2017 PhD studies by O. Coffee, UQ.

These diet studies included:

- Food samples collected opportunistically from the mouths of turtles at capture.
- Gastric lavage samples (Forbes and Limpus, 1993) collected from a representative sample of turtles of all size classes across the study sites to assess dietary variation within the Port Curtis region.
- Skin tissue and blood samples (Owens and Ruiz, 1980) collected from these turtles as well as samples of food species for stable isotope analysis to assess food web dynamics.
- Samples preserved and taken to UQ for analysis.

A detailed analysis and reporting of diet samples and stable isotope analysis is reported within Dr Owen's PhD thesis (2020).

The specific methods, analyses and results of the diet study are reported in Chapter 6.

### **Chapter 7: Toxicology of Port Curtis Green turtles**

This is an extension of a collaborative study that commenced in 2011 between DES QTC Project, Entox (with Dr C. Gaus as co-leader) and University of Queensland School of Veterinary Science (with Dr M. Flint as co-leader). The study commenced in response to public concerns regarding expanded dredging and associated infrastructure development within Port Curtis that was occurring synchronously with the elevated strandings of dead and moribund Green turtles and dugong following two of the highest floods in 100 years. These floods occurred during the previous summer prior to the commencement of the major dredging program of 2011 within Port Curtis (Gaus *et al.* 2012; Limpus *et al.* 2012; Meager and Limpus, 2012; Flint *et al.* 2014).

These studies continued in 2013 following another 100-year record flood event. At this time, the emphasis of the study expanded to include sequential sampling of individual turtles across years to explore changing levels of metal contaminants in Green turtles in Port Curtis in response to industrial activity in the Port and/or environmental influences such as floods (Flint, 2015; Flint *et al.* 2017).

Commencing in 2016, a range of separate studies that could inform on the health of the turtles were facilitated within the framework of the current GPC ERMP funded study, including a collaborative study between DES and Griffith University led by Dr J. van de Merwe investigating several aspects of trace element uptake in Green turtles foraging within Port Curtis:

- Temporal changes in trace elements in Green turtles using samples collected from selected turtles captured on multiple occasions across the years 2011-2019.
- Toxicology of contaminants in Green turtles using cell culture techniques.

During this time, Entox ceased use of Queensland Health facilities in Brisbane and the banked tissue samples and associated post graduate students were relocated to Griffith University.

The specific methods, analyses and results for the toxicology study are reported in Chapter 7.

#### Additional turtle health studies

Building on the GPC ERMP funded studies, the LNG LTTMP via ELA provided additional funding support to DES Queensland Turtle Conservation Project and UQ School of Veterinary Science to expand these health studies to include:

- Turtles captured cross a range of sites within Port Curtis during 2017-2019 examined for external indicators of their health and blood samples collected for blood chemistry and haematological assessment of Green turtle health within Port Curtis.
- Blood and carapace scute samples taken for investigating toxicological assays to assess the effects of chemical contaminants in turtles.
- Necropsy and pathology investigation of freshly dead stranded turtles and euthanised moribund turtles captured from the Port.

These health studies have yet to be made available from the University of Queensland School of Veterinary Sciences. It is expected that the result of these studies will be reported through the LNG LTTMP via post graduate studies within the UQ School of Veterinary Sciences.

#### Permits and Animal Research Ethics approvals

All turtle research activities were undertaken in accordance with the standard practices approved under the Department of Agriculture and Fisheries (DAF) Animal Experimentation Ethics Committee: **Queensland Turtle Conservation Project SA 2018-11-660, 661, 662, 663, 664.**

The use of nets for the capture of turtles was in accordance with DAF General Fisheries Permit 191182, issued to EHP/DES.

Collaborating GU, JCU and UQ research teams had their own University AEC approvals for aspects of the work not addressed under EHP-DES approvals.

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The tasks undertaken within this study was dependant on the participation of a diverse range of skills within the study team:

**Coxswains.** DES: Dr Colin Limpus, Dr Nancy FitzSimmons, Dr Ian Bell, Dr Justin Meager, Duncan Limpus, John Sergeev.

**Veterinarians.** DES: Allan McKinnon. UQ School of Veterinarian Sciences: Dr Mark Flint, Christobelle Hammon.

**Toxicologists.** UQ Entox: Dr Caroline Gaus, Griffith University, School of Environment (GU): Dr Jason van de Merwe, Dr Kimberley Finlayson, Alexander Villa, Arthur Barraza, Gulsah Dogruer

**Satellite telemetry specialists.** JCU College of Earth and Environmental Science: Dr Mark Hamann, Dr Taka Shimada, Emily Webster, Hector Barrios Garrido. DES: Dr Colin Limpus.

**Sea grass ecologists.** University of Queensland: Owen Coffee, JCU Centre for Tropical Water & Aquatic Ecosystem Research (TropWATER): Dr Michael Rasheed, Abbi Scott.

**Eco Logical Australia (ELA):**

Miles Yeates, Rebecca Hide, Jeni Morris, Renee Whitchurch, and James Leonard.

**Gidarjil Land and Sea Rangers:** Des Purcell, and Greg Appo, and R. Blair, C. Coleman, T. Eggmolesse, W. Eggmolesse, B. Fletcher, T. Flinn, R. Geary, D. Hodges, J. Holland, M. Johnson, E. Mallie, L. Mathers, B. Ross, L. Salam, E. Saltner, Q. Springham, C. Stow, I. Twist, L. Watson, and A. Wimbis.

**Queensland Turtle Conservation Volunteers:**

Numerous DES registered volunteers from the Port Curtis area and further afield and students from JCU and Griffith University assisted with the capture and data gathering throughout the project.

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1.1A. Port Curtis and surrounding waters

+



1.1B. Study sites within Port Curtis

Figure 1.1. Port Curtis and environs.





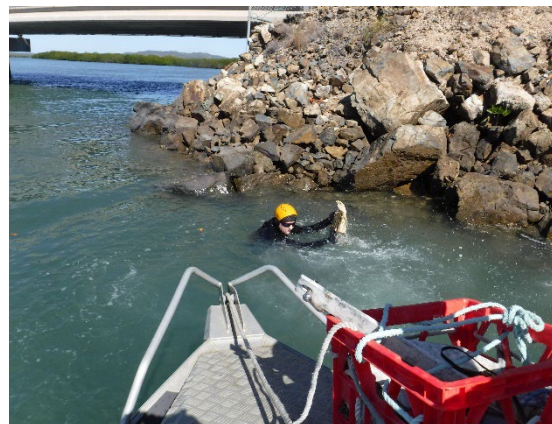
**1.2A. Launching a turtle catch boat within the Gladstone marina.**



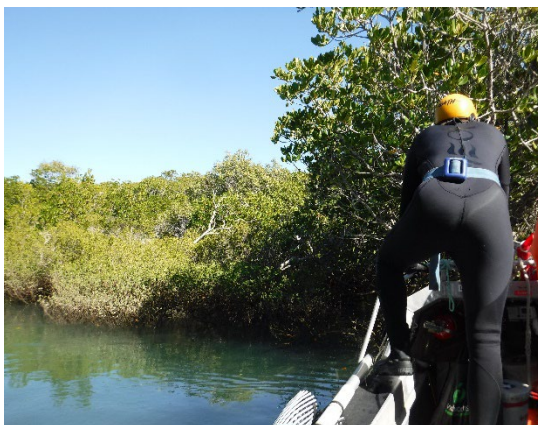
**1.2B. Turtle catch boat, *Turtle Research 7*.**



**1.2C. Dr K. Finlayson with a recently recruited juvenile Green turtle captured on shallow intertidal flats.**



**1.2D. J. Sergeev capturing a juvenile Green turtle foraging at rock infrastructure.**



**1.2E. Searching the margin of mangroves for turtles.**



**1.2F. J. Sergeev lifting an adult sized Green turtle into a catch boat with the aid of ropes attached to the front flippers.**

**Figure 1.2. Turtle rodeo methods used for turtle capture in Port Curtis studies.**



**Figure 1.3A Netting for turtles on the Pelican Banks intertidal flats.**



**1.3B. D. Purcell removing a turtle entangled in the blocking net.**



**Figure 1.3C Netting for turtles on the Wiggins Island intertidal flats.**

Figure 1.3. Use of netting with turtle capture in Port Curtis studies.



**1.4A. Small turtles were held in plastic bins while waiting for processing.**



**1.4B. Large turtles were moved around the work area on Turtle barrows,**



**1.4C. Large turtles were held on their backs on soft matting while waiting for processing.**



**1.4D. Large and medium sized turtles were restrained on turtle barrows for gonad examination, blood sampling and gastric lavage.**

Figure 1.4. Care and handling of turtles at the QPWS Workshop.



**1.5A. Titanium flipper tag attached in the axial tagging position of a flipper and cutting the margin of the last large scale adjacent to body.**



**1.5B. Weighing a small turtle less than 32 kg on an electronic scale.**



**1.5C. Gonad examination using laparoscopy to determine the gender of an immature turtle by A. McKinnon.**



**1.5D. Gonad examination using ultra-sonography to determine the breeding status of an adult female turtle by Dr C. Limpus using a computer and USB linked ultrasound probe.**



**1.5E. Blood sampling of a juvenile turtle by C. Hammon using her purpose-built turtle cradle.**



**1.5F. Collecting a sample of recently ingested food using gastric lavage with a small turtle.**

**Figure 1.5. Illustrations of research methods within the Port Curtis Green turtle population dynamics studies.**



Figure 1.6. Juvenile *Chelonia mydas* captured in western Shoalwater Bay in June 2004, illustrating the colour characteristics of a recently recruited juvenile.

The turtle on the right has recently recruited from the pelagic habitat to residency in the bay. The turtle on the left has been in residence in coastal waters for many months or longer. Image copied from Limpus *et al.* 2005, Fig. 4.

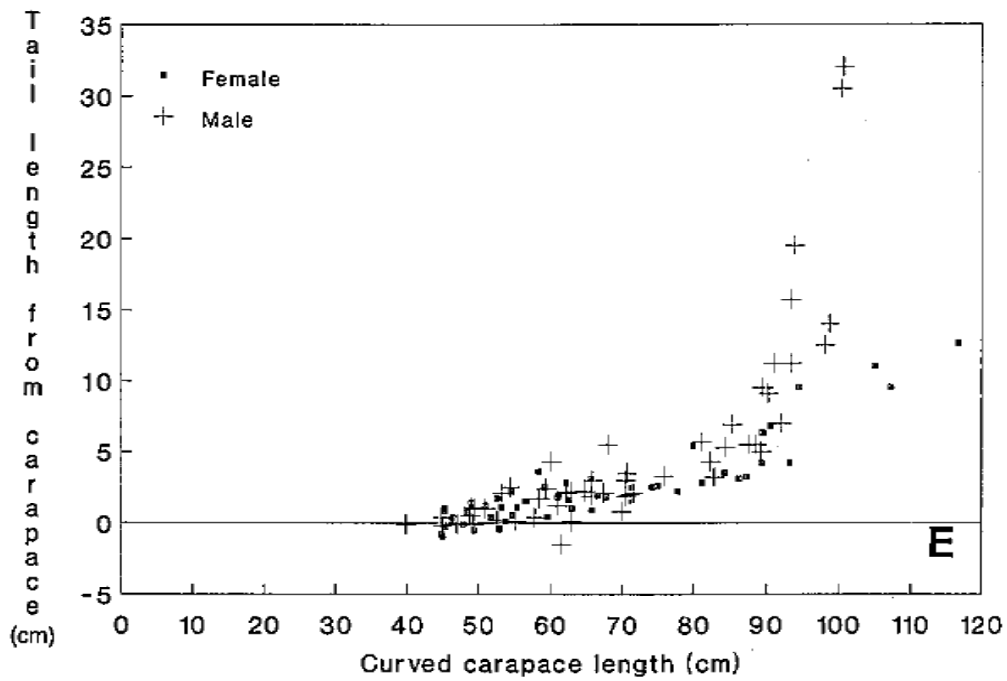
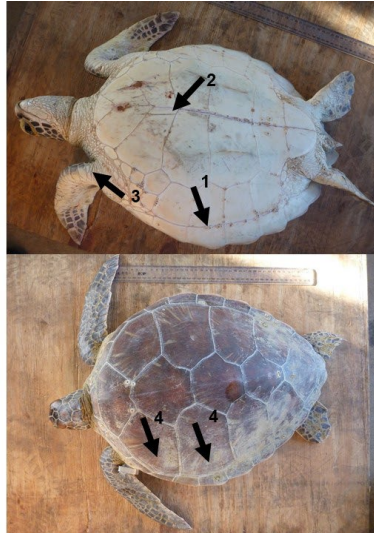


Figure 1.7. Relationship between curved carapace length and tail length beyond the carapace recorded for Green turtles, *Chelonia mydas*, in Moreton Bay, from Limpus *et al.* (1994, Figure 7)

**TURTLE HEALTH**

**BODY CONDITION INDEX = P4**

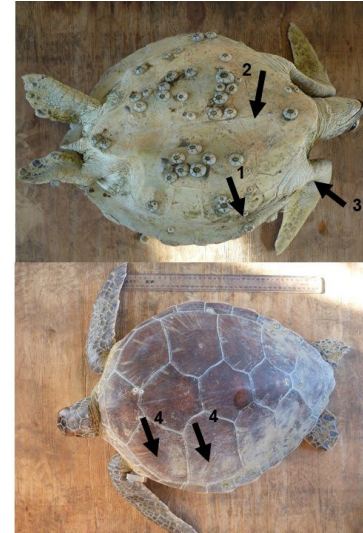
1. plastron well rounded with no concavity under the infra-marginal scutes adjacent to marginal scutes;
2. no concavity in the anterior midline of the plastron behind the ectoplastron bone
3. well muscled proximal portion of the front flipper;
4. no concavity under the costal scutes adjacent to the marginal scutes



**TURTLE HEALTH**

**BODY CONDITION INDEX = P3**

1. flattened plastron profile, with slight concavity under the infra-marginal scutes adjacent to marginal scutes;
2. flattened midline of the plastron behind the ectoplastron bone
3. good muscle in proximal portion of the front flipper;
4. no concavity under the costal scutes adjacent to the marginal scutes



**TURTLE HEALTH**

**BODY CONDITION INDEX = P2**

1. plastron concaved profile, with shallow concavity under the infra-marginal scutes adjacent to marginal scutes;
2. slight concavity long midline of the plastron behind the ectoplastron bone
3. reduced muscle in proximal portion of the front flipper;
4. Obvious concavity under the costal scutes adjacent to the marginal scutes



**TURTLE HEALTH**

**BODY CONDITION INDEX = P1**

1. severe concavity under the infra-marginal scutes adjacent to marginal scutes;
2. Very concave midline of the plastron behind the ectoplastron bone
3. Very reduced muscle in proximal portion of the front flipper;
4. obvious concavity under the costal scutes adjacent to the marginal scutes



**BODY CONDITION INDEX= P0:** More emaciated than P1 and with bones protruding from the plastron.

Figure 1.8. Definition of the qualitative indices of body condition.



## CHAPTER 2

### THE GREEN TURTLE, *Chelonia mydas*, FORAGING POPULATION IN PORT CURTIS

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While recent studies on marine turtles in Port Curtis have focussed primarily on the Green turtle, six species of marine turtle have been recorded foraging within Port Curtis – Port Alma since 2000. The limited number of other species recorded was influenced by the ERMP emphasis on the investigation of Green turtles and the habitats they frequented rather than a random sampling of the entire suite of habitats within the port.

#### Methods

The general methods applied in the capture, tagging, data recording for the investigation of this foraging Green turtle population in Port Curtis are described in Chapter 1.

Age class-specific sex ratio (proportion of females) and the gender-specific breeding rate (proportion breeding) for the year and the 95% highest posterior density interval (HDI) was estimated for each year and site by sampling from a binomial likelihood with a Bayes-Laplace prior (Tuyl et al 2008) using the binom R package (Dorai-Raj 2014).

#### Results

##### Species

All six species of marine turtles that occur in Australia were recorded foraging within Port Curtis since the commencement of tagging studies in 2000.

##### Cheloniidae

- Loggerhead turtles, *Caretta caretta*:

Nine Loggerhead turtles were recorded within Port Curtis since 2000, ranging in size from large immatures to adults. One was recorded in the Western Basin in the shallow subtidal waters at the entrance to Fisherman's Landing Northern Extension prior to closure of the bund wall (for Environmental, 2011). Eight of these Loggerhead turtles were observed in the deeper waters outside of tidal habitats including the main dredged channel into the port. Two

were captured and tagged, including one that had been recently injured by boat-strike and subsequently euthanized.

- Green turtles, *Chelonia mydas*:

In all years, Green turtles have been the most abundant foraging turtle recorded. They were observed mostly in shallow tidal and subtidal waters. Since tagging studies began in Port Curtis in 2000, a total of 1716 individual Green turtles have been tagged, with a total of 2107 recorded captures of these turtles (Table 2.1). The majority of these captures (n = 1565) occurred during the present 2016-2019 study (Table 2.2).

The extensive data recorded for Green turtles within Port Curtis and the Narrows are reported below.

- Hawksbill turtles, *Eretmochelys imbricata*

Nine Hawksbill turtles were recorded within Port Curtis since 2000, ranging in size from small immature turtles that had recently recruited from the open ocean pelagic foraging life history phase, to large adult sized turtles. Hawksbill turtles were observed foraging on the subtidal rocky reefs of the Port. Two small juveniles were captured and tagged, including one that originated from a head-starting project in Vanuatu.

- Olive Ridley turtles, *Lepidochelys olivacea*

One Olive Ridley turtle was recorded within Port Curtis-Port Alma since 2000, an adult sized turtle killed in the main shipping channel by very large propeller cuts.

- Flatback turtles, *Natator depressus*

Nineteen Flatback turtles were recorded within Port Curtis-Port Alma since 2000, ranging in size from small pelagic foraging port-hatchlings through a range of sizes of benthic foraging immature and adult turtles. This species occupied the subtidal waters of the port including the main dredged channel. Four adult females were captured that had been originally tagged while nesting on Curtis Island or Peak Island. Thirteen post-hatchlings Flatback turtles were observed foraging on macro-plankton in surface waters.

#### Dermochelyidae

- Leatherback turtle, *Dermochelys coriacea*

Two large Leatherback turtles were reported at the surface in offshore waters of Port Curtis by members of the public since 2000.

#### Green Turtles

##### Green turtle aggregated foraging area

During the four years 2016-2019, a total of 3423 sightings of Green turtles were recorded within the Port Curtis Study area, from which a total of 1232 individually tagged turtles were captured (Table 2.1). During the four years, there were 1576 captures from among these tagged turtles. Table 2.1 summarises the tagging history of turtles captured during each of the four years of the study. Table 2.2 summarises the number of turtles by their tagging history captured in each study area of the Port during each of the 17 study trips within Port Curtis.

Capture locations for Green turtles foraging within Port Curtis are illustrated in Figure 2.1a. Locations where turtles were observed but not captured are illustrated in Figure 2.1b and include areas where the turbidity and/or depth of the water, precluded the capture of turtles.

Green turtles do not forage randomly within the port. Green turtles were found to consistently aggregate for foraging in five localised sites within Port Curtis, in areas bordering approximately 18 km of shoreline as follows:

- **outflow of Colosseum Creek;** Adjacent intertidal and subtidal flats and the adjacent intertidal flats of the estuary; 5 km of shoreline (Figure 2.1Av).
- **outflow of Boyne River:** Adjacent intertidal and subtidal flats and rocky reefs; 1.5 km of shoreline (Figure 2.1Aiv).
- **outflow of South Trees inlet:** Adjacent intertidal and subtidal flats and mangrove margins that are part of the distributary outflow from the Boyne catchment; 4 km of shoreline (Figure 2.1Aiii).
- **outflow of Calliope River** into the Western Basin adjacent to Wiggins Island: Intertidal and subtidal flats; 1.5 km of shoreline (Figure 2.1Ai).
- **Pelican Banks** - intertidal and subtidal flats, rocky reefs and mangrove margins of Chinaman and Curtis Islands that are adjacent to the out flow of waters from Port Curtis via the channel between Curtis Island and Facing Island; 6.3 of shoreline km (Figure 2.1Aii)

All of these areas with elevated concentrations of foraging Green turtles are adjacent to outflows from rivers and creeks or, in the case of the Pelican Banks, with outflow from the Port and where there are regular reversals of strong tidal currents and associated settlement of sediments to form wide shallow flats supporting seagrass meadows and algal pastures.

Repetitive vessel-based transects along the coastal shallows between these aggregated foraging areas, equivalent to approximately 60 km of shoreline, failed to locate comparable concentrations of foraging Green turtles in alternative areas:

- Colosseum Creek to Boyne River: 8 km
- Boyne River to South Trees: 6.5 km
- South Trees wharves to RG Tanna Wharf (southern margin of Port Curtis): 8.5 km
- Wiggins Island Coal Terminal conveyor infrastructure to the western side of Fisherman's Landing (Western Basin): 13.6 km
- Mangrove margin and rocky reefs along southern side of Facing Island: 17 km
- Wide expanse of intertidal and subtidal flats bounded by the North Channel adjacent to Pelican Banks, Facing Island in the east, the dredged Shipping Channel in the south and Quoin Island in the west: an approximately rectangular area 6 km x 4 km.

Intermittent vessel-based transects in the southern, central, and northern sections of The Narrows and Grahams Creek failed to detect any concentrated aggregations of foraging turtles.

Figure 2.2 pictorially illustrates habitats utilised by foraging Green turtles within Port Curtis. Green turtles were most abundant in the lower estuarine reaches of the Fitzroy Catchment distributary and the adjacent oceanic coastal margin within Port Curtis. However, Green turtles also foraged upstream into the rivers and inlets draining into the Port. Adult and immature Green turtles were recorded foraging as far upstream as the railway bridge at Benaraby, 13 km upstream in the upper tidal reaches of the Boyne River (Figure 2.2Di). At this Benaraby bridge site, the turtles were foraging on filamentous red algae growing on the gravel riverbed.

Three industrial commodities shipped through Port Curtis were observed to contribute to contamination of the port:

- On windy days, coal dust with associated metals and organic compounds settles on surface waters of the port after being blown from the coal storage heaps and loading structures associated with RG Tanner Wharf and the Wiggins Coal Terminal (Figure 2.2Ei).
- Aluminium oxide dust blows onto surface waters from the Queensland Alumina Limited export wharf at South Trees (Figure 2.2Eii).
- Bauxite dust with associated aluminium oxide and ferric oxide is distributed by wind from the South Trees unloading facility and adjacent storage mounds at Queensland Alumina Limited.

For the study sites sampled across the four years, 2016-2019, most turtles were captured on the Pelican Banks (42.8% of total captures; n = 674) and the Boyne Island intertidal flats and Boyne estuary upstream to the Benaraby Bridge (20.9%; n = 333). The western side of Facing Island (6.3%; n = 99), Quoin Island (2.3%; n = 37), Western Basin (3.4%; n = 54) and The Narrows adjacent to Laird Point (0.2%; n = 3) consistently gave limited access to catchable turtles. The two study sites introduced in 2018 supported appreciable numbers of foraging turtles: South Trees (17.1% of total captures; n = 270) and off Wild Cattle Island and Colosseum Creek estuary (6.3%; n = 100). The low proportion of captures off Wild Cattle Island does not accurately reflect the abundance of large Green turtles foraging on that seagrass meadow. The site was only sampled during the last two trips in 2018 and the four trips in 2019 and the distance involved in relocating numerous large turtles to a suitable research base using our existing vessels limited the number of turtles that could be brought ashore.

#### Identified Breeding Areas

Seventeen Green turtles (adult female: n = 16; adult male: n = 1) foraging in Port Curtis during 2016-2019 had additional capture histories that link them to their respective breeding sites (Table 2.3). There were 21 additional flipper tag recoveries prior to 2016 from turtles (18 female; 3 male) foraging in Port Curtis that had known breeding sites as documented in the QTC database. Figure 2.3 summarises the documented breeding distributions of adult female (A) and adult male (B) Green turtles that forage in Port Curtis:

- **Nesting:**

- Adult females have been recorded nesting on islands of the Capricorn-Bunker Group of the southern GBR (Northwest, Wreck, Heron and Lady Musgrave Islands), Sandy Cape on northern Fraser Island and Wreck Rock on the mainland coast.
- **Courtship:**
  - Adult females have been recorded in courtship at a Capricorn-Bunker Reef (Llewellyn Reef) and the Sandy Cape courtship area.
  - Adult males have been recorded in courtship at Capricorn-Bunker Reefs (Heron, Wistari, Fitzroy, Llewellyn Reefs).

#### Foraging Area Fidelity

Table 2.4 summarises the fidelity of Green turtles to their respective foraging areas based on flipper tag recoveries from turtles tagged within Port Curtis since flipper tagging studies began in 2000. In summary:

- **Between year recaptures:** Turtles with recapture intervals spanning across one or more years totalled 227, equivalent to a recapture rate of 14.4% of all captures.
  - The majority (n = 220) of these turtles recaptured across study years were at the same Port Curtis study site as where they had been previously captured, displaying a 96.9% fidelity to a particular foraging site across study years.
- **Within year recaptures:** There were 165 recaptures within the same year, equivalent to 10.4% of all recaptures.
  - The majority (n = 164) of these turtles recaptured within the same study year were at the same Port Curtis study site where they had been previously captured, displaying a 99.4% fidelity to a particular foraging study site within the same study year.

Based on the flipper tag recovery data, Green turtles foraging within Port Curtis have displayed high fidelity to their respective foraging sites during the four years of the present study.

#### Developmental Migration

The progressive change in foraging area by marine turtles as they grow has been identified as “developmental migration” and is a prominent feature in the life history of some marine turtle populations globally (Meylan *et al.* 2011). During the 46 years of in-water foraging area studies of Green turtles in Queensland, there have been only two recapture records of foraging Green turtles within Port Curtis that had made a major shift in foraging area from their respective initially recorded foraging sites:

##### **Small immature (gender not recorded) Green turtle (X13348):**

- **16 February 1979**, captured by rodeo jump while foraging on Heron Island Reef within the southern GBR; approximately 23.433°S, 151.917°E.
  - **6 September 1986**, recaptured on a Queensland Shark Safety drum line hook off Tannum Sands within Port Curtis (QTC data base), 23.95°S,

151.383°S; 81 km from the original capture site and 8.6 yr since originally tagged.

**Large immature (gender not recorded) Green turtle (K21641):**

- **8 January 1999**, captured by rodeo jump while foraging on Lady Musgrave Reef within the southern GBR; CCL = 62.0 cm; 23.900°S, 152.383°E.
  - **21 July 2011** recaptured during the Fisherman's Landing Northern Expansion Bund closure, in the Western Basin of Port Curtis (frc Environmental, 2011), 23.784°S, 151.167°S; CCL = 82.5 cm; 170 km from the original capture site and 12.5 yr since originally tagged.

No Green turtle originally tagged while foraging in Port Curtis has been physically recaptured while foraging at an alternate foraging area. However, several Port Curtis foraging Green turtles tracked by satellite telemetry have been recorded shifting many kilometres to foraging areas distant from the Port (Babcock *et al.* 2015; Hamann *et al.* 2017). These telemetry results will be examined in more detail in Chapter 4.

**Gender and Maturity**

Gender and maturity, determined by gonad examination and/or morphology, body condition, and size are summarised for Green turtles captured at seven study sites within Port Curtis during 2011-2019 (Tables 2.5, 2.6, 2.7; Figures 2.4, 2.5, 2.6)

- Pelican Banks, intertidal and subtidal flats, and mangrove margin (Figure 2.4A).
- South Trees, intertidal and subtidal flats, and mangrove margin (Figure 2.4B).
- Boyne Island, intertidal and subtidal flats, and adjacent Boyne River estuary (Figure 2.4C).
- Colosseum Creek, intertidal and subtidal flats, and adjacent Colosseum Creek estuary (Figure 2.4D).
- Western side of Facing Island, mangrove margins and rocky reefs (Figure 2.4E).
- Western Basin, intertidal flats and mangrove margin, including Wiggins Island flats and The Narrows adjacent to Laird Point (Figure 2.4F).
- Quoin Island and western margin of Chinaman Island, mangrove margins (Figure 2.4G).

For the 1,391 turtles examined for gender determination, there was only one small immature turtle that had gonads that were not clearly definable as either testes or ovaries. This turtle has been scored within the QTC data base as gender = "intersex". This turtle was not included in the sex ratio analyses.

While all study sites displayed a higher proportion of females than males within the population, there is some evidence of a shift towards an increased female proportion in the population across the decades. For Green turtles across the combined study areas within Port Curtis (Table 2.6; Figure 2.5A, 2.5B):

- Adult turtles, representing turtles that were hatched more than 30 years before the current study period, had an approximately equal female:male sex ratio = 0.51 (n = 313), with the largest adult sample, from the Pelican Banks, being male biased with a sex ratio = 0.45 (n = 224).

- Large immature turtles, representing turtles that were hatched at an estimated 15-30 yr before the current study period, had a female biased sex ratio = 0.62 (n = 83). The apparent male biased sex ratio among the large immature turtles in the Western Basin is presumed to be the result of the very small sample size.
- Small immature turtles after their 1<sup>st</sup> year of residency, representing turtles estimated to have hatched 8-15 yr before the current study period, had a female biased sex ratio = 0.63 (n = 106).
- Small immature turtles in their 1<sup>st</sup> year of residency in Port Curtis and representing turtles at approximately 8 yr of age, had a female biased sex ratio = 0.64 (n = 226). The apparent male biased sex ratio with small immature turtles in the Western Basin is presumed to be the result of the small sample size.

### Size of Turtles

The size of turtles captured at the various study sites within the Port are summarised in Figure 2.4. Turtles across the full spectrum of expected size range, from those recently recruited to coastal foraging up to large adults, were accessible for capture on the Pelican Banks and off Wild Cattle Island. At the mouths of the Boyne River and South Trees Inlet, the accessible turtles were strongly biased to small immature turtles. At these latter sites, while large turtles often were seen in the adjacent deeper waters, they were not easily captured by the turtle rodeo method. Small immature turtles dominated those seen in the Western Basin and in the rocky shore and mangrove margin of the western shoreline of Facing Island and in the vicinity of Quoin Island.

Green turtles recruited from the open ocean dispersal life history phase to residency as benthic foraging turtles within Port Curtis with mean CCL = 43.2cm (Table 2.5D). There was no significant inter-annual difference in the size at which either female or male immature turtles recruited to this coastal foraging area. There was no significant difference with respect to gender in the size at which immature Green turtles recruited to benthic foraging in Port Curtis (Table 2.5D).

As a measure of the rate of recruitment of new immature turtles into the population, the proportion of recent recruits among small pre-pubescent turtles (CCL ≤ 65.0 cm) was calculated. During 2016-2019, there were 122 recently recruited immature turtles identified from among a total 862 small pre-pubescent Green turtles captured, giving a mean annual juvenile recruitment rate = 0.14 (95% CI = 0.138 - 0.145).

Adult female Green turtles resident in Port Curtis commenced breeding at a mean CCL = 99.2 cm (Table 2.5B). There was a marked difference in the size between genders for adult Green turtles: adult male mean CCL = 95.3 cm; adult female mean CCL = 102.6 cm (Table 2.5A). There was no significant difference in CCL across the seven years from 2013 to 2019 for either adult females or males within Port Curtis (Table 2.5).

During 2016-2019, a total of 8 adult females were recorded in vitellogenesis in preparation for their first breeding season among 84 individual adult females captured and whose gonads were examined by laparoscopy. This represents a mean annual recruitment rate of first-time breeders into the female breeding population = 0.10 (95% CI = 0.089-0.101).

### Breeding Biology

Three adult Green turtles were tracked via satellite telemetry from their home foraging area on the Pelican Banks to distant breeding sites. These telemetry studies provided limited data on the timing of breeding migrations when breeding adults are absent from their home foraging area:

- **Adult male Green turtle (K93087):** Captured while foraging on the Pelican Banks and deployed with a Sirtrack satellite tag (ARGOS PTT 96777) during preparation for his breeding migration in mid 2010 (Figure 2.6).
  - **19 September 2010** commenced his breeding migration and departed from the Pelican Banks foraging area.
    - Migrated to his presumed courtship area on Llewellyn and Fitzroy Reefs in the Capricorn-Bunker Group of reefs.
  - **3 December 2010** returned back to the Pelican Banks after approximately a 3.5 month absence.
- **Adult female Green turtle (QA34782):** 1 May 2013, captured while foraging on the Pelican Banks and deployed with a Vemco acoustic tag (27928) during a CSIRO telemetry study in 2013 (Babcock *et al.* 2015):
  - **2 May – 25 September 2013**, recorded daily within the Pelican Banks acoustic recording array.
  - **25 September 2013** disappeared from the acoustic array as she began her breeding migration.
    - **25 December 2013** recaptured when ashore for nesting on Lady Musgrave Island within the Bunker Group of islands.
  - **18 February 2014** return from her breeding migration was documented when she next was recorded within the Pelican Banks acoustic array after approximately a 4.5 month absence.
  - **18 February – mid September 2014**, recorded daily within the Pelican Banks acoustic recording array until the last download of the acoustic array.
  - **6 July 2017** recaptured foraging on the Pelican Banks.
- **Adult female Green turtle (QA64318):** captured while foraging on the Pelican Banks and deployed with a Wildlife Computers GPS satellite tag (ARGOS PTT 133758) as a young adult preparing for her first breeding season in mid 2017 (Figure 2.7).
  - **31 August 2017** departed from her Pelican Banks foraging area; commenced her breeding migration.
    - Migrated to her presumed courtship area on Llewellyn Reef in the Capricorn-Bunker Group of reefs before continuing her migration to Lady Musgrave Island.
  - **1 October 2017** went ashore for her first attempted nesting on Lady Musgrave Island.



Further understanding of the breeding behaviour and biology of adult Green turtles in Port Curtis was obtained during the 2016-2019 courtship periods from recorded courtship activity (Table 2.8) and summaries of the reproductive status of turtles captured within Port Curtis during the courtship and breeding season:

#### **2016: 7-14 October study period**

- 127 Green turtles were captured, and an additional 137 sightings were made of Green turtles that were not captured. None of these 264 observations of Green turtles involved turtles engaged in courtship/mating behaviour.
- None of the adult Green turtles captured (n = 25) were in breeding condition for the 2016-2017 breeding season:
  - None of the 11 adult males were in a sperm production/sperm storage phase of a reproductive cycle as would occur if they were males that had aggregated for courtship.
  - None of the 14 adult females were very fat and none carried mature (fully yolked) ovarian follicles as would occur if they were females that had aggregated for courtship on route to their nesting beaches and none of them had fresh courtship injuries on their anterior carapace.

#### **2017: 20-29 September study period**

- 145 Green turtles were captured, and an additional 111 sightings were made of Green turtles that were not captured. Only one of these 256 observations of Green turtles involved turtles engaged in courtship/mating behaviour and members of the public reported two other Green turtle courting pairs (Table 2.8).
  - During the same period, numerous courting pairs of Green turtles were reported from recognised courtship areas at Masthead Island and Lady Musgrave Island (unpublished DES QTC field records).
- During September 2017, when the majority of the breeding males were expected to have departed to their respective courtship areas, only 3 (17%) of the 18 adult males were in breeding condition.
  - This contrasted with the April-June sampling prior to the commencement of breeding migration when there were eight (44%) of the 18 adult males preparing for breeding.
- During September 2017, none of the 15 adult females were very fat and none carried mature (fully yolked) ovarian follicles as would occur if they were females that had aggregated for courtship on route to their nesting beaches and none of them had fresh courtship injuries on their anterior carapace.
  - This contrasted with the April-June sampling prior to the commencement of breeding migration when there were two (20%) of the 10 adult females preparing for breeding.

#### **2018: 3-12 October study period**

- 145 Green turtles were captured, and an additional 231 sightings were made of Green turtles that were not captured. None of these 376 observations of Green turtles involved turtles engaged in courtship/mating behaviour and members of the public reported one Green turtle courting pair in Rodds Bay (Table 2.8).
- During 3-12 October 2018, when the majority of the breeding males were expected to have departed to their respective courtship areas, none of the nine adult males sampled were in breeding condition.
  - This contrasted with the April – June sampling prior to the commencement of breeding migration when there were two (13%) of the 15 adult males preparing for breeding (sperm production in progress; one in each of April and May).

- During October 2018, no adult females were captured.
  - During the April – June 2018 sampling prior to the commencement of breeding migration, two (13%) of the 16 adult females were vitellogenic in preparation for breeding.

#### **2019: 13-24 September study period**

- 91 Green turtles were captured, and an additional 172 sightings were made of Green turtles that were not captured. Three of these 263 observations of Green turtles involved turtles engaged in courtship/mating behaviour (Table 2.8).
- During **13-24 September** 2019, when the majority of the breeding males were expected to have departed to their respective courtship areas, none of the three adult males were in breeding condition.
  - During the April – May 2019 sampling prior to the commencement of breeding migration there were 4 (29%) of the 14 adult males were spermatogenic in preparation for breeding.
- During September 2019, three of the four adult females sampled were not carrying mature (fully yolked) ovarian follicles as would occur if they were females that had aggregated for courtship on route to their nesting beaches and none of these had fresh courtship injuries on their anterior carapace. The fourth female was carrying mature ovarian follicles and had very recent courtship injuries to the anterior carapace and neck, consistent with a female that had recently engaged in courtship.
  - During the April – July 2019 sampling prior to the commencement of breeding migration there was 1 (6%) of the 17 adult females that was very fat and in vitellogenesis, in preparation for breeding.

#### **In summary:**

Based on telemetry tracking of three adult turtles that migrated from foraging in Port Curtis to distant breeding sites, the breeding turtles:

- Departed from Port Curtis during 31 August – 25 September.
- Arrived back in Port Curtis on 3 December (1x adult male) and 18 February (1x adult female).

Based on observations of eight courtship groups of Green turtles within the Port Curtis area during 2016-2019:

- Courtship activity was observed during 26 August-16 October.

Based on the annual observations of breeding behaviour of adult Green turtles captured within Port Curtis during September – October in 2016 to 2019:

- Non-breeding adult Green turtles for the year did not migrate but remained within their home foraging areas in Port Curtis during the summer breeding season.
- The majority of adult Green turtles preparing for their respective breeding seasons had already migrated to breeding locations outside of Port Curtis by late September.
- Adult turtles sampled during late September – October were predominantly local residents that were not breeding for the year.
- Substantial numbers of breeding males and female from other foraging areas did not migrate to aggregate in Port Curtis for courtship or nesting.

Overall, it is concluded that Port Curtis is not an important courtship area for Green turtles in eastern Australia.

#### **Turtle Health**

The number of turtles recorded with external evidence of health problems are summarised in Table 2.8 from among the 1232 individually tagged turtles captured in Port Curtis during 2016-2019. The turtles recorded with these injuries are representative of turtles that had survived the injurious events and do not provide a measure of the full extent of injuries from anthropogenic sources.

#### Emaciated turtles

There were 7.9% of turtles (n = 97; 95% CI  $\pm 1.5\%$ ) scored with reduced body condition (body condition = P1 or P2), being partly to extremely emaciated.

The majority of the emaciated turtles were small immature turtles, and the frequency of their encounter appears to be associated with the abundance of small immature turtles at the study site rather than with hot spots for poor health.

Emaciated turtles were interpreted as turtles that had suffered from protracted poor health. Without an indepth health assessment or necropsy and pathology, the underlying cause of illness with these turtles remains undetermined. There were occasional exceptions:

- **QA86086:** 30 May 2018, adult female, CCL = 113.3 cm; a debilitated turtle that was recaptured alive and floating in the Boyne estuary; sent to rehabilitation and defecated braided fishing line two days after capture (Figure 2.8A).

#### Turtles with fractured carapaces or heads

There were 3.2% of turtles (n = 39; 95% CI  $\pm 3.1\%$ ) with fractures to the carapace and/or head from interactions with vessels. These injuries, ranging from recent to well-healed fractures, were consistent with injuries from propeller cuts or blunt force fractures from boat strike. Non-fatal fractures that damage the spinal column have the potential for causing paralysis of the rear limbs (Figure 2.8B).

None of the Green turtles that had survived vessel damage had injuries that would be inflicted by large propellers like those on tugs and freighters. The injuries were consistent with those that would have come from the smaller propellers, skegs and lower legs of outboard motors.

The majority (69%) of these fractured turtles were captured on the Pelican Banks.

During 2018-2019, stranded dead turtles reported with boat strike injuries within Port Curtis during the individual study trips were examined. One Green turtle killed by boat strike was examined in 2018 and four in 2019 (Figure 2.9). Injured turtles included:

- **QA64333: large immature female**
  - **10 October 2018:** floating dead near South Trees; freshly dead, five propeller cuts along right rear carapace from a medium sized outboard motor.
- **untagged:** immature turtle
  - **22 May 2019:** floating dead south east of Quoin Island; fractured carapace probably from impact of lower leg of an outboard. Reported from member of the public.
- **QA91698:** adult female in non-breeding year

- **7 July 2019:** beach washed dead at Lilley's Beach, Boyne Island; freshly dead, four propeller cuts along the right side of carapace from a large outboard motor travelling at speed.
- **QA91758:** large immature turtle
  - **15 September 2019:** floating dead offshore of Quoin Island; CCL = 76.6 cm; deep propeller cuts to the anterior carapace from an outboard motor.
- **QA61524:** presumed adult female boat strike:
  - **11 September 2019:** Beach washed dead at Wild Cattle Island (advanced decomposition); CCL = 102.7 cm. deeply fractured carapace from impact with outboard motor (fracture from gearbox, a long slice from the skeg and multiple propeller cuts to the mid right carapace).

Damage to these turtles was consistent with that caused by medium to large outboard powered vessels moving at speed.

Entangled in fishing gear and marine debris

Across all years and study sites, 0.7% of captured turtles (n = 8; 95% CI  $\pm 0.4\%$ ) were entangled in fishing gear.

Entangled fishing line and hooks were recovered from seven turtles.

- Only three were considered healthy enough for immediate release.
- Four were severely injured from the entanglements and sent to a local turtle rehabilitation facility. Two of these died while under rehabilitation care.

The fishing line and hooks removed from these turtles was typical of the lightweight gear used by recreational fishers in shallow coastal waters (Figure 2.8C).

One turtle was recorded drowned in a crab pot:

- **QA66759:13 October 2016:** small immature turtle captured by turtle rodeo while foraging on the Boyne Island intertidal flats adjacent to Boyne estuary.
  - **7 January 2018:** recently dead inside a recreational crab pot ashore in Boyne estuary.

Fibropapillomatosis

A total of 3.6% of turtles (n = 44; 95% CI  $\pm 3.6\%$ ) presented with external tumours from Fibropapillomatosis (FP) resulting from herpes virus infection (Figure 2.8D). An additional five turtles presented with healed tumours consistent with having recovered from the infection.

Turtles with external fibropapilloma tumours were most frequently encountered at the Boyne Island and South Trees study sites and very infrequently encountered at the other study sites including the upper estuary of the Boyne River at Benaraby.

Discussion

Although six species of marine turtles forage within Port Curtis, with Green turtles comprising 99.5% of observed turtles within the intertidal and shallow subtidal waters, adjacent mangrove margins and shallow rocky reefs of Port Curtis during the

present study, Green turtles are the dominant species utilising the shallow water around the margins of Port Curtis.

The majority of observations and captures of foraging Green turtles within Port Curtis were aggregated within five localised areas of the Port. These areas of elevated concentrations of foraging Green turtles represent only a small proportion of the total areas of the port characterised as being adjacent to outflows from rivers and creeks: Colosseum Creek, Boyne River, South Trees Inlet and Calliope River. In the case of the Pelican Banks, there is outflow from the Port and regular reversals of strong tidal currents and associated settlement of sediments to form wide shallow flats supporting seagrass and algal pastures.

The genetic stocks breeding within the SW Pacific region have been defined by FitzSimmons and Limpus (2014). Migration data derived from flipper tag recoveries and limited satellite telemetry results from foraging turtles from within Port Curtis demonstrate that Port Curtis is populated from the sGBR Green turtle genetic stock that breeds at the southern Great Barrier Reef courtship areas and nesting beaches. The genetic relationship of Green turtles foraging within Port Curtis will be explored further in Chapter 3.

Developmental migration records within the QTC database contains only 32 instances of the flipper-tag recapture of a foraging Green turtle that has made a major change in foraging areas during its recorded life. These 32 recaptures are derived from over 100,000 Green turtles that were originally tagged in numerous widely scattered foraging areas in south and central Queensland during 1974-2019. Of these 32 developmental migration recaptures, there were 22 females, 2 males and 8 for which the gender was not recorded. All of these developmental migrations of Green turtles have been immature individuals and two of these were of turtles that migrated from foraging within the Capricorn Bunker Group to forage in Port Curtis.

There is currently no evidence to support the hypothesis of regular developmental migration (Meylan *et al.* 2011) across successive foraging areas during the growth of Green turtles in eastern Australia. Where developmental migration has been recorded, it has been associated with immature age class turtles.

Based on the flipper tag recovery data, Green turtles foraging within Port Curtis have displayed high fidelity to their respective localised foraging sites. However, the results of two telemetry studies in Port Curtis that were initiated prior to commencement of the current study (Babcock, 2015; Hamann *et al.* 2017) detected substantial movement by some turtles between study sites within and external to the Port. The significance of these telemetry results will be examined further within the investigation of habitat use and foraging area fidelity in Chapter 4.

The combined observations of low density Green turtle courtship activity within Port Curtis and the timing of departures of the annual breeding cohort from the Port indicate that the Port is not a significant area for Green turtles to aggregate for courtship and mating. However, these observations do not preclude the possibility that a small proportion of the local resident population may mate locally within Port Curtis or that occasional visitors from other foraging areas may migrate for courtship within Port Curtis.

The gender of marine turtles is not determined by sex chromosomes but by the temperature of the nest within the middle few weeks of incubation (Miller and Limpus, 2003). There is for each genetic stock of marine turtles a theoretical temperature

(pivotal temperature) that should produce approximately equal proportions of male and female hatchlings. This has been confirmed for Green turtles breeding within the GBR, with a higher pivotal temperature (by 1.7°C) for the nGBR genetic stock nesting in the northern GBR –Torres Strait region than for the sGBR stock nesting in the southern GBR region (29.3 vs. 27.6°C; Miller and Limpus, 1981; Limpus, 2008). With changing beach temperatures in response to environmental variables including orientation to the sun, rainfall, shade and sand colour, different sex ratios can be expected to be produced within and between the individual nesting beaches within a single breeding season and across successive breeding seasons (Limpus *et al.* 1983; Booth and Astill, 2001a, b; Chu *et al.* 2008; Sims *et al.* 2014; Woods *et al.* 2014). With the foraging Green turtles within Port Curtis being primarily from the sGBR genetic stock, the sex ratio of these foraging turtles can be expected to reflect the temperature regimes at the multiple nesting beaches that supports this foraging population. In addition, young immature turtles that have recently recruited to residency are expected to have originated during temperature regimes within the last decade, while larger turtles (presumed older) will have hatched from beach thermal conditions further back in time. In the extreme, adult turtles will have hatched more than 30 years ago, possibly up to 70 years ago, before they were sampled (unpublished age determination data, QTC database. See also Jensen *et al.* (2018) for a comparable assumption of age structure within the Howicks Reef foraging population).

The sex ratio of Green turtles sampled during 2011-2019 from all study sites collectively varied across the age classes within Port Curtis: adults had approximately equal proportions of female and males (51% females; 1.03:1 ratio) while large immatures, small immatures and recently recruited very small immature turtles showed an overall increased proportion of females in the younger age classes (to 64% female; 1.77:1 ratio). Interpretation of these sex ratio data will be made in the context of existing comparative data from other study site dominated by Green turtles from the sGBR stock in eastern Australia.

The sex ratio by age class of wild caught foraging Green turtles has been recorded at numerous locations throughout eastern Australia since visual inspection of gonads via laparoscopic examination was introduced into the QTC program in 1983 (Table 2.10; Figure 2.10). It is apparent that where there has been a dominance of sGBR genetic stock, there has been an adult female biased sex ratio that has varied around approximately 2:1, female:male (66.7% female) at Shoalwater Bay and Moreton Bay for more than 2 decades. In contrast, at Heron-Wistari Reefs and Port Curtis the adult sex ratio has approximated to 1:1, female:male (50% female) or lower. There is no evidence to suggest a higher rate of mortality for adult females at either location, so additional hypotheses are needed. The lower adult female biased sex ratios are associated with sampling sites in close proximity to the focal courtship and nesting region for the sGBR genetic stock (Figure 2.10A). In contrast, the female biased adult sex ratio is highest at foraging areas 3° or more in latitude away from the core breeding area for the sGBR stock (Figure 2.10A). One hypothesis is that males may select foraging grounds that are closer to breeding sites as adults to reduce energetic costs of more frequent breeding migrations (FitzSimmons, 1997 FitzSimmons *et al.* 2000), although tag recovery data have not supported this idea. The sex ratio of small immature Green turtles from sGBR stock dominated foraging areas has fluctuated mostly within the range of 0.6 to 0.8 female across recent decades and with no obvious tendency towards increased feminisation within the stock (Figure 2.10B). Superficially, may be even a tendency towards increased maleness.

In contrast, foraging areas dominated by the nGBR Green turtle genetic stock have supported a highly biased female sex ratio across all age classes for many decades at multiple foraging areas (Table 2.10; Figure 2.10B). A recent study of sex ratios by genetic stock within the Howick Reefs foraging population has produced results consistent with progressive feminisation of the nGBR genetic stock attributed to climate change (Jensen *et al.* 2018).

To enhance capacity for understanding the impact of climate change on our turtle populations, the strongly female biased sex ratio that has been operational within the nGBR stock for decades, the lower female biased sex ratio recorded for foraging areas dominated by the sGBR stock across the same time period and the apparent extreme recent feminisation of the Howick Reef foraging population warrants a comprehensive reinvestigation of sex ratio by genetic stocks in Green turtle foraging areas from Torres Strait to Moreton Bay. In addition, given the variable adult sex ratios between foraging areas within the foraging range for the sGBR stock, the possibility of differential movement of adult males to foraging areas in close proximity to the core breeding area and/or of adult females moving away from these core breeding areas for the stock warrants investigation. The sex ratio by genetic stock of recently recruited small juvenile Green turtles to benthic foraging in Port Curtis is investigated further in Chapter 3.

The resident Green turtle population foraging within Port Curtis ranged from very small immature turtles recently recruited from the open ocean dispersal life history phase to benthic foraging residency across the full spectrum of age classes to mature adults. As with other foraging populations investigated with the dispersal range of the sGBR stock, the Port Curtis population is dominated by small immature turtles. Very small immature turtles recruited to residency in Port Curtis with a mean CCL = 43.2 cm (Table 2.5D), with no detectable differences in the size at which they recruited with respect to gender or year of recruitment. Very small immature Green turtles recruited into the Port Curtis foraging population at a mean annual proportional rate = 0.14 of the small pre-pubescent Green turtle resident population present in the Port.

While typical for the species, adult female Green turtles in Port Curtis were larger than adult males and there was no detectable difference in size of either the adult females or males across the seven years of study. The adult females within Port Curtis commenced breeding at a relatively small mean CCL = 99.2 cm.

During 2016-2019, based on laparoscopic and ultrasound examination of gonads to determine gender, maturity and breeding condition, the annual recruitment rate of first-time breeders into the female breeding population (rate = 0.10) was low (Chapter 5, Figure 5.8, 5.9).

External examination of foraging turtles in Port Curtis identified only low incidence of compromised health among those turtles captured: partly or extremely emaciated turtles = 7.9%; turtles fractured from vessel interactions = 3.2%; entangled in fishing gear and marine debris = 0.7%; Fibropapillomatosis = 3.6%.

Fractured turtles can provide information regarding the size of the impacting propellers and vessels based on the dimensions of injuries and areas of the Port where turtles are at risk from vessel interactions based on the distribution of fractured turtles. Observed injuries to fractured turtles were consistent with damage caused primarily by a medium to large outboard powered vessels moving at speed, not from

the larger commercial vessels such as tugs and freighters using the Port infrastructures. In addition, the majority of fractured turtles (69%) were captured on the Pelican Banks. This is a wide area of intertidal flats adjacent to a meandering North Channel where outboard powered vessels on the plane were regularly observed taking shortcut across the flats during the upper tidal heights and water skiing off the South End shoreline. These data represent only turtles that survived after having been hit by vessels. They do not include the unquantified number of turtles that died as a result of the vessel related injuries. The introduction of a go-slow zone over the Pelican Banks within vessel management policy for Port Curtis has the potential for substantial reduction of vessel related injuries and mortality within the Port. By restricting vessel speeds to 10 knots or lower over the Pelican Banks, but not including the North Channel, would reduce high speed traffic over the shallow flats where turtles aggregate for feeding but would not impact on large vessel commercial traffic operations within the Port.

Fibropapillomatosis (FP), also referred to as Green turtle fibropapilloma disease, has been considered to represent a major threat to the survival of Green turtle populations (Davidson, 2001). However, the 2015 International Summit on Fibropapillomatosis in Hawaii (Hargrove *et al.* 2016) that assessed the global status, trends, and population impacts of the disease on turtle populations concluded:

- Globally, FP has long been present in wild sea turtle populations.
- FP primarily affects medium-sized immature turtles in coastal foraging pastures.
- Expression of FP differs across ocean basins and to some degree within basins. Turtles in the Southeast US, Caribbean, Brazil, and Australia rarely have oral tumours (inside the mouth cavity), whereas they are common and often severe in Hawaii. Internal tumours (on vital organs) occur in the Atlantic and Hawaii, but only rarely in Australia. Liver tumours are common in Florida but not in Hawaii.
- Recovery from FP through natural processes, when the affliction is not severe, has been documented in wild populations globally.
- FP causes reduced survivorship, but documented mortality rates in Australia and Hawaii are low. The mortality impact of FP is not currently exceeding population growth rates in some intensively monitored populations (e.g., Florida and Hawaii, USA and Southern Great Barrier Reef stock Queensland, Australia) as evidenced by increasing nesting trends despite the incidence of FP in immature foraging populations.
- Pathogens, hosts, and potential disease and environmental cofactors have the capacity to change; while we are having success now, there needs to be continued monitoring to detect changes in the distribution, occurrence, and severity of the disease.
- While we do not have clear evidence to provide the direct link, globally, the preponderance of sites with a high frequency of FP tumours are areas with some degree of degradation resulting from altered watersheds. Watershed management and responsible coastal development may be the best approach for reducing the spread and prevalence of the disease.



- Future research efforts should employ a multi-factorial ecological approach (e.g., virology, parasitology, genetics, health, diet, habitat use, water quality, etc.) since there are likely several environmental cofactors involved in the expression of the disease, which is still thought to be caused by a herpesvirus.
- Minimum FP data collection in new areas should include: individual identification (photo ID, PIT tags, etc.), standard measurements (length and weight), presence/absence of tumours, tumour severity, body condition, oral examination, method of capture, and effort.

Limpus *et al.* (2016) reviewed the history and distribution of FP in Australia: FP has a widespread distribution in turtle populations in eastern and Northern Australia, especially with Green turtles and most frequently in inshore waters with restricted tidal flushing and with altered catchments. The low incidence of the disease as recorded by the presence and severity of external tumours in Port Curtis during 2016-2019 (3.6%) is at the lower range of the incidence of tumoured turtles in coastal bays in Queensland (Moreton Bay, 27°S, 1990–2014: annually recorded frequencies ranged 5–20%; Shoalwater Bay, 22°S, 1986–2012: annually recorded frequencies ranged 2–5%) and the incidence of FP tumoured turtles in Port Curtis was slightly higher than that recorded at Heron-Wistari Reefs on the outer margin of the GBR (23°S, 1984–1999: annually recorded frequencies ranged < 1%) (Limpus *et al.* 2016). As recorded for Moreton Bay and Shoalwater Bay (Limpus *et al.* 2005, 2016), turtles in Port Curtis have displayed a capacity for recovery from FP.

Even though FP has been documented as a widespread disease in foraging areas for the sGBR Green turtle stock, this stock has been documented as a strongly recovering stock at Port Denison (20°S; Hof *et al.* 2017); Heron-Wistari Reefs (23°S; Chaloupka and Limpus, 2001); Hervey Bay (25°S; Twaddle, 2014) and Moreton Bay (27°S; Limpus *et al.* 2016). Based on these results and the conclusions drawn from the 2015 International Summit on Fibropapillomatosis, FP is not considered to represent a significant threat to the foraging Green turtle population in Port Curtis. Continued monitoring of this disease in the Port is considered desirable, particularly at the outflow areas of the Boyne River and South Trees Outlet where the highest incidence of the disease was identified within the Port.

## Recommendations

The Green turtle population within Port Curtis would benefit from improved habitat management of the five localised aggregated foraging areas identified in this study.

The introduction of a go-slow zone over the Pelican Banks within vessel management policy for Port Curtis has the potential for substantial reduction of vessel related injuries and mortality within the Port. By restricting vessel speeds to 10 knots or lower over the Pelican Banks but not including the North Channel would reduce high speed traffic over the shallow flats where turtles aggregate for feeding but would not impact on large vessel commercial traffic operations within the Port.

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Table 2.1. Summary of the tagging status and tagging history of foraging Green turtles (*Chelonia mydas*) captured in Port Curtis during 2016-2019.

Tagging status and history of turtles	Number of turtles			
	2016	2017	2018	2019
• 1 <sup>st</sup> time tagged turtles	309	274	391	233
• Recaptured turtles from previous years from the same area	18	83	59	46
• Recaptured turtles from elsewhere in the Port	1	-	3	2
• Recaptured turtles with tags scars indicating a turtle that has been previously tagged but lost its tag(s)	0	5	2	3
• Recaptured after release from rehabilitation	0	1	2	0
• Recapture of a turtle tagged at a breeding site.	3	5	4	0
<b>TOTAL TURTLES</b>	<b>331</b>	<b>368</b>	<b>461</b>	<b>284</b>
• <b>Recaptured turtles from the same year from the same area</b>	35	34	43	20
<b>TOTAL CAPTURES</b>	<b>366</b>	<b>402</b>	<b>504</b>	<b>304</b>

Table 2.2. Summary of Green turtles (*Chelonia mydas*) captured by tagging history and study areas in Port Curtis (PC) and adjacent waterways for the four years of the GPC funded study: 2016 to 2019. Study areas added in 2018 or later are highlighted in blue text.

Month	Pelican Banks			Quoin Island			Facing Island			Western Basin & Narrows			South Trees			Boyne River & offshore			TOTAL
	New tagging	recapture from PC (within year recapture)	Migration recapture from a breeding area	New tagging	recapture from PC (within year recapture)	Migration recapture from a breeding area	New tagging	recapture from PC (within year recapture)	Migration recapture from a breeding area	New tagging	recapture from PC (within year recapture)	Migration recapture from a breeding area	New tagging	recapture from PC (within year recapture)	Migration recapture from a breeding area	New tagging	recapture from PC (within year recapture)	Migration recapture from a breeding area	
<b>2016</b>																			
Mar-May	17	-	-	4	-	-	17	-	-	8	1	-	-	-	-	30	1	-	79
June	52	4 (1)	1	9	- (2)	-	30	- (1)	-	5	-	-	-	-	-	7	- (3)	-	108 (7)
September	22	2 (4)	-	-	-	-	4	-	-	1	-	-	-	-	-	7	- (5)	-	36 (9)
October	71	8 (15)	2	1	-	-	2	-	-	-	-	-	-	-	-	22	2 (4)	-	108 (19)
<b>TOTAL</b>	<b>162</b>	<b>14 (20)</b>	<b>3</b>	<b>14</b>	<b>- (2)</b>	<b>-</b>	<b>53</b>	<b>- (1)</b>	<b>-</b>	<b>14</b>	<b>1</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>66</b>	<b>3 (12)</b>	<b>-</b>	<b>331 (35)</b>
<b>2017</b>																			
April-May	27	11	-	-	-	-	-	2	-	-	-	-	-	-	-	32	10	-	82
June	45	14 (2)	-	1	1	-	9	3	-	1	-	-	-	-	-	5	1 (3)	-	80 (5)
September	80	19 (7)	1	1	-	-	1	-	-	-	-	-	-	-	-	23	5 (8)	-	130 (15)
November	54	17 (14)	3	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	76 (14)
<b>TOTAL</b>	<b>206</b>	<b>61 (23)</b>	<b>4</b>	<b>3</b>	<b>1</b>	<b>-</b>	<b>10</b>	<b>5</b>	<b>1</b>	<b>1</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>60</b>	<b>16 (11)</b>	<b>-</b>	<b>368 (34)</b>

Table 2.2 Continued.

Month	Pelican Banks & General Port area			Quoin Island & West Chinaman Is.			Facing Island			Western Basin & Narrows			South Trees			Boyne River & offshore & Wild Cattle Is flats			TOTAL
	New tagging	recapture from PC (within year recapture)	Migration recapture from a breeding area	New tagging	recapture from PC (within year recapture)	Migration recapture from a breeding area	New tagging	recapture from PC (within year recapture)	Migration recapture from a breeding area	New tagging	recapture from PC (within year recapture)	Migration recapture from a breeding area	New tagging	recapture from PC (within year recapture)	Migration recapture from a breeding area	New tagging	recapture from PC (within year recapture)	Migration recapture from a breeding area	
<b>2018</b>																			
Jan-April	15	1	-	-	-	-	-	-	-	-	-	-	-	-	-	51	16 (7)	2	85 (7)
May	56	22 (3)	1	6	-	-	6	6	-	8	0 (1)	-	22	1	-	15	5 (5)	-	148 (9)
June	6	4	-	2	-	-	5	2	-	1	-	-	67	-	-	10	2 (8)	-	99 (8)
August	-	-	-	-	-	-	-	-	-	-	-	-	14	0 (3)	-	3 26	- 0+1	- -	42 (4)
October	9	3 (3)	-	3	0 (1)	-		0 (1)		8	-	-	36	0 (7)		7 20	0 (2) 0 (1)	- 1	87 (15)
<b>TOTAL</b>	86	30 (6)	1	11	0 (1)	-	13	6 (1)	-	17	0 (1)	-	139	1 (10)	-	86 46	23 (22) 0 (1)	2 1	461 (43)
<b>2019</b>																			
April	16	3 1	-	1	-	-	6	-	-	7	1	-	26	4	-	3 1	2 -	-	71
May	7	3	-	-	-	-	-	-	-	-	-	-	25	5 (2)	-	10 28	9 (2) 1	-	88 (4)
June-July	-	-	-	1	-	-	-	-	-	6	2	-	25	8 (3)	-	1 2	-	-	44 (3)
September	12 3	7 (2)	-	3 9	-	-	2	0 (1)	-	7		-	14	2 (7)	-	2 19	0 (2) 0+1	-	80 (13)
<b>TOTAL</b>	35 2	13 (2) 1	-	5 9	-	-	8	0 (1)	-	20	3	-	90	19 (12)	-	16 50	11 (4) 1+1	-	284 (20)

Table 2.3. Breeding migration records for adult Green turtles, *Chelonia mydas*, that were recorded foraging within Port Curtis during 2016-2019.

Black text denotes foraging records; red text denotes nesting records; blue text denotes courtship records.

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### 2.3A. FEMALE MIGRATION BETWEEN NESTING AND FORAGING

**T30096:** CCL = 99.9 cm, 32 yr history within QTC data base

- Originally tagged when nesting at Northwest Island, 1986-1987 breeding season
- Recaptured after 6 yr, nesting at Northwest Island, 1992-1993 breeding season
- Recaptured foraging on the Pelican Banks, 18 May 2018

**T70052:** CCL = 99.8 cm, 26 yr history within QTC data base

- Originally tagged when nesting at Lady Musgrave Island, 1992-1993 breeding season
- Recaptured after 10 yr, nesting at Lady Musgrave Island, 2002-2003 breeding season
- Recaptured foraging on the Pelican Banks, 20 May 2011
- Recaptured after 11 yr, nesting at Lady Musgrave Island, 2013-2014 breeding season
- Recaptured foraging on the Pelican Banks, 7 June 2016, 4 November 2017, 20 May 2018
  - Not breeding for 5 consecutive breeding seasons, 2014-2018

**T70804:** CCL = 108.7 cm, 24 yr history within QTC data base

- Originally tagged when nesting at Wreck Island, 1994-1995 breeding season
- Recaptured foraging on the Pelican Banks, 3 November 2017

**T84899:** CCL = 105.0 cm, 24 yr history within QTC data base

- Originally tagged when nesting at Northwest Island, 1994-1995 breeding season
- Recaptured foraging at the mouth of the Boyne River, 9 April 2018

**K4240:** 23 yr history within QTC data base

- Originally tagged when nesting at Lady Musgrave Island, 1996-1997 breeding season
- Recaptured foraging on the Pelican Banks, 1 January 2000
- Recaptured after 23 yr, nesting at Lady Musgrave Island, 2019-2020 breeding season

**T83025:** 23 yr history within QTC data base

- Originally tagged when nesting at Lady Musgrave Island, 1994-1995 breeding season
- Recaptured after 6 yr, nesting at Lady Musgrave Island, 2000-2001 breeding season
- Recaptured Basking on Facing Island Beach, 14 October 2017

**T82632:** CCL = 98.2 cm, 23 yr history within QTC data base

- Originally tagged when nesting at Lady Musgrave Island, 1994-1995 breeding season
- Recaptured foraging on the Pelican Banks, 3 November 2017

**T88971:** CCL = 98.6 cm, 21 yr history within QTC data base

- Originally tagged when nesting at Northwest Island; 1995-1996 breeding season
- Recaptured foraging on the Pelican Banks, 8 October 2016
  - Gonad examination result: not bred in the last two breeding seasons and not preparing to breed in coming 2016 breeding season

**K306:** CCL = 101.9 cm, 15 yr history within QTC data base

- Originally tagged when nesting at Heron Island; 2002-2003 breeding season
- Recaptured after 11 yr, nesting at Heron Island, 2013-2014 breeding season



- Recaptured foraging on the Pelican Banks, 5 November 2017

### 2.3A. FEMALE MIGRATION BETWEEN NESTING AND FORAGING (continued)

**K57038:** CCL = 108.4 cm, 16 yr history within QTC data base

- Originally tagged when nesting at Heron Island; 2002-2003 breeding season
- Recaptured foraging offshore of Colosseum Creek, 4 October 2018

**K98103:** CCL = 104.2 cm, 9 yr history within QTC data base

- Originally tagged when nesting at Heron Island; 2008-2009 breeding season
- Recaptured foraging on Pelican Banks, 3 November 2017

**QA34792:** CCL = 104.2 cm, 4yr history within QTC data base

- Originally tagged when foraging on Pelican Banks, 1 May 2013
- Recaptured when nesting at Lady Musgrave Island; 2013-2014 breeding season
- Recaptured foraging on Pelican Banks, 6 November 2017

**QA64318:** CCL = 94.5 cm, 1yr history within QTC data base

- Originally tagged when foraging on Pelican Banks, 24 June 2017
- Recaptured when nesting at Lady Musgrave Island; 2017-2018 breeding season

### 2.3B. FEMALE MIGRATION BETWEEN COURTSHIP AND FORAGING

**T96692:** CCL = 106.3 cm; 21 yr history within QTC data base

- Originally tagged at the courtship area of Sandy Cape, Fraser Island, 19 November 1996
- Recaptured when foraging on the Pelican Banks, 27 September 2017

**QA52698:** CCL = 106.3 cm; 16 yr history within QTC data base

- Originally tagged at the courtship area of Sandy Cape, Fraser Island, when in her first breeding season, 29 October 2016
- Recaptured foraging on the Pelican Banks, 08 April 2018

**QA64318:** CCL = 94.5 cm; 1 yr history within QTC data base

- Originally tagged when foraging on the Pelican Banks, 24 June 2017; preparing for her first breeding season
- Tracked by satellite telemetry to the courtship area at Llewellyn Reef, mid September 2017
- Tracked by satellite telemetry to nesting beach on Lady Musgrave Island, 2017-2018 breeding season

### 2.3C. MALE MIGRATION BETWEEN COURTSHIP AND FORAGING

**K28651:** CCL = 94.8 cm; 18 yr history within QTC data base

- Originally tagged at the courtship area of Heron Island Reef, 27 October 1999
- Recaptured foraging on the Pelican Banks, 15 July 2015, 7 November 2017

Table 2.4. Fidelity of foraging Green turtles to their respective foraging areas based on tagging and recaptured data for 20 years of study 2000 to 2019.

Study sites highlighted in yellow were introduced to the study in 2018; study sites highlighted in blue were introduced to the study in 2019.

**Study site of recapture between years (within year)**

Study site where tagged	Pelican Banks	Facing Island mangrove & reefs	South Trees	Boyne River & offshore	Colosseum Ck, estuary offshore	South trees inlet to Charles Tanner wharf	Wiggins Is flats	Western Basin	The Narrows	Chinaman Is, western	Quoin Is.	Rodds Bay
<b>Total # turtles tagged</b>	786	85	229	362	96	1	56	36	3	9	34	19
<b>Pelican Banks</b>	128 (52)	2		2	1							
<b>Facing Island mangrove &amp; reefs</b>		11 (3)										
<b>South Trees</b>			18 (21)			- (1)						
<b>Boyne River &amp; offshore</b>			2	58 (74)								
<b>Colosseum Ck, estuary offshore</b>					0 (5)							
<b>South trees inlet to RG Tanner wharf</b>						-						
<b>Wiggins Is flats</b>							4 (1)					
<b>Western Basin</b>								0 (5)				
<b>The Narrows</b>									-			
<b>Chinaman Is, western</b>										-		
<b>Quoin Is.</b>											1 (3)	
<b>Rodds Bay</b>												-
<b>TOTAL CAPTURES</b>	<b>966</b>	<b>101</b>	<b>270</b>	<b>496</b>	<b>102</b>	<b>2</b>	<b>61</b>	<b>41</b>	<b>3</b>	<b>9</b>	<b>38</b>	<b>19</b>

Table 2.5. Summary of curved carapace length of definable age class cohorts of the foraging Green turtles (*Chelonia mydas*) in Port Curtis.

**2.5A. Adult females, all ages.**

	<b>Curved carapace length (cm)</b>			
<b>Year</b>	<b>Mean</b>	<b>SD</b>	<b>Range</b>	<b>Sample</b>
<b>2013</b>	103.6	6.13	88.5 – 114.3	21
<b>2014</b>	103.8	6.98	84.4 – 116.6	31
<b>2015</b>	101.7	9.26	95.1 – 108.2	2
<b>2016</b>	102.3	4.88	96.0 – 114.6	24
<b>2017</b>	101.2	5.52	93.0 – 118.5	39
<b>2018</b>	101.8	5.29	91.5 – 113.3	29
<b>2019</b>	103.5	4.84	95.0 - 117.4	22
<b>One way analysis of variance: <math>F_{6,161} = 0.917</math>; <math>p &gt; 0.50</math>. not significant. Sample: 7 years; 168 turtle records.</b>				
<b>Combined 7 yrs</b>	102.5	5.77	84.4 – 118.5	168

**2.5B. Adult females in their first breeding cycle.**

<b>Cohort</b>	<b>Curved carapace length (cm)</b>			
	<b>Mean</b>	<b>SD</b>	<b>Range</b>	<b>Sample</b>
<b>Combined 7 yrs</b>	99.2	4.50	93.0 – 106.5	8

**2.5C. Adult males, all ages.**

<b>Cohort</b>	<b>Curved carapace length (cm)</b>			
<b>Year</b>	<b>Mean</b>	<b>SD</b>	<b>Range</b>	<b>Sample</b>
<b>2013</b>	96.6	4.24	90.3 – 106.0	18
<b>2014</b>	94.6	5.38	86.4 – 103.6	29
<b>2015</b>	91.8	2.62	89.1 – 94.1	3
<b>2016</b>	95.6	4.85	85.9 – 105.3	27
<b>2017</b>	94.9	4.01	86.9 – 104.2	56
<b>2018</b>	95.6	4.46	87.7 – 104.8	30
<b>2019</b>	95.5	3.41	89.5 – 101.3	19
<b>One way analysis of variance: <math>F_{6,175} = 0.799</math>; <math>p &gt; 0.50</math>; not significant. Sample: 7 years; 182 turtle records.</b>				
<b>Combined 7 yrs</b>	95.3	4.40	85.9 – 106.0	182

**Table 2.5 Continued**

**2.5D. Small immature turtles recently recruited to coastal foraging from the pelagic foraging life history phase.** \* Sample includes some turtles for which sex was not determined.

Cohort	Curved carapace length (cm)				
	Year	Mean	SD	Range	Sample
<b>2015</b>					
Female					0
male	43.7	0.28	43.5 – 43.9		2
combined sex*	43.7	0.28	43.5 – 43.9		2
<b>2016</b>					
Female	42.6	3.10	40.3 – 46.1		3
male	43.7	0.35	43.4 – 43.9		2
combined sex*	44.8	6.12	40.3 – 59.3		8
<b>2017</b>					
Female	43.2	2.72	39.8 - 48.1		14
male	42.5	1.33	40.4 – 44.3		8
combined sex	42.9	2.30	39.8 – 48.1		22
<b>2018</b>					
Female	43.4	1.04	37.6 – 46.2		29
male	43.4	2.05	40.3 – 46.9		10
combined sex*	43.3	1.90	37.6 - 46.9		42
<b>2019</b>					
Female	43.1	2.14	38.7 – 47.0		33
male	43.5	2.23	39.8 – 47.0		16
combined sex*	43.2	2.12	38.7 – 47.0		47
<b>One way analysis of variance:</b>					
<b>Female: <math>F_{3,75} = 0.237</math>; <math>p &gt; 0.50</math>; not significant. Sample: 4 years; 79 turtles.</b>					
<b>Male: <math>F_{4,33} = 0.478</math>; <math>p &gt; 0.5</math>; not significant. Sample: 5 years; 38 turtles.</b>					
<b>Combined years</b>					
Female	43.2	1.98	37.6 – 48.1		79
male	43.3	1.91	39.6 – 47.0		38
<b>One way analysis of variance: <math>F_{1,115} = 0.081</math> <math>p &gt; 0.50</math>; not significant.</b>					
<b>Sample: both sexes across 5 years; 117 turtle records.</b>					
combined sex*	43.2	1.95	37.6 – 48.1		117

Table 2.6. Comparison of sex ratio by age class and study year for Green turtles, *Chelonia mydas*, within Port Curtis.

Sex ratio quantified with 95% Confidence limits.

YEAR	ADULT					IMMATURE, CCL > 65 CM					IMMATURE, CCL = 65 TO 47.5 CM					IMMATURE CCL < 47.6 CM (RECENTLY RECRUITED TO FORAGING)				
	Female	Male	Total by gender	Gender not identified	Sex ratio Proportion female	Female	Male	Total by gender	Gender not identified	Sex ratio Proportion female	Female	Male	Total by gender	Gender not identified	Sex ratio Proportion female	Female	Male	Total by gender	Gender not identified	Sex ratio Proportion female
2011	3	-	3	1	-	-	-	-	5	-	10	8	18	21	0.55 0.34-0.76	27	29	56	96	0.48 0.36-0.61
2012	-	-	-	-	-	-	-	-	2	-	-	-	-	1	-	-	-	-	13	-
2013	22	18	40	1	0.55 0.40-0.69	12	39	51	11	0.25 0.13-0.36	9	6	15	16	0.62 0.36-0.81	6	3	9	20	0.64 0.37-0.89
2014	32	29	61	3	0.52 0.40-0.65	17	10	27	30	0.62 0.45-0.79	6	6	12	17	0.50 0.25-0.75	11	6	17	20	0.63 0.42-0.84
2015	2	4	6	-	-	24	9	33	-	0.71 0.57-0.86	21	8	29	1	0.71 0.55-0.86	7	5	12	8	0.57 0.32-0.81
2016	24	29	53	-	0.45 0.33-0.58	32	20	52	24	0.61 0.48-0.74	26	17	43	40	0.60 0.46-0.74	37	32	69	53	0.54 0.42-0.65
2017	40	50	90	-	0.45 0.35-0.55	80	41	121	-	0.66 0.57-0.74	53	21	74	-	0.71 0.61-0.81	48	34	83 #	1	0.58 0.47-0.68
2018	29	29	58	1	0.50 0.37-0.63	47	46	93	11	0.51 0.41-0.61	76	41	117	11	0.65 0.56-0.73	107	45	153#	14	0.70 0.62-0.77
2019	24	20	44	-	0.54 0.40-0.68	32	19	51	45	0.62 0.49-0.75	45	28	73	1	0.61 0.50-0.72	74	36	115	3	0.64 0.55-0.73

# includes 1 turtle that was neither male nor female.





Table 2.7. Comparison of sex ratio by age class and study site for Green turtles, *Chelonia mydas*, within Port Curtis.

STUDY SITE	ADULT					IMMATURE, CCL > 65 CM					IMMATURE, CCL = 65 TO 47.5 CM					IMMATURE CCL < 47.6 CM (RECENTLY RECRUITED TO FORAGING)				
	Female	Male	Total by gender	Gender not identified	Sex ratio Proportion female	Female	Male	Total by gender	Gender not identified	Sex ratio Proportion female	Female	Male	Total by gender	Gender not identified	Sex ratio Proportion female	Female	Male	Total by gender	Gender not identified	Sex ratio Proportion female
Pelican Banks	101	123	224	2	0.45 0.39-0.52	166	89		61	0.65 0.59-0.71	80	34	114	26	0.70 0.61-0.78	48	26	75#	22	0.64 0.54-0.75
Facing Island	1	-	1	-	-	-	-	-	-	-	10	4	14	17	0.69 0.47-0.90	19	8	27	30	0.69 0.52-0.85
South Trees	7	4	11	-	0.62 0.36-0.86	17	13	30	2	0.56 0.39-0.73	48	28	76	4	0.63 0.52-0.73	73	27	100	7	0.73 0.64-0.81
Boyne Estuary	16	10	26	1	0.62 0.43-0.78	7	4	11	4	0.62 0.36-0.86	37	33	70	25	0.53 0.41-0.64	101	70	171	79	0.59 0.52-0.66
Colosseum Estuary	18	14	32	1	0.56 0.39-0.72	14	11	25	6	0.56 0.37-0.74	10	6	16	4	0.61 0.39-0.82	7	6	13	-	0.53 0.29-0.77
Western Basin & Narrows	5	-	5	-		6	10	16	5	0.39 0.18-0.61	11	10	21	-	0.52 0.32-0.72	5	6	11	4	0.46 0.21-0.72
Quoin Island area	-	-	-	-		-	1	1	-	-	4	2	6	-	-0.62 0.32-0.92	12	8	20	15	0.59 0.39-0.79
<b>TOTAL ENTIRE PORT</b>	159	154	313	6	0.51 0.45-0.56	213	129	342	83	0.62 0.57-0.67	200	117	317	106	0.63 0.58-0.68	267	151	418 #	226	0.64 0.59-0.68

# includes 2 turtles that was neither male nor female.

Table 2 8. Observations of Green turtle (*Chelonia mydas*) courtship within the Port Curtis region during 2016-2019.

<b>2016</b>	
	No observations of courtship
<b>2017</b>	
26 August	<p>Mounted pair of Green turtles in the Boyne River estuary (23.97010°S, 151.34620°E).</p> <ul style="list-style-type: none"> <li>• Photograph by Peter Tremul.</li> </ul> 
23 September	Mounted pair of Green turtles observed offshore of Rat Island adjacent to South End, Curtis Island (23.76578°S, 151.31785°E). No photograph taken.
28 September	Courting pair of Green turtles observed on the eastern margin of the Pelican Banks (23.78093°S, 151.30630°E). No photograph taken.
16 October	<p>Mounted pair stranded on the intertidal flats on the Pelican Banks at low tide.</p> <ul style="list-style-type: none"> <li>• Photographed during a helicopter seagrass survey by TropWATER</li> </ul> 
<b>2018</b>	
05 October	Mounted pair of Green turtles offshore in Rodds Bay (24.04117°S, 151.60985°E).



	<ul style="list-style-type: none"><li>• No photograph available</li></ul>
--	---

**Table 2.8. Continued**


<b>2019</b>	
05 September	<p>Courting pair of Green turtles observed offshore from Tannum Sands (23.94170°S, 151.45100°E):</p> <ul style="list-style-type: none"> <li>• Video clip of the courtship behaviour taken.</li> </ul>
12 September	<p>Mounted pair of Green turtles observed offshore from Lilley's Beach (23.87530°S, 151.332987°E):</p> <ul style="list-style-type: none"> <li>• No photograph taken</li> </ul>
22 September	<p>Mounted pair of Green turtles captured over the intertidal flats off Lilley's Beach (23.91717°S, 151.34441°E):</p> <ul style="list-style-type: none"> <li>• QA91899: Male, CCL = 101.1 cm.</li> <li>• QA91900: Female, CCL = 93.8 cm; image below shows the characteristic bite wounds inflicted on the neck and flippers of females by the males during courtship</li> </ul> 

Table 2.9. Summary of the number of foraging Green turtles (*Chelonia mydas*) captured in Port Curtis with external evidence of health problems categorised by study area, year, and health problem.

Grey shading denotes that study area was not sampled. Stranded dead turtles are in addition to turtles captured in the study sites.

	Pelican Banks	Facing Island	South Trees	Boyne Island & Boyne River	Off Wild Cattle Island	Quoin Island area	Western Basin	Total
<b>Turtles showing emaciation:</b> a = moribund to veterinarian, b = rescued to rehabilitation								
<b>Turtles in very poor health</b>								
2016	7	6		8		6	1b	27+1b
2017	11+ 1a	1		7+ 1b		1		20+1a,1b
2018	5		5	8	1	3		22
2019	6		11+ 1a	3+ 1a	1	2		23+2a
<b>Total</b>	29+ 1a	7	16+ 1a	18+ 1a, 1b	2	12	1b	92+3a,2b
<b>Turtles with vessel related injuries</b> S = additional stranded dead turtle								
2016	14						2	16
2017	10	1						11
2018	2		1	4	1		(1S)	6 (1S)
2019	1		0+ 2S		1+ 1S	1+ 1S	1	4+ 4S
<b>Total</b>	27	1	1+ 2S	4	2+ 1S	1+ 1S	3+ 1S	39+ 5S
<b>Turtles entangled in fishing gear</b> (x = sent to rehabilitation. a = moribund, to veterinarian. S = stranded dead turtle)								
2016				2+ 1xa			1	3+ 1xa
2017	1x	1x						2x
2018				1x 1S				1x 1S
2019								nil
<b>Total</b>	1x	1x		2+ 1x 1xa 1S			1	3 +3x 1xa 1S
<b>Fibropapillomatosis</b> (R = additional turtles recovered from FP)								
2016	1			14			1	16
2017				12				12
2018			3 (1R)	6	(1R)			9 (2R)
2019	1		3	3			(3R)	7 (3R)
<b>Total</b>	2		6 (1R)	35	(1R)		1 (3R)	44 (5R)

Table 2.10. Proportion of females (sex ratio) within populations of foraging Green turtles in eastern Australia by genetic stock and age classes within each foraging area.

“n” denotes sample size. Shaded adult sex ratios from study sites in close proximity to focal breeding areas for the sGBR genetic stock. Areas dominated by nGBR are in blue type.

Foraging area	Latitude	Year	Sex ratio			Reference
			Immature, small (n)	Immature, large (n)	Adult (n)	
<b>Foraging areas strongly dominated by nGBR stock</b>						
Daru Market, TS	9°S	1983-84	0.82 (158)	-	-	Limpus. 2008
Milman Island Reef	11°S	1996	0.81 (31)	0.75 (12)	-	QTC data base
<b>Foraging areas with strong mix of both nGBR &amp; sGBR stock</b>						
Clack Reef	14°S	1988-97	0.81 (-)	0.69 (-)	0.68 (-)	Limpus <i>et al.</i> 2009
Howick Reefs	14°S	decades				
• nGBR stock			0.99 (-)	1.0 (-)	0.87 (-)	Jensen <i>et al.</i> 2018
• sGBR stock			0.68 (-)	0.65 (-)	0.69 (-)	Jensen <i>et al.</i> 2018
<b>Foraging areas strongly dominated by sGBR stock</b>						
Green Island	16°S	1998-2005	0.80 (138)	0.67 (98)	-	QTC data base
Port Dennison	20°S	<1990	0.67 (9)	-	-	Limpus, 2008
Repulse Bay	20°S	1988-93	0.75 (55)	0.80 (111)	0.84 (154)	QTC data base
Shoalwater Bay	22°S	<1990	0.72 (129)	-	0.61 (93)	Limpus, 2008
		2000-04	0.64 (738)	0.77 (637)	0.64 (620)	Limpus <i>et al.</i> 2005

**Table 2.10 Continued**

Foraging area	Latitude	Year	Sex ratio			Reference
			Immature, small (n)	Immature, large (n)	Adult (n)	
		2006	0.66 (254)	0.66 (150)	0.66 (119)	QTC data base
• new recruits			0.59 (17)			QTC data base
		2007	0.63 (296)	0.72 (157)	0.62 (98)	QTC data base
		2008	0.58 (320)	0.67 (160)	0.59 (153)	QTC data base
		2012	0.64 (317)	0.67 (205)	0.56 (131)	QTC data base
• new recruits			0.81 (16)			QTC data base
Port Curtis	23°S	2011-19	0.63 (317)	0.62 (342)	0.51 (313)	This study (Table 2.7)
• new recruits			0.64 (418)	-	-	This study (Table 2.7)
Heron-Wistari Reef	23°S	1984-85	0.54 (145)		0.38 (42)	Limpus & Reed, 1985a
		1984-92	0.63 (342)	0.59 (377)	0.40 (235)	Chaloupka & Limpus, 2001
		1996	0.66 (89)	0.66 (295)	0.49 (67)	QTC data base
		1997	0.70 (109)	0.60 (161)	0.43 (86)	QTC data base
		1998	0.63 (59)	0.62 (182)	0.36 (99)	QTC data base
		1999	0.62 (42)	0.63 (120)	0.42 (104)	QTC data base

Table 2.10 Continued

Foraging area	Latitude	Year	Sex ratio			Reference
			Immature, small (n)	Immature, large (n)	Adult (n)	
Moreton Bay: eastern	27°S	1990-93	0.63 (536)		0.84 (51)	Limpus <i>et al.</i> 1994
Banks		1996	0.75 (138)	0.67 (90)	0.58 (31)	QTC data base
		1997	0.63 (150)	0.62 (104)	0.59 (29)	QTC data base
		1998	0.62 (86)	0.75 (122)	0.67 (45)	QTC data base
		1999	0.71 (112)	0.67 (72)	0.75 (24)	QTC data base
		2012	0.61 (153)	0.60 (95)	0.71 (38)	QTC data base
• new recruit			0.76 (37)			QTC data base
		2013	0.64 (74)	0.65 (60)	0.83 (29)	QTC data base
• new recruit			0.81 (16)			QTC data base



2.1A. Port Curtis.



2.1Ai. Western Basin and Wiggins Island



2.1Aii. Pelican Banks, western Facing Island and Quoin Island

Figure 2.1A Distribution of captures of Green turtles, *Chelonia mydas*, within Port Curtis during 2010-2019.

Yellow dots denote location of captures of Green turtles. Grey areas denote the dredged channels. Concentrated foraging areas are outlined in red.



2.1Aiii. South Trees and western Facing Island.



2.1Aiv. Boyne Island and Boyne River.



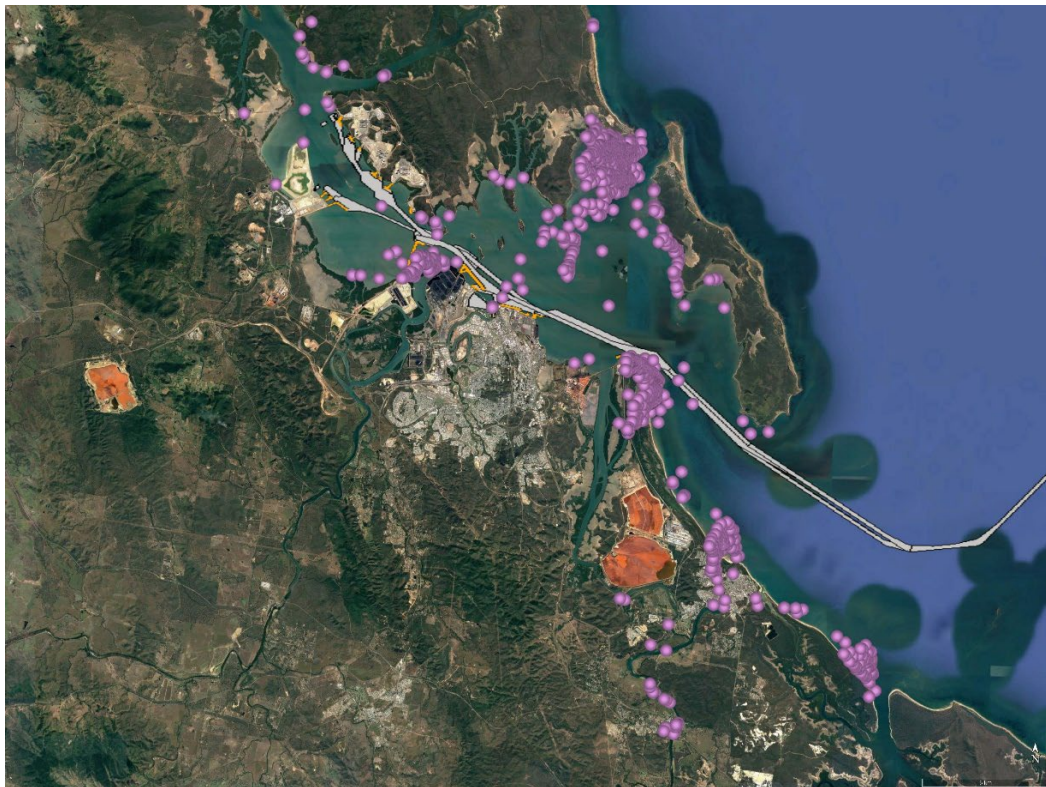
2.1Av. Colosseum Creek and Wild Cattle Island

2.1A Continued.





2.1Bi. Port Curtis and Port Alma



2.1Bii. Port Curtis.

Figure 2.1B. Distribution of captures of Green turtles, *Chelonia mydas*, within Port Curtis during 2010-2019. Purple dots denote location of sightings of turtles not captured. Grey areas denote the dredged channels.

**2.2A. Pelican Banks Green turtle foraging area**



**2.2Ai. June 2017: Pelican Banks, sand-mud flats with dugong feeding trails**



**2.2Aii. May 2016: Pelican Banks, Mangrove lined stream and gutter at high tide in the mangrove forest**



**2.2Aiii. June 2017: Pelican Banks, *Halophila ovalis* and algae**



**2.2Aiv. June 2017, Pelican Banks, seagrass, and algae**



**2.2Av. May 2016: Pelican Banks: Seagrass, *Zostera spp.*, and algae, *Ulva polyclada*, with molluscs on the Pelican Banks.**



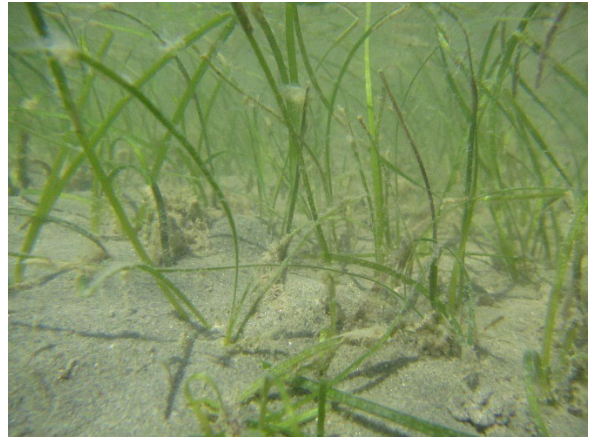
**2.2Avi. June 2018: Pelican Banks, *Zostera spp.* overgrown by *Ulva polyclada*,**

Figure 2.2. Turtle habitats of Port Curtis.

**2.2A. Pelican Banks Green turtle foraging area**



**2.2Avii. June 2018: Pelican Banks, mollusc egg mass and *Zostrea spp.***



**2.2Aviii. June 2018: Pelican Banks, *Zostrea spp.***

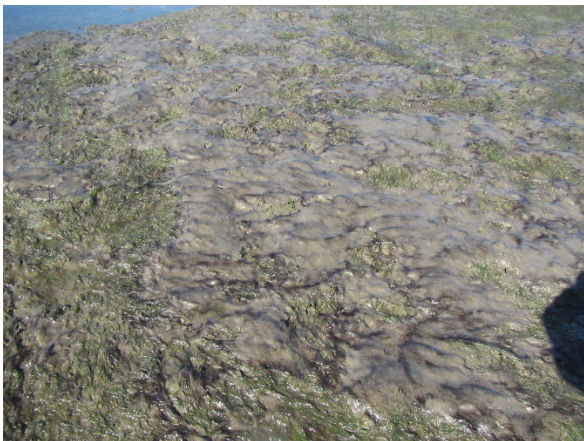
**2.2B. SouthTrees Green turtle foraging area**



**2.2Bi. June 2018: South Trees, sand-mud flats with dugong feeding trails**



**2.2Bii. June 2018: South Trees, *Zostera muelleri.***



**2.2Biii. June 2018: South Trees, *Lyngbya sp.* bloom on *Zostera muelleri.***



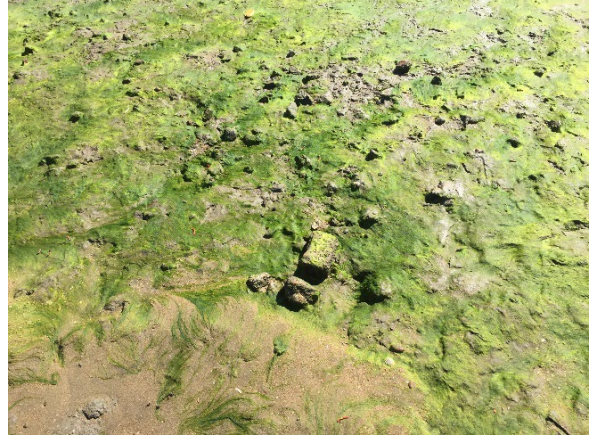
**2.2Bv. June 2018: South Trees, erosion and/or die back of *Zostera muelleri.***

**Figure 2.2. Turtle habitats of Port Curtis (Continued)**

**2.2C. Wiggins Island flats Green turtle foraging area**



**2.2Ci. May 2019 May 2019: Wiggins Island flats, *Ulva polyclada* on the gravel substrate deposited on Wiggins Island intertidal flats by the early 2017 floods.**



**2.2Cii. May 2019: Wiggins Island flats, *Ulva polyclada* on the gravel substrate deposited on Wiggins Island intertidal flats by the early 2017 floods.**



**2.2Ciii. Aug 2018: May 2019: Wiggins Island flats, *Lyngbya* sp. and *Ulva polyclada* on a mud substrate.**



**2.2Di. Aug 2018: upper Boyne River estuary at Benaraby bridge, recreational fishing**



**2.2Dii. May 2016: Western Facing Island, *Rhizophora stylosa* fringe to mangrove forest**

Figure 2.2. Turtle habitats of Port Curtis (Continued)

**2.2E. Sources of industrial pollution.**

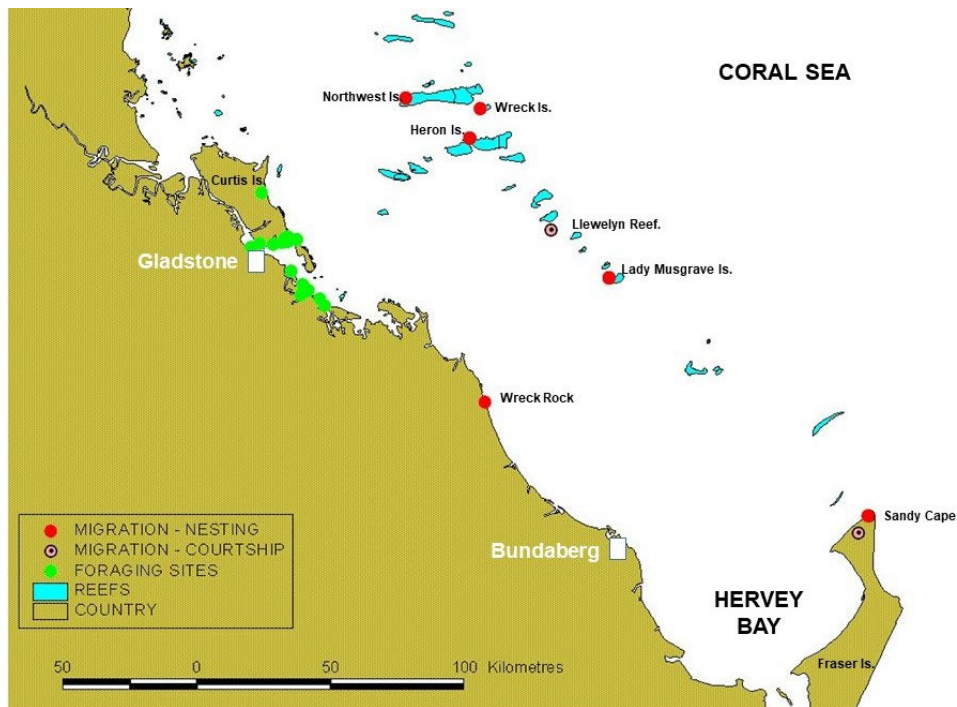


**2.2Ei. May 2019: Wind-blown coal dust pollution adjacent to Wiggins Island**

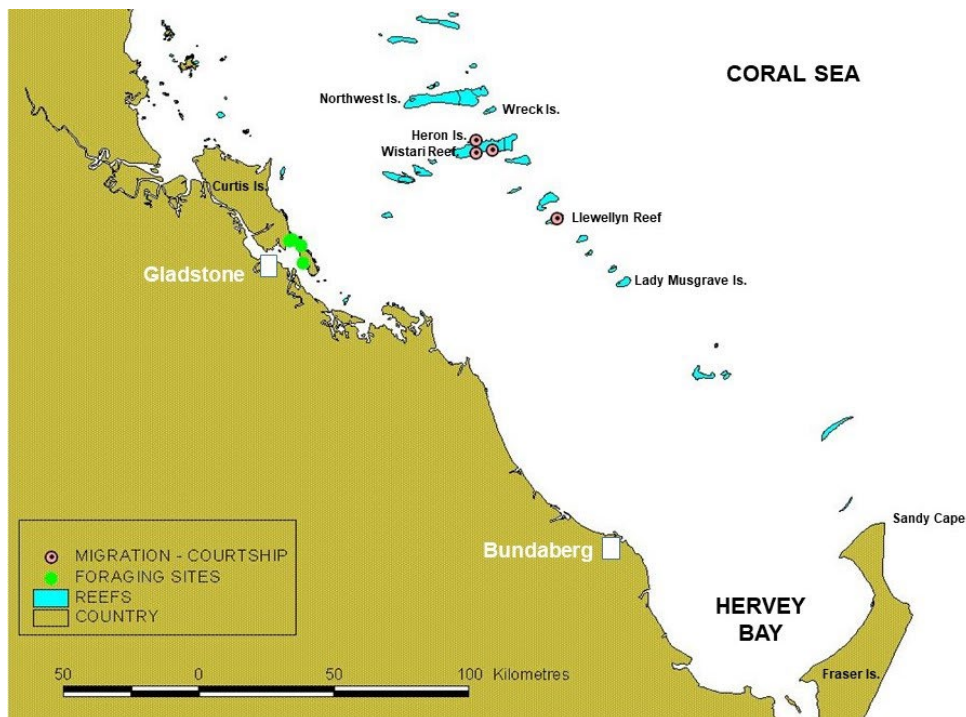


**2.2Eii. April 2019: Wind-blown alumina dust haze coming from vessel loading at Queensland Alumina Limited. South Trees wharf, South Trees.**

**Figure 2.2. Turtle habitats of Port Curtis (Continued)**



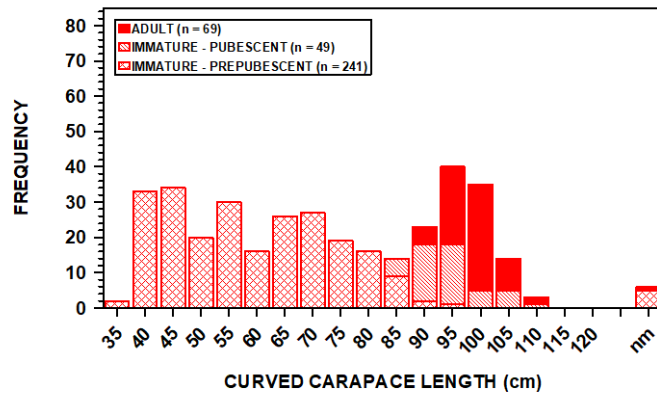
**2.3A. Females (n = 32)**



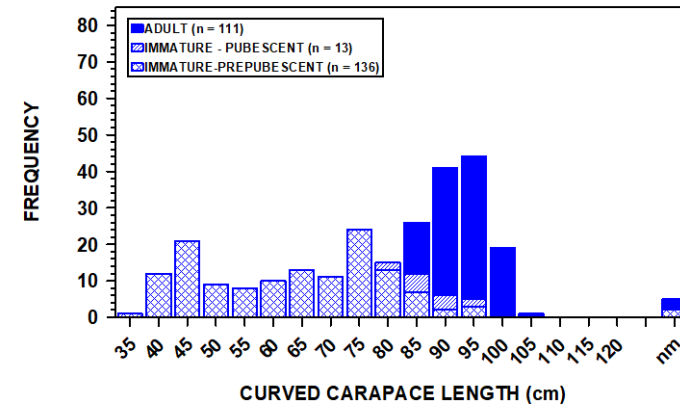
**2.3B. Males (n = 4).**

Figure 2.3. Identified breeding sites for adult Green turtles, *Chelonia mydas* that forage in Port Curtis. These migration data linking breeding sites (nesting beaches and courtship areas) with foraging areas were identified through flipper tag recoveries and telemetry tracking data within the QTC Data Base.

**GREEN TURTLES, *Chelonia mydas* : PELICAN BANKS  
FEMALE: AGE CLASS by SIZE**



**GREEN TURTLES, *Chelonia mydas* : PELICAN BANKS  
MALE by AGE CLASS & SIZE**



**GREEN TURTLES, *Chelonia mydas* : PELICAN BANKS  
GENDER by SIZE**

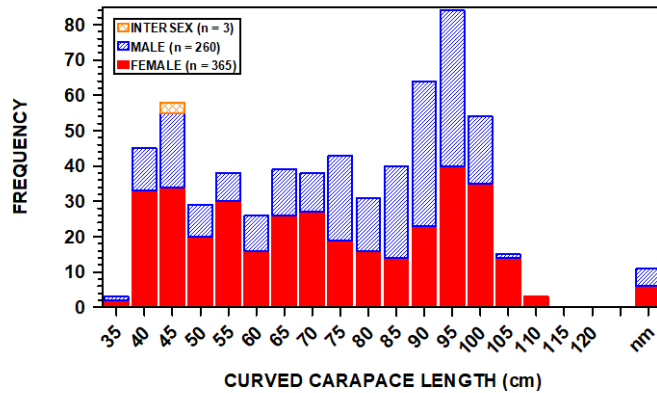
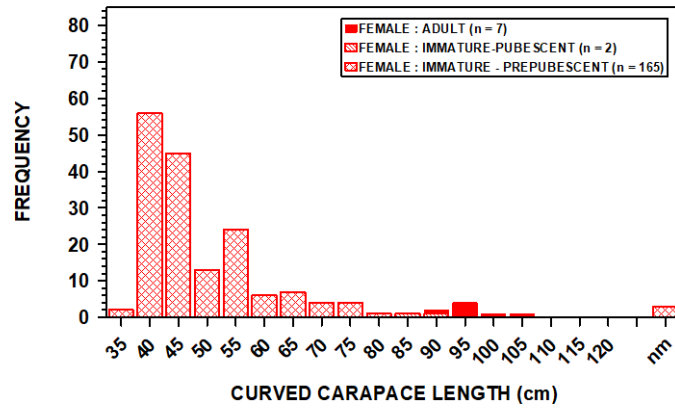
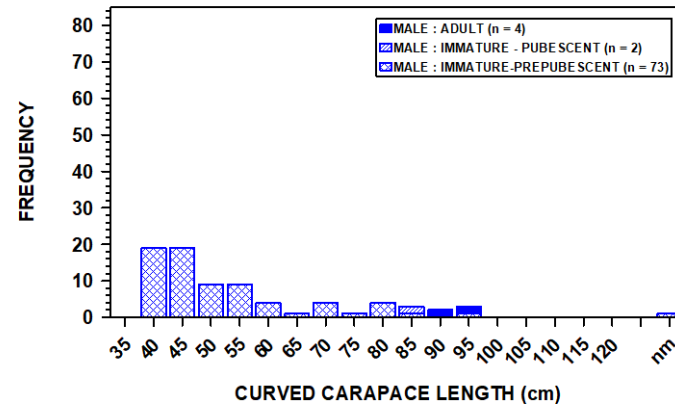


Figure 2.4A. Pelican Banks intertidal flats and adjacent subtidal waters in north-eastern Port Curtis adjacent to Curtis Island.

**GREEN TURTLES, *Chelonia mydas* : SOUTH TREES  
FEMALE by AGE CLASS & SIZE**



**GREEN TURTLES, *Chelonia mydas* : SOUTH TREES  
MALE by AGE CLASS & SIZE**



**GREEN TURTLES, *Chelonia mydas* : SOUTH TREES  
GENDER by SIZE**

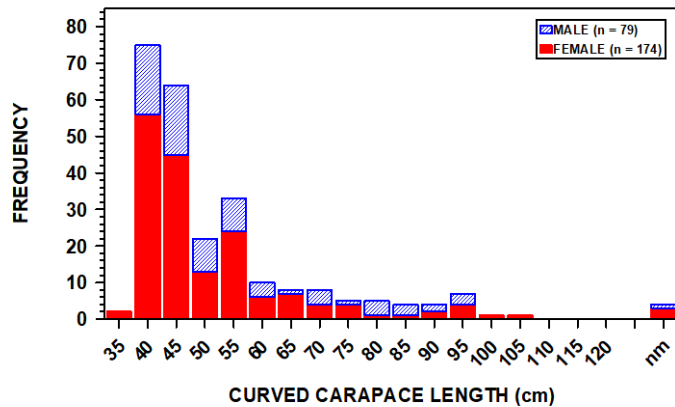
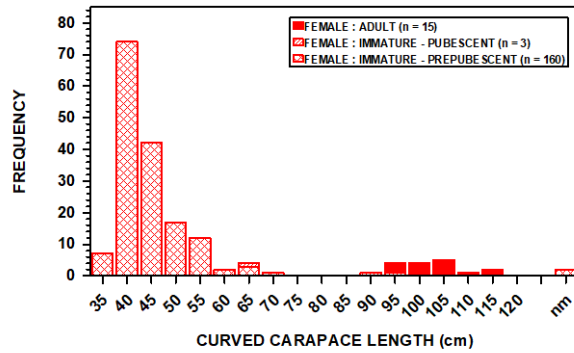


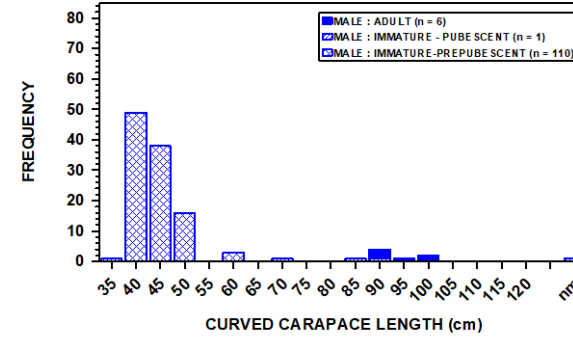


Figure 2.4B. South Trees intertidal flats and adjacent subtidal waters.

**GREEN TURTLES, *Chelonia mydas* : BOYNE**  
**FEMALE by AGE CLASS & SIZE**



**GREEN TURTLES, *Chelonia mydas* : BOYNE**  
**MALE by AGE CLASS & SIZE**



**GREEN TURTLES, *Chelonia mydas* : BOYNE**  
**GENDER by SIZE**

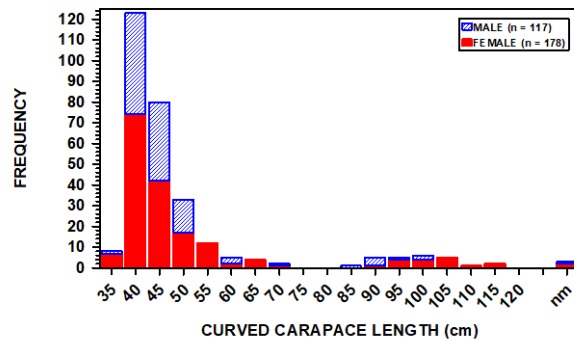
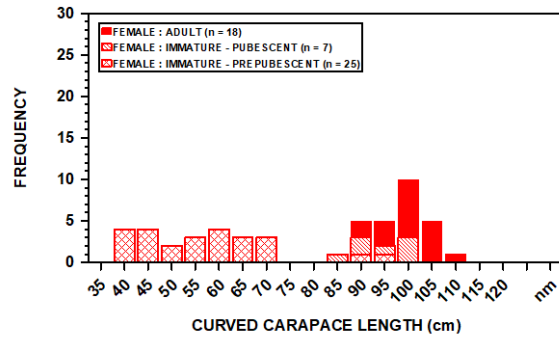
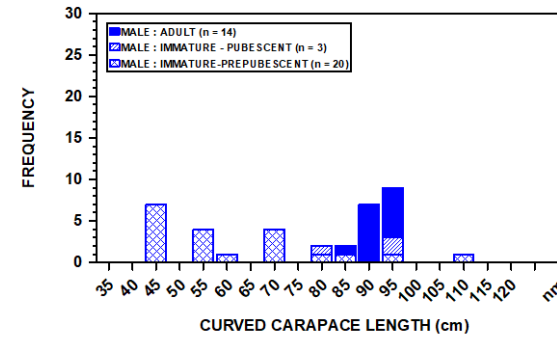


Figure 2.4C. Boyne River and adjacent inshore intertidal flats and adjacent subtidal waters Island at Boyne Island.

**GREEN TURTLES, *Chelonia mydas* : COLOSSEUM  
FEMALE by AGE CLASS & SIZE**



**GREEN TURTLES, *Chelonia mydas* : COLOSSEUM  
MALE by AGE CLASS & SIZE**



**GREEN TURTLES, *Chelonia mydas* : COLOSSEUM  
GENDER by SIZE**

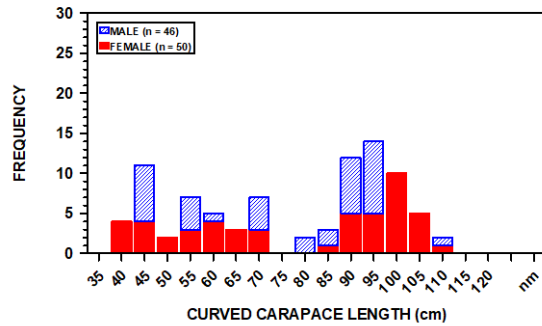


Figure 2.4D. Colosseum Creek estuary and adjacent inshore intertidal flats and subtidal waters.

**GREEN TURTLES, *Chelonia mydas* : FACING ISLAND  
GENDER by AGE CLASS & SIZE**

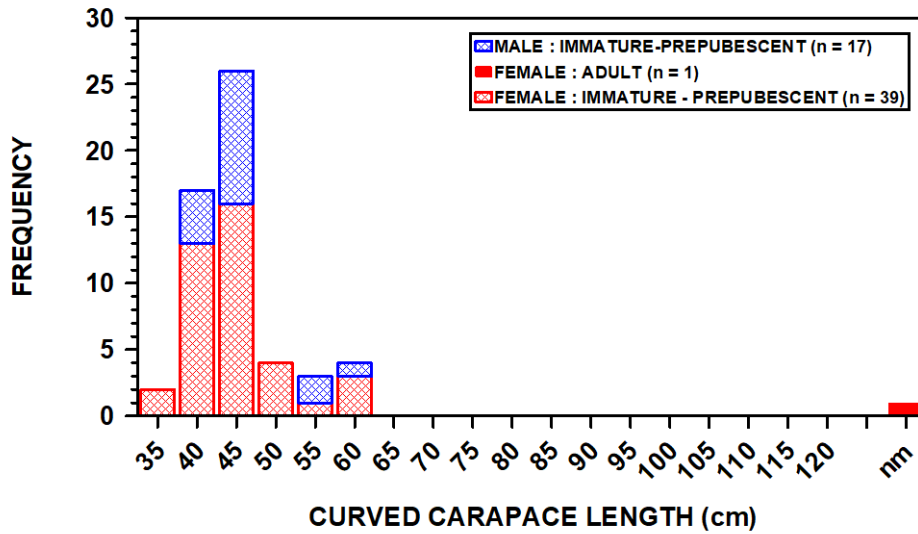


Figure 2.4E. Intertidal flats, rocky reefs and mangrove margins on the western side of Facing Island.

**GREEN TURTLES, *Chelonia mydas* : WESTERN BASIN  
GENDER : AGE CLASS by SIZE**

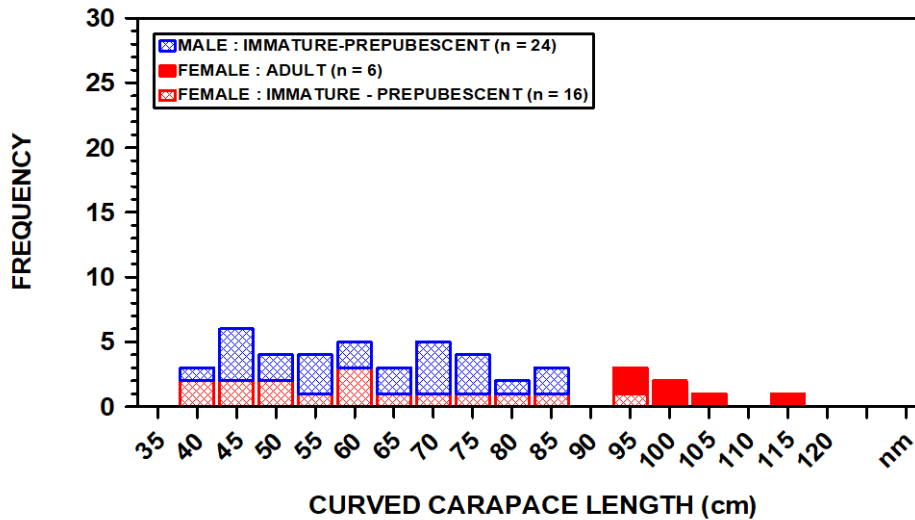


Figure 2.4F. Intertidal flats and mangrove margins of the Western Basin, Port Curtis, including Wiggins Island flats and The Narrows.

**GREEN TURTLES, *Chelonia mydas* : QUOIN & CHINAMAN IS.  
GENDER : AGE CLASS by SIZE**

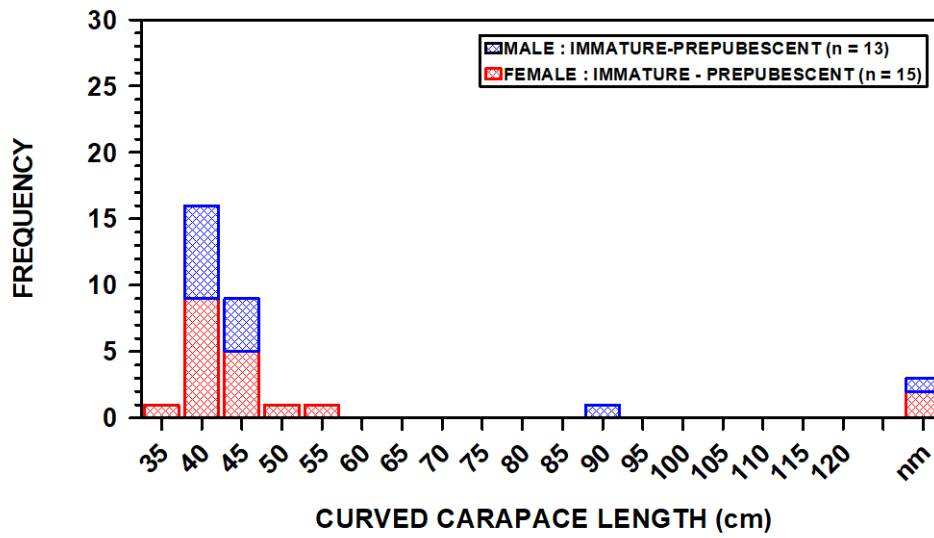


Figure 2.4G. Intertidal flats and subtidal waters and rocky reefs adjacent to Quoin island and the western margin of Chinaman Island.

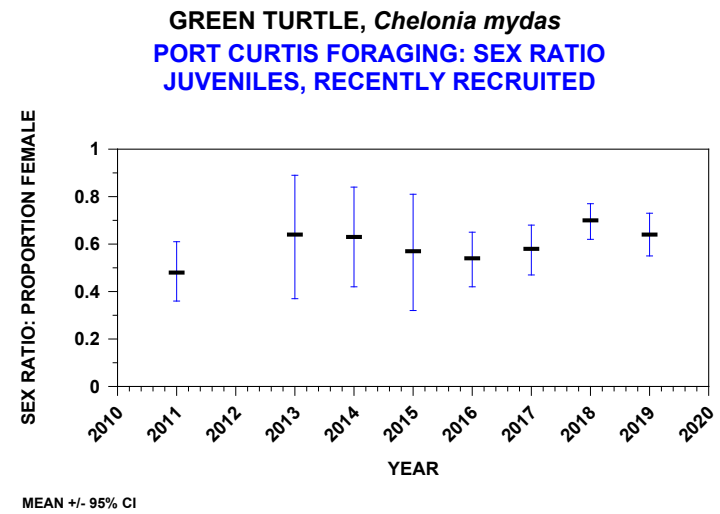
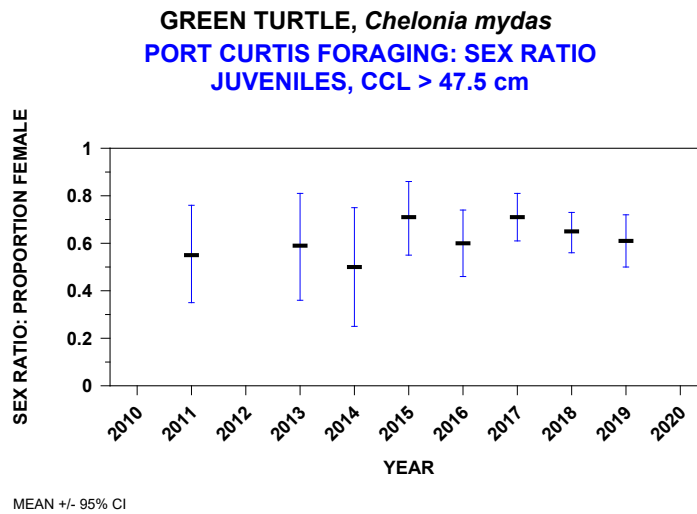
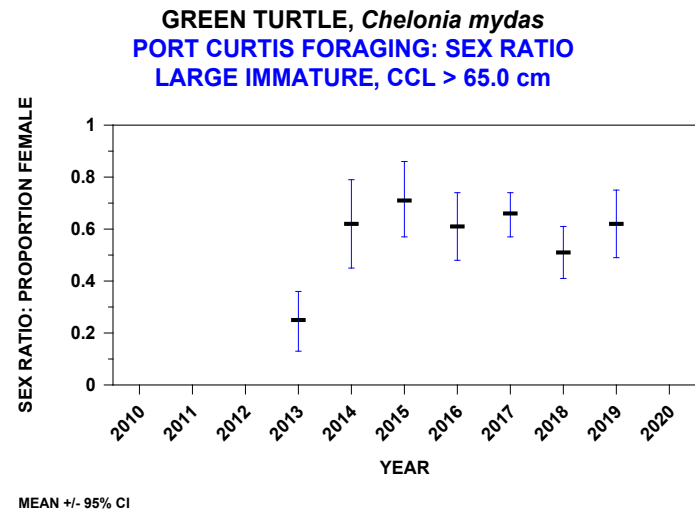
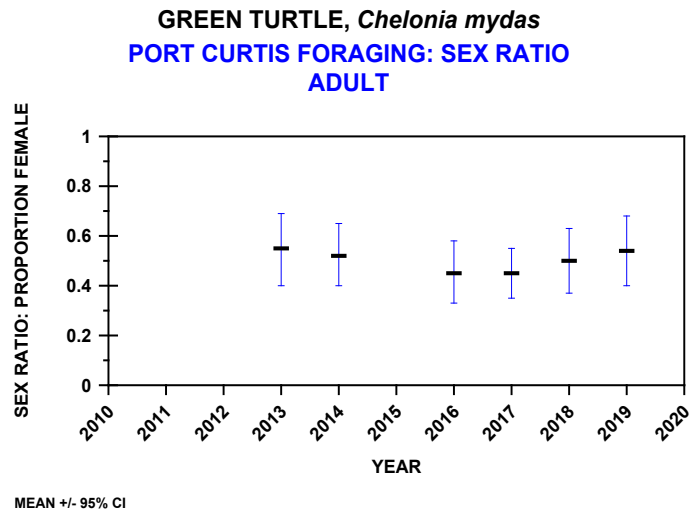


Figure 2.5A. Sex ratio by age class and years for Green turtles, *Chelonia mydas*, foraging in Port Curtis.



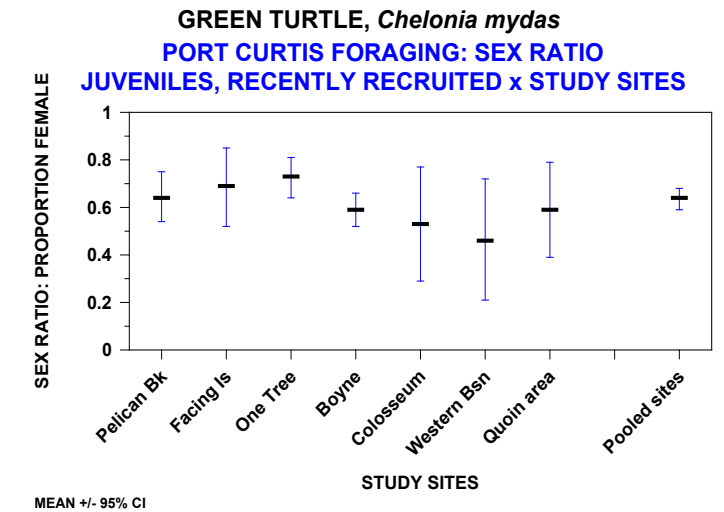
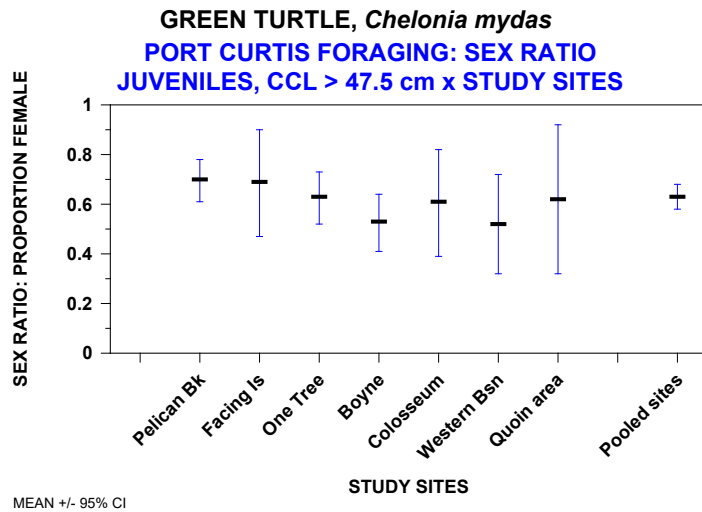
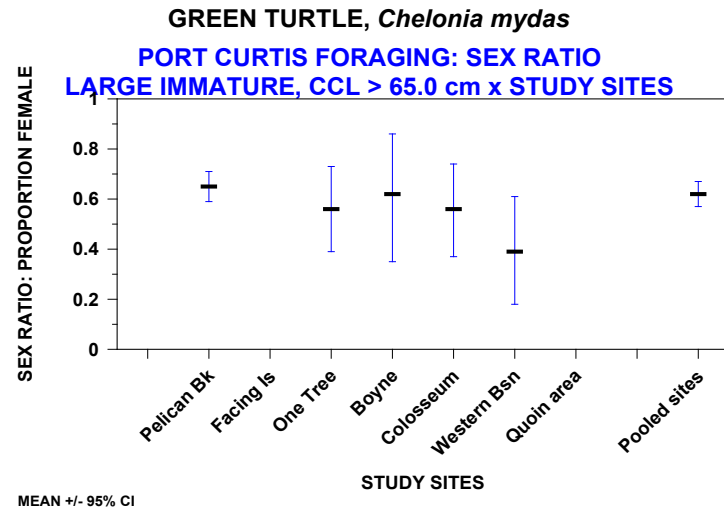
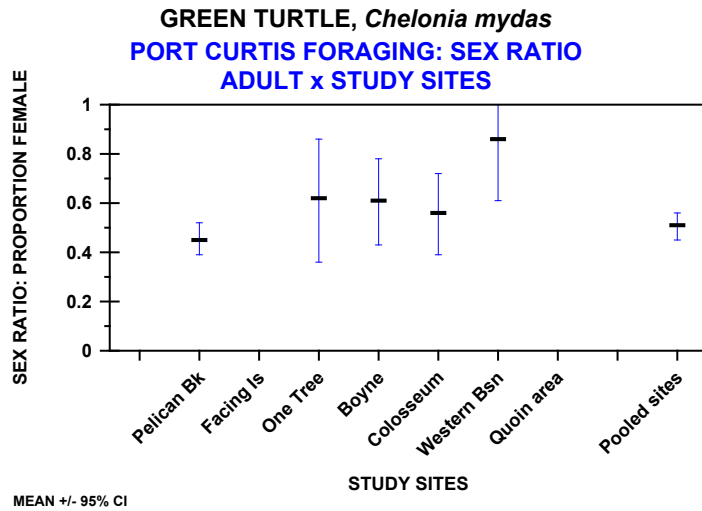




Figure 2.5B. Sex ratio by study sites for Green turtles, *Chelonia mydas*, foraging in Port Curtis.

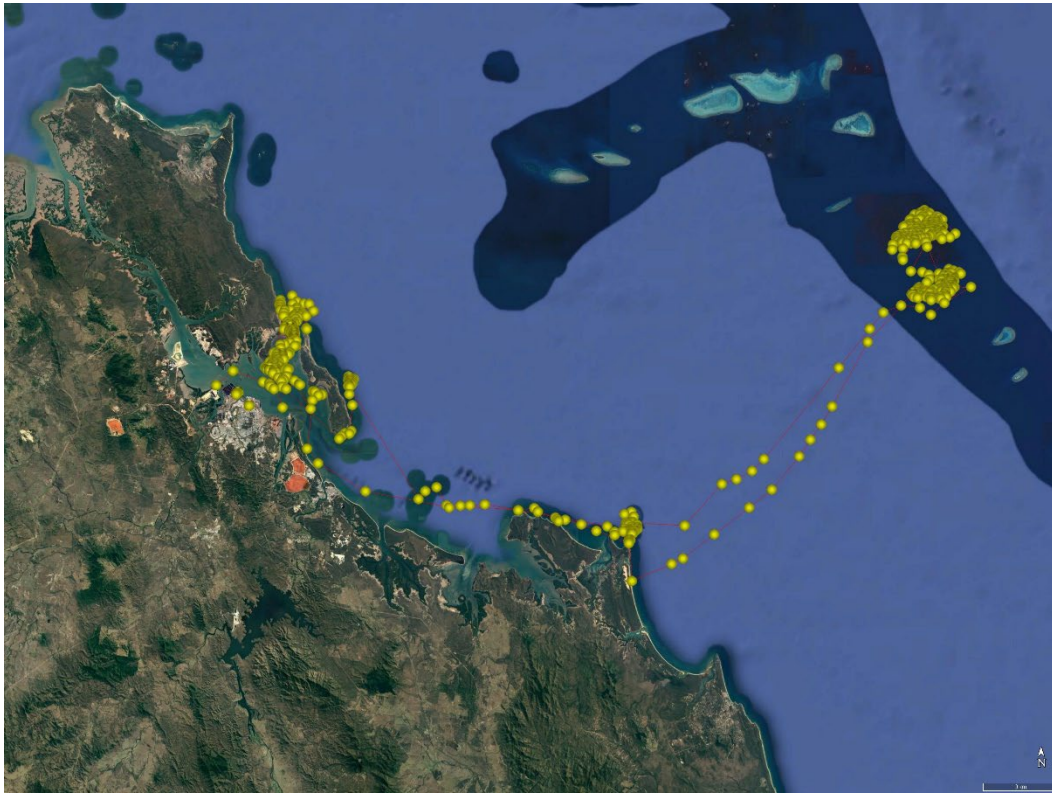
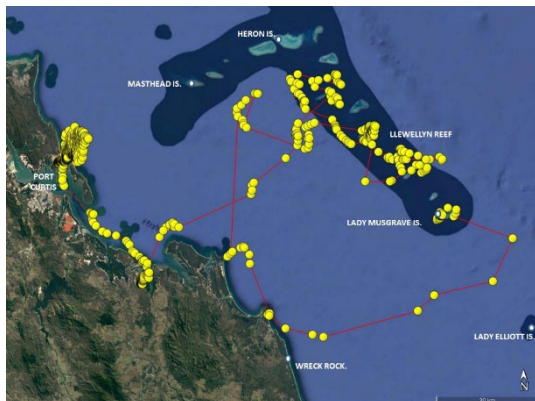


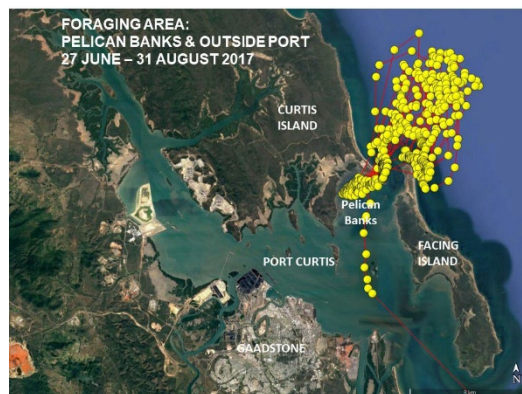
Figure 2.6. Satellite telemetry summary for adult male Green turtle (K93087) tracked from his home foraging area on Pelican Banks during its 2010 breeding migration to courtship on lagoon reefs in the southern Great Barrier Reef (Llewellyn and Fitzroy Reefs) and his subsequent post breeding migration back to the Pelican Banks (data from collaborative study by DES and Dr Rachel Groom; QTC Project)



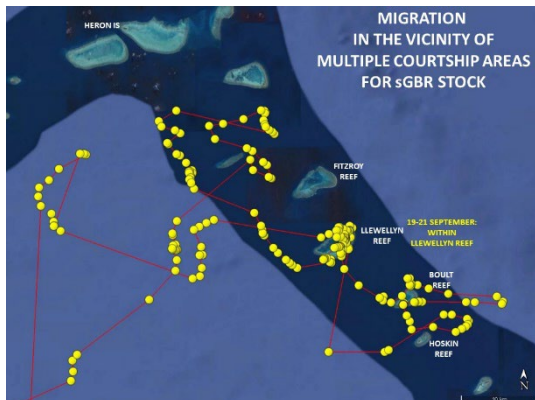
**2.7A. Release of QA64318 on the Pelican Banks**



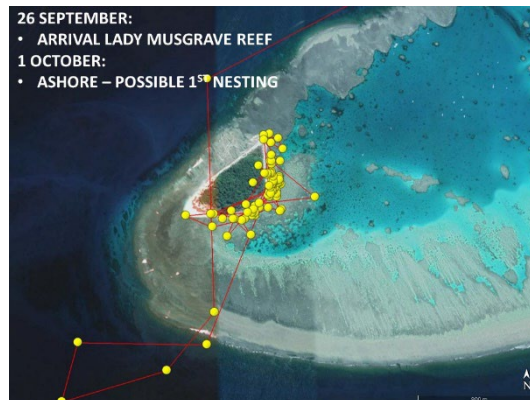
**2.7B. Migratory path from foraging in Port Curtis to courtship at Llewellyn Reef and on to coming ashore at Lady Musgrave Island.**



**2.7C. Locations recorded within foraging area on the Pelican Banks and offshore of Curtis Island**



**2.7D. Migratory path in vicinity of courtship areas in vicinity of Llewellyn Reef.**



**2.7E. Locations recorded in the inter-nesting habitat on the reef before coming ashore at Lady Musgrave Island.**

Figure 2.7. Satellite telemetry summary for adult female Green turtle QA64318 during her preparation for her first breeding season in 2017 and migration to courtship at Llewellyn Reef and on to her nesting beach at Lady Musgrave Island.



**2.8A. QA86086: 30 May 2018, Boyne estuary, adult female, CCL = 113.3 cm; defecated braided fishing line 2 days after captures as a debilitated floating turtle that was sent to rehabilitation**



**2.8B. QA87223: 22 May 2019, South Trees estuary, small immature female, CCL = 45.2 cm, both rear flippers paralysed from skeg-cut fracture.**



**2.8C. QA80266: 6 November 2017, Pelican Banks, adult female, CCL = 101.9 cm; entangled recreational fishing line cutting into the bone of right front flipper; sent to rehabilitation**



**2.8D. QA77327: 29 September 2017, offshore of Boyne Island, small immature female, CCL = 43.9 cm; fibropapilloma tumour on ventral surface of right front flipper**

Figure 2.8. Illustration of reduced health of Green turtles, *Chelonia mydas*, resulting from anthropogenic impacts.



**2.9A. QA64333: 10 October 2018, South Trees, large immature female, CCL = 76.0 cm.**



**2.9B. QA91698: 7 July 2019, One Tree estuary, adult female.**



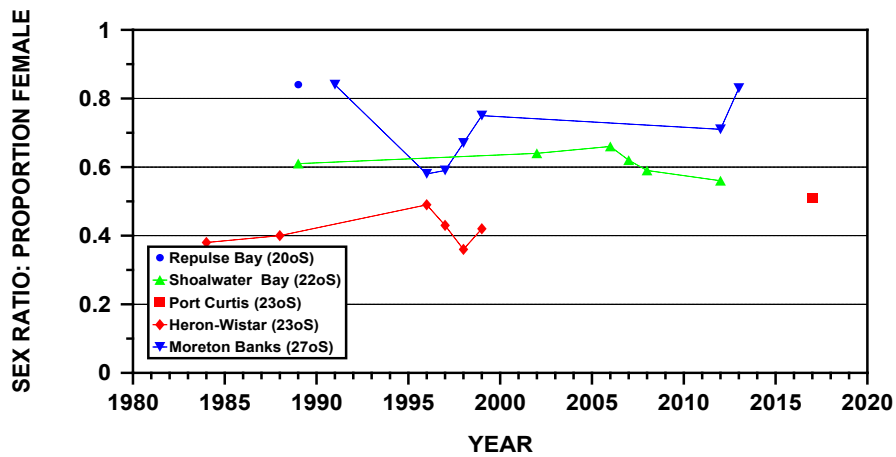
**2.9C. QA91758: 15 September 2019, Quoin Island, large immature, CCL = 76.6 cm.**



**2.9D. QA61524: 11 September 2019, Wild Cattle Island; adult sized female, CCL = 102.7 cm.**

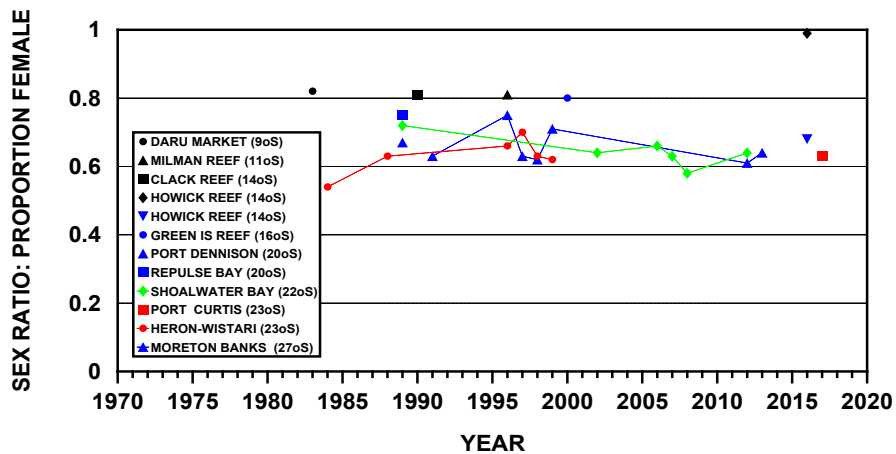
Figure 2.9. Dead Green turtles examined within Port Curtis with propeller cuts and outboard motor damage

**sGBR GREEN TURTLE FORAGING POPULATIONS  
ADULT SEX RATIO: PROPORTION FEMALE**



**2.10A. Adult sex ratio.**

**sGBR GREEN TURTLE FORAGING POPULATIONS  
SMALL IMMATURE SEX RATIO: PROPORTION FEMALE**



**2.10B. Small immature sex ratio.**

Figure 2.10. Sex ratio, proportion female, recorded at green turtle foraging areas in eastern Australia.

See Table 2.10 for the sources of the data. Black symbols denote foraging areas predominated by nGBR Green turtle stock. Other colours denote primarily sGBR Green turtle stock foraging areas. Red symbols denote foraging areas at 23°S in close proximity to the core sGBR stock breeding areas; green symbols denote Shoalwater Bay at 22°S and blue symbols denote foraging areas 3 degrees of latitude or more away from the core breeding area.

## CHAPTER 3

### POPULATION GENETICS OF THE GREEN TURTLE, *Chelonia mydas*, POPULATION FORAGING IN PORT CURTIS

**Nancy N. FitzSimmons, Colin J. Limpus, Nathan McIntyre**

Effective conservation management of marine turtle populations requires our ability to identify the various rookeries that comprise each population, and to determine the different foraging grounds used by each population. Population genetic studies, analysed in combination with data from flipper tagging and satellite telemetry, have contributed to the quantification of this knowledge for Green turtles in Queensland. Initial studies confirmed what had been observed from flipper tagging, that Green turtles nesting in the Gulf of Carpentaria, northern Great Barrier Reef (nGBR) and southern Great Barrier Reef (sGBR), are each independent populations, comprised of multiple rookeries (Norman et al. 1994). This was later confirmed by more detailed analyses (Dethmers et al. 2006) that showed a very high level of genetic distinction between the nGBR and sGBR populations and defined independently functioning management units (*sensu* Moritz 1994) for several Indo-Pacific Green turtle populations.

This characterisation of the genetic diversity within each population has provided the baseline data for conducting mixed stock analyses at foraging grounds to determine the proportions of different populations that use particular foraging areas. Analyses of six foraging grounds from Torres Strait to Moreton Bay quantified how the proportion of nGBR and sGBR Green turtles shifted from a predominance of nGBR turtles in the north, to that of sGBR turtles to the south (Jensen et al. 2016). The foraging grounds nearest to Port Curtis (Shoalwater Bay to the north and Moreton Bay to the south) indicated that 85% - 96% of turtles came from a grouping of the sGBR and Coral Sea genetic stocks, 4% -16% came from other unspecified rookeries, and 0% - 4% came from the nGBR. Additionally, the study compared the genetic results among small immature, medium immature, and large immature, or adult turtles, as well as to flipper tag data. These comparisons indicated that across all foraging grounds, there had been a decline in the proportion of nGBR turtles among the small and medium immature turtles. In the southern foraging grounds there were indications of an increased proportion of turtles from unspecified rookeries. These observations suggested a decline in productivity of the nGBR rookeries, as previously noted (Limpus et al. 2003, Limpus 2008), relative to other rookeries. Further studies at a foraging ground in the northern Howicks Group also found that among juvenile and subadult turtles, <1% of the males had originated from nGBR rookeries, indicating a feminisation of the nGBR population (Jensen et al. 2018), as expected due to increasing temperature from climate change (Fuentes et al. 2010).

Therefore, as part of a larger effort to understand the origins of juvenile turtles at foraging grounds used by the GBR stocks, we took skin samples for genetic analysis from all newly recruited and smaller juvenile turtles, most of which had been sexed via laparoscopy, to compare results to the previous studies.

#### Methods

The general methods applied in the capture, tagging, genetic sampling, and data recording for the foraging Green turtle population in Port Curtis are described in Chapter 1.

#### DNA extraction and amplification

Genomic DNA was extracted from tissue samples using a chelex protocol in which the tissue was digested overnight with proteinase K in a 5% chelex solution of 1 x TE (10 mM Tris, 1 mM EDTA, pH 8.0) and heated to 95°C for five minutes prior to use. DNA amplification via

polymerase chain reaction (PCR) was conducted using primers H950g and LCM15832 (Abreu-Grobois et al. 2006). PCR reactions were done in 25  $\mu$ l solutions with 0.34  $\mu$ M of each primer, ~40 ng template DNA, and 1.0 units of MyTaq solution with buffers (Bioline). Amplification conditions were 95°C for 4 min, 35 cycles of 95°C for 25 s, annealing temp for 25 s, and 72°C for 30s followed by a final extension at 72°C for 2 min. The annealing temperatures were 2 cycles at 50°C, 2 cycles at 51°C, and 31 cycles at 52°C. PCR products were sent to Macrogen Inc. (South Korea) for purification and Sanger sequencing. All samples with unique haplotypes and samples with any questionable nucleotides were sequenced in both directions.

## Genetic analyses

Sequences were aligned using Geneious 6.1.8 (Biomatters Ltd.) and visually checked. Haplotypes were determined by alignment to a data file of 307 known Indo-Pacific Green turtle haplotypes. New sequences were determined by using the nucleotide 'blast' function in the international DNA database GenBank. Estimates of haplotype and nucleotide diversity were calculated in Arlequin 3.5.2.2 (Excoffier and Lischer 2010). Haplotype frequencies in the Port Curtis sample were compared to those of contributing stocks and other regional foraging grounds, using exact tests of population differentiation (Raymond and Rousset 1995) and a Markov chain approach in Arlequin 3.5.2.2 (Excoffier and Lischer 2010). Comparative data on haplotype frequencies in Indo-Pacific Green turtle genetic stocks and Queensland foraging grounds were taken from Jensen et al. (2016) and Read et al. (2015).

A mixed stock analysis was conducted in Bayes (Masuda and Pella 2002), which uses a Bayesian method of analysing genetic data (Pella and Masuda 2001) to determine population origins of foraging animals if the contributing stocks have been characterised. Published data on the haplotype frequencies of 24 genetic stocks (Read et al. 2015; Jensen et al. 2016), which shared at least one haplotype with the Port Curtis foraging turtles, were used for mixed stock analysis. These genetic stocks were: sGBR, northern Coral Sea/Chesterfield Islands, New Caledonia (d'Enrecateaux Islands), northern Papua New Guinea, nGBR, Aru, Gulf of Carpentaria, Vanuatu, Marshall Islands, Micronesia, Palau, Northern Mariana Islands/Guam, American Samoa, eastern Borneo, northeastern Borneo, Sulu Sea, western Borneo, Peninsular Malaysia, Ashmore Reef, Scott/Browse Reef, West Java, Cobourg Peninsula, Northwest Shelf, and Cocos 'Keeling' Island. New haplotype frequency data from 14 samples from northern Coral Sea rookeries (sampled in the 2019/2020 season) were added to the data set (FitzSimmons unpublished data). Three sets of priors were tested in the analyses: (1) uniform priors where all genetic stocks contributed equally, (2) population size priors, where priors represented the proportion of the total population estimate for all genetic stocks, and (3) distance, where priors represented a proportional inverse of the distance from Port Curtis. Population size estimates were the same as used in Jensen et al. (2016) for consistency. In these analyses, 24 chains were run with 40000 Markov chain Monte Carlo steps and a burn-in of 20,000 steps. Convergence of the chains was determined by the Gelman and Rubin shrink factor using a threshold value of >1.2.

## Results

### Genetic diversity

A total of 93 samples were successfully sequenced across ~787 bp of the mtDNA control region from juvenile Green turtles in the Port Curtis region, 54 of these were from females, 35 from males and 4 from turtles whose sex had not been determined (Table 3.1). Among these samples, 18 haplotypes were observed, with haplotype CmP47.1 being the most



common, which was found in 56 (60.2%) turtles. This haplotype is also the most common haplotype in the sGBR and Coral Sea genetic stocks and in small immature Green turtles foraging in Edgecombe Bay, Shoalwater Bay and Moreton Bay (Table 3.2; data from Jensen et al. 2016). The most common haplotype found in the nGBR genetic stock was only observed in three (3.2%) turtles in the Port Curtis sample, in two females and one male. One new haplotype was observed in one of the juvenile males. An exact test for genetic differentiation indicated no significant differences between the haplotype frequencies of juvenile males and females ( $p = 0.699$ )

Haplotype diversity, which is a measure of the number of haplotypes and their frequencies within the samples, was notably higher ( $h = 0.62$ ) among juvenile Green turtles foraging in Port Curtis, than among small immature Green turtles foraging in Edgecombe Bay ( $h = 0.35$ ), Shoalwater Bay ( $h = 0.33$ ) and Moreton Bay ( $h = 0.35$ ) (Table 3.3). In comparison to regional genetic stocks, haplotype diversity in the Port Curtis sample was considerably higher than in the sGBR ( $h = 0.16$ ), indicative of additional contributions from stocks other than the sGBR. Nucleotide diversity, which considers the genetic distance among haplotypes, as well as the haplotype frequency, was somewhat higher than the other foraging grounds, and notably higher than the sGBR stock. Tests for haplotype frequency differences between the Port Curtis sample and regional genetic stocks indicated significant differences in each comparison, as expected if the Port Curtis sample had multiple contributing stocks. Haplotype frequencies in the Port Curtis sample were not significantly different from samples of small immature Green turtles foraging in Edgecombe Bay, Shoalwater Bay and Moreton Bay.

#### Mixed Stock Analysis

As suggested from the genetic diversity indices, the mixed stock analysis found multiple origins for the juvenile Green turtles foraging in Port Curtis. Estimated contributions and their confidence intervals were generally similar regardless of the prior information used in the Bayesian analysis (Table 3.4). The mixed stock analysis indicated that the greatest contributor to the foraging population was the sGBR genetic stock, with an estimated mean contribution ranging from 55.6%– 59.0%, depending on the prior information used in the Bayesian analysis (Table 3.4). Estimated contributions from the New Caledonia genetic stock ranged from 20.8% – 21.6%, and from the Coral Sea the range was 14.0% – 16.6%, depending on the priors used. The estimated contribution from the nGBR genetic stock was <1% and from all other stocks combined ranged from 4.4% – 6.9%. Confidence intervals were large for the sGBR and Coral Sea estimates. For example, considering uniform priors the 95% confidence intervals were 22.0% - 76.5% for sGBR and 0% - 52.2% for Coral Sea stocks.

#### Discussion

##### Genetic Composition

Genetic diversity was higher in the juvenile turtles foraging in Port Curtis in comparison to foraging small immature turtles in Edgecombe Bay, Shoalwater Bay and Moreton Bay due to the presence of several haplotypes that were only found in 1 - 2 individuals. There were at least twice as many haplotypes found in the Port Curtis sample, although in comparison to the Shoalwater Bay sample, that may be explained by a considerably smaller sample size ( $n = 44$ ) from Shoalwater Bay. Because the two predominant haplotypes observed in the Port Curtis sample were also the most commonly observed at the other foraging grounds, the foraging grounds were not found to be genetically differentiated.

Haplotypes that were observed in the Port Curtis sample, but not in the three main contributing stocks have been found in a wide range of genetic stocks. The haplotype CmP20.1 is the predominant haplotype in northern New Guinea, the Marshall Islands, Micronesia, Palau, the Northern Mariana Islands and Guam, but is also common at Ashmore Reef in the Timor Sea (Jensen et al. 2016). CmP22.1 is found in the Marshall Islands and is common in American Samoa, and CmP91.1 is the common haplotype in Aru and Vanuatu and has been found at several locations including the Gulf of Carpentaria, Cobourg Peninsula, Peninsular Malaysia, Micronesia, and Borneo. The presence of these haplotypes in the Port Curtis sample form an important component of the ~5% contribution attributed to the combined 'other' genetic stocks.

Some of the haplotypes found in the Port Curtis sample are known as 'orphan' haplotypes, meaning they have not yet been identified at any rookeries. These were not included in the mixed stock analysis. That includes CmP55.1, known from foraging grounds in Torres Strait, Clack Reek and the Howicks Group, CmP165.1 and CmP166.1, found in one individual each in Moreton Bay, and CmP80.4, found in a turtle foraging in New Zealand (Godoy 2016).

### Mixed Stock Analysis

Mixed stock analyses estimated that the largest contributor to the foraging juvenile turtles in Port Curtis was the sGBR genetic stock, followed by the New Caledonia and Coral Sea stocks. These results were little affected by the type of priors used in the analyses. Population size was not a main determinant, given the very low estimated contribution from the nGBR stock, which the largest stock in the region. The sGBR stock is the second largest stock among the main contributors, and also the closest (50-100 km) to Port Curtis. In these analyses, and those done by Jensen et al. (2016), the New Caledonia rookery sample (reported in Read et al. 2015) was from turtles nesting at the d'Enrecateaux Islands (19.7° S, 163.6°E) ~1360 km from Port Curtis. The Coral Sea sample included samples from northern rookeries (16.9° S, 149.5 E), approximately 780 km north of Port Curtis, and from the Chesterfield Islands (20.9° S, 158.9° E), located approximately 850 km northwest of Port Curtis. Although these later two rookeries are approximately 1000 km apart, they have been grouped together as a single stock based on genetic analyses (Read et al. 2015, Jensen et al. 2016). Turtles from the main contributing stocks therefore, would have migratory distances of from 50 - 1360 km distant if they maintain fidelity to the Port Curtis foraging areas through to maturity.

Mixed stock analysis assumes that all contributing stocks have been adequately sampled and that the haplotype frequencies of the stocks are significantly differentiated from each other. The range of the confidence intervals is an indication of how reliable the results are, and these were large for the sGBR and Coral Sea estimates. One issue is when there are apparently unique haplotypes found at very low frequencies at a single stock. Their occurrence in these stocks, and not others, may be a sampling artefact and this can inflate their estimated contribution in mixed stock analysis. Possible examples of such haplotypes are CmP180.1, only found in two Coral Sea turtles, or CmP118.1 and CmP160.1, only found in a single individual each in the New Caledonia stock. As a test of how reliant the results were on low frequency haplotypes, post hoc analysis was run by removing three individuals from the Port Curtis sample that were the sole representatives of haplotypes: CmP180.1, previously mentioned; CmP49.1, which was found in one Coral Sea and one New Caledonia sample as well as from the 13 'other' rookeries; and CmP83.1 found in one Coral Sea sample, and from five 'other' rookeries. In an analysis with uniform priors, estimated contributions were increased for the sGBR to 71.1%, New Caledonia to 22.2% and 'other' stocks to 6.9%, decreased for the Coral Sea to 0.6%, and did not change the nGBR

estimate. Thus the sGBR and Coral Sea estimates are closely linked, and were the most sensitive to low frequency haplotypes in the samples.

These two stocks are also problematic because they have relatively similar haplotype frequencies. One measure of genetic differentiation is the  $F_{ST}$  value (Wright 1969), which ranges from 0 to 1. In post hoc pairwise comparisons between the nGBR, Coral Sea, sGBR, New Caledonia and Papua New Guinea stocks,  $F_{ST}$  values ranged from 0.16 to 0.59, except for the sGBR and Coral Sea comparison, in which  $F_{ST}$  was only 0.086. In Jensen et al. (2016), they concluded that individual stock estimates for these two stocks were not reliable and they combined the sGBR and Coral Sea estimates. This was also done by Jones et al. (2018), who studied three foraging grounds in north Queensland at Low Isles, Green Island and Magnetic Island. If done for the Port Curtis analysis, the combined sGBR/Coral Sea contribution ranges from 72.2% - 73.5% depending on the priors used. At present, this is the recommended way to consider the results until such time in the future when new genetic techniques might be applied to the samples to provide finer resolution, such as being able to discriminate genetic diversity within common haplotypes across genetic stocks.

In comparison to previous mixed stock analyses of Green turtles foraging at sites along the central coast of Queensland (Jensen et al. 2016), there has been an apparent relative decline in the contribution of the combined sGBR/Coral Sea stocks, while the contribution from New Caledonia has increased. These comparisons to the previous study are to small immature turtles sampled in the early 2000s at Edgecombe Bay, approximately 540 km north of Port Curtis; in the 1990s at Shoalwater Bay, approximately 230 km north of Port Curtis; and in the 1990s at Moreton Bay, approximately 470 km south of Port Curtis. In comparing results from Bayesian analyses using uniform priors across both studies, the combined sGBR/Coral Sea stocks were estimated to contribute 72.2% to Port Curtis, 94% to Edgecombe Bay, 88% to Shoalwater Bay and 85% to Moreton Bay foraging grounds. The New Caledonia stock was estimated to contribute approximately 20.8% to Port Curtis, 9.1% to Edgecombe Bay, 6.7% to Shoalwater Bay and 13.7% to Moreton Bay foraging grounds. These data thus suggest a 12% - 22% decrease in the relative contribution of the sGBR/Coral Sea stocks to the region since the 1990s and early 2000s, countered by an average increased contribution of 7% - 14% from the New Caledonia. The contribution of all other stocks combined was also somewhat higher in the Port Curtis sample at 6.9% in comparison to the average contribution of 3.9% from other stocks to the Edgecombe Bay, Shoalwater Bay and Moreton Bay foraging sites. Estimated contributions from the nGBR genetic stock were uniformly low (<0.5%) across all foraging grounds. The only variation in the two sets of analyses was the addition of 14 samples to the Coral Sea sample.

One hypothesis put forth by Jensen et al (2016) to explain a decline over time in the contribution of the nGBR stock to the northern foraging grounds was an increase in the sGBR, Coral Sea and 'other' genetic stocks. This hypothesis was supported by data indicating an increase in the sGBR population since the 1980s (Chaloupka and Limpus 2001, Chaloupka et al. 2008), but countered by an argument that the rate of population increase was not sufficient to explain their results. In contrast, the Port Curtis data are counter to this trend, and not readily understood. As noted in Jensen et al. (2016) there are limited data to assess population trends at remote rookeries in the Coral Sea. A recent regional report by the Marine Turtle Specialist group that included Tuvalu, Vanuatu, New Caledonia, PNG and the Solomon Island stated that population trends were only available for Vanuatu, which indicated a decline (Work et al. 2020). Recent data on the Coral Sea genetic stock from the 2019-2020 nesting season, which was a high density season, suggested that the number of turtles (turtles/night) were similar to previous high density

years, but given the high seasonal fluctuations seen at Green turtle rookeries throughout the region (Limpus and Nichols, 1988, 1994, 2000), a population trend was not determined.

A second hypothesis of Jensen et al. (2016) to explain differences between the haplotype frequencies and mixed stock analyses of adult versus small immature turtles was that small immature turtles might shift residency to feeding grounds closer to their breeding grounds and nesting beaches as they mature. If the hypothesis is applied to the Port Curtis turtles, this would suggest that many of the juvenile turtles originating from the Coral Sea, New Caledonia and 'other' stocks would only be temporary residents of Port Curtis. However, tag recovery data (n = 37) show that Green turtles nesting in New Caledonia have resident foraging grounds along the Queensland coast from Torres Strait to Moreton Bay (Read et al. 2014), suggesting that many turtles have not shifted foraging grounds as they mature. Similarly, satellite telemetry and flipper tag recoveries of nesting females at the northern Coral Sea rookeries also strongly indicate the use of GBR foraging grounds by adults (Bell et al. 2020). Although field data show that juvenile turtles foraging along the east coast of Queensland show strong fidelity to their foraging grounds as they mature (Limpus et al. 1992), the hypothesis is still worth testing further.

### Summary and Recommendations

In summary, genetic analyses of foraging juvenile Green turtles residing in Port Curtis indicate that:

- There were no significant differences in haplotype frequencies between juvenile male and female turtles.
- The genetic diversity of juvenile turtles was greater in Port Curtis than observed at foraging grounds at Edgecombe Bay, Shoalwater Bay and Moreton Bay due to the presence of several haplotypes observed at low frequencies.
- Although genetic differences were not significant between the Port Curtis sample and the Edgecombe Bay, Shoalwater Bay and Moreton Bay foraging samples, results from the mixed stock analysis were notably different.
- Mixed stock analyses estimated that juvenile turtles originate primarily (72.2% - 73.5%) from the combined sGBR/Coral Sea stock, with contributions from New Caledonia (20.8% - 21.6%), and a combination of other (4.4% - 6.9%) genetic stocks.
- In comparison to previous mixed stock analyses of small immature turtles during the 1990s and early 2000s at the Edgecombe Bay, Shoalwater Bay and Moreton Bay foraging grounds, the results were unexpected: indicating an average 16.8% decrease in the relative contribution of the sGBR/Coral Sea stock, and average increased contributions of 11.0% from the New Caledonia stock and 3.0% increase in 'other' stocks.

These results show the value in comparative genetic studies and highlight a need to analyse new genetic samples from adult Green turtles from Port Curtis and well as from juvenile and adult turtles foraging at the Capricorn Bunker reefs. This additional information is needed to better understand the dynamics of the observed shifts in haplotype frequencies and estimated contributions of genetic stocks to the foraging turtles in Port Curtis.

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Table 3.1. Haplotype frequencies of mtDNA control region haplotypes in juvenile Green turtles (*Chelonia mydas*) in Port Curtis.

<b>Haplotype</b>	<b>Females</b>	<b>Males</b>	<b>Unknown sex</b>
CmP20.1	1	1	1
CmP22.1	-	1	-
CmP44.1 <sup>1</sup>	2	1	-
CmP47.1	35	20	1
CmP49.1	-	1	-
CmP55.1	-	1	-
CmP68.1	1	-	-
CmP80.1	5	5	1
CmP80.4	2	-	-
CmP83.1	1	-	-
CmP85.1	1	1	-
CmP91.1	1	-	-
CmP118.1	3	1	-
CmP160.1	-	1	1
CmP165.1	-	1	-
CmP166.1	1	-	-
CmP180.1	1	-	-
CmP-new	-	1	-
<b>Total</b>	<b>54</b>	<b>35</b>	<b>4</b>

<sup>1</sup>predominant haplotype in the nGBR

Table 3.2. Haplotype frequencies of Green turtles (*Chelonia mydas*) sampled at rookeries of the contributing genetic stocks and at regional foraging grounds, used in analyses of exact population differences.

Haplotype	Genetic Stock <sup>1</sup>				Foraging Grounds			
	nGBR	Coral Sea	New Caledonia	sGBR	Port Curtis	Edgecombe Bay <sup>1</sup>	Shoalwater Bay <sup>1</sup>	Moreton Bay <sup>1</sup>
CmP20.1					3	2	1	
CmP22.1					1			1
CmP31.1		2	2					1
CmP44.1	46		11		3	1	1	2
CmP44.2			2					
CmP44.4	1							
CmP44.5	1							
CmP47.1	2	76	9	93	56	67	36	67
CmP47.5		1	1					
CmP49.1		1	1		1			
CmP55.1					1			
CmP57.2						1		
CmP68.1	2			1	1			1
CmP80.1	3	23	14	8	11	10	4	5
CmP80.4					2			
CmP81.1	7							
CmP83.1		1			1			
CmP84.1	4							
CmP85.1			18		2	2	1	4
CmP91.1					1			
CmP98.1	8							
CmP108.1	2							
CmP117.1			1					
CmP118.1			1		4			
CmP134.1	1							



**Table 3.2. continued**

Haplotype	Genetic Stock <sup>1</sup>				Foraging Grounds			
	nGBR	Coral Sea	New Caledonia	sGBR	Port Curtis	Edgecombe Bay <sup>1</sup>	Shoalwater Bay <sup>1</sup>	Moreton Bay <sup>1</sup>
CmP160.1			1		2			1
CmP164.1		1						
CmP165.1					1			
CmP166.1					1			1
CmP168.1		1						
CmP169.1	1							
CmP170.1	2							
CmP171.1	1							
CmP173.1			2					
CmP174.1			1			1		
CmP180.1		2			1			
CmP191.1							1	
CmP193.1		1						
CmP211.1		1						
CmP-new1					1			
CmP-new2		1						
Total	81	111	64	102	93	84	44	83

<sup>1</sup> from Jensen et al. (2016)

Table 3.3. Estimates of haplotype and nucleotide diversity for mtDNA control region haplotypes in juvenile Green turtles and levels of genetic differentiation between Port Curtis foraging juvenile Green turtles to their source genetic stocks and to small immature turtles foraging elsewhere in Queensland.

<b>Location</b>	<b>Genetic haplotype diversity (<i>h</i>) (std. dev.)</b>	<b>Genetic nucleotide diversity (<math>\pi</math>) (std. dev.)</b>	<b>Genetic difference w/ Port Curtis <i>p</i>-value</b>
<b>Stock</b>			
nGBR	0.6614 (0.0057)	0.0083 (0.0044)	<0.0001
sGBR	0.1640 (0.0473)	0.0063 (0.0034)	<0.0001
Coral Sea	0.4914 (0.0492)	0.0159 (0.0080)	0.0023
New Caledonia	0.8323 (0.0238)	0.0241 (0.0120)	<0.0001
<b>Forage ground</b>			
Edgecombe Bay	0.3523 (0.0634)	0.0121 (0.0062)	0.213
Shoalwater Bay	0.3277 (0.0889)	0.0097 (0.0051)	0.960
Port Curtis	0.6237 (0.0564)	0.0197 (0.0099)	n/a
Moreton Bay	0.3453 (0.0668)	0.0119 (0.0061)	0.171

Table 3.4. Estimated origins of juvenile Green turtles foraging in Port Curtis determined by Bayesian mixed stock analysis with either uniform priors, relative population size priors, or relative distance priors.

Parameter	Contributing Stock					
	nGBR	sGBR	Coral Sea	Combined sGBR/Coral Sea	New Caledonia	other
<b>Uniform Prior</b>						
mean (s.d.)	0.0013 (0.0059)	0.5556 (0.1397)	0.1661 (0.1409)	0.7217	0.2079 (0.0583)	0.0690 (0.0041)
median	0	0.5785	0.1324		0.2037	n/a
2.5%	0	0.2202	0		0.1066	n/a
97.5%	0.0161	0.7654	0.5228		0.3334	n/a
<b>Population Size Prior</b>						
Mean (s.d.)	0.0039 (0.0098)	0.5905 (0.1446)	0.1402 (0.1456)	0.7307	0.2158 (0.0619)	0.0444 (0.0051)
median	0.0001	0.6176	0.0991		0.2109	n/a
2.5%	0	0.2284	0		0.1095	n/a
97.5%	0.0338	0.7941	0.5253		0.3503	n/a
<b>Distance Prior</b>						
Mean (s.d.)	0.0009 (0.0046)	0.5717 (0.1298)	0.1634 (0.1315)	0.7351	0.2116 (0.0595)	0.0463 (0.0043)
median	0	0.5899	0		0.2068	n/a
2.5%	0	0.1298	0		0.1088	n/a
97.5%	0.0102	0.7746	0.4902		0.3397	n/a

## CHAPTER 4

### GREEN TURTLE HABITAT USE AND SITE FIDELITY IN PORT CURTIS

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**Release of an immature female Green turtle fitted with a satellite transmitter, in front of Wiggins Island Coal Terminal at Port Curtis, July 2019.**

#### Introduction

Green turtles occur throughout environments within the continual shelf off the Queensland coast. They are generally slow growing and show long-term fidelity to their foraging areas (Chaloupka *et al.* 2004; Shimada *et al.* 2016a; Shimada *et al.* 2019). In nearshore environments that are adjacent to urban areas, or areas of high use by people, Green turtles are variously impacted by vessel strike, degraded water quality, variation in the abundance and distribution of food and increased use of marine areas by people. As the patterns of human use change along coastal areas, coupled with an increase in the population size of Green turtles, increased frequency of interactions is possible. Hence there is a need to better understand the degree to which turtles use their habitats and could be impacted by

existing and future use. Telemetry is one of the modern technologies that can improve our understanding of how turtles utilise habitats in relation to human use.

Studies examining the habitat use and home range of Green turtles in Port Curtis began in 2009. The first deployments of satellite tags on Green turtles were part of an environmental survey conducted by GHD in relation to planned infrastructure and dredging developments in Port Curtis. The tags were deployed as part of a collaborative project with Department of Environment and Science (DES) and data was made available to James Cook University (JCU) for analysis in various research projects (e.g., Shimada *et al.* 2016a).

With extensive expansion of Port Curtis port facilities continuing in 2013, with dredging operations in progress and construction of the Wiggins Island Coal Terminal and loading facilities for Liquid Natural Gas (LNG) plants on the southern bank of Curtis Island, CSIRO deployed satellite and acoustic tags on Green turtles in Port Curtis as part of the Gas Industry Social and Economic Research Alliance (GISERA) marine environmental research program. In the same year, funding was provided to Environment and Heritage Protection (EHP) following a Magistrate's Court of Queensland at Gladstone decision to direct a portion of an imposed fine to fund a satellite telemetry study of Green turtles foraging in Port Curtis. Gladstone Ports Corporation's (GPC) Ecosystem Research and Monitoring Program (ERMP) provided funding for a three-year study conducted by JCU in collaboration with EHP, now DES, from 2014-2017. In these years, tags were selectively deployed on turtles of mixed age and sex classes, primarily on the Pelican Banks, but also at Wiggins Island, Quoin Island, and the Boyne Estuary. Initial examination of data from deployments 2013-2017 revealed that tagged individuals had spatially confined home ranges. Effort was made to target individuals from other areas in subsequent tagging studies, in order to provide information on habitat use by marine turtles in the wider port. The area around Wiggins Island, which coincides with shipping vessel traffic in proximity to loading facilities for the LNG plants and the Wiggins Island and RG Tanna coal terminals, was designated as a high significance area. In 2018, four tags funded by JCU TropWater were deployed for studies by JCU Seagrass at South Trees and Wild Cattle Island. DES also deployed two tags in 2018. Funding for a further 12 satellite tags to be deployed at Wiggins Island and South Trees in 2018 and 2019 was provided by Eco Logical Australia (ELA) as part of the Queensland Curtis LNG Long Term Turtle Management Plan (LTTMP), for study of fine-scale habitat use of Green turtles in the vicinity of Port infrastructure. JCU has been sub-contracted for analysis of these data.

The telemetry component of this study focussed on establishing spatial distributions of tracked individuals, and to quantify spatial overlap between these individuals and features of existing and planned developments in Port Curtis. It examined individuals' tracks to explore foraging site fidelity, spatial and temporal scales of observed movement, environmental parameters associated with range shifts and potential threats to turtles related to their distribution. This chapter summarises data collected from satellite tags deployed on 73 Green turtles in 2010-2019 within the Port Curtis study area to increase understanding of how turtles use habitats within this region.

## Methods

### Turtle capture

Capture and release of turtles for satellite telemetry studies took place during field trips in July and December 2010, November 2013, May and December 2014, July 2015, May and October 2016, May 2017, June, August and October 2018 and July and September 2019. All turtles were caught in the intertidal or immediately adjacent subtidal habitats using either blocking nets or turtle rodeo (see Chapter 1 for details). Study sites where turtles were captured for satellite telemetry studies included Pelican Banks, Wiggins Island flats (Western Basin), Boyne River and Quoin Island, and South Trees and Wild Cattle Island (see Chapter 1 for details). Each turtle was tagged with titanium flipper tags and a GPS location was recorded for the capture location.

The satellite tags used in Port Curtis (2010-2016) were mostly deployed on turtles captured in the Pelican Banks. This was largely due to the assumption that individual Green turtles would occupy habitat throughout Port Curtis and the consistency of sightings of turtles on the seagrass flats during study trips, and the relative ease of capture by rodeo of turtles in the shallow, clearer water. For representation of habitat use in the wider port, several individuals with satellite tags were opportunistically deployed off shore of the Boyne River, Western Basin, Quoin Island, Wild Cattle Island and South Trees study sites 2014-2018 as part of GPC and DES funded studies (Table 4.1). The telemetry data from these initial studies suggested that individuals from the Pelican Banks were not utilising habitats in the wider port. In response, priority for deployment of satellite tags in 2018-2019 shifted to the vicinity of Port Infrastructure at South Trees and the Western Basin.

#### Transmitter Attachment

Captured turtles were transported to the Queensland Parks and Wildlife Service (QPWS) workshop at the Gladstone Marina for processing. Each turtle was weighed, measured, and examined for external signs of injury or disease (see Chapter 1). Where capable personnel were present, sex and breeding status were determined for newly captured turtles (not previously tagged) using gonad examination (via ultrasonography or laparoscopy) or external morphology for adult males. In the absence of gonad assessment, age class was determined based on Curved Carapace Length (CCL): juveniles >35 up to 65cm; subadults >65cm up to maturity. (see Chapters 1 and 5). Only turtles exceeding minimum weight requirements were selected for satellite telemetry to ensure that tags weighed less than 5% of an individual's body weight. Prior to transmitter attachment, the anterior portion of the carapace was first cleaned with a paint-scraper to remove flaking scute material and epibionts, including barnacles. Sandpaper and ethanol were used to remove remaining algal growth and dirt. The transmitters were positioned to overlap the 1<sup>st</sup> and 2<sup>nd</sup> vertebral scutes and adjacent costal scutes and attached with Sika (@Anchor Fix 3) two-part epoxy with imbedded strips of fibreglass tape. In 2013, transmitters were attached with spacing blocks to increase their height above the carapace in an attempt to import drying of the saltwater switch when the turtle surfaced for a breath. When touch-dry, both tag and epoxy were painted with Micron 66 anti-foul paint and the turtles were kept in shallow tubs overnight to allow the epoxy to harden. Transmitters were either activated manually prior to release of the turtle or activated automatically in response to rapid change in water depth post-release. Most turtles were released close to their capture location, but others were released near Quoin Island, from the beach at Spinnaker Park or at the entrance to Gladstone Marina (Figure 4.1). No turtle was tracked twice.

In 2013, CSIRO deployed both acoustic and satellite tags on Green turtles at the Pelican Banks (n=33 acoustic; n=5 satellite) and Wiggins Island (n=16 acoustic; n=5 satellite) as part of the GISERA marine environmental research program (Babcock *et al.* 2015). Acoustic receiver arrays were deployed on the intertidal and subtidal sandflats at the Pelican Banks, and at Wiggins Island close to LNG loading facilities and the Wiggins Island Coal loading terminal which were under construction at the time. SPLASH10-F-296A and SPLASH10-F-296C satellite transmitters (n=10) were attached as above and set to transmit 254 times per day. Data were not available for inclusion in this report but reported results will be compared with findings from DES 2013 deployments.

### Transmitter Settings

All transmitters collected both Fastloc GPS (FGPS) as well as ARGOS PTT location data. Each of these is assigned a quality index. ARGOS data have a maximum accuracy of ~250 m, while FGPS can be accurate to within ~30 m (Dujon *et al.* 2014). All were set to record turtle locations 24/7 until transmitter failure or loss from the turtle, with FGPS locations being collected at intervals between 15 minutes to one hour.

### Data Filtering

FGPS obtained from at least four satellites and high-quality ARGOS (LC 3, 2, 1) fixes were combined in order to maximise data retention. The data were filtered with the R package SDL filter (Shimada *et al.* 2012; Shimada *et al.* 2016b) to remove spatial and temporal duplicates and spurious locations identified as exceeding biological maximum travel speeds ( $9.9 \text{ kmh}^{-1}$ ) and turning angles ( $90^\circ$ ) for Green sea turtle trajectories, and fixes recorded above the high tide line (i.e., on land). Travel to courtship areas by two individuals was removed and is discussed in detail in Chapter 2. For each individual we excluded the first six hours of data to account for acclimation of turtles post-release, particularly where release occurred away from the capture site. Data was not received from adult female K70229 between July 9 and December 2, 2014 and after December 2, 103 ARGOS and 300 FGPS points were received from this tag, of which only 24 were retained after filtering. These data were discarded in subsequent analyses.

### Home Range Calculation

We defined an individual's home range as the 95% kernel utilisation distributions (UD) obtained from the movement-based Kernel density estimator based on biased random bridges with least squares cross validation (Benhamou 2011). This method was selected in order to deal with spatial autocorrelation of the tracking data. The 50% UD is referred to as the core use area. The 95% and 50% UD delineate the area used by an individual during 95% and 50% of the tracking period respectively. We calculated home ranges for all individuals, overlaying these to visually depict the overlap in home ranges for each year of turtle tracking 2010-2019. A minimum of 30 locations post-processing is required for kernel density estimation, an adult male QA45408 had insufficient data and was removed. Home range calculation was conducted with the R package adehabitatHR (Calenge 2006). Home range size was compared for turtles deployed at the same study site for each year. The relationship between estimated home range size and total number of tracked days was also assessed.

We compared the area of individual home ranges (95% UD) across study sites, years and age classes with linear models. Home range areas were log transformed to meet model

assumptions prior to analyses. Turtles were described as staying “home” if they had one contiguous area in their home range, or as “shifting” habitats if they used more than one discrete area as part of the home range (Appendix 1).

## Results

From 2010-2019, 73 Green turtles including 39 adults (19 males and 20 females), 17 sub-adults (7 male, 7 female and 3 sex not determined) and 17 juveniles (5 male, 3 females and 9 sex not determined) were released in Port Curtis with satellite transmitters (Appendix 4.1.). Tracking was unsuccessful for only one of these turtles. Average CCL was 99.8 cm (85.6-116.6) for all adults and 66.1 cm (42.1-99.7) for immatures. Over the eight sampling years, two adults were tracked from Boyne River, 32 adults and 14 immatures from Pelican Banks, one juvenile from Quoin Island, one adult and four immatures from South Trees, three adults and 14 immatures from Western Basin sites including Wiggins Island flats, and one adult and one subadult from Wild Cattle Island (Table 4.2). There was considerable variation in release locations relative to capture sites (Figure 4.2). Adult male K93087 departed the Pelican Banks in September 2010 for breeding and returned to the Pelican Banks post-breeding for a further 87 days before transmission ceased. Home ranges were generated separately for pre and post-breeding and then compiled for this individual.

### Transmitter Performance

FGPS positions were received for an average  $135\pm 7$  days after deployment, and ARGOS for an average  $147\pm 7$  days. ARGOS locations were consistently received after FGPS ceased (for up to 72 days), except for two individuals for which ARGOS ceased 1 day prior to cessation of FGPS (adult male QA45689 and female juvenile QA64251). Transmitters yielded an average of  $660\pm 48$  FGPS and  $1634\pm 106$  ARGOS locations, or  $5.7\pm 0.5$  and  $13.0\pm 0.9$  locations per day respectively. This represented a significantly higher number of ARGOS locations received compared to FGPS locations ( $t=8.95$ ,  $df=72$ ,  $p<0.001$ ). The amount of data received was not significantly different between age classes, sexes or capture sites. Significantly less ARGOS data was received in 2019 compared to other years, and significantly more was received in 2015. For FGPS, significantly more data was received in 2019 than in other years (Figure 4.3.). In 2018, one individual was deployed with a Wildlife Computers SPLASH-10 device designed for deployment on seals. The design may not have been optimal for turtles but does not appear to have significantly affected performance in terms of data acquisition. For individuals, the number of both ARGOS and FGPS fixes received decreased gradually with time after deployment (Figure 4.4.).

Total distance travelled by tracked turtles ranged from 29.1 to 2210.3 km (mean= $551.2\pm 47.1$  km) and the maximum linear displacement recorded from the first retained location ranged 1.9 to 153.5 km (mean  $17.4\pm 2.6$  km, Appendix 4.2.). We did not detect differences in average distance travelled by turtles of different age class or sex.

### Foraging home range and site fidelity

Home ranges (95% UD) of tracked individuals were small, averaging  $20\pm 3$  km<sup>2</sup> (1-115 km<sup>2</sup>). Home ranges were generally located near outflows from the Calliope and Boyne Rivers, Colosseum Ck, the South Trees inlet and the northeast channel connecting Port Curtis to outside the bay (Figure 4.5). Characterisation of these areas is provided in Chapter 2. Half of the tracked turtles had home ranges less than 11 km<sup>2</sup>. Most turtles appeared to adhere to



home ranges close to the study site where they were captured, with 60 of 72 resettling there soon after release. Homing behaviour was also observed at other coastal foraging grounds (Shimada *et al.* 2016b). Of these 60 individuals:

- 45 remained in this “home” area for the entire tracking period (Figures 4.6A & 4.6B).
- 7 performed either “loop” trips (n=3; <3 days) or “settlement” to secondary areas (n=4; >3 days) before returning to the capture area (Table 4.4, Figure 4.6C & 4.6D).
- 2 turtles (1 male (K93087) and 1 female (QA64318)) captured on the Pelican Banks initially returned home but departed for breeding migrations. Male K93087 was later tracked back to the Pelican Banks.
- 6 individuals that resettled home immediately, later shifted to discrete areas (not contiguous with the rest of their 95% UD) before transmission ceased (Table 4.4, Figure 4.6E)

There were 10 individuals that resettled in the capture area after several days (Table 4.3), of which six remained for the duration of tracking and four utilised other areas as part of their 95% UD (Table 4.4). Turtles that displayed “fidelity” by remaining at or returning to the capture area (i.e., that did not completely depart from the area) represent 62 of 72 tracked individuals (86.1%).

Only two individuals did not resettle to the area of capture:

- an adult male captured on the Pelican Banks in 2014 who travelled south ~20 km to Tannum Sands, and
- an adult male tracked in 2015 from the Pelican Banks approximately 37 km south to Rodds Bay (Figure 4.6F & Figure 4.6G).

One adult female, originally captured on the Pelican Banks, departed the Pelican Banks in November 2016, and travelled ~150 km north along the coast over 6 days towards Shoalwater Bay, where she remained for the remainder of the tracking (Table 4.4, Figure 4.6H). She had been examined via laparoscopy at the time of deployment and she was not vitellogenic in preparation for the coming breeding season.

Several “typical” patterns of movement were identified. Home ranges of 17 individuals extended into estuary habitat (Figure 4.6I). Three individuals occupied The Narrows for either transitory movement (n=2) or longer settlement (n=1, Figure 4.6J). Of the 46 individuals captured at the Pelican Banks, 32 underwent multiple trips out of Port Curtis via the northeast channel between Curtis and Facing Islands, to offshore reef habitats within the same home range (see example Figure 4.6A). Some individuals departed the Pelican Banks and returned the same day, and others travelled periodically, spending up to a month at a time inside or outside the bay. Five individuals, two of which also travelled in and outside the bay, switched between discrete areas across the Western Basin and Pelican Banks, returning after visits of several days to several months (Figure 4.6E). Seven individuals remained entirely on the Curtis Island Reef - Pelican Banks foraging home range (Figure 4.6B). The remaining four either did not return to Pelican Banks post-release (n=2) or departed and spent most of the tracking duration at another location (n=2).

Individuals with large home ranges were mostly adults, and adults in general had significantly larger home range areas than juveniles and subadults (Figure 4.7A). There was a significant interaction between the effect of study site and age on home range area. We did not detect a difference in home range area between sexes. The relationship between home range area and both number of days tracked, and number of FGPS locations, was weak ( $R^2=0.032$  and  $R^2=0.117$  respectively). Home range area was significantly lower on average in 2018 and 2019 than in other years (Figure 4.7B, Kruskal-Wallis chi-squared=33.169,  $df=7$ ,  $p<0.001$ ). In these years, there was a disproportionate number of immature turtles tracked. In 2019, all turtles were sampled from Wiggins Island; however, we found no interaction between the effects of year and capture site on home range area. On average, turtles captured at the Pelican Banks had significantly larger home ranges than at all other sites, except Wiggins Island (Table 4.6, Figure 4.7C, Kruskal-Wallis chi-squared=36.197,  $df=6$ ,  $p<0.001$ ).

Home ranges of 53 tracked turtles extended outside of the Port Curtis boundary by at least 2% of the 95% UD. Home ranges of 28 individuals overlapped with dredged channels (Figure 4.6K, Appendix 1). Locations contributing to individual home ranges were located in a variety of habitats, including estuaries, intertidal and subtidal flats, rocky reef, and in and on the edges of dredged channels.

Babcock *et al.* (2015) reported larger detection timespan for acoustic tags than satellite tags at both Wiggins Island and Pelican Banks 2013, with 100% of acoustic-tagged turtles still detected within the Wiggins Island receiver array 6 months after deployment. Home ranges were given as kernel utilisation distributions (50% UD and 95% UD) generated with the reference smoothing parameter function (Van Winkle 1975; Worton 1989) in the R package *adehabitat HR* (Calenge 2006). KUDs were generated separately from acoustic data for individuals that were detected for more than 30 days on at least two receivers and from “raw” FGPS positions. One satellite transmitter deployed at Wiggins Island malfunctioned and data was not received. Average home range area was  $1.3 \text{ km} \pm 0.2 \text{ km}^2$  (50% KUD) and  $6.7 \pm 0.8 \text{ km}^2$  (95% KUD) from acoustic tags. Excluding turtles that shifted between sites, 50 and 95% KUDs were  $1.4 \pm 0.3 \text{ km}^2$  and  $6.7 \pm 0.9 \text{ km}^2$  respectively at Pelican Banks and  $0.7 \pm 0.1 \text{ km}^2$  and  $3.8 \pm 0.4 \text{ km}^2$  at Wiggins Island. KUDs from acoustic tags were generally greater in area than those generated from satellite tag data for turtles that remained within the receiver array for the duration of tracking. This difference was attributed to higher detection frequency of acoustic transmitters. Acoustic tags emit a “ping” containing location information with higher frequency than satellite tracker relocations are transmitted, thus their detection probability is higher. Additionally, in less sheltered water, where saltwater switch is less likely to activate, satellite tags sent fewer relocations resulting in smaller KUDs than acoustic tags. Satellite transmitters conversely had the advantage of detecting movements outside of receiver arrays, including outside of the bay and at upstream estuary habitats. Acoustic locations are generated by mean position-generating algorithms that are highly dependent on the design of the receiver array and the spacing between receivers. It is reasonable to expect some differences in the location data and KUDs generated from the two transmitter types.

At Pelican Banks, three of the five turtles satellite-tagged by CSIRO departed from the receiver array shortly after release and travelled >150 km along the coast. One of these was an adult female that moved to courtship areas (refer to Chapter 2). Long-range movement not associated with breeding was only observed once in 72 DEH deployments 2010-2019

(adult female QA66526, 2016). Of EHP deployments in 2013 (n=10 at Pelican Banks and n=3 at Wiggins Island), one adult female K70229 departed the Pelican Banks and made two separate trips ~40km south to Rodds Bay where transmission ceased. For turtles that were satellite-tagged by CSIRO and remained at the capture site (n=6), average home range was very similar to those calculated from EHP deployments in 2013 (n=9, Table 4.6). Differences can be attributed to the KUD calculation method, data filtering process and differences in individual behaviour.

Of 49 deployments by CSIRO in 2013, six individuals were detected at both the Pelican Banks and Western Basin receiver arrays, including one satellite tracked individual. Of 72 satellite tag deployments by DES 2010-2019, five individuals have been recorded moving between habitats in the Western Basin and Pelican Banks after post-release settlement. The frequency of DES deployed turtles that switched between contiguous parts of their home range, was somewhat larger in 2013 and 2017 than in other years, but this difference was not significant (Figure 4.8).

## Discussion

From 2010-2019, data was received from and analysed for 72 Green turtles deployed with satellite transmitters in Port Curtis. Tags yielded an average of  $660 \pm 48$  FGPS and  $1634 \pm 106$  ARGOS locations, or  $5.7 \pm 0.5$  and  $13.0 \pm 0.9$  locations per day respectively, with ARGOS transmission consistently continuing after FGPS ceased. FGPS capability appears to have improved somewhat with tag model development. FGPS was favoured over ARGOS for home range calculation due to substantially higher location accuracy. Home range areas were between 1 and 115 km<sup>2</sup>, which is comparable to home ranges calculated for the species at coastal foraging aggregations in Moreton Bay, Sandy Strait, Shoalwater Bay and Torres Strait, Queensland (Whiting and Miller 1998; Gredzens *et al.* 2014; Shimada *et al.* 2016a; Hamann *et al.* 2017; Shimada *et al.* 2017). Turtles occupied a variety of habitats including estuaries, intertidal and subtidal flats, mangroves, rocky reefs and deeper channels and channel edges. Home ranges were generally associated with habitats adjacent to river outflows, highlighting the importance of these areas for foraging Green turtles in Port Curtis. Individuals from the Pelican Banks moved regularly in and out of the bay via northeast channel, spending between hours or weeks on rocky reefs habitats. Further assessment is needed to determine foraging resources available to turtles outside the port, and the drivers of this intermittent movement.

Tracked turtles demonstrated short-term fidelity to foraging sites, with small home range areas and 86.1% remaining associated to their capture area throughout the tracking period. Most individuals returned to their area of capture (Shimada *et al.* 2016b) and remained there for the duration of tracking, for a maximum 260 days (female subadult QA64251). Others performed short-term movements (n=11, travelling 4-55 km) and returned to the capture area. Though the present study refers to individuals' association to capture site, this was done for ease of interpretation of the tracking data. Site of capture does not necessarily represent a "home" area but is a position within the broader spatial range of the turtles, which is likely to be comprised of other additional areas that may or may not be visited within the tracking period. However, the observed tendency for turtles to track "home" to capture sites, implies that relocation of turtles is not a viable option for mitigating the impacts of local industrial development (Shimada *et al.* 2016b).

Short-term fidelity to foraging areas, based on a flipper tagging capture-mark-recapture study was recorded within Port Curtis (Chapter 2) and reinforced in the present study.; Mark-recaptures at other coastal foraging sites in Queensland suggest that few individuals switch between foraging grounds after recruitment (Limpus *et al.* 1994; Brand-Gardner *et al.* 1999; Limpus, 2008). Remaining faithful to foraging sites is likely to confer an advantage over relocating to unfamiliar sites with uncertain long-term foraging viability or different levels of exposure to predators.

Tracking at a few sampled “representative” sites is unlikely to depict habitat use across the wider Port Curtis, as individuals have small home ranges (less than 11 km<sup>2</sup> for half the sampled individuals) and few visit multiple sites. When studies are directed to understanding turtle habitat use relative to Port infrastructure, sampling needs to be targeted to sites of interest. This may pose a significant challenge at Port Curtis shipping channels and close to infrastructure as the established capture methods require shallow and/or relatively clear water.

Turtles that did not remain near the capture site during tracking either initially returned there before departing to other areas (n=8, travelling ~11-150 km), did not return to the capture site at all (n=2) or travelled to courtship areas (n=2, one of which was tracked back to the capture area). Some individuals’ home ranges may encompass multiple non-contiguous sites for diel or seasonal exploitation of resources for foraging and resting. It is also possible that turtles that used multiple sites may have done so in response to resource availability and access to sites. In 2012-2013, seagrass distribution and biomass were depleted following major flooding caused by ex-tropical cyclone Oswald. Seagrass cover was described as patchy at all sites, including the Pelican Banks which had previously been considered to support greater biomass than other sites within the port. Sparse seagrass beds were reported throughout the Western Basin, Quoin Island, and The Narrows in this year. Though it appeared that frequency of “shifting” between multiple non-contiguous sites was higher in years with large floods (2013, 2017), this difference was not statistically significant. Indeed, some turtles “shifted” between habitats in years when seagrass was in good conditions, bypassing nearby suitable habitats. Extreme weather events were likewise not demonstrated as significantly affecting seasonal home range size of satellite tracked turtles in Moreton Bay or Port Curtis (Shimada *et al.* 2016a). Babcock *et al.* (2015) drew links between turtle movements and tidal movement in 2013, with turtles on the Pelican Banks moving onto intertidal flats at high tide & retreating to channel/channel edges at low tide. At Wiggins Island, turtles were detected most frequently on channel edges at low tide, and in mangrove drains at high tide. The relationship with tides could be assessed because of the volume of acoustic tracking data. There is some evidence that turtles may move in response to changes in resource availability including changes in quality of forage and access to shallow intertidal habitats. This warrants further study.

Long range movements of tracked turtles from foraging sites that are not part of a breeding event are very uncommon. Based on data from our tracking and a long-term mark recapture of Green turtles at several sites the departure of two individuals from the Pelican Banks to sites >180 km away along the coast recorded by CSIRO in 2013 are very rare in Queensland. The drivers of this movement are unknown. It is possible that these individuals experienced high disturbance due to attachment of both acoustic and satellite tags, which required drilling of one or two holes into the marginal scutes of the carapace, as well as prolonged retention for epoxy to dry prior to release. However, all 10 individuals in this study,

and 10 others deployed by DES in the same year underwent the same procedure. The attachment protocol is standard for acoustic tagging of sea turtles. Such long-range movements were not observed in the other DES satellite tag deployments.

There was some overlap in home ranges of individuals captured at the same study sites. Home ranges were larger on average for turtles captured at the Pelican Banks than the other study sites, except for Wiggins Island, and were generally larger for adults than juveniles and subadults. However, a higher proportion of adult turtles was sampled at the Pelican Banks than at other sites, and sampling effort at the different study sites was inconsistent across sampling trips. Larger turtles may forage across larger areas, as was found in a radio tracking project in Repulse Bay (Whiting and Miller 1998). Mark-recapture of Moreton Bay and Shoalwater Bay turtles likewise suggests there are differences in spatial distribution and habitat use by turtles of different age classes (Limpus *et al.* 1994, 2005). There are also differences in diet associated with age classes (Forbes 1996; Chapter 6). We did not examine fine scale variability in the size and shape of individuals' KUDs, and its relationship to body size, but this is a potential avenue for investigating dietary preferences, vulnerability to changes in resource availability, and adaptation to threatening processes.

Few turtles crossed areas designated for dredging or infrastructure developments, including the Channel Duplication Project. Though 28 individual home ranges overlapped somewhat with dredged channels, this overlap accounted for less than 10% of 25 individuals' home ranges. For the individuals that crossed shipping channels as part of loop trips or periods of settlement at habitats in both the Western Basin and Pelican Banks, crossing these channels may pose a collision risk. While commercial vessels, especially those >50 m in length are largely restricted to the designated shipping channels, recreational, or smaller vessels are able to move over the shallower areas and their use of these sections is not regulated. Risk of vessel strike is a concern for turtles that reside in sections of shallower water such as the Pelican Banks, where high-speed recreational vessel traffic was regularly observed during the study period. Home ranges were not closely linked to the location of weirs.

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Table 4.1. Summary of satellite transmitters deployed on Green turtles in Port Curtis 2010-2019.

All tags had Fastloc GPS capability, and no individual was tracked twice in the study period

<b>Year</b>	<b>n</b>	<b>Capture Site</b>	<b>Tag Type</b>	<b>Deployed by</b>	<b>Funding Source</b>
2010	5	Pelican Banks	Sirtrack Fastloc	GHD	GPC
2013	10	Pelican Banks- 5 Wiggins Island- 5	SPLASH10-F-296A and SPLASH10-F-296C	CSIRO	GISERA Marine project
2013	13	Pelican Banks- 10 Wiggins Island- 3	SPLASH10-F-296A and SPLASH10-F-297A	EHP + JCU	ORICA
2014	12	Pelican Banks- 10 Boyne River- 1 Quoin Island- 1	SPLASH10-F-296A	JCU	GPC
2015	11	Pelican Banks	SPLASH10-F-297A	JCU	GPC
2016	11	Pelican Banks- 6 Western Basin- 4 Boyne River- 1	SPLASH10-F-297A	JCU	GPC
2017	3	Pelican Banks	SPLASH10-F-297A	EHP	EHP
2018	4	Wild Cattle Island- 2 South Trees- 2	SPLASH10-BF-344E	JCU Seagrass (Mike Rasheed)	TropWater (JCU)
2018	2	Pelican Banks- 1 South Trees- 1	SPLASH10-BF-297B	EHP	EHP
2018	2	South Trees	SPLASH-10-F-334D	EHP	Eco Logical Australia
2019	10	Wiggins Island	SPLASH10-F-385	DES + JCU	Eco Logical Australia



Table 4.2. Details of sex and simplified age classes of 73 Green turtles deployed with satellite transmitters at Port Curtis study sites 2010-2019.

Sex: F = female; M = male; I = indeterminate, not examined for sex. This table does not include 10 tags deployed by CSIRO in 2013.

	2010	2013	2014	2015	2016	2017	2018	2019	Total
<b>Boyne River</b>			1		1				2
<b>Adult</b>			1		1				2
F			1						1
M					1				1
<b>Pelican Banks</b>	5	10	10	11	6	3	1		46
<b>Adult</b>	4	7	8	6	6	1			32
F	1	5	4	2	3	1			16
M	3	2	4	4	3				16
<b>Immature</b>	1	3	2	5		2	1		14
F				3		1			4
I	1	3	2						6
M				2		1	1		4
<b>Quoin Island</b>			1						1
<b>Immature</b>			1						1
I			1						1
<b>South Trees</b>							5		5
<b>Adult</b>							1		1
M							1		1
<b>Immature</b>							4		4
I							2		2
M							2		2
<b>Western Basin (including Wiggins Island)</b>		3			4			10	17
<b>Adult</b>					3				3
F					3				3
<b>Immature</b>		3			1			10	14
F								6	6
I		3							3
M					1			4	5
<b>Wild Cattle Island</b>							2		2
<b>Adult</b>							1		1
M							1		1
<b>Immature</b>							1		1
M							1		1
<b>Total</b>	5	13	12	11	11	3	6	10	73

Table 4.3. Summary details for 10 Green turtles that returned to capture site after several days.

<b>Primary Tag</b>	<b>Year Deployed</b>	<b>Age Class</b>	<b>Capture Site</b>	<b>Sites Visited</b>	<b>Time Before Resettlement (days)</b>
K28651	2015	A	Pelican Banks	Quoin Island	30
K93088	2010	A	Pelican Banks	The Narrows	7
QA33327*	2013	A	Pelican Banks	Wiggins Island	54
QA33342*	2013	A	Pelican Banks	Wiggins Island	7
QA33350*	2013	J	Pelican Banks	Western Basin (Diamantina and Picnic Islands)	22
QA33368	2013	J	Wiggins Island	Bushy Island Reef	7
QA58206*	2015	SA	Pelican Banks	Western Basin (South Bank of Curtis Island)	4
QA58210	2015	SA	Pelican Banks	Sable Chief Rocks Reef & North Point Reef (Facing Island)	35
QA87017	2018	SA	South Trees	Facing Island Reef	5
T83097	2010	A	Pelican Banks	The Narrows, Quoin Island	4

\*Also see Table 4

Table 4.4 Summary details for turtles that after moving back to near their capture location, used discrete secondary areas as part of their 95% UD or performed loop trips.

Primary Tag	Tracked Year	Age Class	Capture Site	Other Sites Visited	% Tracked days	Visits	Loop "L" (<3 days) or settlement "S" (>3)	Return before end transmission (Y/N)
K70229	2013	A	Pelican Banks	Rodds Bay (~40km south)	62.7	3	S	N
QA33327	2013	A	Pelican Banks	Wiggins Island (~10km west)	91.0	3	S	Y
QA33342	2013	A	Pelican Banks	Wiggins Island (~10km west)	22.6	4	S&L	Y
QA33348	2013	A	Pelican Banks	South Trees (~15km south)	5.6	2	L	Y
QA33350	2013	J	Pelican Banks	Curtis Island Reef (~10km north)	66.7	1	S	Y
QA36875	2014	A	Pelican Banks	Western Basin (~12km west)	9.3	1	S	N
QA43063	2013	A	Pelican Banks	The Narrows (~45km northwest)	9.1	1	S	Y
QA45566	2014	A	Pelican Banks	Colosseum Ck (~28km southeast)	44.9	2	S	N
QA45627	2014	J	Pelican Banks	Hummock Hill Island (~28km southeast)	92.8	1	S	N
QA58206	2015	SA	Pelican Banks	South Bank of Curtis Island (~5km west)	2.2	2	L	Y
QA58284	2015	A	Pelican Banks	The Narrows (~11km east)	76.4	2	S	N
QA64291	2017	SA	Pelican Banks	Port Alma (~55km northwest)	10.7	1	S	Y

QA66526	2016	A	Pelican Banks	Shoalwater (~150km northwest)	54.1	1	S	N
QA86189	2018	A	South Trees	Manning Reef & Bushy Island Reef (~4km northeast)	17.6	8	L	Y
QA86302	2018	A	Wild Cattle Island	Upstream Colosseum Ck towards Hummock Hill Is (~7km southeast)	70.4	1	S	Y
QA91605	2019	SA	Wiggins Island	South Bank of Curtis Island & Quoin Island (~5km northeast, ~6km northwest)	4.0	2	S&L	Y
T88971	2016	A	Pelican Banks	South Bank of Curtis Island (~9km southwest)	4.5	2	S	Y

Table 4.5 Average 95% and 50% UD area for Green turtles deployed with satellite transmitters in Port Curtis 2010-2019 according to capture site.

Capture Site	Adults (n)	Immatures (n)	95% UD area (km <sup>2</sup> )	50% UD area (km <sup>2</sup> )
Boyne River	2	0	4.7±1.4	1.2±0.5
Pelican Banks	31	14	28.7±3.9	3.4±0.4
Quoin Island	0	1	5.3	0.7
South Trees	1	4	3.2±0.9	0.4±0.1
Western Basin	3	1	6.5±2.7	1.2±0.4
Wiggins Island	0	13	5.2±1.3	0.8±0.2
Wild Cattle Island	1	1	7.3±0.3	1.1±0.3
<b>TOTAL</b>	<b>38</b>	<b>34</b>	<b>19.9±2.8</b>	<b>2.4±0.3</b>

Table 4.6 Kernel Utilisation Distribution (UD) area (km<sup>2</sup>) generated from satellite transmitters deployed in 2013 by CSIRO and DES (n in brackets), excluding individuals that departed the capture site.

<b>Capture Site</b>	<b>95%UD CSIRO</b>	<b>95%UD DES</b>	<b>50%UD CSIRO</b>	<b>50%UD DES</b>
Pelican Banks	33.8±30.4 (2)	15.2±3.6 (9)	1.9±0.4 (2)	1.9±0.4 (9)
Wiggins Island	4.8±1.9 (4)	11.4±3.4 (2)	0.9±0.6 (4)	1.5±0.9 (2)
Average	14.5±10.0 (6)	14.2±2.8 (11)	1.9±1.1 (6)	1.8±0.3 (11)



**4.1A. Positioning of Wildlife Computers SPLASH-10 tag between 1<sup>st</sup> and 2<sup>nd</sup> vertebral scutes of subadult Green turtle. Anterior portion of the carapace has been cleaned. Initial attachment with epoxy and held in place with adhesive tape.**



**4.1B. Reinforcement and expansion of attachment with fibreglass tape and epoxy connecting to adjacent costal scutes.**



**4.1C. Painting of tag and epoxy with antifouling paint.**



**4.1D. Release into Western Basin.**

Figure 4.1. Attachment of satellite tags to Green turtles, *Chelonia mydas*, in Port Curtis.

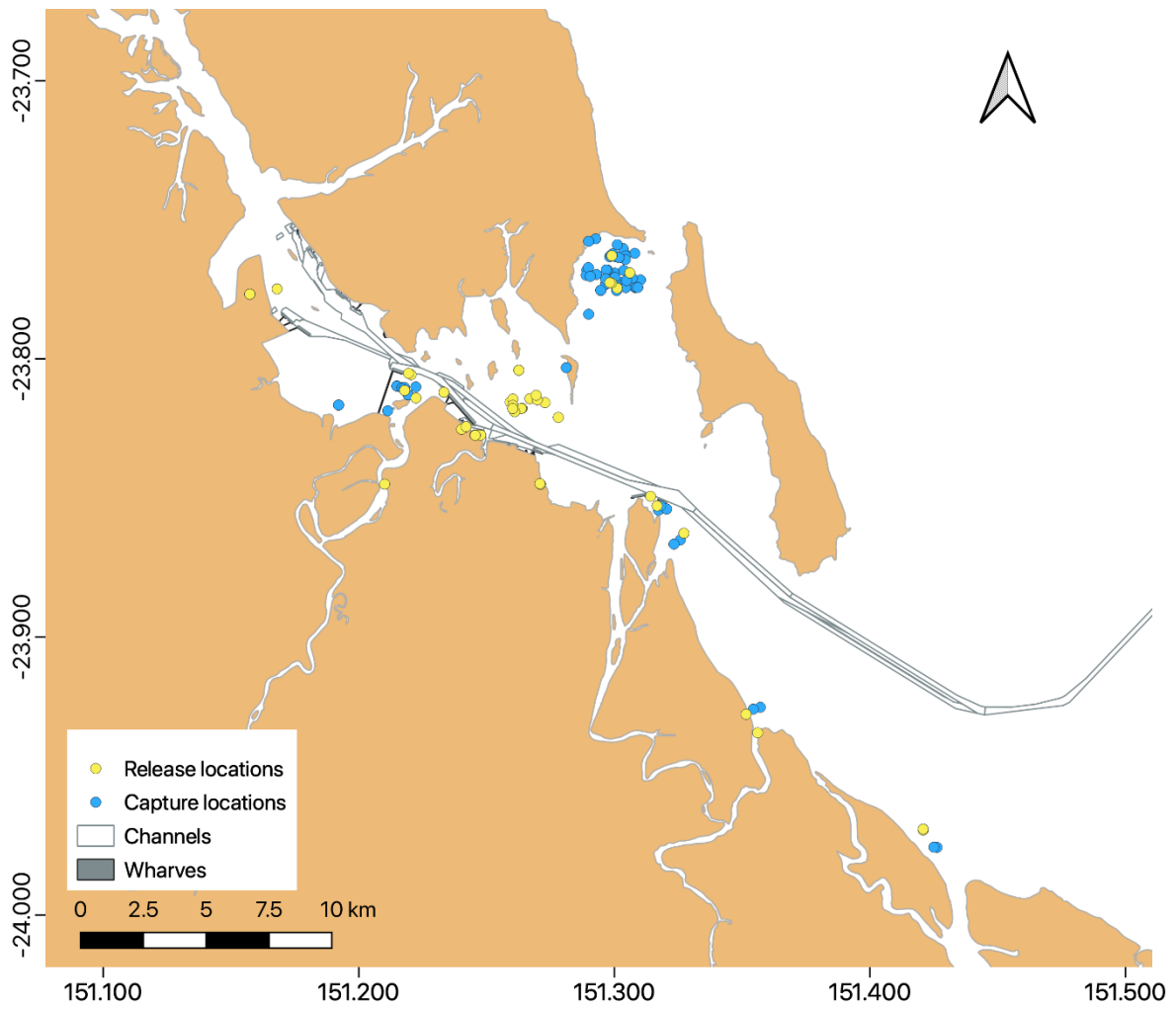


Figure 4.2. Map of capture and release locations of 73 Green turtles deployed with satellite transmitters in Port Curtis 2010-2019, showing locations of shipping channels and wharf structures. Data missing for two captures and five releases. Wiggins Coal Terminal and three LNG wharves were constructed after 2013.

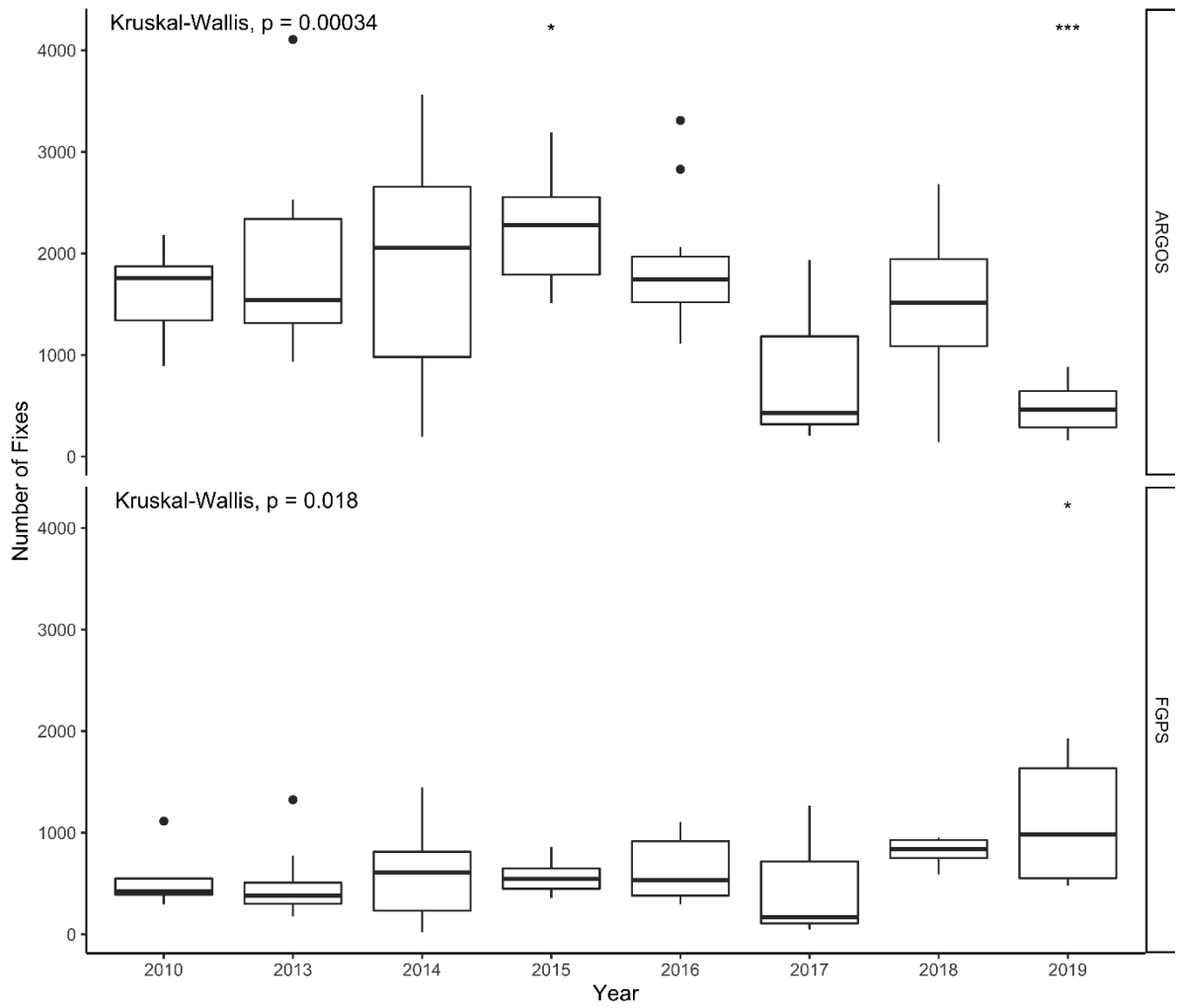


Figure 4.3. Number of ARGOS PTT and FGPS positions received from satellite transmitters deployed on Green turtles in Port Curtis 2010-2019.



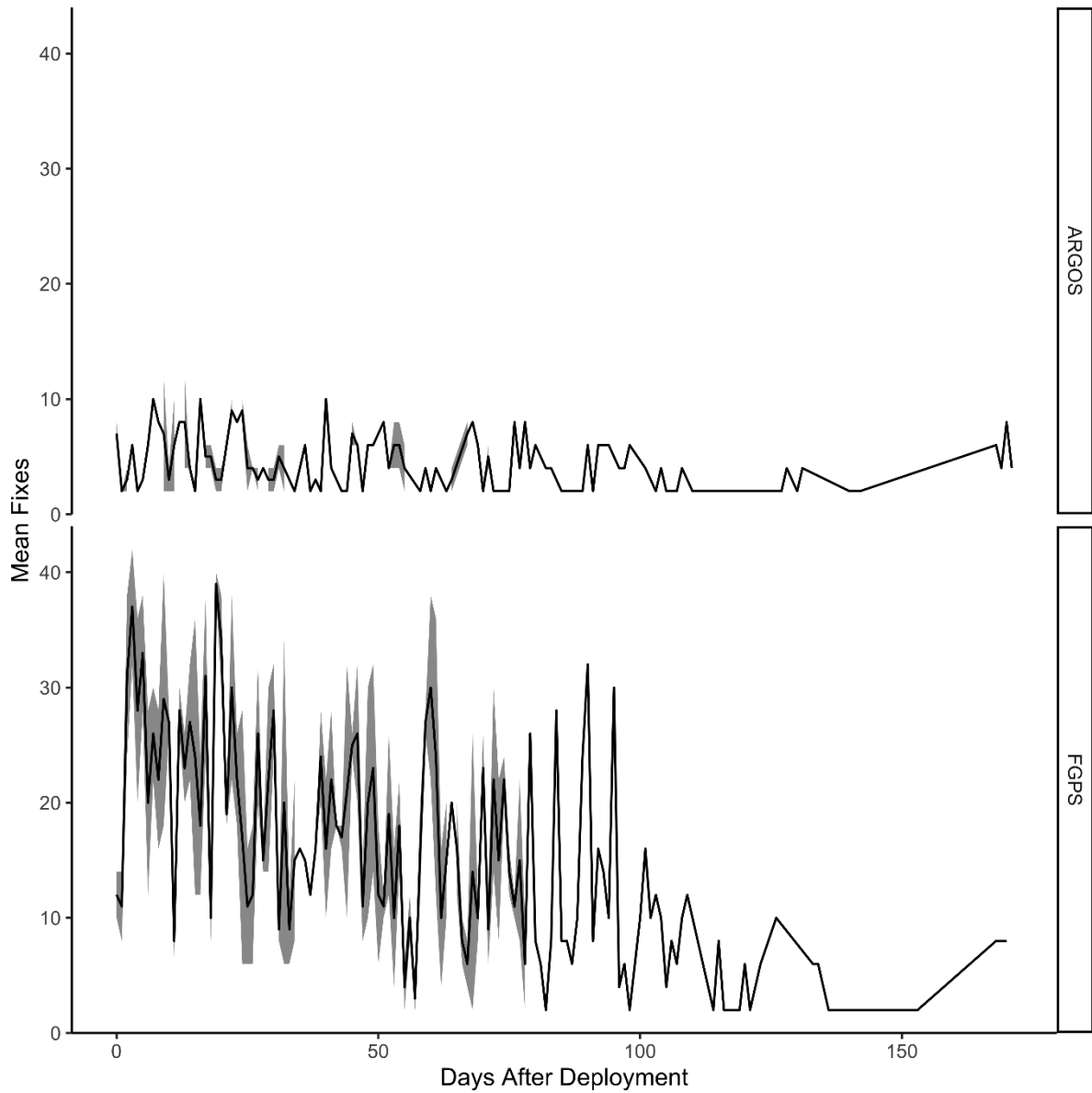
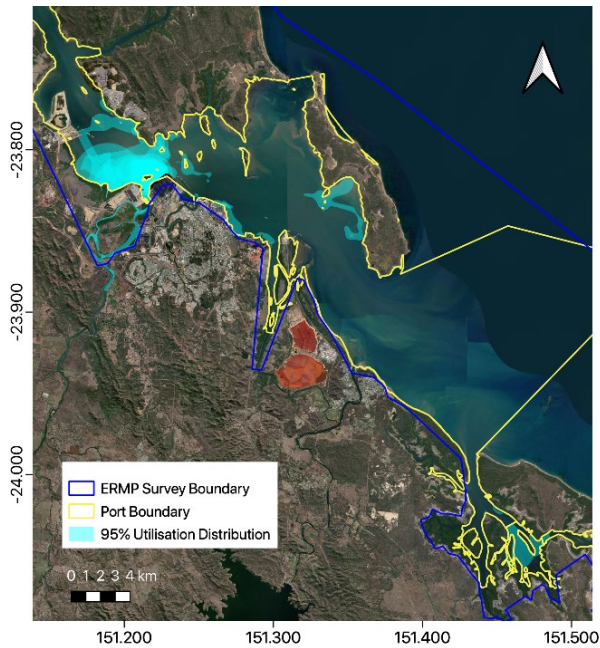
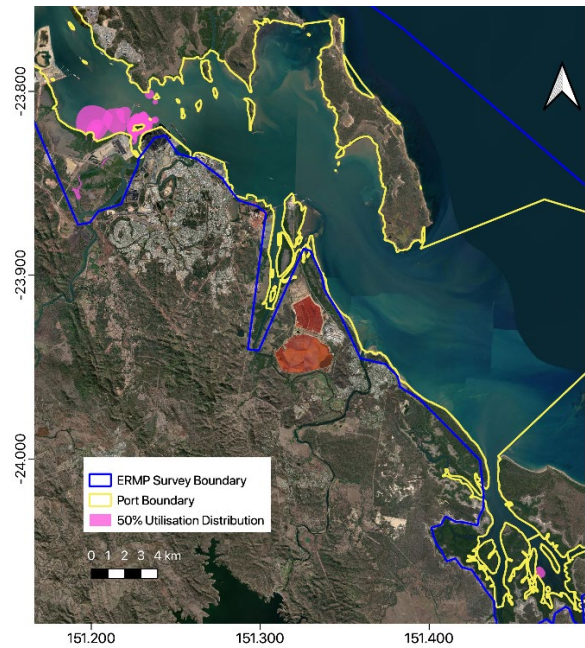


Figure 4.4. Mean and standard error of number of Fastloc GPS and ARGOS PTT locations received from satellite transmitters deployed on Green turtles in Port Curtis each day after deployment. Fixes were recorded more than 300 days after deployment for only one individual - adult female K70229.

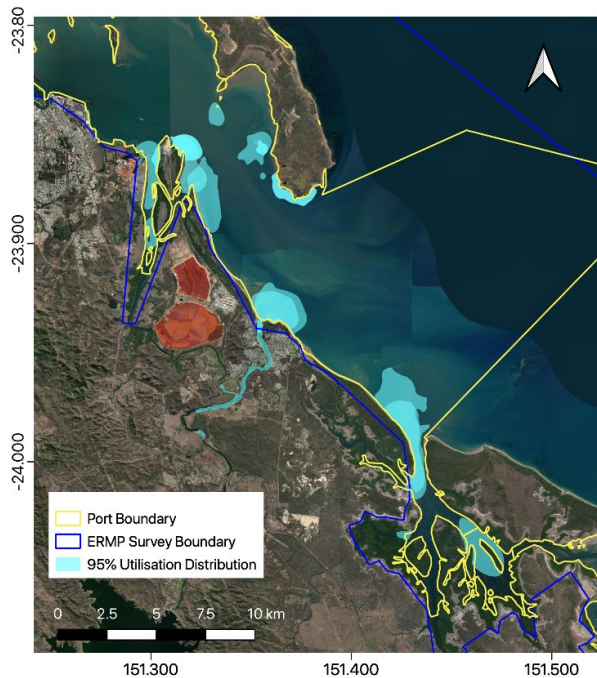
**Figure 4.5. Utilisation Distribution of Green turtles tagged with satellite transmitters within Port Curtis. Satellite and aerial imagery from ESRI World Imagery base map 2020.**



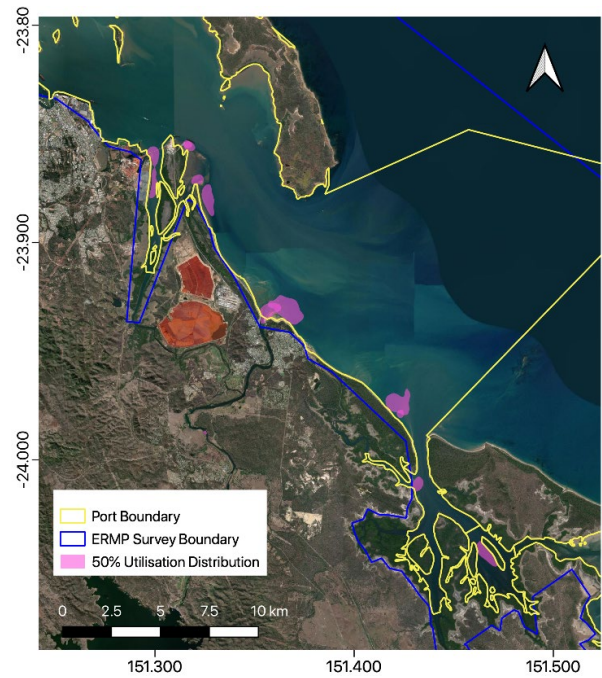
**Figure 4.5A. 95% Utilisation Distribution (UD) of 17 Green turtles tagged with satellite transmitters in Western Basin, Port Curtis.**



**Figure 4.5B. 50% Utilisation Distribution (UD) of 17 Green turtles tagged with satellite transmitters in Western Basin, Port Curtis.**



**Figure 4.5C. 95% Utilisation Distribution (UD) of 9 Green turtles tagged with satellite transmitters at Boyne River, South Trees and Wild Cattle Island, Port Curtis.**



**Figure 4.5D. 50% Utilisation Distribution of 9 Green turtles tagged with satellite transmitters at Boyne River, South Trees and Wild Cattle Island, Port Curtis.**

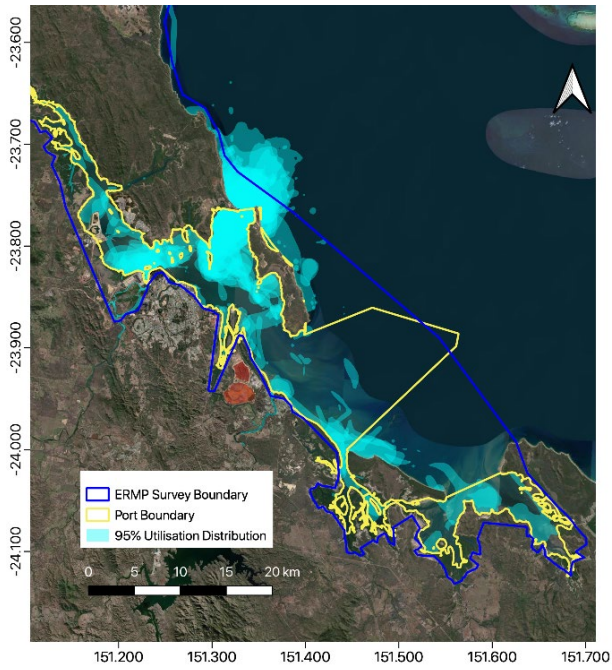


Figure 4.5E. Close up of Port Curtis showing 95% Utilisation Distribution of 47 Green turtles tagged with satellite transmitters at Pelican Banks and Quoin Island, Port Curtis.

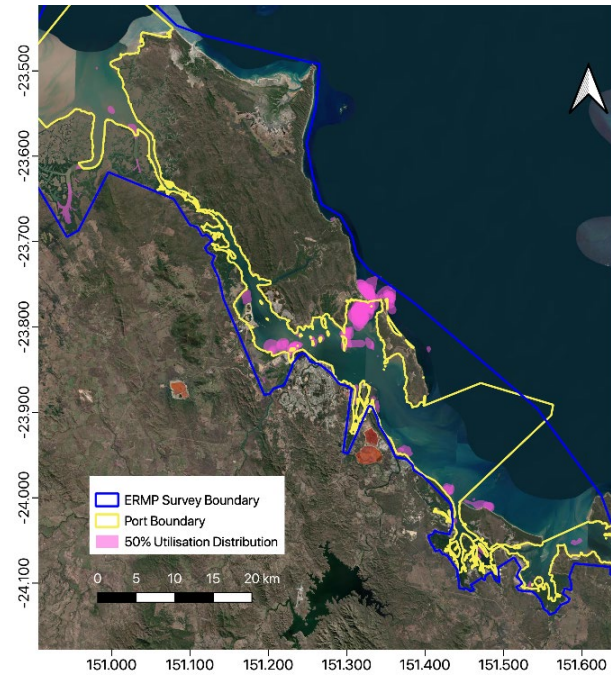


Figure 4.5F. Close up of Port Curtis showing 50% Utilisation Distribution of 47 Green turtles tagged with satellite transmitters at Pelican Banks and Quoin Island, Port Curtis.

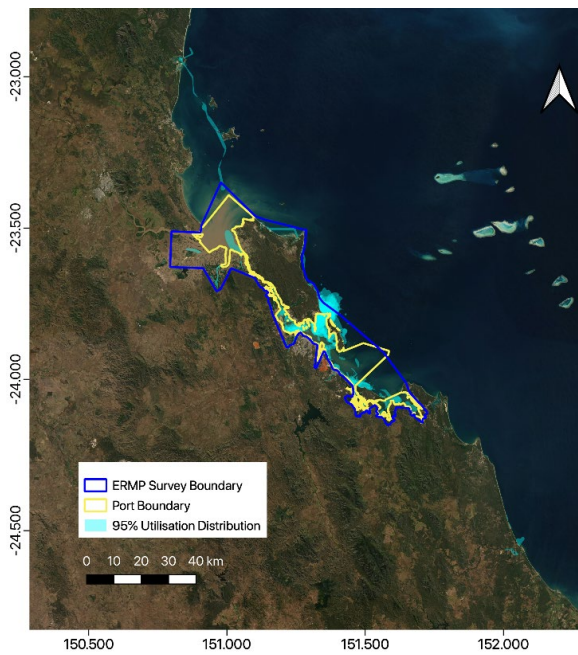


Figure 4.5G. 95% Utilisation Distribution of 46 Green turtles tagged with satellite transmitters at Pelican Banks and Quoin Island.

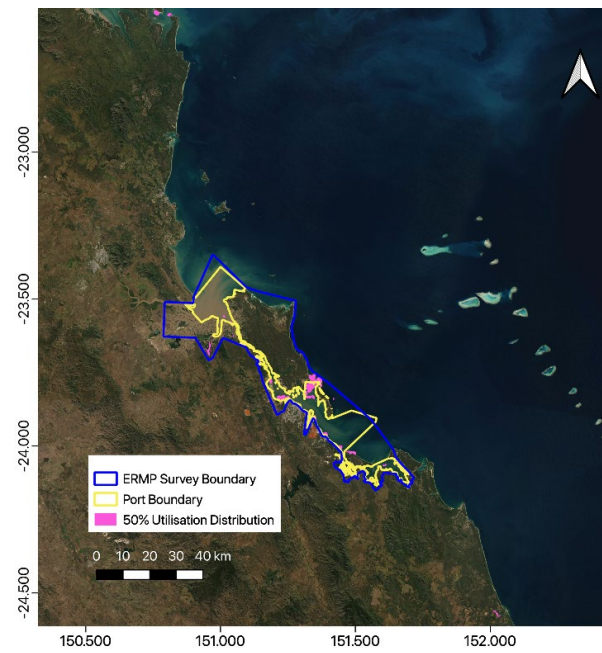


Figure 4.5H. 50% Utilisation Distribution of 46 Green turtles tagged with satellite transmitters at Pelican Banks and Quoin Island.

Figure 4.6. Examples of the behaviour of Green turtles foraging within Port Curtis illustrated by satellite telemetry tracking.

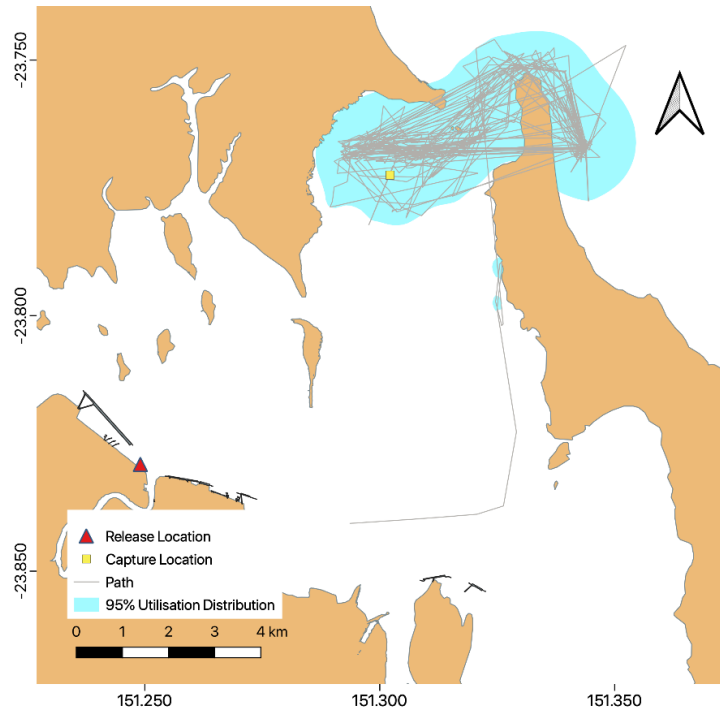


Figure 4.6A. Example of turtle resettling at capture site at Pelican Banks and moving in and out of northeast passage. Trajectory, capture release locations, and 95% Utilisation Distribution. Subadult QA45601, 2014.

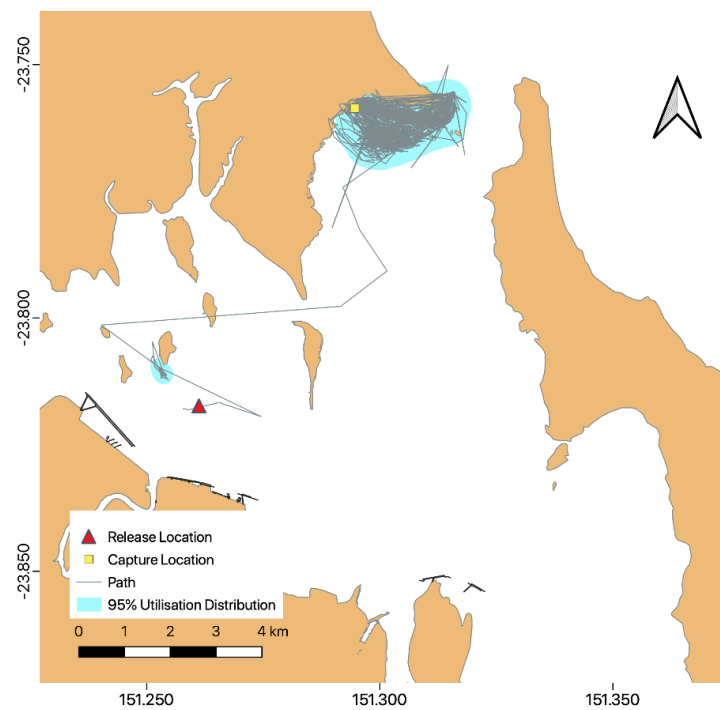


Figure 4.6B Example of turtle resettling and remaining at capture site and remaining on Pelican Banks and Curtis Island Reef. Juvenile QA33349, 2013.

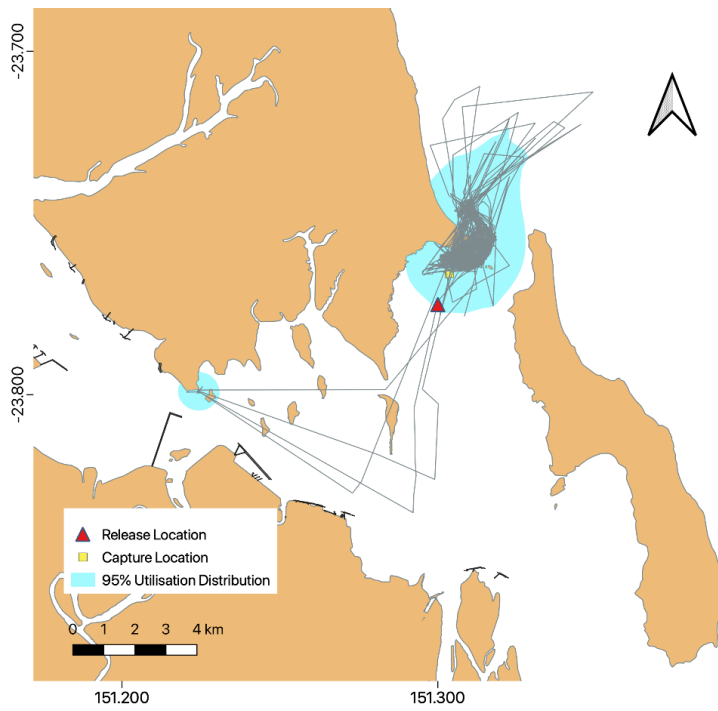


Figure 4.6C. Example of turtle performing “loop” trips before returning to capture site. Adult female T88971, 2016.

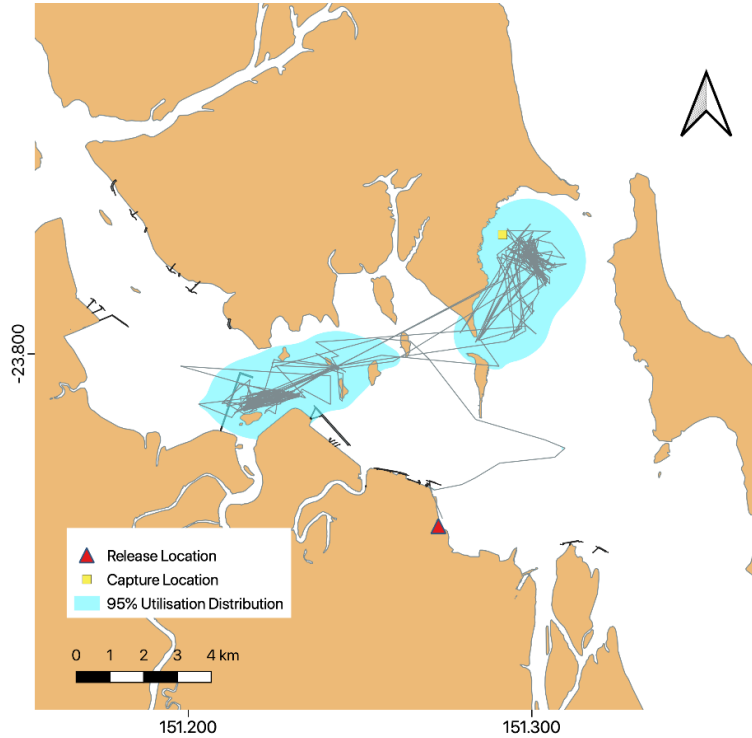


Figure 4.6D. Example of turtle “shifting” between discrete areas of utilisation distribution. Adult female QA33342, 2013

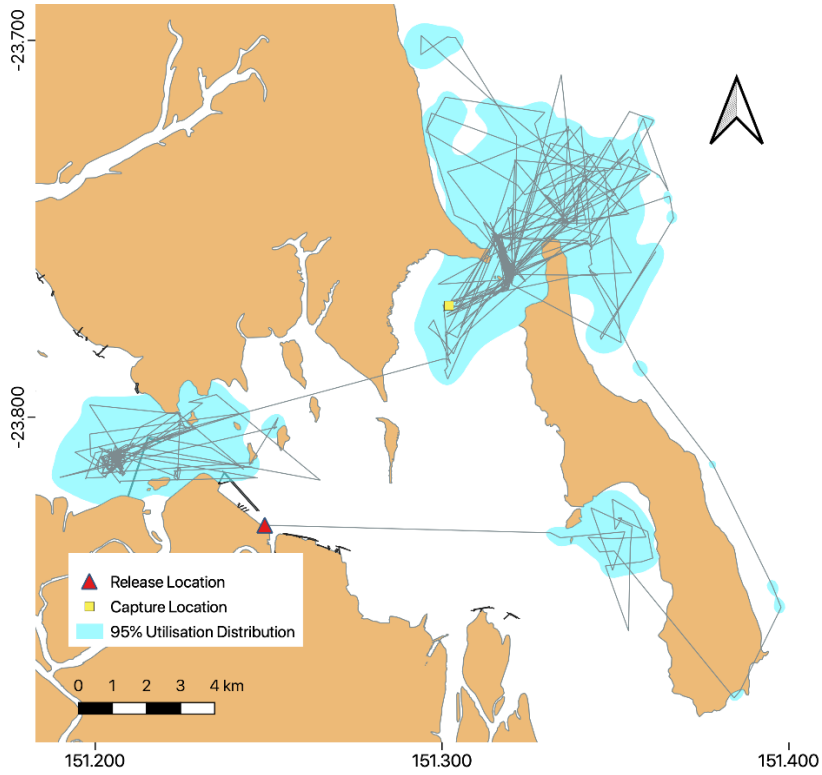


Figure 4.6E. Example of turtle “shifting” between discrete areas of the utilisation distribution. Adult male QA36875, 2014.

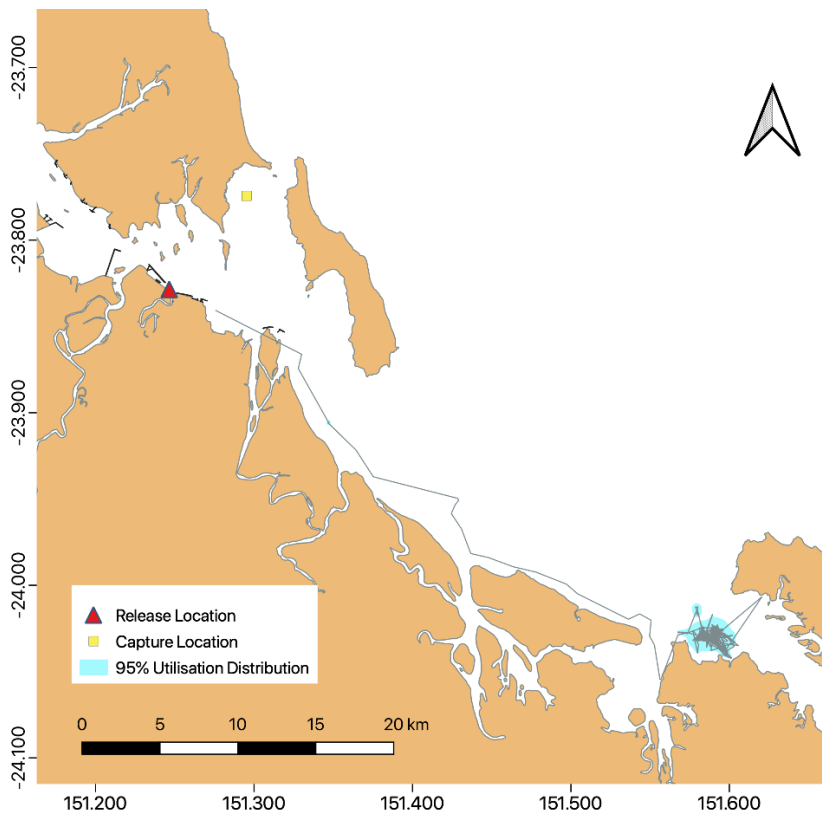


Figure 4.6F. Example of turtle that did not return to capture site. Adult male QA45689, 2014.

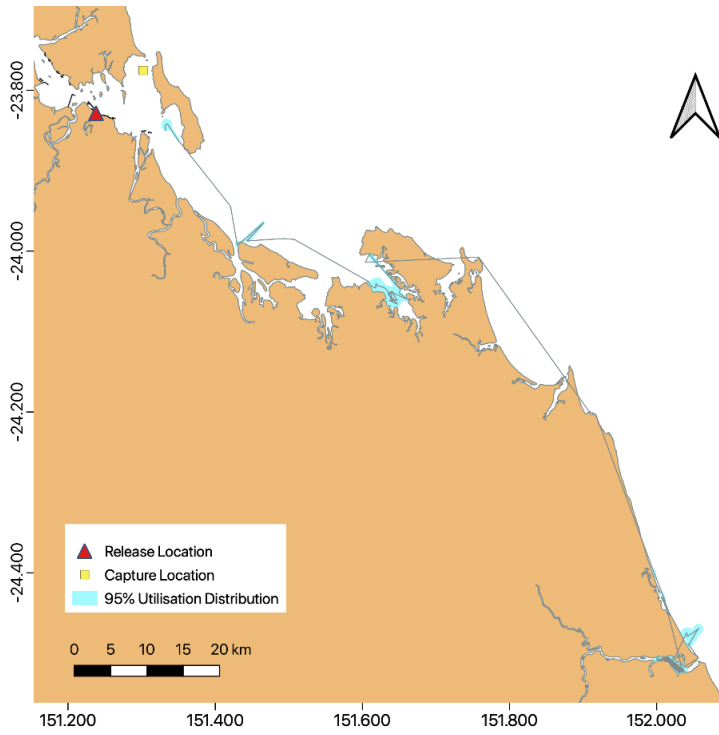


Figure 4.6G. Example of turtle that did not return to capture site. Adult male QA58291, 2015.

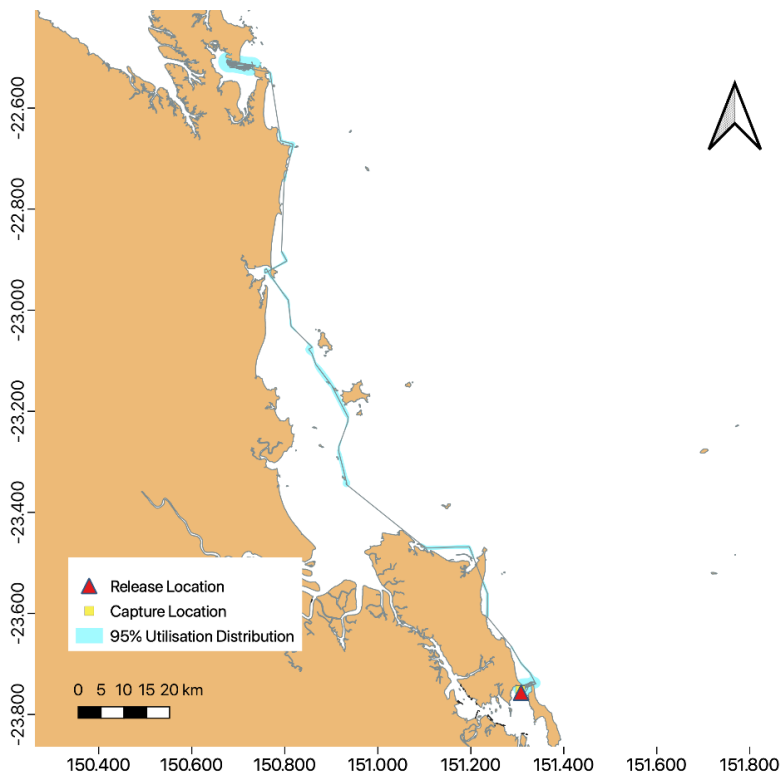


Figure 4.6H. Example of turtle that performed a long, northward movement from the Pelican Banks. Adult female QA66526, 2016.

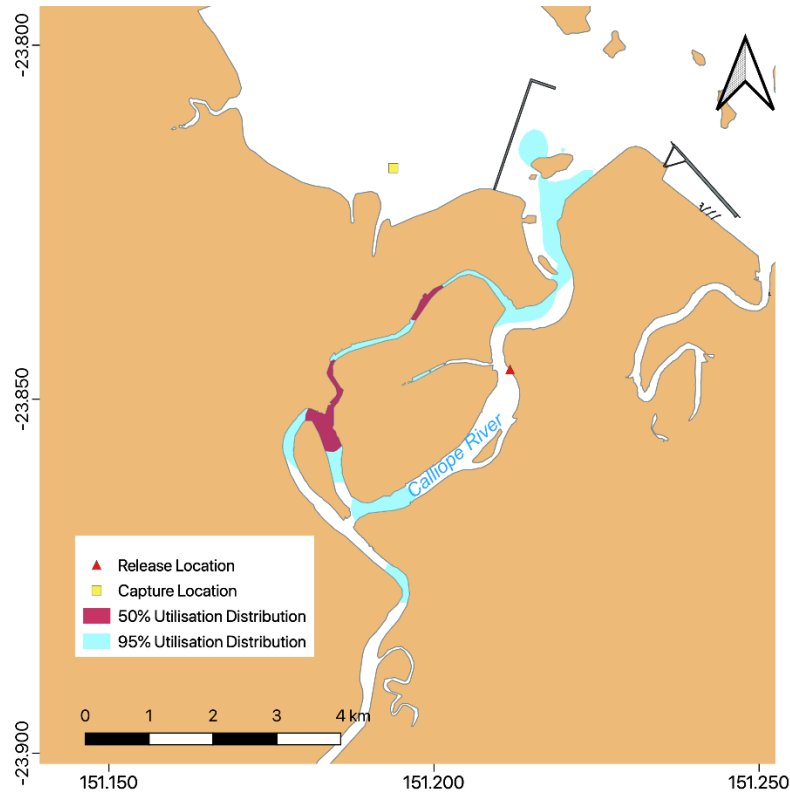


Figure 4.6I. Example of turtle using estuary habitat upstream of Wiggins Island in the Calliope River. Subadult female QA81603, 2019.

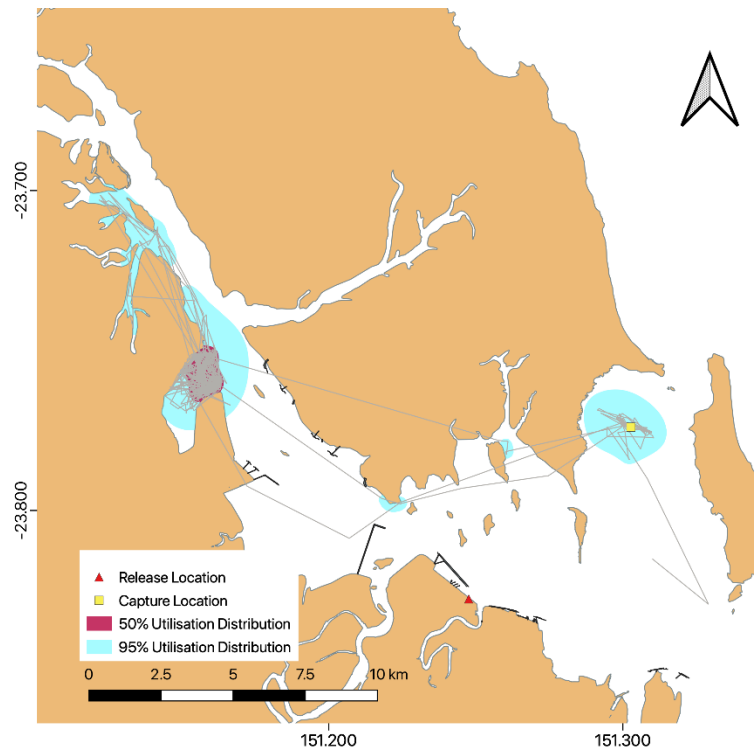




Figure 4.6J. Example of use of habitats in the Narrows  
Adult male QA58284, 2015.

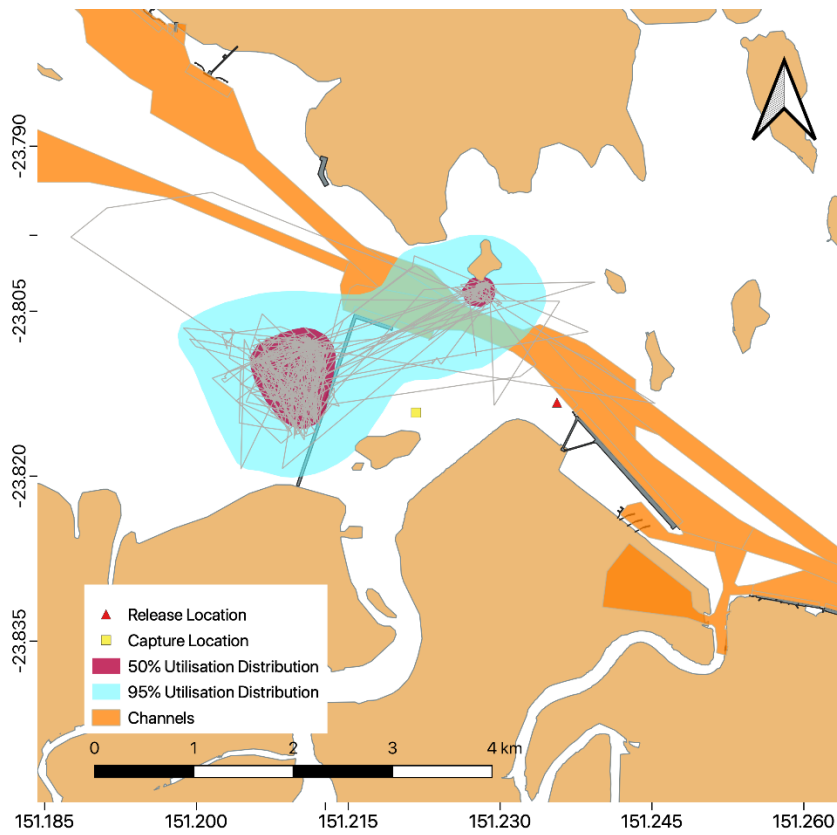


Figure 4.6K. Example of turtle with 95% Utilisation distribution overlapping shipping channels. Adult female QA64932, 2016.



Figure 4.7. Graphical summary of home range analyses for green turtles tracked by satellite telemetry within Port Curtis during 2010-2019.

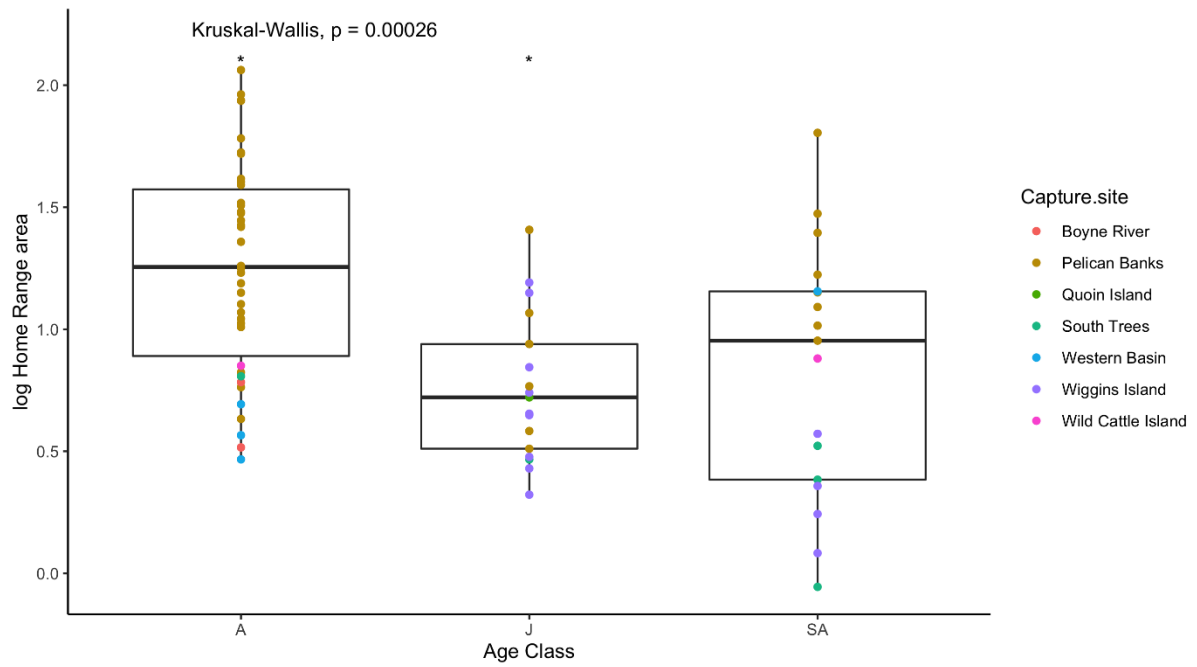


Figure 4.7A. Log home range area compared across age classes for Green turtles tracked in Port Curtis 2010-2019 indicating study sites.

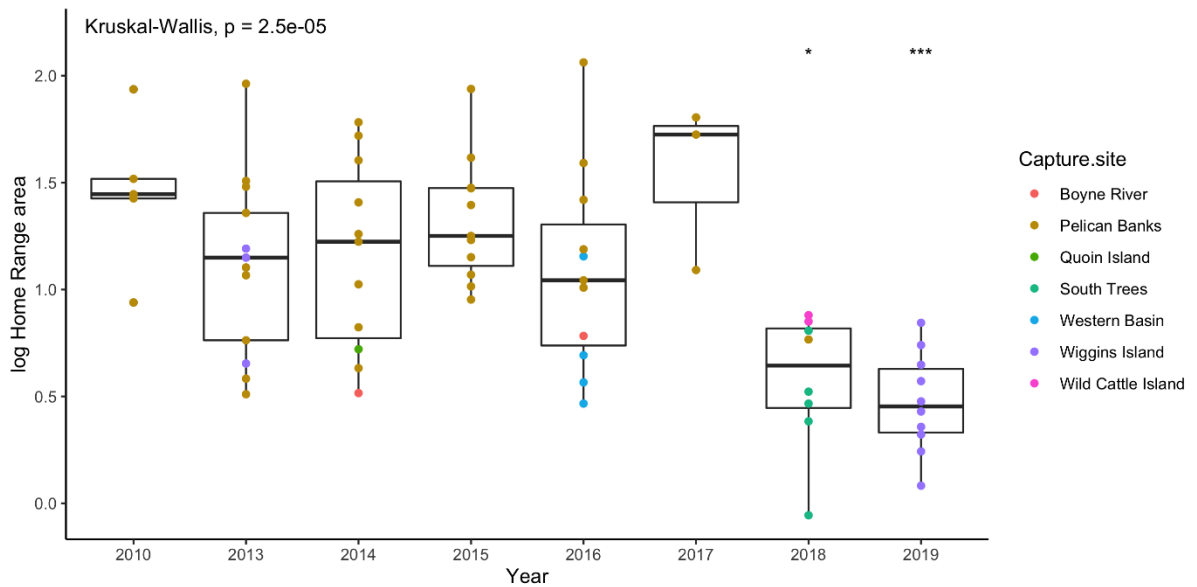


Figure 4.7B. Log home range area compared across deployment years for Green turtles tracked in Port Curtis 2010-2019 indicating study sites.

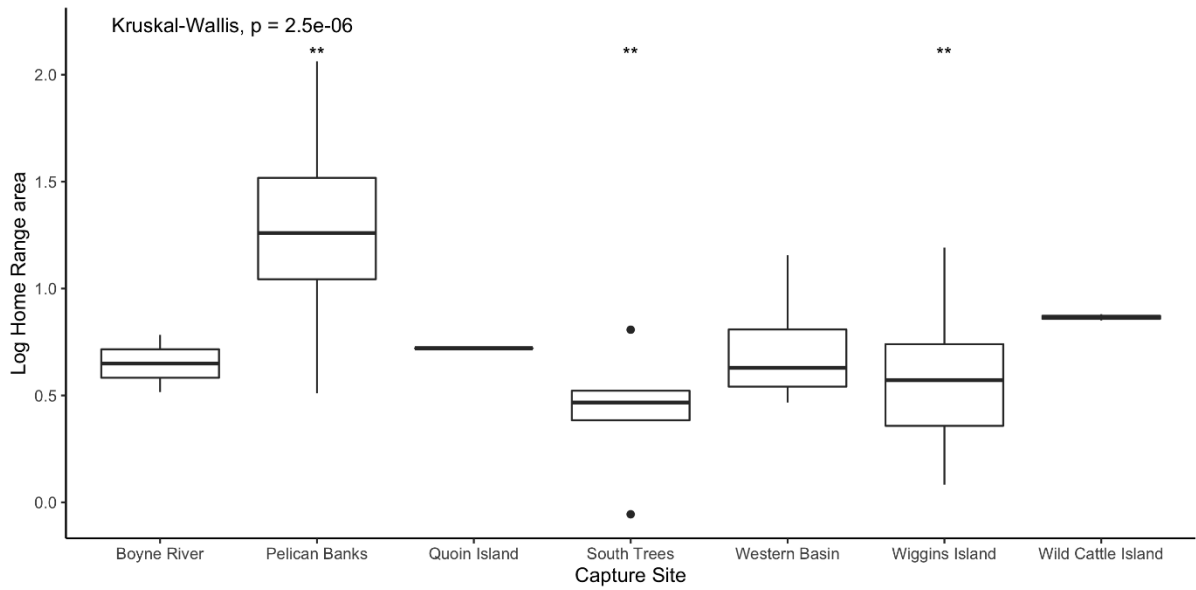


Figure 4.7C. Log home range area compared across study sites for Green turtles tracked in Port Curtis 2010-2019.

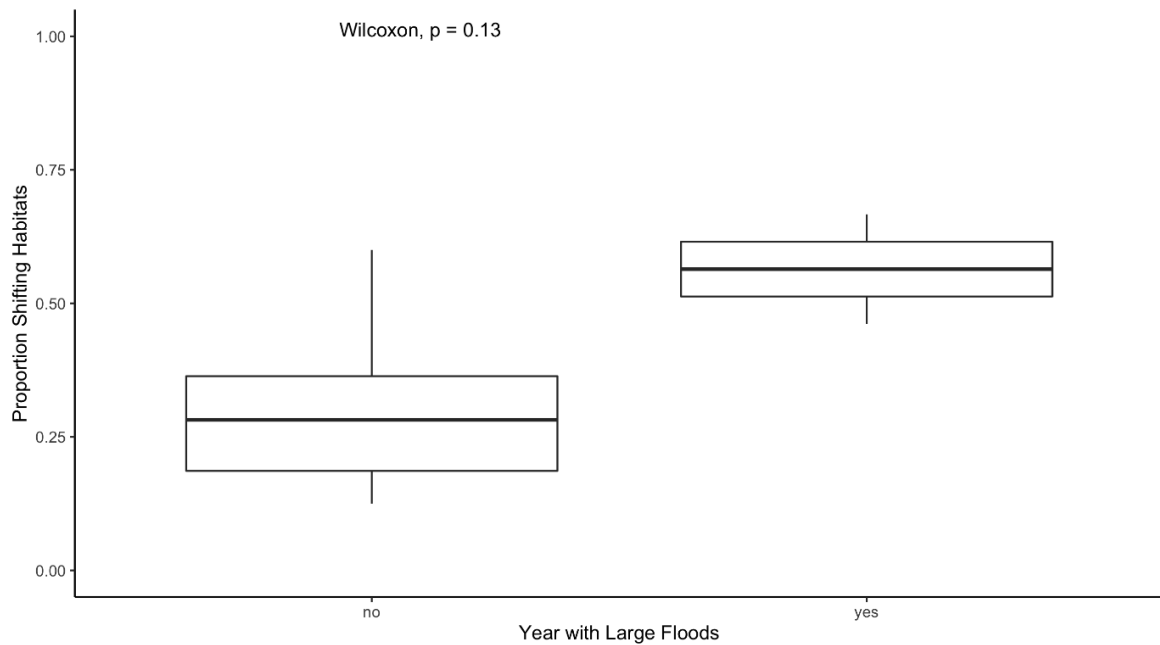


Figure 4.8. Comparison of proportion of individuals ( $n=72$ ) that “shifted” between habitats i.e. did not remain in the capture area for the duration of tracking.

Years with large floods were 2013 & 2017. In 2011 there were major floods but no tracking was conducted .

## Appendices

Appendix 1. Tag and capture details of Green turtles deployed with satellite transmitters in Port Curtis 2010-2020, giving deployment and home range summaries.

Sexes are abbreviated as male= "M", female= "F", and "I" refers to sex not determined. Age classes are adult= "A" and juvenile = "J". Max. Displacement is the square root of the maximum squared linear displacement from the initial fix.

Primary Tag	Satellite Tag	Deployment Date	Capture Site	Sex	CCL (cm)	Age Class	Track Length (km)	Max. Displacement (km)	Home Range Area (km <sup>2</sup> , 95%UD)	Core Use Area (km <sup>2</sup> , 50%UD)	95%UD lying outside Port (% area)	95%UD overlapping shipping channels (% area)
K283	143704	28/12/14	Pelican Banks	F	98.1	A	701.47	10.28	18.18	2.66	58.37	0.00
K28651	149082	15/7/15	Pelican Banks	M	98.9	A	641.34	15.83	17.81	1.98	26.53	0.00
K70229	133767	6/11/13	Pelican Banks	F	105.7	A	438.78	40.69	91.70	5.73	40.47	0.49
K93085	72448	30/6/10	Pelican Banks	I	51.3	J	503.28	5.09	8.70	1.61	12.93	0.00
K93086	96781	30/6/10	Pelican Banks	M	85.6	A	742.34	4.73	32.93	4.89	35.53	0.00
K93087*	96777	30/6/10	Pelican Banks	M	104.3	A	529.59	11.60	86.36	10.81	31.44	0.01
K93088	96778	30/6/10	Pelican Banks	M	92.2	A	898.69	18.67	26.67	3.02	16.96	0.00
QA13938	134182	13/5/14	Pelican Banks	M	95.4	A	662.34	17.70	10.58	1.70	0.96	0.00
QA32523	133762	5/11/13	Wiggins Island	I	49.1	J	94.39	5.13	15.54	3.27	19.01	0.19
QA33327	133765	4/11/13	Pelican Banks	M	96.5	A	276.33	8.33	14.12	1.53	0.81	7.82

**Appendix 1. continued.**

Primary Tag	Satellite Tag	Deployment Date	Capture Site	Sex	CCL (cm)	Age Class	Track Length (km)	Max. Displacement (km)	Home Range Area (km <sup>2</sup> , 95%UD)	Core Use Area (km <sup>2</sup> , 50%UD)	95%UD lying outside Port (% area)	95%UD overlapping shipping channels (% area)
QA33335	134180	13/5/14	Pelican Banks	F	89	A	80.01	2.62	4.29	0.87	0.00	0.00
QA33342	133764	4/11/13	Pelican Banks	F	111	A	349.63	9.25	22.82	3.59	0.80	5.01
QA33348	133769	4/11/13	Pelican Banks	F	107.3	A	297.41	11.12	32.19	3.92	4.11	0.29
QA33349	133759	4/11/13	Pelican Banks	I	42.6	J	904.38	10.10	3.83	1.08	10.73	0.00
QA33350	133763	4/11/13	Pelican Banks	I	42.1	J	221.18	16.69	11.66	1.49	26.07	1.55
QA33368	133760	4/11/13	Wiggins Island	I	46	J	203.39	16.57	14.10	0.76	1.33	7.42
QA33394	133758	8/11/13	Pelican Banks	I	43.6	J	194.64	6.00	3.24	0.56	1.25	0.00
QA34529	133761	4/11/13	Wiggins Island	I	47.9	J	226.99	10.48	4.51	0.41	4.41	0.00
QA36875	134178	13/5/14	Pelican Banks	M	97.6	A	711.81	15.10	60.56	6.25	51.09	2.71
QA43023	133770	7/11/13	Pelican Banks	F	102.7	A	631.97	9.24	5.79	1.09	0.00	0.00
QA43063	133768	6/11/13	Pelican Banks	M	93.5	A	743.39	41.38	30.26	2.02	9.42	4.60
QA43066	133766	6/11/13	Pelican Banks	F	105.7	A	315.89	7.63	12.68	2.15	32.80	0.00
QA43123	149087	12/7/15	Pelican Banks	F	108.2	A	820.15	22.13	86.70	9.40	66.97	0.00

**Appendix 1. continued.**

Primary Tag	Satellite Tag	Deployment Date	Capture Site	Sex	CCL (cm)	Age Class	Track Length (km)	Max. Displacement (km)	Home Range Area (km <sup>2</sup> , 95%UD)	Core Use Area (km <sup>2</sup> , 50%UD)	95%UD lying outside Port (% area)	95%UD overlapping shipping channels (% area)
QA45408	134179	16/5/14	Pelican Banks	M	103.3	A	29.06	8.86	NA	NA	NA	NA
QA45524	134183	13/5/14	Pelican Banks	F	101.7	A	432.14	17.17	52.41	6.76	67.43	0.00
QA45554	134184	15/5/14	Boyne River	F	116.6	A	489.03	9.80	3.28	0.69	6.95	0.00
QA45566	134188	16/5/14	Pelican Banks	F	110.9	A	243.85	27.30	40.20	4.54	34.68	0.00
QA45601	134185	14/5/14	Pelican Banks	I	79	SA	564.29	6.43	16.74	3.96	42.28	0.00
QA45627	134181	14/5/14	Pelican Banks	I	63.1	J	846.98	29.15	25.56	2.85	37.18	0.08
QA45654	134186	15/5/14	Quoin Island	I	50.2	J	80.98	3.98	5.26	0.67	3.85	0.00
QA45689	134187	16/5/14	Pelican Banks	M	102.5	A	304.88	31.36	6.66	0.84	3.29	0.00
QA58206	149088	12/7/15	Pelican Banks	F	81.5	SA	308.06	13.47	24.84	1.19	26.26	0.44
QA58209	149081	12/7/15	Pelican Banks	M	89.1	A	388.96	12.52	11.73	2.32	27.83	0.00
QA58210	149086	12/7/15	Pelican Banks	M	80.1	SA	338.46	12.41	29.76	2.36	46.58	0.00
QA58211	149085	13/7/15	Pelican Banks	F	99.7	SA	256.32	3.47	14.17	2.03	2.35	0.00
QA58221	149080	15/7/15	Pelican Banks	F	95.1	A	865.75	6.90	41.36	7.16	73.99	0.00

**Appendix 1. continued.**

Primary Tag	Satellite Tag	Deployment Date	Capture Site	Sex	CCL (cm)	Age Class	Track Length (km)	Max. Displacement (km)	Home Range Area (km <sup>2</sup> , 95%UD)	Core Use Area (km <sup>2</sup> , 50%UD)	95%UD lying outside Port (% area)	95%UD overlapping shipping channels (% area)
QA58239	149083	16/7/15	Pelican Banks	M	77.8	SA	329.74	4.70	8.98	2.16	40.18	0.00
QA58284	149084	13/7/15	Pelican Banks	M	92.2	A	476.59	19.72	17.05	1.85	8.19	0.00
QA58291	149090	11/7/15	Pelican Banks	M	94.1	A	385.15	104.06	29.90	1.61	46.20	0.00
QA58295	149089	11/7/15	Pelican Banks	F	83.8	SA	393.31	4.33	10.35	1.86	21.98	0.00
QA64251	152716	1/5/17	Pelican Banks	F	75.7	SA	192.62	4.81	12.34	2.12	58.37	0.00
QA64291	152717	1/5/17	Pelican Banks	M	76.2	SA	200.50	54.88	63.81	12.26	37.05	0.00
QA64318*	133758	24/6/17	Pelican Banks	F	94.6	A	1220.65	43.32	53.04	5.36	80.73	0.12
QA64830	157925	5/5/16	Boyne River	M	94.7	A	144.99	8.04	6.07	1.61	31.09	0.00
QA64930	157926	7/5/16	Western Basin	F	108.7	A	379.58	2.39	2.93	0.72	1.40	0.29
QA64931	157927	7/5/16	Western Basin	F	96.8	A	496.80	7.39	3.68	0.97	1.10	0.26
QA64932	157929	7/5/16	Western Basin	F	103.5	A	252.72	4.17	4.93	0.72	0.01	11.35
QA64933	157928	7/5/16	Western Basin	M	85.3	SA	623.51	37.85	14.30	2.19	2.82	5.49
QA65085	157933	8/10/16	Pelican Banks	M	96.4	A	447.75	45.32	39.05	5.61	49.76	0.00



**Appendix 1. continued.**

Primary Tag	Satellite Tag	Deployment Date	Capture Site	Sex	CCL (cm)	Age Class	Track Length (km)	Max. Displacement (km)	Home Range Area (km <sup>2</sup> , 95%UD)	Core Use Area (km <sup>2</sup> , 50%UD)	95%UD lying outside Port (% area)	95%UD overlapping shipping channels (% area)
QA65088	157931	8/10/16	Pelican Banks	M	101.7	A	425.81	6.88	26.28	4.55	60.91	0.00
QA65177	157930	7/10/16	Pelican Banks	F	114.6	A	253.93	5.81	11.05	1.44	62.96	0.00
QA66466	157934	9/10/16	Pelican Banks	M	92.9	A	306.86	4.08	10.21	1.76	46.55	0.00
QA66526	157935	9/10/16	Pelican Banks	F	97.3	A	436.91	153.52	115.41	6.12	93.23	0.00
QA84342	181367	3/7/19	Wiggins Island	M	45.1	J	1208.04	19.74	5.50	1.09	0.86	1.93
QA86025	40934	17/6/18	South Trees	M	96	SA	270.71	5.57	2.93	0.83	14.24	0.00
QA86189	61689	16/8/18	South Trees	M	98	A	429.39	6.07	6.42	0.70	0.59	0.00
QA86190	61690	16/8/18	South Trees	M	70.3	SA	285.94	2.66	2.42	0.26	2.30	0.00
QA86202	176006	19/6/18	Pelican Banks	M	77.2	SA	678.34	11.19	5.84	1.06	32.73	0.00
QA86247	61692	18/8/18	Wild Cattle Island	M	71.5	SA	858.40	5.00	7.59	1.44	2.60	0.00
QA86302	61691	18/8/18	Wild Cattle Island	M	91.3	A	504.75	8.98	7.09	0.75	5.64	0.00
QA87017	64747	6/10/18	South Trees	I	67.2	SA	124.81	10.53	3.33	0.22	4.01	7.96
QA87018	64748	6/10/18	South Trees	I	67.4	SA	68.74	1.91	0.88	0.14	3.25	14.13

**Appendix 1. continued.**

Primary Tag	Satellite Tag	Deployment Date	Capture Site	Sex	CCL (cm)	Age Class	Track Length (km)	Max. Displacement (km)	Home Range Area (km <sup>2</sup> , 95%UD)	Core Use Area (km <sup>2</sup> , 50%UD)	95%UD lying outside Port (% area)	95%UD overlapping shipping channels (% area)
QA87129	181368	3/7/19	Wiggins Island	M	76.6	SA	2210.29	32.78	2.28	0.47	1.72	0.00
QA91173	181369	2/7/19	Wiggins Island	M	47.1	J	1946.13	27.65	1.21	0.21	3.43	0.00
QA91603	181370	3/7/19	Wiggins Island	F	60.4	J	618.47	10.70	2.10	0.32	66.27	0.00
QA91605	181366	3/7/19	Wiggins Island	F	71.5	SA	1206.97	7.74	3.73	0.37	1.82	0.55
QA91639	194464	17/9/19	Wiggins Island	F	64.1	J	1255.32	37.72	4.45	0.86	1.01	12.01
QA91766	194460	14/9/19	Wiggins Island	F	99.0	SA	992.51	10.37	1.75	0.52	1.76	0.02
QA91767	194461	14/9/19	Wiggins Island	M	49.2	J	1034.30	11.50	3.00	0.57	1.32	0.00
QA91768	194462	14/9/19	Wiggins Island	F	45.1	J	615.25	5.64	2.69	0.27	0.14	0.00
QA91791	194463	17/9/19	Wiggins Island	F	83.4	J	1229.77	8.76	6.99	1.01	0.59	0.84
T83097	96780	30/6/10	Pelican Banks	F	104.4	A	655.56	18.65	27.92	3.15	36.15	2.49
T88971	157932	8/10/16	Pelican Banks	F	98.6	A	736.64	16.29	15.43	2.24	51.47	0.81

\*Travelled to courtship areas during tracked period. Breeding migration was excluded from data summaries.

Appendix 2. Summary of satellite tag data received pre-processing for Green turtles deployed in Port Curtis 2010-2020

Primary Tag	Satellite Tag	ARGOS Fixes	FGPS Fixes	Total Days Tracked (FGPS,ARGOS)	Mean ARGOS Fixes per Day	Mean FGPS Fixes per Day
K283	143704	2540	774	102, 101	25	8
K28651	149082	3166	858	181, 176	17	5
K70229	133767	1719	300	399, 397	4	1
K93085	72448	1873	295	214, 211	9	1
K93086	96781	1757	390	179, 174	10	2
K93087*	96777	1341	1114	241, 240	6	5
K93088	96778	2181	423	222, 219	10	2
QA13938	134182	3093	1448	202, 194	15	7
QA32523	133762	935	177	47, 41	20	4
QA33327	133765	1543	405	73, 67	21	6
QA33335	134180	713	121	102, 78	7	2
QA33342	133764	2339	509	122, 106	19	5
QA33348	133769	1314	231	58, 53	23	4
QA33349	133759	4106	1324	145, 140	28	9
QA33350	133763	1208	574	46, 42	26	14
QA33368	133760	1836	301	67, 62	27	5
QA33394	133758	1302	366	105, 67	12	5
QA34529	133761	1351	380	61, 55	22	7
QA36875	134178	2222	637	163, 162	14	4
QA43023	133770	2529	401	72, 67	35	6
QA43063	133768	2345	775	71, 66	33	12
QA43066	133766	1449	371	72, 64	20	6
QA43123	149087	2750	446	221, 217	12	2
QA45408	134179	192	22	88, 74	2	0
QA45524	134183	1639	260	168, 165	10	2
QA45554	134184	3005	859	202, 199	15	4
QA45566	134188	955	184	100, 78	10	2
QA45601	134185	2002	576	188, 184	11	3
QA45627	134181	3565	942	210, 209	17	5
QA45654	134186	989	249	260, 256	4	1
QA45689	134187	2107	797	120, 121	18	7
QA58206	149088	2279	379	142, 136	16	3

**Appendix 2. Continued.**

Primary Tag	Satellite Tag	ARGOS Fixes	FGPS Fixes	Total Days Tracked (FGPS,ARGOS)	Mean ARGOS Fixes per Day	Mean FGPS Fixes per Day
QA58209	149081	1745	546	97, 93	18	6
QA58210	149086	1511	547	185, 168	8	3
QA58211	149085	1838	356	132, 126	14	3
QA58221	149080	2359	604	175, 173	13	3
QA58239	149083	2148	450	163, 148	13	3
QA58284	149084	2321	761	127, 123	18	6
QA58291	149090	1630	530	144, 141	11	4
QA58295	149089	3192	693	159, 154	20	5
QA64251	152716	430	167	259, 260	2	1
QA64291	152717	206	48	191, 178	1	0
QA64318*	133758	1938	1267	98, 97	20	13
QA64830	157925	1479	682	126, 125	12	5
QA64930	157926	1831	922	145, 141	13	7
QA64931	157927	2065	911	86, 83	24	11
QA64932	157929	1139	533	78, 75	15	7
QA64933	157928	2829	1107	171, 166	17	7
QA65085	157933	1744	384	109, 107	16	4
QA65088	157931	1873	453	156, 154	12	3
QA65177	157930	1559	312	123, 114	13	3
QA66466	157934	1658	379	150, 148	11	3
QA66526	157935	1113	295	78, 74	14	4
QA84342	181367	299	590	188, 143	2	4
QA86025	40934	1388	591	125, 91	11	6
QA86189	61689	1423	839	75, 74	19	11
QA86190	61690	1906	931	178, 152	11	6
QA86202	176006	2058	753	189, 188	11	4
QA86247	61692	2685	952	114, 112	24	9
QA86302	61691	1611	835	116, 115	14	7
QA87017	64747	184	926	142, 138	1	7
QA87018	64748	141	746	171, 170	1	4
QA87129	181368	883	1075	242, 177	4	6
QA91173	181369	283	522	264, 192	1	3
QA91603	181370	732	1673	201, 137	4	12

**Appendix 2. Continued.**

Primary Tag	Satellite Tag	ARGOS Fixes	FGPS Fixes	Total Days Tracked (FGPS,ARGOS)	Mean ARGOS Fixes per Day	Mean FGPS Fixes per Day
QA91605	181366	654	1930	197, 151	3	13
QA91639	194464	159	480	158, 127	1	4
QA91766	194460	616	1924	104, 73	6	26
QA91767	194461	492	886	126, 95	4	9
QA91768	194462	213	539	121, 88	2	6
QA91791	194463	430	1519	127, 98	3	16
T83097	96780	894	549	168, 158	5	3
T88971	157932	3309	1108	160, 156	21	7

## CHAPTER 5

### GREEN TURTLE POPULATION DYNAMICS IN PORT CURTIS

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#### Introduction

The sGBR genetic stock that breeds on islands of the southern Great Barrier Reef (sGBR) and adjacent mainland dominates the Green turtle foraging populations on reefs, in coastal bays and estuaries and mangrove forests along the eastern Australian coast from approximately Cooktown southward to New South Wales. (Limpus *et al.* 2013). The spatial distribution of breeding sites of the sGBR stock has been defined (Dithmers *et al.* 2006; FitzSimmons *et al.* 1997a,b) along with the spatial distribution of the foraging areas supporting this stock: based on flipper tag recoveries (Limpus *et al.* 2009, 2013; Read *et al.* 2014) and by genetic stock identification (Norman *et al.* 1994; Read *et al.* 2015; Jensen *et al.* 2016). There is high fidelity of adult turtles to their respective localised foraging areas in which the turtles grew to maturity. This high site fidelity is maintained across multiple breeding migrations (Shimada *et al.* 2020).

This sGBR genetic stock is one of the most comprehensively studied Green turtle stocks globally with respect to demographic characteristics. The population structure with respect to gender, age class and breeding status has been described for multiple foraging areas: Shoalwater Bay (Limpus *et al.* 2005), Heron-Wistari Reefs (Limpus and Reed, 1985), Moreton Bay (Limpus *et al.* 1994). Gender and age class specific growth rates have been modelled for these localised foraging areas (Limpus and Chaloupka, 1997; Chaloupka, 2001a; Chaloupka *et al.* 2004). Analysis of long term capture-mark-recapture tagging data from selected localised foraging populations have provided estimates of a range of demographic parameters including population abundance and trends, recruitment, survivorship, and disease prevalence: Edgecombe Bay (Hof *et al.* 2017), Heron Reef (Chaloupka and Limpus, 2001, 2005), Hervey Bay (Twaddle, 2014), Moreton Bay (Limpus *et al.* 2016). Long term trends in annual abundance of nesting females at Heron Island has been described (Chaloupka *et al.* 2008). Limpus and Nicholls (2000) described the response of annual breeding abundance for sGBR Green turtles to the El Nino-Southern Oscillation climate cycle and Flint *et al.* (2017) have identified the regional role of river runoff as the primary driver of Green turtle mortality and strandings along the urban coast of Queensland. Chaloupka (2001b,c, 2002a,b, 2004) developed stochastic simulation population models for the sGBR Green turtle stock, integrating the comprehensive range of data for this genetic stock and facilitating exploration of management options for the stock.

Monitoring key demographic processes are essential for understanding the health status of marine turtle populations (Chaloupka 2003, Chaloupka 2004). One of those demographic processes parameters that can influence population dynamics include the body condition and somatic growth rates of individual turtles. Marine turtle body condition reflects the health status of an individual turtle and has important implications for reproductive output and long-term survival (Bjorndal *et al.* 2000). Body condition is usually estimated indirectly as a ratio of body mass and length using the Fulton condition index (Ricker 1975) — although other indices are possible (Falk *et al.* 2017). The Fulton body condition index is simple to estimate

and widely used in fisheries research (Ricker, 1975, Nash *et al.* 2006) and for exploring the density-dependent population dynamics of marine turtles (Bjorndal *et al.* 2000).

Marine turtle somatic growth dynamics are an accumulated response to a range of nutrition, population density and environmental factors (Chaloupka *et al.* 2004; Bjorndal *et al.* 2002; Bjorndal *et al.* 2017). Monitoring Green turtle somatic growth rates supports evidence-based conservation advice concerning the status of the sGBR Green turtle foraging populations (Chaloupka 2003) — including those populations that forage in the Port Curtis region.

Abundance is also a key demographic process for monitoring the health status of marine turtle populations exposed to anthropogenic hazards — and especially for diagnosing population trends (Bjorndal *et al.* 2000, National Research Council 2010), assessment of long-term population viability (Chaloupka, 2003b, 2004) and development of recovery plans (Chaloupka 2003a). Nearly all estimates of marine turtle trends and population status are based on beach-based monitoring of nesting females (Chaloupka *et al.* 2008) but marine turtles spend most of their lives in pelagic or coastal marine habitats (National Research Council 2010). Yet despite being subject to a long history of exploitation there are few reliable in-water estimates of abundance for any marine turtle population (Chaloupka and Limpus 2001; Bjorndal *et al.* 2005; Hof *et al.* 2017).

Recruitment is also fundamental to understanding the population dynamics of a long-lived species such as marine turtles that are exposed to a range of anthropogenic hazards (Chaloupka 2003b). There are surprisingly few estimates of age- or stage-specific recruitment for any marine turtle population (Parmenter *et al.* 1995; Chaloupka, 2003a, b; Dobbs *et al.* 2007; National Research Council 2010; Caillouet *et al.* 2011). Most attempts to estimate recruitment to the breeding component of a marine turtle population have used laparoscopy to determine whether an adult-sized female turtle had either bred in the previous season or was preparing to breed in the coming season (Limpus and Limpus, 2002; Limpus *et al.* 2005; Dobbs *et al.* 2007). Recruitment measures that are applicable to the modelling marine turtle population dynamics (Chaloupka 2003a, b, 2004) can be derived from a capture-mark-recapture-based sampling coupled with the reverse-time or temporal symmetry modelling approach developed originally by Pradel (1996) — and furthered by Pradel *et al.* (1997) and Nichols *et al.* (2016).

The population dynamics of the Green turtle foraging population within Port Curtis will be explored within the context of this broad understanding of the population dynamics of the sGBR Green turtle stock.

## Methods

The general methods applied in the capture, tagging, data recording for description of this foraging Green turtle population in Port Curtis are described in Chapter 1.

### Trends in Body Condition

The Fulton body condition index (BCI) was calculated using body mass (kg) and Curved Carapace Length (CCL) (cm) for 1068 Green turtles of known age class, sex and habitat sampled in the Port Curtis region from 2016-2019. Age class- and sex-specific BCIs were also calculated for 3474 Green turtles sampled from 2 additional coastal foraging populations of the sGBR Green turtle genetic stock for comparison — at Shoalwater Bay and Moreton Bay (see Chaloupka *et al.* 2004, 2004).

A generalised linear mixed model (GLMM, Wood 2006) with a Student-*t* likelihood was fitted to the Port Curtis Green turtle BCI data accounting for year, age class, sex and spatial

(habitat) effects. A random-effects model (GLMM) was used to account for turtle-specific variability since ca 17% of the 1275 BCIs represented multiple recordings for some turtles. . The GLMM was fitted within a Bayesian modelling framework using the Stan computation engine (Carpenter *et al.* 2017) via the brms R package interface (Bürkner 2017). A Student-*t* likelihood was used to minimise outlier effects on parameter estimates (see Bjoerndal *et al.* 2017, 2019) and was a significantly better fit than using a GLMM with Gaussian likelihood. The probability of an effect was determined using the effect existence metrics proposed by Makowski *et al.* (2019a) and implemented in the bayestestR package for R (Makowski *et al.* 2019b). All model fit summaries are displayed using the ggplot2 package for R (Wickham 2016).

### Somatic Growth

The data set comprised 177 growth rate estimates for 164 uniquely tagged Green turtles sampled in the Port Curtis region. Absolute growth rates were derived from the capture-mark-recapture profiles for individual turtles captured from 2010 through 2019 and with a minimum of eight months between successive captures. The turtles ranged in size from juvenile recruits at 40 cm CCL to mature adults at 118 cm CCL. Recapture intervals ranged from around 1-7 years. Negative growth rates were included as there is no cause to discard them (Chaloupka *et al.* 2004). This is a small data set and so modelling based on Bayesian inference presented below is especially applicable here.

A generalised additive mixed model (GAMM, Wood 2006) with a Student-*t* likelihood was fitted to the Port Curtis Green turtle somatic growth rates accounting for year, year within sex, body size (proxy for age) and recapture interval. The specific class of GAMM used here is a distributional regression form of GAMM to simultaneously model both the mean growth and the growth variance (Stasinopoulos *et al.* 2018). A random-effects structure was also included to account for turtle-specific variability due to multiple growth measurements for most turtles. Thin plate regression spline smooths (Wood 2006) were used to account for any nonlinear functional form between mean somatic growth rates and the potentially informative covariates such as sampling year within sex and body size (age). This distributional GAMM was fitted here within a Bayesian modelling framework using the Stan computation engine (Carpenter *et al.* 2017) via the brms R package interface (Bürkner 2017). A Student-*t* likelihood was used to minimize any outlier effects on parameter estimates and was a better fit based on the leave-one-out cross-validation approach (Vehtari *et al.* 2017) than using a distributional GAMM with Gaussian likelihood. See Bjoerndal *et al.* 2019 for a recent example for modelling Green turtle growth dynamics using this approach. All model fit summaries are displayed using the ggplot2 package for R (Wickham 2016).

### Population Size

Capture-mark-recapture (CMR) histories were compiled for 810 Green turtles caught and tagged within Port Curtis region foraging habitats over the 7-year period from 2013-2019. The 810 Green turtles were of known age class/sex and sampled specifically from the Boyne Island and Pelican Banks areas. Each turtle was also assigned to a 2-level size class factor: (1)  $\leq 65$  cm CCL or (2)  $> 65$  cm CCL to simplify analysis for a small data set.

A random-effects Cormack-Jolly-Seber (CJS) statistical modelling approach that accounts for individual heterogeneity in survival and/or recapture was used to estimate key demographic parameters (Gimenez and Choquet 2010). There are no established procedures for assessing CJS random-effects model goodness-of-fit (Gimenez and Choquet 2010). Therefore, an *ad-hoc* approach based on comparison with various fixed effects CJS-type models (Lebreton *et al.* 1992) that have known goodness-of-fit metrics was used to help



assess model fit. Specifically, CJS model assumptions such as transience (seen once and never again), capture heterogeneity (known as trap-dependence) and goodness-of-fit were evaluated using various test procedures (Choquet *et al.* 2009) implemented in the R2ucare package for R (Gimenez *et al.* 2017) as well as the Fletcher  $\hat{c}$  estimate of goodness-of-fit (Fletcher 2012) in the program MARK (White *et al.* 2006). A time-since-marking model structure was also used to account for transient behaviour with separate survival probability estimates for newly and previously tagged turtles (Chaloupka and Limpus 2002). The random effects model approach (Gimenez and Choquet 2010) was used to account for capture heterogeneity. All random and fixed effects CJS models were fitted using program MARK (White *et al.* 2006) via the RMark package for R (Laake 2013). Model selection was based on an information-theoretic approach with the Akaike Information Criterion corrected for sample size to determine model parsimony and support statistical inferences (Burnham *et al.* 2011). The best-fit model was used to estimate recapture and apparent survival probabilities. Annual population size was estimated by applying a Horwitz-Thompson-type estimator using those recapture probabilities (Chaloupka and Limpus 2001, Bjørndal *et al.* 2005) with nonparametric bootstrap-based variance estimates of the annual population size estimates (Madon *et al.* 2013: with recent R code corrections by O. Gimenez).

### Recruitment

A simple recruitment metric was defined here for convenience to refer to any entry into the population between marking periods of any unmarked turtles. Thus recruitment measures the first time that a previously undetected or unmarked turtle of any age class or gender was estimated to have entered the Port Curtis resident Green turtle population. This is also known as a per capita recruitment rate, which in this study, there is no distinction between either age class or gender.

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A range of Pradel temporal symmetry models parameterized in terms of per capita recruitment and accounting for individual capture heterogeneity were fitted to the 810 capture histories (Pradel *et al.* 2009, Marescot *et al.* 2011). All models were fitted using the MARK computation back-end (White *et al.* 2006) via the RMark package for R (Laake 2013). Model selection was then based on using an information-theoretic approach with the Akaike Information Criterion corrected for sample size to determine model parsimony and support statistical inferences (Burnham *et al.* 2011).

### Breeding Biology

The size of annual breeding populations for Green turtles throughout eastern Australia and extending into southeast Asia fluctuates in response to the El Niño-Southern Oscillation (ENSO) climate fluctuations (Limpus and Nicholls, 1988, 2000; Limpus *et al.* 2003, 2005, 2013; Chaloupka, 2001d):

- In eastern Australia there is approximately an 18 month lag between the climate signal and the proportion of the breeding female population coming ashore on beaches for nesting.

- There is strong synchrony in fluctuations in annual breeding numbers across widely spatially separated sGBR and nGBR genetic stocks.
- There is strong synchrony in annual fluctuations in Green turtle breeding numbers at nesting beaches supporting the sGBR genetic stock.
- There is strong synchrony in annual fluctuations in the proportion of adult female and male Green turtles preparing for breeding from dispersed foraging areas and the size of the annual breeding population at Heron Island, the primary index beach for monitoring breeding within the sGBR genetic stock.

Green turtles from numerous widely dispersed foraging areas migrate each year to breed on the sGBR Capricorn-Bunker cays (Limpus *et al.* 2013). Heron Island, within the Capricorn Group, is the primary index site for monitoring trends in the size of the annual sGBR Green turtle breeding population (Limpus, 2007).

Therefore, the annual fluctuations in the annual Green turtle breeding population at Heron Island will be used as the index of the annual fluctuations for the combined breeding populations across the entire foraging range for the sGBR genetic stock. The long-term breeding census data from the Heron Island nesting population and from Shoalwater Bay and Moreton Bay as representative foraging populations within the sGBR genetic stock are derived from the DES Queensland Turtle Conservation (QTC) Data Base. These data will form the basis for comparison of the variations in annual breeding rates of Green turtles foraging within Port Curtis with those of the more widely distributed foraging population for the sGBR genetic stock.

## Results

### Trends in Body Condition

The GLMM with Student-*t* likelihood was a good fit to the Port Curtis BCI data accounting for > 55% (95% credible interval: 50% - 60%) of the model deviance (Bayesian R-squared, see Gelman *et al.* 2019). The age class:sex interaction was a significant effect and shown in the top panel of Figure 5.1 (with 95% credible or uncertainty intervals). A sex-specific effect is apparent for the adult age class but there was no sex-specific difference for juveniles. BCI was significantly higher for Green turtles sampled in the Western Basin than in the 4 other areas (bottom panel of Figure 5.1). In fact, there was a > 98% probability that the expected (mean) Western Basin BCI was higher than at One Tree (the next highest area for BCI (bottom panel of Figure 5.1). There was only limited temporal variation in expected BCI over the 4-yr period (2016-2019) with a > 94% probability of a lower mean BCI in 2018 compared to the other years. Figure 5.2 shows the summary for the similar model fit to the 3 sGBR coastal foraging populations (Shoalwater Bay, Moreton Bay, Port Curtis). Age class specific BCI was higher for the Moreton Bay foraging population and then lower for Shoalwater Bay when compared to the population resident in the Port Curtis region.

### Somatic Growth

Despite the limited sample size, the distributional GAMM with Student-*t* likelihood was a good fit to the Port Curtis somatic growth rate data accounting for > 46% (95% credible interval: 21% - 63%) of the model deviance (Bayesian R-squared, see Gelman *et al.* 2019). There was no sex-specific effect (Figure 5.3a, c) and only a weak annual effect (Figure

5.3d). There was some evidence that the variance of the growth rates increased over the years (Figure 5.3f). Importantly, the expected size-specific growth rate function was non-monotonic increasing rapidly from ca 40 cm CCL at recruitment to the benthic habitat and peaking at around 60 cm CCL (Figure 5.3b) which is a similar general trend to other sGBR Green turtle foraging populations (Chaloupka, 2004). The expected size-specific growth rate function derived from this model (Figure 5.3b) was then integrated numerically to derive the size-at-age curve (Figure 5.4a) and that size-at-age curve was then differentiated numerically to derive the age-specific growth rate curve shown in Figure 5.4b (see Chaloupka *et al.* 2004 for details). Figure 5.5 shows the size-specific growth rate curve for the Port Curtis Green turtle population compared with sizes-specific growth rate curves for sGBR Green turtle foraging populations at Moreton Bay and Shoalwater Bay, and one foraging population at Clack Reef, which comprises ~50% of Green turtles from the sGBR stock (Jensen *et al.* 2016).

### Population Size

There were no significant departures from the time-dependent CJS model assumptions (TESTS 2 + 3:  $X^2 = 14.4$ ,  $df = 12$ ,  $P = 0.27$ ). The best-fit model comprised: (1) survival rates that were a function of size class and time-since-marking and (2) time-dependent recapture probabilities with significant individual capture heterogeneity ( $\hat{\alpha}_p = 0.96$ , 95% CI: 0.49-1.89). The Fletcher  $\hat{c}$  estimate for this model was 1.05, suggesting an adequate fit to the 810 individual CMR histories. The overwhelming weight of evidence was in support of this model compared to the other 22 models fitted to these CMR histories. The best-fit model accounted for > 57% of the weight of evidence for these data (next best model = 21%). The time-dependent recapture probabilities from that model were used to derive the annual population size estimates for the two specific sampling sites within the Port Curtis region (Boyne Island, Pelican Banks). The same recapture probabilities were used for the population estimates since there was no evidence to support the need for site-specific recapture probabilities. The site-specific population size estimates from 2016 onwards are shown in Figure 5.6 since the recapture probabilities for those years were estimated with higher precision compared to the earlier years with sparse CMR histories.

### Recruitment

The best-fit Pradel temporal symmetry model comprised: (1) size class-specific survival, (2) time-dependent recapture probabilities and (3) sample-site-specific per capita recruitment rates. The overwhelming weight of evidence (> 99%) was in support of this model compared to the other nine models fitted to these CMR histories. The site-specific recruitment rates derived from this model are shown in Figure 5.7a with the annual recruitment rates shown in Figure 5.7b. It is apparent that the estimated rate of detecting a previously undetected (unmarked) Green turtle was high, and especially so at the Boyne Island sampling site (Figure 5.7a). The annual recruitment rate estimates combined for both sampling sites ranged from ca 0.20 to 0.70 (Figure 5.7b), which are also very high and not reflective of demographically relevant estimates of recruitment (see Chaloupka, 2003a, b, 2004; National Research Council, 2010). These unreasonably high “recruitment rate” estimates are a function of low recapture probabilities that are themselves due to a large resident population.

### Breeding Biology

The proportion of resident adult female and male Green turtles foraging within Port Curtis that were in preparation for breeding (commencing vitellogenesis for females, commencing spermatogenesis for males) during the 2013 to 2019 breeding seasons ranged from 10% to 29% for females and from 12% to 49% for males (Table 5.1).

The modest annual sample sizes of captured adult male and female Green turtles limited the capacity for comprehensive analyses of breeding rates by site specific foraging areas within the Port at this time.

As expected, the variability in annual breeding rate of adult male Green turtles foraging within Port Curtis (Table 5.1, Figure 5.8A) fluctuated approximately in synchrony with the variation in annual breeding rate of Green turtles recorded at Heron Island, the primary index site for monitoring sGBR Green turtle breeding.

In contrast, the annual breeding rates of adult female Green turtles foraging in Port Curtis did not fluctuate in synchrony with the fluctuations in breeding rates of the adult males during 2016-2019, nor did they fluctuate in approximate synchrony with the variation in annual breeding rates of Green turtles recorded at Heron Island (Figure 5.8B). However, the fluctuations in annual breeding rates of the adult females foraging in Port Curtis fluctuated approximately in synchrony with the variation in annual breeding rates of Green turtles recorded at Heron Island during 2013-2016.

## Discussion

### Trends in Body Condition

Body condition of foraging turtles is influenced by the quality of available food resources, aspects of foraging behaviour and underlying health issues. There was a broad range in the body condition index (BCI) within size classes, with no difference between sexes for the juvenile and subadult turtles, but adult females had a greater BCI compared to adult males. This applied for all foraging areas examined. Spatial and temporal variation in BCI was detected within Port Curtis. Spatially, BCI was significantly higher for Green turtles sampled in the Western Basin than elsewhere in the Port followed by turtles at South Trees. While there was only limited temporal variation in estimated BCI over the 4 yr period (2016-2019), there appeared to be a lower mean BCI in 2018 than in the other years within Port Curtis, in comparison to two other coastal foraging areas also used predominantly by the sGBR stock. Whether the lower BCI for Port Curtis in 2018 was a consequence of the localised high flood level in Gladstone in early 2017 was not investigated.

Port Curtis displayed an intermediate BCI across all three age classes with age class-specific BCI highest in Moreton Bay and lowest in Shoalwater Bay when compared to the resident foraging population in Port Curtis. A previous study of immature Green turtles foraging in the Caribbean found that body condition was positively correlated to individual growth rates (Bjørndal *et al.* 2000), and negatively correlated to population density. We did not have adequate data to analyse whether these correlations apply to turtles in Port Curtis. Differences in diet at the Port Curtis study sites will be examined in Chapter 6.

### Somatic Growth

Juvenile Green turtles within the sGBR stock recruit from the open ocean pelagic life history stage to benthic foraging at an approximately uniform size across to all foraging areas sampled from 20°S to 27°S (Table 5.2). As a consequence, Green turtles within south-central Queensland foraging areas can be expected to commence growth within coastal habitats at a uniform starting size.

The Green turtles resident in foraging habitats of Port Curtis grew more slowly at any given size or age compared with turtles foraging in Moreton Bay, Heron-Wistari Reefs and Shoalwater Bay, which are also predominantly turtles from the sGBR Green turtle genetic stock (Figure 5.5).

The mean size at which Green turtles commence their breeding life is significantly different depending on the specific foraging area in which the turtles live and grow (Table 5.3). There appears to be no longitudinal gradient, with a presumed north-south temperature gradient, across these foraging areas that could account for this variability of size at 1<sup>st</sup> breeding. Green turtles living in Port Curtis and Shoalwater Bay commence breeding at a smaller size than those living in Heron-Wistari Reefs and Moreton Bay. Comparing growth rates and size at 1<sup>st</sup> breeding from these sites and Clack Reef (14°S. Chaloupka *et al.* 2004; present study Figure 5.5) it appears that Green turtles commence breeding at a larger mean size in habitats that support rapid growth while they commence breeding at a smaller size in habitats which support slow growth. Therefore, turtles growing to maturity in habitats supporting fast growth rates and large CCL at 1<sup>st</sup> breeding may not be very different in age at first breeding than turtles growing to maturity in habitats supporting slow growth rates and small CCL at 1<sup>st</sup> breeding.

This poor growth performance and associated small size at commencement of breeding might reflect suboptimal foraging habitats in Port Curtis.

### Population Size

Moderate to high water turbidity which is a long term, pervasive feature of Port Curtis and the temporal variability in availability of turtles within the study sites resulted in sub-optimal numbers of turtles being captured and tagged at most study areas within Port Curtis. Given these limitations, it was only possible to estimate population size for turtles using the foraging grounds at Pelican Banks and the Boyne Island area. It has been established that there are significant numbers of Green turtles resident within these areas of Port Curtis and the site-specific abundance trends have been relatively constant over the 4-year period from 2016 onwards. The overall 4-year mean population size combined for both sites (Figure 5.6) is estimated at 1170 Green turtles (95% credible interval: 1154-1186). The other foraging area study sites within Port Curtis, apart from Boyne Island and the Pelican Banks, were inadequately sampled for effective CMR analyses. However, based on sightings of turtles, it appears that there are also large number of turtles using especially the South Trees and Wild Cattle sites. It is evident that the total resident foraging population within Port Curtis will number in the many thousands of Green turtles.

In contrast with the other sGBR Green turtle foraging populations in south and central Queensland that are increasing in population size, the foraging population at Pelican Banks and off Boyne Island is stable at best (Table 5.4).

### Recruitment

Recruitment has not been a well estimated demographic parameter for the Port Curtis Green turtle foraging population using the current capture-mark-recapture-based sampling program. For the purpose of the analyses, recruitment was considered to have occurred for any turtle upon its first capture.

However, using identification of recently recruited juvenile Green turtles from the open ocean, which have shifted from a pelagic life history phase to benthic foraging phase within Port Curtis, the proportion of new recruits to the small immature age class of the population occurred at the rate of 0.14 per annum during 2016-2019 (Chapter 2).

Similarly, using visual examination of gonads of adult females via laparoscopy to identify females in vitellogenesis for their first breeding season (Limpus and Limpus, 2003; Dobbs *et al.* 2007), the rate of recruitment of new breeding females into the adult female foraging population of Port Curtis during 2016-2019 was 0.10 per annum (Chapter 2).

## Breeding Biology

Across Australasia the proportion of adult Green turtles that prepare for breeding in any one year is strongly linked to the regional ENSO climate cycle (Limpus and Nicholls, 2000; Limpus *et al.* 2003; Chaloupka, 2001d). Breeding rates decline 1.5 to 2 years following La Nina events (flood years) and increase 1.5 to 2 years after El Nino event (drought years). Heron Island is the principal index monitoring site for breeding of the sGBR Green turtle genetic stock (Limpus, 2007; Limpus *et al.* 2013). The turtles nesting at Heron Island migrate from throughout the foraging range for the stock and provide an index of the annual pooled breeding rates from all represented foraging areas. The annual breeding numbers at Heron Island are strongly correlated with the variation in ENSO climate data (Limpus and Nicholls, 2000). Quantified breeding rates of adult males and females recorded in foraging areas for the sGBR stock show approximate synchrony of annual fluctuations between the sexes (Limpus *et al.* 2005, 2013).

There has been close synchrony of the annual fluctuations in male and female breeding rates within the sGBR Green turtle stock recorded at multiple foraging areas (Shoalwater Bay and Moreton Bay) and the annual fluctuations in the size of the female breeding population nesting at Heron Island (Limpus *et al.* 2013; Figure 5.9). The annual fluctuations in adult male breeding rates recorded in Port Curtis show comparable synchrony with the previous records of male Green turtles foraging in Shoalwater Bay and Moreton Bay and the female breeding rate recorded at Heron Island (Figure 5.8A, 5.9). In contrast, there has been a marked lack of synchrony of fluctuations in adult female breeding rate within Port Curtis during the three year period 2017-2019 and the approximately synchronous fluctuations in annual breeding rates previously recorded for females foraging at Shoalwater Bay and Moreton Bay and nesting at Heron Island. (Figure 5.8B, 5.9)

The adult female foraging population in Port Curtis has displayed anomalous breeding rates during 2017-2019 relative to other monitored populations within the sGBR stock. No such anomalous breeding rate is evident for the adult male population within Port Curtis during the same period.

In summary, although a substantial number of foraging Green turtles continue to reside within Port Curtis, collectively the population has functioned at a suboptimal level during the last decade, at least:

- Low somatic growth across all age classes.
- Lack of synchrony between adult female breeding rates within Port Curtis and adult female breeding rates at other foraging areas supporting sGBR Green turtle foraging populations.
- Non-increasing foraging population size within Port Curtis.
- Lower Body Condition Index in Port Curtis than in Moreton Bay but higher than in Shoalwater Bay.

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Table 5.1. Annual breeding rate of adult female and male Green turtles (*Chelonia mydas*) that forage in Port Curtis.

<b>Samples</b>	<b>No. of adults preparing for breeding.</b>	<b>Total no. of turtles assessed</b>	<b>Proportion of adults breeding (95% CI)</b>
<b>Females</b>			
<b>2013</b>	6	22	0.29 (0.12-0.47)
<b>2014</b>	3	37	0.10 (0.02-0.20)
<b>2015</b>	4	50	0.10 (0.03-0.18)
<b>2016</b>	9	74	0.13 (0.06-0.21)
<b>2017</b>	9	76	0.13 (0.06-0.20)
<b>2018</b>	4	44	0.11 (0.03-0.20)
<b>2019</b>	2	22	0.12 (0.02-0.25).
<b>Males</b>			
<b>2014</b>	4	25	0.19 (0.05-0.33)
<b>2015</b>	-	-	-
<b>2016</b>	4	26	0.18 (0.05-0.32)
<b>2017</b>	19	49	0.39 (0.26-0.53)
<b>2018</b>	2	29	0.10 (0.01-0.20)
<b>2019</b>	5	12	0.43 (0.19-0.68)

Table 5.2. Comparison of the curved carapace length (CCL, cm) for male and female Green turtles (*Chelonia mydas*) recorded as recent recruits to foraging in Port Curtis (present study) with recent recruits to other coastal foraging areas in central and eastern Queensland.

(after Limpus et al. 2013 - QTC turtle database).

Locality	Latitude	Diet	Female CCL			Male CCL		
			Mean $\pm$ SD	Range	N	Mean $\pm$ SD	Range	N
Shoalwater Bay	22°S	Mostly seagrass + algae & mangrove	43.58 $\pm$ 2.705	37.7 – 67.3	77	43.10 $\pm$ 2.179	38.8 – 47.5	39
Heron-Wistari Reefs	23°S	Algae	43.58 $\pm$ 2.705	37.4 – 48.6	50	43.67 $\pm$ 2.463	36.5 – 49.3	41
Port Curtis	23°S	Mostly seagrass + algae & mangrove	43.18 $\pm$ 1.975	37.6 – 48.1	79	43.29 $\pm$ 1.906	39.6 – 47.0	38
Moreton Bay	27°S	Mostly seagrass + algae & mangrove	44.23 $\pm$ 3.966	38.2 - 73.4	98	45.06 $\pm$ 3.236 *	39.4 – 55.4	54
<b>ANALYSES</b>								
Testing gender vs site		One way ANOVA	<b>F<sub>3,300</sub> = 1.846; 0.5 &gt; p &gt; 0.2; not significant</b>			<b>F<sub>3,168</sub> = 5.375; 0.005 &gt; p &gt; 0.002; significant</b>		
			43.39 $\pm$ 3.037	37.4 – 73.4	304	43.89 $\pm$ 2.678	36.5 – 55.4	172;
Testing all females vs all males								
		One way ANOVA	<b>F<sub>1,474</sub> = 0.555; p &gt; 0.5; not significant</b>					
				<b>Mean <math>\pm</math> SD</b>	<b>Range</b>	<b>N</b>		
				43.761 $\pm$ 2.911	36.5 – 73.4	476		

Table 5.3. Comparison of the size at which adult female Green turtles (*Chelonia mydas*) from the sGBR genetic stock commence their breeding life in eastern Queensland foraging areas.

Data derived from Limpus *et al.* 2013 (QTC data base) and the present study (Chapter 2, Table 2.4B).

Foraging area (study years)	Latitude	Diet	Curved carapace length (cm)			
			Mean	SD	Range	N
<b>Repulse Bay</b> (1987-1989)	20°S	Mostly seagrass + algae & mangrove	103.66	4.940	96.2-107.8	5
<b>Shoalwater Bay</b> (1987-2007)	22°S	Mostly seagrass + algae & mangrove	98.75	5.790	87.1-115.5	92
<b>Heron-Wistari Reefs</b> (1984-1999)	23°S	Algae	102.72	3.225	96.0-109.6	35
<b>Port Curtis</b> (2013-2019)	23°S	Mostly seagrass + algae & mangrove	99.18	4.502	93.0-106.5	8
<b>Moreton Bay</b> (1990-2007)	27°S	Mostly seagrass + algae & mangrove	108.69	4.555	95.1-116.6	32
<b>ANALYSIS</b>			One way ANOVA	<b>F<sub>4,167</sub> = 23.78 p &lt; 0.001; significant</b>		

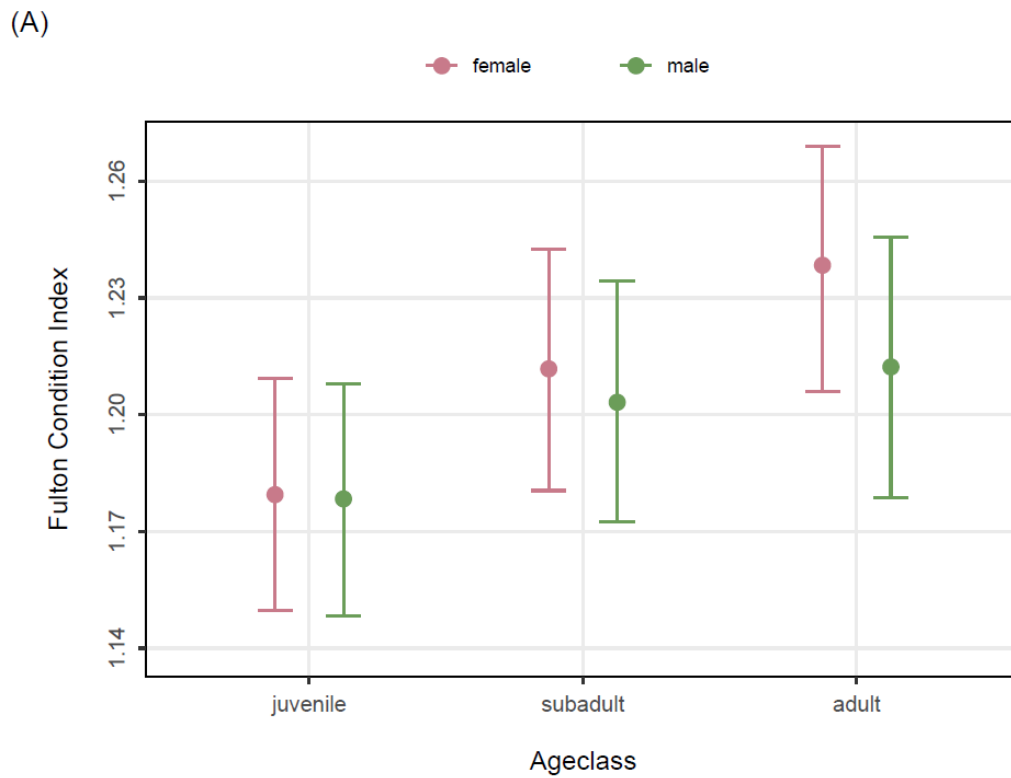
Table 5.4. Demographic parameters quantified by capture-mark-recapture flipper tagging studies in foraging areas in south and central Queensland dominated by sGBR Green turtle (*Chelonia mydas*) genetic stock.

Foraging area	Edgecombe Bay	Heron-Wistari Reefs	Port Curtis: Pelican Banks & Boyne Is flats	Hervey Bay: Booral	Moreton Bay: Eastern Banks
References	Hof <i>et al.</i> 2017	Chaloupka & Limpus, 2001, 2005 Limpus <i>et al.</i> 2005	Present study	Twaddle, 2014	Limpus, Jones & Chaloupka, 2016
Latitude	20°S	23°S	23°S	25°S	27°S
Study period	2003 - 2014	1985–1992.	2016-2019	2006 - 2013	1991 - 2014
Population trend	Increasing at 8.3% pa	Increasing at 11% pa	-	increasing	increasing very strongly
Adult	-	Increasing: 14.4% pa; 95% CI: 11.4–17.5	Pelican Banks: declining Boyne Island: stable	Stable?: +7.8% pa; 95% CI: -10.1% - 29.3%	-
subadult	-	Increasing: 6% pa; 95% CI: 4.3–7.8			-
Juvenile	-	Increasing: 14% pa; 95% CI: 12.9–15.1			significant increase: 32.3% pa; 95% CI:17.0% - 49.9%

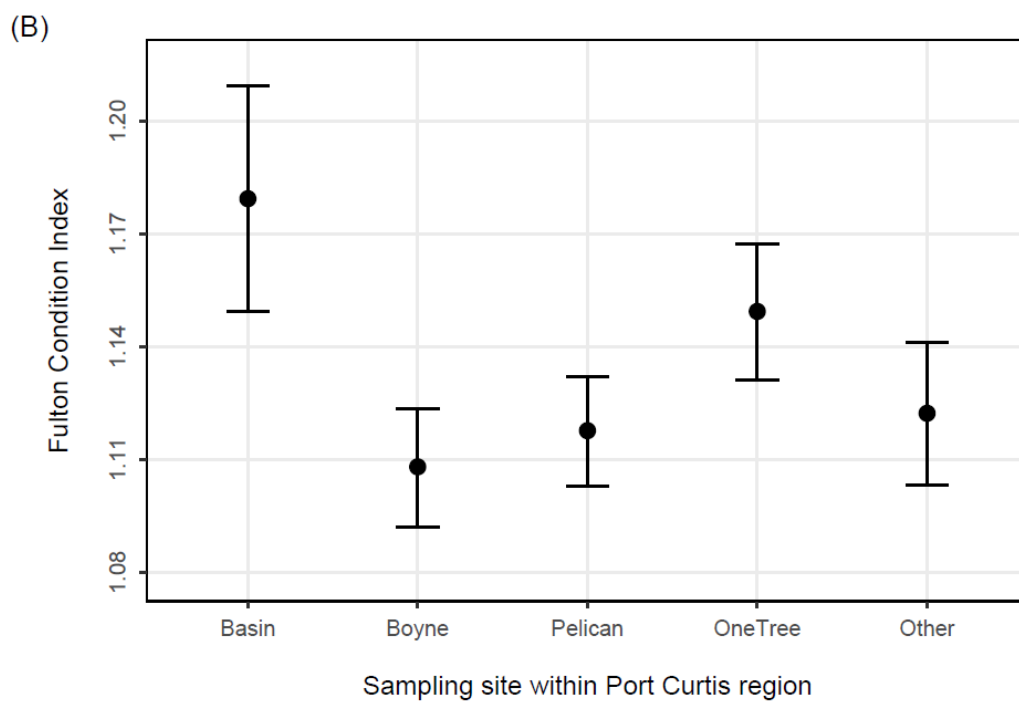
Table 5.4 continued

Foraging area	Edgecombe Bay	Heron-Wistari Reefs	Port Curtis: Pelican Banks & Boyne Is flats	Hervey Bay: Booral	Moreton Bay: Eastern Banks
<b>Survivorship per annum</b>	0.90, combined ages	no sex-specific differences by age class	-	-	no sex-specific differences by age class
<b>Adult</b>	-	0.95 (95% CI: 0.92–0.98)	-	0.961 (95% Ci: 0.94 – 0.98)	0.96 (95% CI: 0.93 - 0.97); without GTFD tumours.
<b>Subadult</b>	-	0.85 (95% CI: 0.79–0.91)	-		0.89 (95% CI: 0.76 - 0.95); with GTFD tumours
<b>Juvenile</b>	-	0.88 (95% CI: 0.84–0.93)	-	0.822 (95% Ci: 0.78 – 0.87)	0.88 (95% CI: 0.86 - 0.89); without GTFD tumours. 0.81 (95% CI: 0.76 - 0.86); with GTFD tumours





**5.1A The estimated age class- and sex-specific body condition index.**



**5.1B. The estimated sample-site-specific body condition index**

Figure 5.1. Body condition index for sampled Green turtles foraging within Port Curtis.

Solid dot = posterior mean, vertical bar = 95% credible interval.

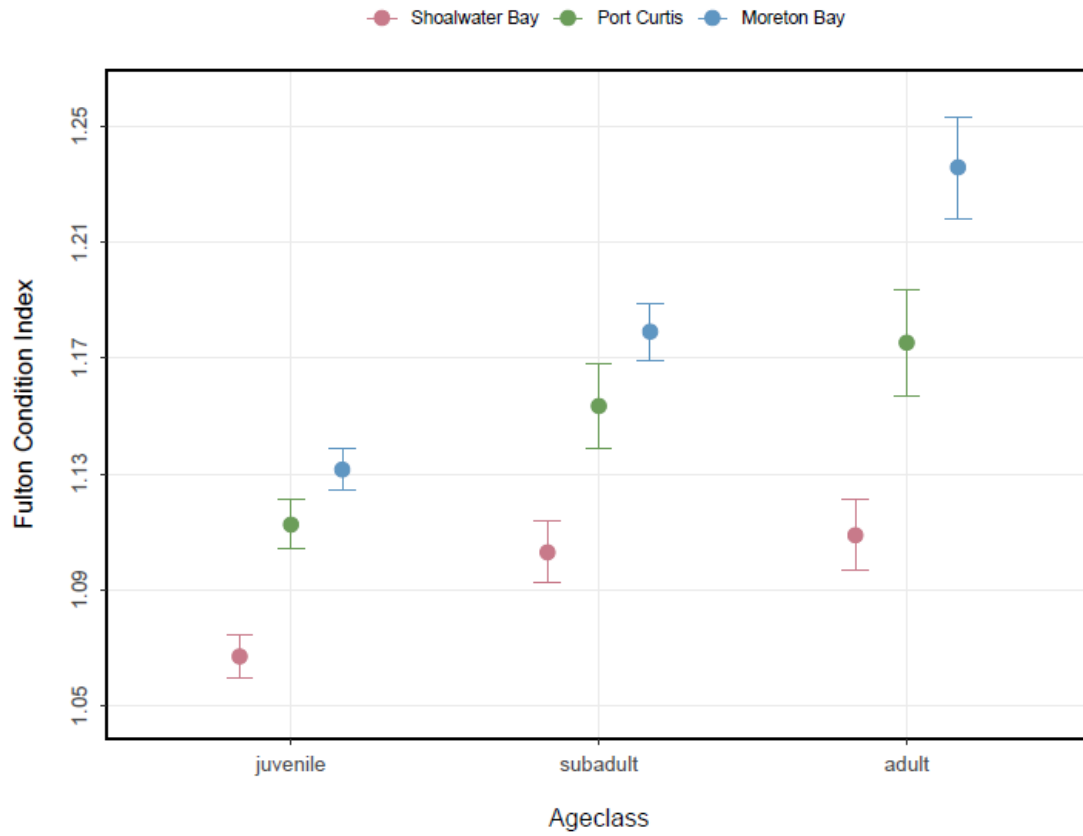


Figure 5.2. Estimated age class-specific body condition index for turtles sampled in three coastal foraging populations of the sGBR Green turtle genetic stock.

Solid dot = posterior mean; vertical bar = 95% credible interval.

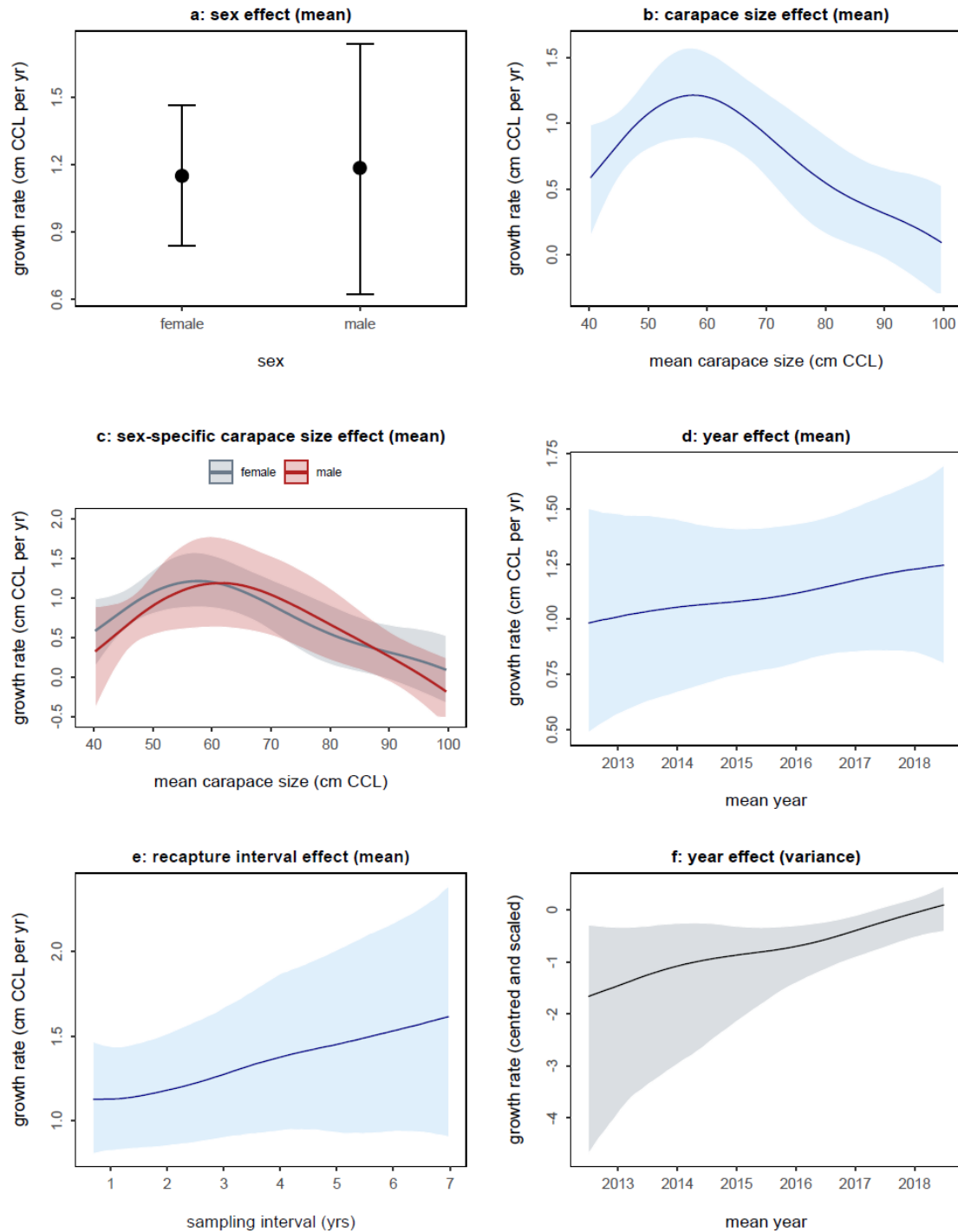
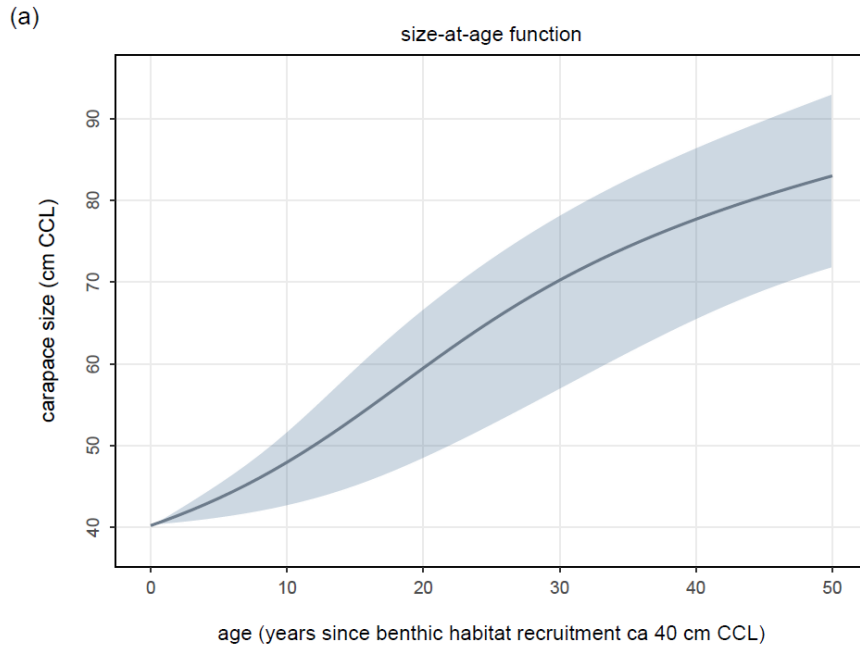
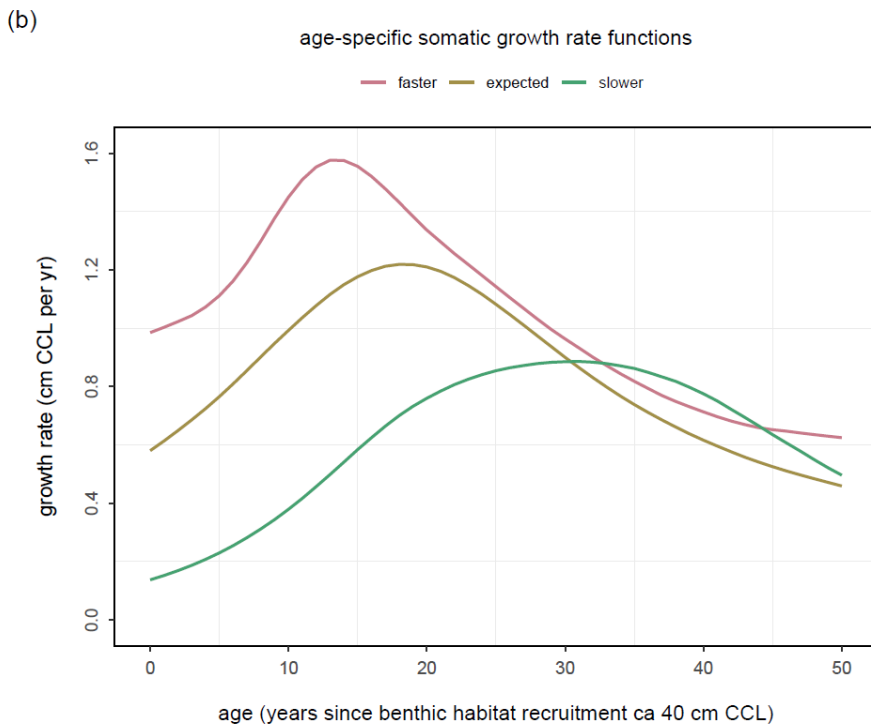


Figure 5.3. Distributional regression model summary for the covariates used as mean predictors (a-e) and variance predictor (f) for Green turtle growth dynamics within Port Curtis.

The response variable for the estimated mean effects are growth rate (cm CCL per year) while the estimated variance effect for year (f) is the growth rate variance that is centred and scaled. Solid curves are the smoothing spline fits conditioned on all other covariates. Shaded areas are bounded by pointwise 95% credible intervals around the fits.



**5.4A Estimated size-at-age curve (solid curve) with the 95% confidence interval shown by the shaded polygon. Age = years since recruitment to the benthic habitat from the oceanic or pelagic developmental phase.**



**5.4B The age-specific growth rate functions derived by numerical integration of the mean curve (expected), upper confidence curve (faster) or the lower confidence curve (slower) shown in panel.**

Figure 5.4. Growth of sampled Green turtles foraging within Port Curtis

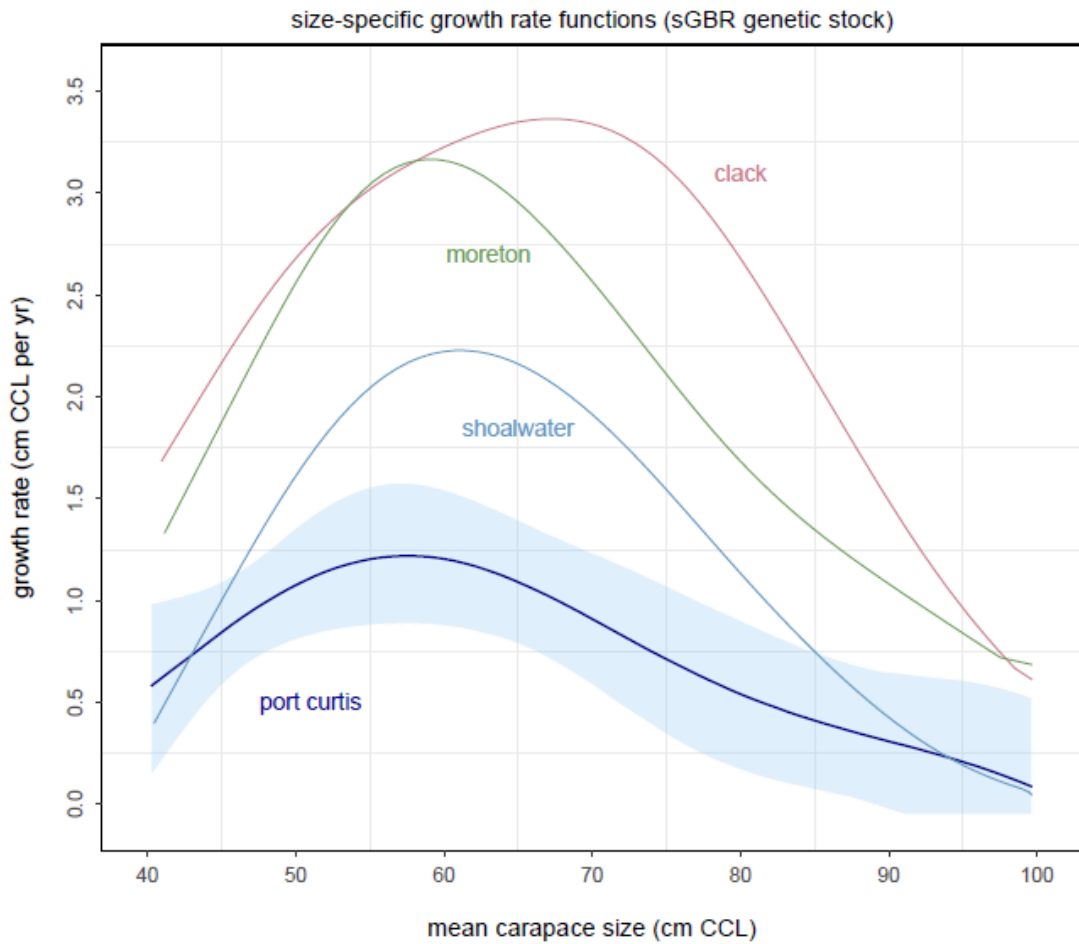


Figure 5.5. Comparison of the size-specific somatic growth rate curves for four foraging populations of the sGBR Green turtle genetic stock including three coastal foraging populations (Port Curtis, Shoalwater Bay, Moreton Bay) and one northern Great Barrier Reef coral reef foraging population (Clack Reef) which includes ~50% of turtles from the sGBR.

Solid curves are the expected population-specific curves (pointwise 95% credible interval included for the Port Curtis but not for the 3 other curves to reduce visual clutter).

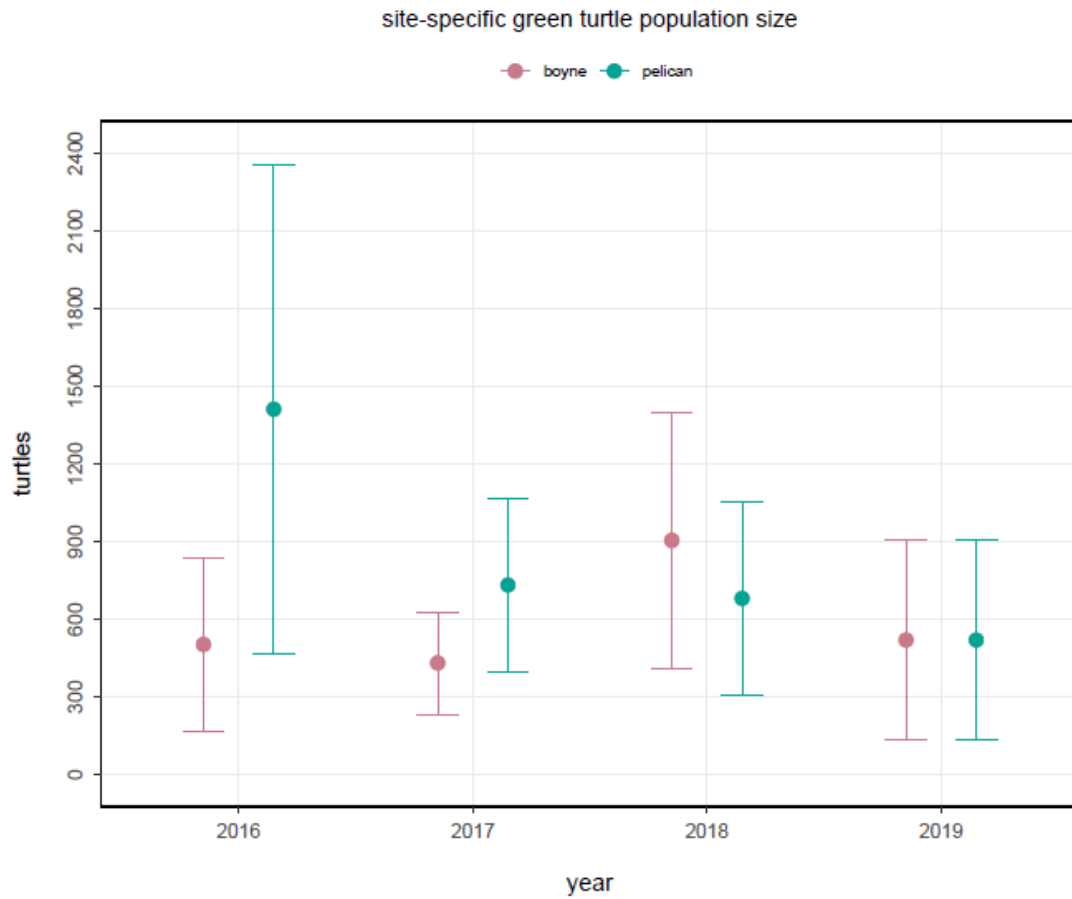
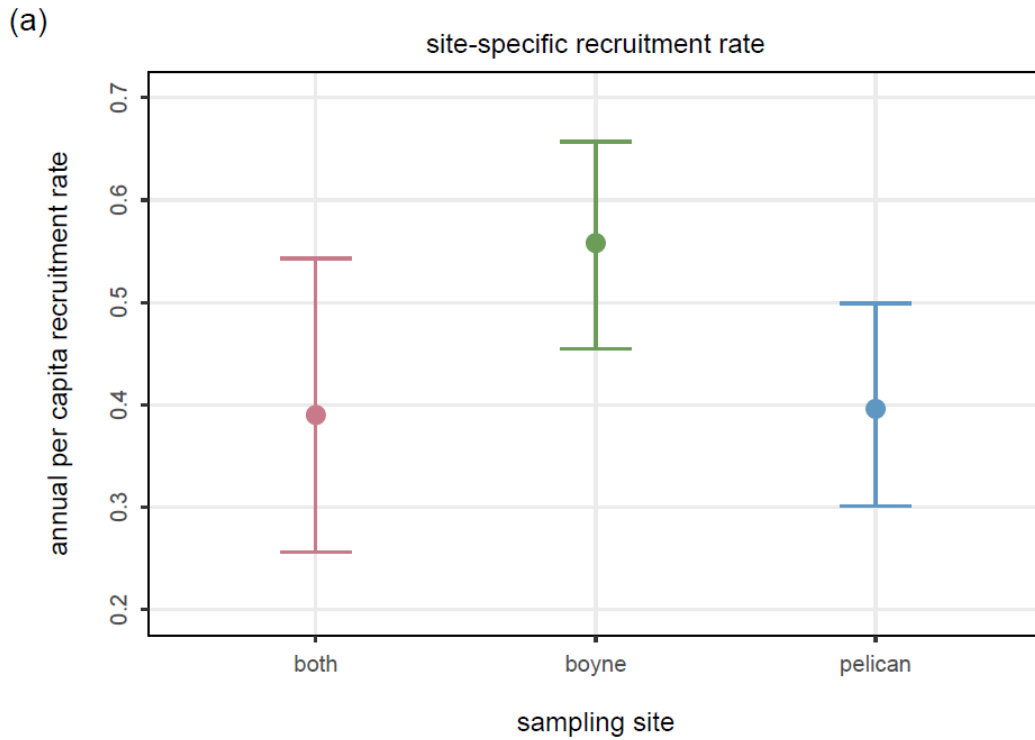


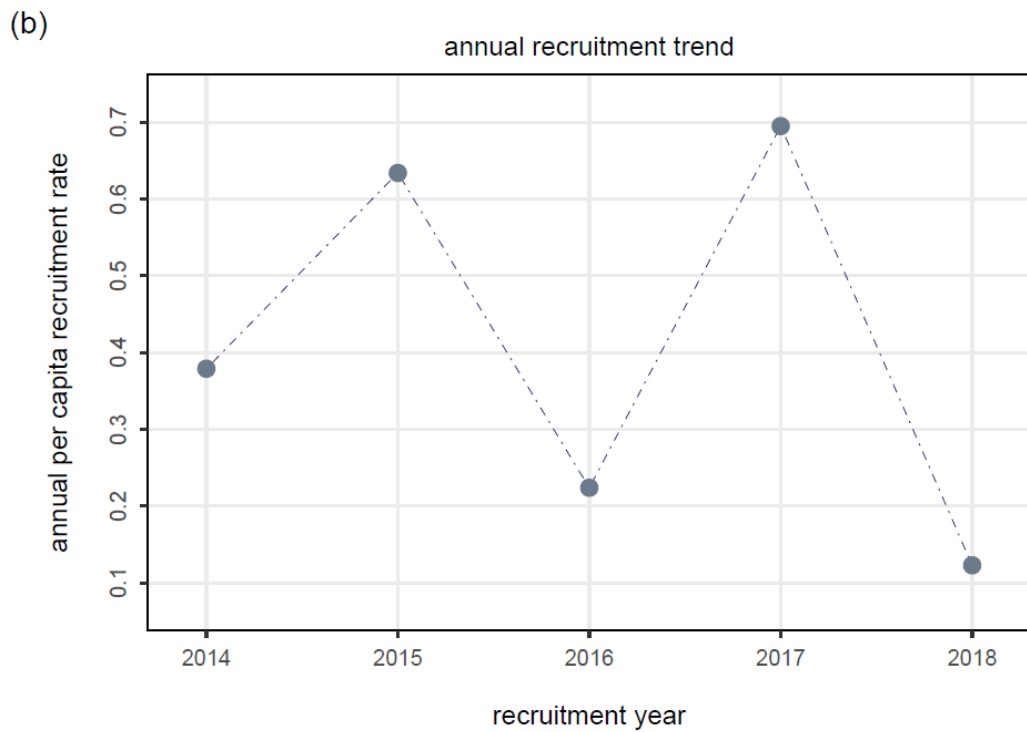
Figure 5.6. Sample-site specific population size estimates for Green turtles foraging in Port Curtis during 2016-2019.

Solid dot = posterior mean, vertical bar = 95% credible interval.



**5.7A Sample-site-specific recruitment rate estimates including an estimate for the two sites combined.**

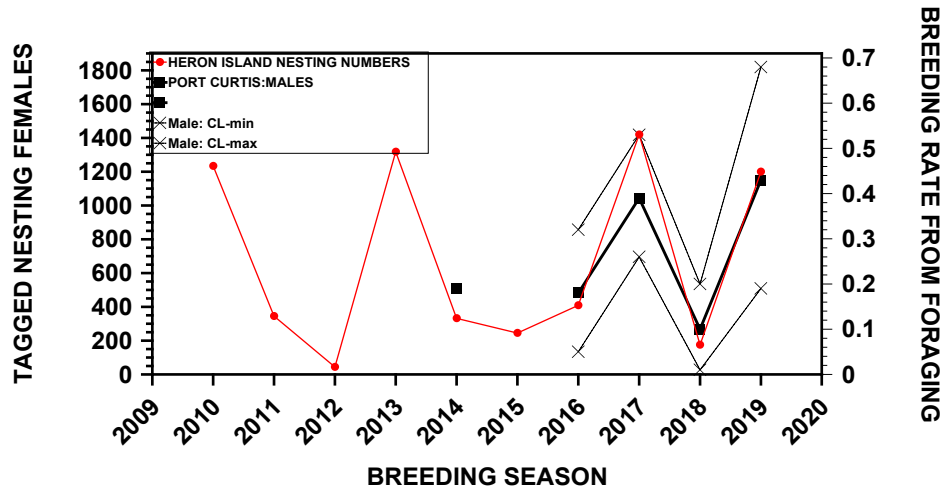
Solid dot = posterior mean, vertical bar = 95% credible interval



**5.7B Trend in the annual recruitment rate estimates for both sites combined.**

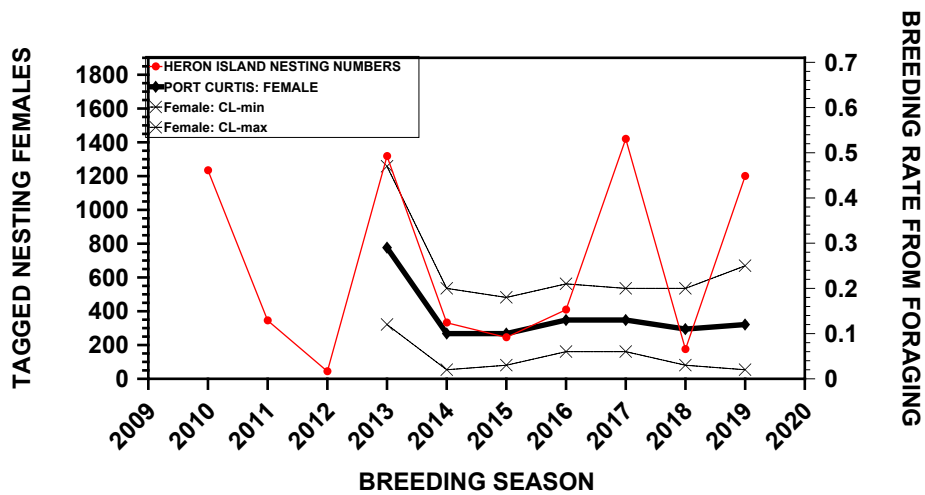
Figure 5.7. Recruitment rate estimates for Green turtles (*Chelonia mydas*) foraging in Port Curtis.

### ANNUAL BREEDING RATE OF GREEN TURTLES FORAGING IN PORT CURTIS & NESTING AT HERON ISLAND



#### 5.8A. Males

### ANNUAL BREEDING RATE OF GREEN TURTLES FORAGING IN PORT CURTIS & NESTING AT HERON ISLAND



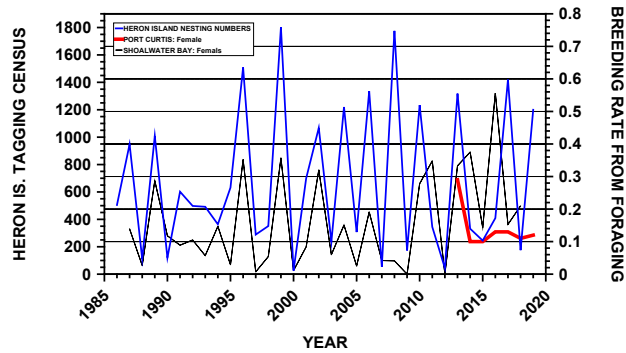
#### 5.8B. Females

Figure 5.8. Annual breeding rates (with 95% CI) of adult male (A) and adult female (B) Green turtles (*Chelonia mydas*) foraging in Port Curtis compared with the annual numbers of Green turtles recorded nesting at Heron Island.

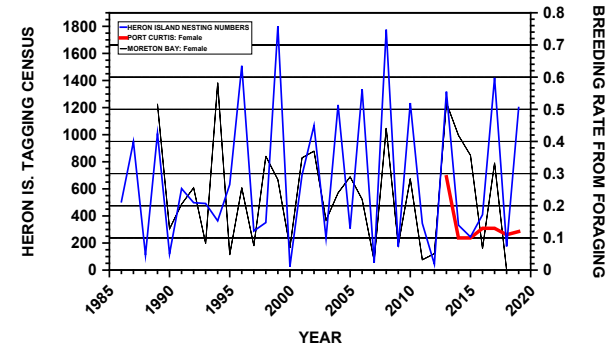




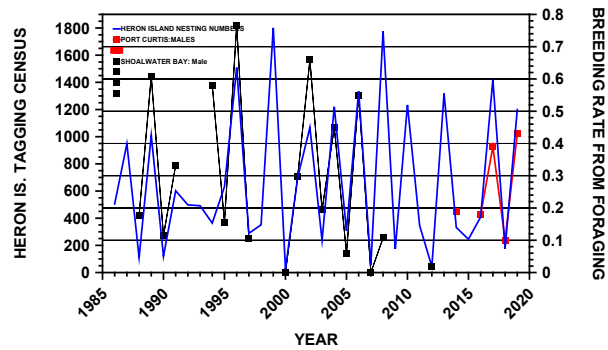
**ANNUAL BREEDING RATE OF GREEN TURTLES  
FORAGING PORT CURTIS & SHOALWATER BAY: FEMALE**



**ANNUAL BREEDING RATE OF GREEN TURTLES  
FORAGING PORT CURTIS & MORETON BAY: FEMALE**



**ANNUAL BREEDING RATE OF GREEN TURTLES  
FORAGING PORT CURTIS & SHOALWATER BAY: MALE**



**ANNUAL BREEDING RATE OF GREEN TURTLES  
FORAGING PORT CURTIS & MORETON BAY: MALE**

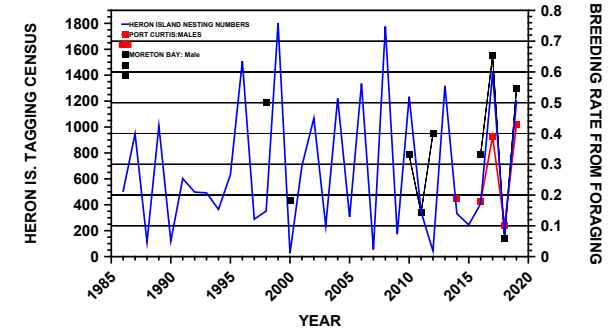


Figure 5.9. Comparison of annual breeding rates off Green turtle (*Chelonia mydas*) foraging populations in Port Curtis, western Shoalwater Bay and eastern Moreton Bay with the annual fluctuations in the annual sGBR Green turtle nesting abundance as measured at the Heron Island index site.

## CHAPTER 6

### DIETARY ECOLOGY OF THE GREEN TURTLES IN PORT CURTIS

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#### Introduction

Green turtles can be significant grazers on seagrass and algae in their foraging grounds and the quality of the forage is critical for turtle health, individual growth, and population dynamics. Individuals forage in a diversity of tidal and subtidal benthic habitats on sandflats and mudflats, coral and rocky reefs, and amongst mangroves, where they eat seagrass and algae as well as mangrove fruits and leaves and some soft-bodied invertebrates (Forbes, 1996, Limpus and Limpus, 2000, Read and Limpus, 2002, Arthur *et al.* 2007). Diets of Green turtles vary across habitat types, partly in response to the available species to forage on (Santos *et al.* 2015b), but there is evidence that turtles are selective foragers (Forbes, 1996; Brand-Gardner *et al.* 1999).

Several studies of Green turtle diets have been conducted in Queensland in habitats where turtles forage on seagrass, and algae (André *et al.* 2005, Brand-Gardner *et al.* 1999), as well as mangroves (Read and Limpus, 2002, Prior *et al.* 2016) or at coral reefs where algae predominates (Forbes 1996). These studies have demonstrated that Green turtles have diet preferences, such as avoiding particularly species of algae (Forbes, 1996) or preferring particular species of seagrass (Whiting and Miller, 1998, Brand-Gardner *et al.* 1999). In algal habitats at Heron Reef, the repeated sampling of the same individuals indicated that some turtles have limited dietary preferences; while others show temporal variation in the algal species they forage on (Forbes, 1996). A previous study of Green turtle diets in Port Curtis used an analysis of “last-bite” food items in 47 turtles at Pelican Banks and 12 turtles at Wiggins Island (Prior *et al.* 2016). There was strong dietary difference between turtles at the two sites, with seagrass dominating the diet at Pelican Banks and red algae predominating in the diets at Wiggins Island, based on the relative mass (Prior *et al.* 2016).

In the Port Curtis region, an extensive analysis of seagrass habitats, and the abiotic factors that affect seagrass communities was reported on for the period 2012-2013 (Babcock *et al.* 2015). In 2014, the study focused in detail on Pelican Banks and the western banks off the northern end Facing Island, due to those areas being identified as important turtle habitat (Babcock *et al.* 2015). Additionally, long-term-monitoring (since 2002) of seagrass communities in Port Curtis was continued at several sites for three years (2015-2017) of the diet study (Davies *et al.* 2016, Rasheed *et al.* 2017, Chartrand *et al.* 2018). Because these research studies were done at or near areas where turtles were captured, it provides a basis for comparing seagrass distribution and turtle diets at several sites.

In Queensland there is evidence that major flood events, which deposit sediment on seagrass meadows and increase turbidity have a negative effect on seagrass and on sea turtle populations. Extensive flooding during 2011 in the Port Curtis region resulted in decreased seagrass biomass (Bryant and Rasheed 2013) and a spike in the number of stranding Green turtles, many of which were in poor body condition (Limpus *et al.* 2012; Meager and Limpus, 2012). Seagrass resilience varied across species and sites, with some sites substantially recovering after one year and other sites still in poor condition after three years (Davies *et al.* 2015).

In addition to investigations of Green turtle diets in Port Curtis using traditional gut content analyses, stable isotope analyses (SIA) of sampled bodily tissues were conducted in 2013 (Prior *et al.* 2016) and in conjunction with this study from 2015-2016 (Coffee, 2020). Stable isotopes are present in natural systems and are assimilated in predictable patterns into the tissues of an organism by cellular processes (Katzenberg, 2008). SIA enables the inference of an organism's dietary composition by comparing the isotopic ratios in the tissues of a sampled individual to potential forage items from their environment (Deniro & Epstein, 1978, 1981; Peterson and Fry, 1987). Consequently, when SIA is used in addition to traditional lavage analyses, dietary composition that is inferred from their most recent meal (oesophageal lavage) can be compared to past dietary inference dependent upon the turnover rates (upwards of 6 months) of the particular tissues (blood, skin) sampled (Seminoff *et al.* 2007; Reich *et al.* 2008; Vander Zanden *et al.* 2012). Results from the recent SIA analyses are presented in Coffee (2020) and the conclusions of that study are summarised in the discussion section of this chapter.

Investigations of marine turtle diets often uncover evidence of the ingestion of marine debris (Schuyler *et al.* 2013; Fukuoka *et al.* 2016; Duncan *et al.* 2018), which can be a threat for some populations or life stages (Witherington *et al.* 2012; Santos *et al.* 2015a). Marine debris is of global concern and the probability of ingestion by most marine turtle species has increased for each of the past four decades, including for Green turtles (Schuyler *et al.* 2013). The most commonly ingested marine debris items are plastics, which can cause blockage and lacerations of the digestive tract, and there is some concern over the availability of toxins in the plastics consumed (Schuyler *et al.* 2013; Bjorndal *et al.* 1994).

This study sampled the diets of Green turtles at nine sites in the Port Curtis area from 2015 – 2019. All size classes of turtles captured were used in the study. As much as possible, turtles were selected for dietary analysis to encompass the range of habitats where they were captured. Due to recaptures of some individuals, opportunistic repetitive sampling of some individuals was possible. The study also allowed for an assessment of the frequency of macro-plastic ingestion within a limited time frame.

## Methods

Turtles were selected for the diet study to encompass the range of study sites within Gladstone Port, the range of habitats used by turtles and the range in the size of turtles captured. No poor condition turtles were sampled for dietary analysis. Sites were located at Pelican Banks, Chinaman Island (western bank), Facing Island, Quoin Island, The Narrows, Wiggins Island, the mouth of the Boyne River, South Trees, and Wild Cattle Island. Turtles were sampled from different habitat types

categorised as; bays or estuaries with shallow soft sediments (BE), bays or estuaries with intertidal or subtidal seagrass flats (BS), rocky reef (RR), mangrove (M), and estuarine (ER). Habitat types sampled at the different sites were as follows: Pelican Banks (BS, RR, M), Chinaman Island (BS, M), Facing Island (BS, M, RR), Quoin Island (BS, M, RR), The Narrows (BE, M, RR), Wiggins Island (BE, BS, M), the mouth of the Boyne River (BE, BS, ER), South Trees (BE, BS, M, RR), and Wild Cattle Island (BS) (Table 6.1).

Collection of dietary items was accomplished using oesophageal lavage. Initial lavages were performed following the protocols outlined in Forbes and Limpus (1993). Inconsistency in the collected volumes of ingesta led to the modification of this method, using only a flexible PVC tubing (0.5 – 1.0 diameter) to provide water to dislodge and flush out the sample, as sufficient volumes of ingesta were obtained without the need for a second larger tube for retrieval. Samples were collected into a bucket positioned under the turtle's head.

Identification and quantification of samples was done using a stereomicroscope. Species were identified to the lowest possible taxonomic level using keys in Lanyon (1986) for seagrass species and in Cribb and Cribb (1985) for algae and mangroves. Estimation of the relative volume of items ingested followed the micro-stereology methods used by Read (1991) to quantify Green turtle diets in Moreton Bay. Each sample was mixed until homogenous and a random subsample was placed into a 90 mm diameter petri dish of sufficient quantity to cover the bottom of the dish in a single layer. For smaller samples, the entire sample was used. Quantification was done using a 19 mm, 42 endpoint, Weibel graticule, which was inserted into the eyepiece of the microscope, and scanning eight fields of view (Read and Limpus 2002). Ingested material that intersected with the graticule points was identified and counted and the relative volume calculated as the number of observations divided by the total number of graticule points that were observed with ingesta. Later, this method was modified by marking out a grid of 160 points directly on the petri dish. For both methods, if fewer than 50% of the points did not have ingesta, that sample was not used to determine relative food volume but was used for determining the frequency of occurrence across all samples. An index of relative importance (IRI), which takes into account both the frequency of occurrence and the relative food volume was calculated for food items using the equation:

$$\%IRI = (FO_i \times M_i / (\sum_{i=1}^n (FO_i \times M_i))) \times 100$$

Where FO = frequency of occurrence, M = average volume, n = the number of observed food items, and *i* = each category of food item. This index reduces the bias associated with food items that have a high frequency of occurrence but low relative food volume and vice versa (Bjorndal 1997).

## Results

Collection of samples was conducted during multiple field trips from 2015-2019 (Table 6.1). Only Pelican Banks and Wiggins Island were sampled in 2015. Wild Cattle was added as a new site in 2018. Samples were analysed for 329 turtles from across the nine sites as follows: Pelican Banks (96), Chinaman Island (6), Facing Island (43), Quoin Island (16), The Narrows (5), Wiggins Island (13), the mouth of the Boyne River (59), South Trees (61), and Wild Cattle Island (19) (Table 6.1). All

samples were analysed for frequency of occurrence, but 51 samples had an insufficient density of food items to account for greater than 50% of the graticule points considered for stereological analysis and determination of mean volume.

Ingested items included one species of mangrove, four species of seagrass, 14 species of red algae (Phylum Rhodophyta), three species of green algae (Phylum Chlorophyta), one species of brown algae (Phylum Ochrophyta), animals in four phyla (Porifera, Mollusca, Cnidaria, and Crustacea) that appeared to be intentionally eaten, and unintentionally eaten items categorised as 'other' (detritus, amphipods and leeches in Ozobranchidae) and plastic debris (Table 6.2). Food items with a high (>50%) frequency of occurrence at more than one site were the seagrasses *Zostera muelleri* subsp. *capricorni*, (herein referred to in the text as *Z. muelleri* for consistency with previous studies), *Halodule pinnifolia*, *Halophila ovalis*, the red algae *Catenella nipae* and *Bostrychia tenella* and red mangrove (*Rhizophora stylosa*) (Table 6.2). Those food items with high (>20%) mean volume across more than one site were the seagrass *Z. muelleri*, the red algae *Catenella nipae* and the green algae *Ulva polyclada* (Table 6.2).

Based on the index of relative importance, the food items of most importance (>30%) per site were as follows: *Z. muelleri* at Pelican Banks, mouth of the Boyne River, and South Trees; *R. stylosa* and *C. nipae* at Facing Island; *C. nipae* at Quoin Island and The Narrows; *U. polyclada* at Wiggins Island; *H. pinnifolia* and *Z. muelleri* at Wild Cattle; and *H. ovalis* at Chinaman Island (Table 6.3). Grouping of food items into higher-level taxonomic forage categories (e.g., Rhodophyta) indicated a strong predominance of seagrass in turtle diets at Pelican Banks, South Trees, and Wild Cattle (Table 6.4, Figure 6.1). Red algae were the dominant food items at Quoin Island and green algae were the primary food ingested at Wiggins Island. Turtles at the mouth of the Boyne River primarily ate red algae and seagrass and turtles at Facing Island ate a diet of red algae and mangroves. Grouping of sites by habitat indicated that seagrass species were the most important food items (56.6% - 81.3%) for Green turtles caught in bays and estuaries, followed by red algae with varied importance (13.3% - 40.4%) (Table 6.5). For turtles caught in mangrove and rocky reef habitats, red algae was the dominant food item (52.9% - 53.3%) followed by seagrass (40%) at rocky reefs, or mangroves (27.7%) and seagrass (17.3%) in mangrove habitats.

Proportional IRI data of the sampled individuals was grouped according to six categories (mangrove, seagrass, red algae, green algae, brown algae, animals) to determine whether dietary composition differed based on site. While ANOVA is resilient to unequal sample sizes, the dataset was reduced to only consider capture sites with > 20 samples (Pelican Banks, Facing Island, Boyne River, and South Trees) for a more balanced model. Given the variety of species observed in the samples, a significant difference was identified in the proportions of the six food groupings on the basis of site (ANOVA  $F_{3, 223} = 10.15$ ,  $p < 0.05$ ; Table 6.4). A Tukey post hoc test identified the turtle diet at Pelican Banks as significantly different ( $p < 0.05$ ) from the diets at the mouth of the Boyne River, South Tree, and Facing Island. Similarly, a significant difference in diet was detected based on foraging habitat (ANOVA  $F_{3, 223} = 2.84$ ,  $p = 0.04$ ; Table 6.5), although a Tukey post hoc test did not reveal specific differences between habitats.

Opportunistic repetitive sampling was possible for 15 captured turtles, all of which were recaptured at the same sites of their original capture (Table 6.6). The length of

time between successive sampling events ranged from six weeks to 23 months. Based on IRI analyses, 8 of the 15 turtles had different predominant food items across the two sampling periods. Of those, five turtles switched between eating seagrass and algae and the other three changed between two species of seagrass (Table 6.6)

Changes in diet reflected the characteristic forage items of their sampled location and habitat as ascertained by the IRI results from all sampled turtles.

Plastic debris was found with a frequency of occurrence ranging from 0% at multiple sites to 12.5% at Quoin Island. Types of ingested debris included plastic fibres, fishing line, and flat, hard plastic and soft plastics such as from plastic bags. All ingested plastic was small, less than 0.5 cm in length and was of insignificant volume.

## Discussion

Green turtles in Port Curtis predominantly fed on three species of seagrass, four species of red algae and one species of mangrove depending upon the location and habitat they were foraging in. Irrespective of habitat, there were differences in the proportions of food items ingested across the locations sampled, particularly at Pelican Banks where a very high proportion of *Z. muelleri* was eaten. Significant differences in turtle diets were also found based on habitat type.

### Temporal Variation- Port Curtis

The previous study of Green turtles in Port Curtis conducted over five days in November 2013 found differences in diets between their sites at Pelican Banks (n = 47) and Wiggins Island (n = 12) (Prior *et al.* 2016), which is also apparent in our study. Previously at Pelican Banks *Z. muelleri* was the dominant dietary item, both in terms of frequency of occurrence (97.9%) and relative mass (93.5%). *Halophila ovalis* was also present at high frequency (72.3%) but contributed little (4.8%) to the relative mass of food eaten. The combined relative mass of seagrass species was 99.9%, with all other food items having a relative mass of <1%. In comparison, our study of 96 turtles at Pelican Banks found a similarly high frequency of ingestion of *Z. muelleri* (96.9%), but a lower mean volume (51.2%), a higher mean volume of *H. ovalis* (12.3%), and a somewhat lower index of relative importance (IRI) for all seagrass combined (80.7%), with red algae having an increased importance (19.0%). Turtle diets at Pelican Banks largely reflect the species that are available to forage on, with surveys of the seagrass beds confirming that *Z. muelleri* is the dominant species, with areas of mixed *Z. muelleri* and *H. ovalis* (Babcock *et al.* 2015).

At Wiggins Island the red algae *C. nipa*e was the most frequent (50.0%) food item in the previous study and it contributed the highest relative mass (34.3%) to the diet. Other food items that contributed >10% to the relative mass included the red algae *Chondria* sp., an unidentified filamentous green algae (11.3%) and the seagrass *H. ovalis* (11.1%). Combining taxa indicated that red algae had a much higher relative mass (68.2%) in comparison to seagrass (14.3%) or green algae (11.3%). All other food items had a relative mass of <1%. In contrast, our study found that the green algae *U. polyclada* (which may have been the previously unidentified filamentous green algae) was the most frequent (66.7%) food item with the largest mean volume (77.0%) and highest IRI (93.9%) and *C. nipa*e played a minor role (IRI

= 1.2%) in the diet. Such variation in the diets of turtles at Wiggins Island may reflect opportunistic foraging on *U. polyclada*, which may have been present in larger quantities in our more recent study. The genus *Ulva* includes early successional species that are able to quickly colonise new substrate after disturbances (Wichard *et al.* 2015), which has been observed to occur over the duration of the several field trips.

Previous analyses of seagrass communities in 2012-2014 at Pelican Banks, Wiggins Island, and South Trees (Babcock *et al.* 2015) were done at turtle capture sites and thus provide comparative data. Seagrass biomass was greatest at Pelican Banks, followed by South Trees, where the biomass was 34% less and there was little seagrass at Wiggins Island (Babcock *et al.* 2015). Long-term monitoring of seagrass communities in Port Curtis since 2002 has documented that over time the quality, distribution and species composition of seagrass meadows has changed at several of the turtle sample sites (Carter *et al.* 2015, Davies *et al.* 2015, 2016, Rasheed *et al.* 2017, Chartrand *et al.* 2018). It is likely, that turtle diets at some sites have also varied across years, although this could not be adequately assessed in this study. Variation in diets of Green turtles across years was observed at Shoalwater Bay, with the most variation due to consumption of the seagrasses *Halodule* sp., *Z. muelleri*, *Halophila* sp., *Cymodocea serrulata*, the red alga *Hypnea* sp. *Gracilaria* sp., and the toxic epiphyte *Lyngbya majuscula* (Arthur *et al.* 2009). Additionally, Green turtles studied at coral reefs in the Great Barrier Reef showed temporal shifting of dietary items, either between different algal species (Forbes, 1996) or between algae and seagrass (Bell *et al.* 2019).

Dietary studies provide a way of understanding patterns of food consumption for a population, and the extent of diet variation among individuals, but it is often challenging to get repetitive sampling of individuals to quantify daily or seasonal variation. A necropsy of an adult Green turtle that had died of unknown causes at Shoalwater Bay examined food from the crop, stomach and at 1m intervals along the intestines (Arthur *et al.* 2009). Seagrass was found throughout the digestive tract, interspersed with mangrove, algae, red sponge and solitary ascidians, indicative of the turtle foraging opportunistically and following the high tide to forage amongst mangroves (Arthur *et al.* 2009).

Therefore, it is not surprising to find some of the turtles in this study had predominantly ingested different food items across two sample periods that varied from six weeks to 23 months. We were not able to assess the extent to which turtle diets varied with the tidal cycles, other than unquantified observations of turtles that were feeding on mangroves during high tides.

#### Spatial variation in diets- population level

Turtles foraging at Port Curtis are part of the sGBR Green turtle genetic stock (see Chapter 3). Individuals from this stock are known to use foraging grounds at coastal habitats and offshore reefs from New South Wales to the Northern Territory, throughout the Great Barrier Reef, and internationally in waters of Papua New Guinea and Fiji (Limpus, 2008). Quantitative diet studies have been conducted on turtles foraging at Heron Island reef, Orman Reefs in Torres Strait, Moreton Bay and Shoalwater Bay.



At Heron Reef, turtles almost exclusively feed on several species of algae. They primarily crop algal turf, which has a varied species composition over time, but they also take advantage of the temporary availability of patches of single species that may rapidly colonise sites (Forbes, 1996). In contrast, turtles at Orman Reefs also had access to seagrass and ate somewhat more seagrass species (55.3%) than algal species (44.5%) (André *et al.* 2005).

In coastal sites, Green turtles forage largely on *Z. muelleri*, followed by *Halodule* spp. and *Gracilaria* spp. at Shoalwater Bay (Arthur *et al.* 2009). Immature Green turtles in Moreton Bay that were sampled at two sites predominated by seagrass primarily ate two species of *Halophila* (*H. ovalis* and *H. spinulosa*), and the red algae *Gracilaria cylindria* and *Hypnea spinella*, based on the relative volume ingested (Read and Limpus, 2002). Mangrove material (*Avicennia marina*) was only ingested at a site with nearby mangroves and consumed with relatively high frequency (40.2%), but low relative volume (8.8%) (Read and Limpus, 2002). Ingestion of animal matter was at a low frequency, but some individuals had consumed a significant amount of the cnidarian *Catostylus mosaicus* (15.4% relative volume) (Read and Limpus, 2002).

#### Ecological considerations

Grazing of seagrass by marine megagrazers can be an important component in the dynamics of seagrass communities if foragers are at high densities. When Green turtles forage on seagrass they crop the leaf blades and rarely disturb the root structure (Lanyon *et al.* 1989; Brand-Gardner *et al.* 1999). This is in contrast the other main predator of seagrass, *Dugong dugong*, which can destroy much of the roots and rhizomes of some species when furrow grazing (Preen, 1992), although they will also crop above ground leaves (Marsh *et al.* 1982; Preen, 1992, 1995). Selective cropping behaviour in Green turtles was confirmed in this study, where roots material was rarely observed, and the length of cropped seagrass was typically around 1cm in length. Simulated grazing experiments in Moreton Bay found that the biomass of *Halophila ovalis* was maintained and was thus able to increase growth rates under grazing pressure by Green turtles (Kuiper-Linley *et al.* 2007). In contrast, two other species of seagrass, *Z. muelleri* and *C. serrulata* had reduced leaf biomass under grazing pressure. A similar result was found in tropical Queensland, where the net above-ground biomass production of *H. ovalis* increased under simulated grazing, but this decreased for *Z. muelleri* (Aragones and Marsh, 2006). Recovery of seagrass after grazing, whether in a simulated experiment, or by dugong, can be relatively short (3-8 months), but repeated grazing by dugong can limit the recovery (Preen, 1992; Aragones and Marsh, 2006). Megagrazers can have broader effects on seagrass communities because variation in the composition, density and above ground biomass of seagrass is shown to influence the species composition and complexity of the fauna associated with these communities (Jinks *et al.* 2019). An extreme example occurred in a marine protected area in Indonesia in which Green turtle densities had become extreme (up to 20/ha), and they were digging to forage on rhizomes, resulting in a 30% loss of underground seagrass biomass, increased patchiness and decreased rates of regrowth that threatened the persistence of the seagrass community (Christianen *et al.* 2014).

There is some evidence to support a hypothesis that Green turtles select young seagrass leaves that may have higher nutritional value due to higher digestibility, higher protein, and lower lignin content (Bjorndal, 1980). However, immature Green

turtles in Moreton Bay did not necessarily select forage that provided the most energy (Brand-Gardner *et al.* 1999). Based on relative volumes, turtles at one site preferred the red algae *Gracilaria* sp. (41.2%) over *Z. muelleri* (19.1%) and *Halophila ovalis* (19.0%), yet *Gracilaria* sp. provided a relatively low gross energy (9.6 KJ/g) in comparison to *Z. muelleri*, (22.1 KJ/g) *Halodule univervis* (20.6 KJ/g) or *Halophila ovalis* (18.4 KJ/g). However, *Gracilaria* sp. had the lowest fibre content suggesting easier digestibility, as well as the highest nitrogen content, which may be factors in diet choice as also shown in the Caribbean (Bjorndal, 1985). Turtles were selectively feeding on *Gracilaria* sp. given that its presence at the site accounted for <1% cover and *Z. muelleri* was the most abundant species (50-70% cover; Brand-Gardner *et al.* 1999). What is not apparent in our study, or in most others is a lack of data on Green turtles foraging on gelatinous invertebrates that float in the water column. However, the use of a crittercams on Green turtles foraging in Moreton Bay found a relatively high rate (0 – 6.1 items/hour) of ingestion of these prey, which were intentionally consumed (Arthur *et al.* 2007).

A study of Green turtles foraging in coastal habitats at Cleveland and Upstart Bays and offshore at the Howicks Group of Reefs, quantified growth rates, and body condition and qualitatively assessed diet samples. It was suggested that higher growth rates in immature turtles might in part be due to higher density of seagrass in the coastal habitat versus the reef environment (Bell *et al.* 2019). Additionally, forage in the reef environment has spatial and temporal variability (Coles *et al.* 2000), which was also suggested by temporal variation in diets, with a predominance of algae in one year and seagrass in the next year (Bell *et al.* 2019). A lack of sufficient forage due to multiple species grazing pressure has been linked to poor body condition and death in Green turtles foraging on algae (see Wabnitz *et al.* 2010) and from smothering of seagrass due to flood events (Amies *et al.* 20013; Davies *et al.* 2015).

#### Stable isotope analyses

Prior *et al.* (2016) investigated the dietary composition of foraging Green turtles from two sample sites (Wiggins Is. and Pelican Banks) within the Port Curtis region using SIA in tandem with oesophageal lavage. In concurrence with prior observations of Green turtle foraging behaviour, oesophageal lavage samples were predominantly composed of forage items characteristic of the habitat where captured. Individuals sampled from Wiggins Island (n = 12), a subtidal flat with adjacent mangrove habitat, foraged mostly on items from the algal phylum Rhodophyta (most commonly *C. nipae*, *Hypnea* spp., *Soliera robusta*, and *Bostrychia tenella*). In contrast, the diet of turtles captured on the Pelican Banks (n= 47), an intertidal sandbank, was predominantly composed of seagrass species *Z. muelleri* and *H. ovalis* (Prior *et al.* 2016). Isotopic analysis (n = 25) of turtle epidermis (slow tissue turnover) could not distinguish significant difference between the diet of turtles from each site, while the isotopic ratios from turtle serum (fast tissue turnover) were significantly different between Wiggins Island and Pelican Banks. A subsequent analysis identified that a subset of individuals from Wiggins (n = 5) and Pelican Banks (n = 6) had distinct isotopic ratios across blood and tissue suggesting that the dietary composition of these individuals had changed between the tissue turnover period of epidermis and serum (Prior *et al.* 2016).

As an expansion on the research of Prior *et al.* (2016) and conducted concurrent to this study (Coffee, 2020) evaluated the stable isotopic composition of foraging Green

turtles from three sites within Port Curtis (Pelican Banks, Facing Island and the mouth of the Boyne River). The sampled foraging turtles from each of these three sites were further categorised based on the habitat of capture (subtidal flats, sand banks, mangroves, and rocky reefs) for isotopic comparisons. Initial comparisons reported significant differences in the isotopic ratios of turtles from different habitats. A significant portion of larger turtles expressed isotopic ratios consistent with diets composed mostly of seagrass. In addition, the isotopic ratios of a significant proportion of juveniles indicated the potential supplementation of their diet with animal material.

Subsequently, reference samples of potential forage items characteristic to each habitat were collected and a Bayesian mixing model was used to infer the approximate dietary composition of turtles from each habitat. It was inferred that the diets of turtles foraging on subtidal flats were composed of predominantly seagrass forage in concurrence with observations based on oesophageal lavage. However, contrary to lavage sampling, a non-trivial proportion of their dietary composition was inferred to originate from animal matter (Coffee, 2020). Turtles inhabiting mangrove habitats had isotopic ratios consistent with a diet composed predominantly of mangrove or red algae with a significant contribution from seagrass. In contrast to observations from lavage sampling, turtles captured amongst rocky reef habitat that had ingested mostly seagrass, had isotopic ratios of sampled plasma indicating a diet principally composed of red algae/mangrove material. For turtles foraging on sandbanks, the outcomes of the isotopic mixing model conformed with oesophageal lavage contents, with a diet primarily composed of seagrasses.

Juvenile turtles were frequently encountered and had dietary compositions and isotopic ratios consistent with focused foraging activity among the shallow subtidal intertidal mangrove and rocky reef habitats. By contrast, large subadult and adult turtles exhibited dietary compositions (based on lavage and isotopic ratios) more consistent with foraging amongst the vast subtidal flats. These datasets indicate that within Port Curtis individuals exhibit a shift in micro-habitat use at the foraging sites as they develop. This supports regular observations that smaller turtles utilize sheltered intertidal and shallow subtidal regions that provide a safe foraging habitat when tides permit access. Additionally, oesophageal contents and isotopic ratios identified variable dietary compositions within the habitats and individuals, potentially reflecting shifts in dietary composition as a reflection of availability, preference or changes in foraging habitat. Finally, it was concluded that foraging individuals from this population continue to supplement their diet with animal material beyond recruitment from their oceanic developmental phase where pelagic turtles are known to consume oceanic macro-zooplankton (Boyle and Limpus, 2008).

#### Marine Debris

Green turtles are particularly susceptible to the ingestion of plastics, including translucent plastics that look like gelatinous soft-bodied invertebrates floating in the water, which Arthur *et al.* (2007) documented as important food items. In a southeast Queensland study of marine turtles that had died and been necropsied, 29% of benthic feeding turtles had ingested marine debris (Schuyler *et al.* 2012), and among Green turtles of all size classes (included some <35 cm), 18.2% had marine debris in their digestive system (Schuyler *et al.* 2013). Surface net tows conducted around much of Australia (not the Kimberley coast, Western Australia or the Northern

Territory) found that most plastics were classed as microplastics and had a median length of 2.8 mm (Reiser *et al.* 2013).

Although plastic ingestion was an insignificant component of turtle diets in Port Curtis, it should be noted that the oesophageal lavage sample of turtles was a biased sample in that no turtles in poor condition were sampled. It is recommended to continue analysing the digestive tract of dead stranded turtles in Port Curtis to determine rates of marine debris ingestion and whether this is associated with mortality of Green turtles in Port Curtis.

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Table 6.1. Total individual Green turtles analysed from each sampling site/habitat within Port Curtis.

	<b>Pelican Banks</b> n=96			<b>Boyne</b> n=59			<b>Facing Is.</b> n=43			<b>Quoin Is.</b> n=16			<b>South Tree</b> n=61				<b>The Narrows</b> n=5			<b>Wiggins Is.</b> n=24			<b>Wild Cattle</b> n=19	<b>Chinaman Is.</b> n=6	
<b>Year</b>	BS	RR	M	BE	BS	ER	BS	M	RR	BS	M	RR	BE	BS	M	RR	BE	M	RR	BE	BS	M	BS	BS	M
<b>2015</b>	24	8																			1				
<b>2016</b>	42		1	25	9	1	12	18			1	2								2					
<b>2017</b>	10		1	2				3	1	1															
<b>2018</b>	8			12		3		5		1		8	1	30	3	1	1	3			11	1	7		
<b>2019</b>	2			6	1		2	2				3	8	10	6	2			1		9		12	2	4

Table 6.2. The frequency of occurrence (%FO) and mean volume ( $\pm$ SD) of identified species observed in the oesophageal lavage samples of foraging Green turtles (n=329) from sample locations within Port Curtis, Queensland.

Grouping	Food Item	% FO by location <sup>1</sup>									Mean Vol. % (SD) by location <sup>1</sup>								
		PB n=9 6	Boy n=5 9	FI n=4 3	Quo n=1 6	ST n=6 1	Nar n=5	WI n=2 4	WC n=1 9	Ch n=6	PB n=87	Boy n=51	FI n=37	Quo n=7	ST n=53	Nar n=4	WI n=15	WC n=18	Ch n=6
Mangrove	<i>Rhizophora stylosa</i>	8.3	1.7	83.7	0	0	80.0	8.3	0	16.7	2.4 (11.1)	0.0 (0.0)	44.5 (31.9)	0.0 (0.0)	0.0 (0.0)	16.1 (12.9)	1.0 (3.6)	0.0 (0.0)	13.8 (0)
Seagrass	<i>Zostera muelleri capricorni</i>	96.9	64.4	30.2	37.5	82.0	0	12.5	63.2	16.7	51.2 (33.5)	30.4 (35.4)	2.2 (6.2)	0.4 (0.7)	59.9 (38.4)	0.0 (0.0)	0.1 (0.2)	40.3 (42.4)	1.7 (4.7)
	<i>Halophila ovalis</i>	63.5	13.6	14.0	31.3	23.0	0	12.5	15.8	50.0	12.3 (17.9)	0.8 (3.3)	1.0 (3.1)	19.6 (31.1)	1.2 (3.4)	0.0 (0.0)	0.0 (0.0)	0.2 (0.4)	41.3 (47.1)
	<i>Halophila pinnifolia</i>	62.5	57.6	4.7	18.8	62.3	20.0	8.3	84.2	0	6.8 (10.2)	18.5 (32.7)	1.0 (6)	0.4 (1.1)	21.9 (30.3)	0.2 (0.3)	0.0 (0.2)	59.1 (42.4)	0.0 (0.0)
	<i>Cymodoce rotundata</i>	6.3	18.6	0	0	0	0	0	0	0	0	1.2 (6.2)	1.2 (2.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Phylum Rhodophyta	<i>Herposiphona secunda</i>	1.0	5.1	0	6.3	0	0	0	0	0	0.4 (4.1)	2.4 (13.6)	0.0 (0.0)	6.1 (16.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	<i>Gracilaria</i> sp.	18.8	59.3	2.3	18.8	6.6	0	4.2	0	0	2.4 (7.4)	17.6 (27.5)	1.0 (6.2)	0.5 (0.8)	1.4 (9.8)	0.0 (0.0)	0.1 (0.2)	0.0 (0.0)	0.0 (0.0)
	<i>Hypnea</i> sp.	47.9	64.4	7.0	31.3	19.7	0	12.5	5.3	33.3	6.8 (15.1)	17.9 (29)	0.2 (0.9)	4.6 (9.7)	6.5 (23.5)	0.0 (0.0)	6.4 (24.4)	0 (0.2)	0.5 (0.8)
	<i>Audouinella</i> sp.	1.0	3.4	0	0	0	0	0	0	0	0.0 (0.3)	1.9 (13.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	<i>Sarconema</i> sp.	4.2	3.4	0	0	0	0	0	0	0	0.3 (2)	0.1 (0.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	<i>Catenella nipae</i>	7.3	0	93.0	87.5	6.6	80.0	8.3	0	33.3	0.9 (6.5)	0.0 (0.0)	32.0 (24)	37.1 (27.5)	1.4 (8.9)	45 (32.6)	6.1 (18.9)	0.0 (0.0)	18.2 (43.4)
	<i>Polysiphonia</i> sp.	10.4	10.2	4.7	31.3	19.7	60.0	37.5	5.3	16.7	1.5 (9.6)	0.1 (0.4)	0.6 (3.3)	12.5 (23.1)	2.1 (10)	4.4 (7.3)	7.4 (25.7)	0.1 (0.3)	6.2 (16.7)
	<i>Chondria</i> sp.	2.1	0	0	0	0	0	0	0	0	0.9 (6.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

Table 6.2. continued

Grouping	Food Item	% FO by location <sup>1</sup>									Mean Vol. % (SD) by location <sup>1</sup>								
		PB n=9 6	Boy n=5 9	FI n=4 3	Quo n=1 6	ST n=6 1	Nar n=5	WI n=2 4	WC n=1 9	Ch n=6	PB n=87	Boy n=51	FI n=37	Quo n=7	ST n=53	Nar n=4	WI n=15	WC n=18	Ch n=6
Phylum Rhodo- phyta	<i>Laurencia</i> sp.	5.2	16.9	2.3	0	0	0	0	0	0	1.6 (10.7)	2.9 (7.8)	0.5 (3.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	<i>Bostrychia tenella</i>	0	0	83.7	12.5	0	60.0	0	0	33.3	0.0 (0.0)	0.0 (0.0)	13.2 (14.3)	0.4 (1.0)	0.0 (0.0)	4.0 (7.5)	0.0 (0.0)	0.0 (0.0)	1.3 (1.3)
	<i>Amansia glomerata</i>	4.2	5.1	0	0	0	0	0	0	0	0.6 (3.0)	0.2 (1.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	unidentified Sp. 1	0	1.7	0	0	0	0	0	0	0	0.0 (0.0)	1.8 (12.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	11.4 (30.5)
	unidentified Sp. 2	0	0	0	12.5	0	0	0	0	0	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	14.0 (37)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	unidentified Sp. 3	2.1	1.7	0	0	0	0	0	0	0	0.1 (0.7)	0.0 (0.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Phylum Chloro- phyta	<i>Ulva lactuca</i>	1.0	5.1	4.7	0	0	0	0	0	0	0.0 (0.1)	0.2 (0.6)	0.5 (2.7)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	<i>Ulva polyclada</i>	2.1	0	0	6.3	3.3	20.0	66.7	0	0	0.4 (3.2)	0.0 (0.0)	0.0 (0.0)	0.1 (0.3)	3.9 (18.9)	25.0 (50)	77.0 (40.5)	0.0 (0.0)	0.0 (0.0)
	<i>Codium</i> sp.	1.0	0	0	0	0	0	0	0	0	0.3 (3.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Phylum Ochro- phyta	<i>Sargassum flavicans</i>	3.1	1.7	0	0	1.6	0	0	5.3	0	0.4 (3.4)	0.3 (2.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Animal	Porifera	1.0	0	0	0	0	0	0	0	0	0.0 (0.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Mollusca	1.0	3.4	0	0	11.5	0	4.2	5.3	0	0.0 (0.2)	0.1 (0.4)	0.0 (0.0)	0.0 (0.0)	0.1 (0.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.2)	0.0 (0.0)
	Cnidaria	1.0	0	2.3	0	0	0	0	0	0	0.2 (1.5)	0.0 (0.0)	0.8 (5.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

Table 6.2. continued

Grouping	Food Item	% FO by location <sup>1</sup>									Mean Vol. % (SD) by location <sup>1</sup>								
		PB n=9 6	Boy n=5 9	FI n=4 3	Quo n=1 6	ST n=6 1	Nar n=5	WI n=2 4	WC n=1 9	Ch n=6	PB n=87	Boy n=51	FI n=37	Quo n=7	ST n=53	Nar n=4	WI n=15	WC n=18	Ch n=6
	Crustacea	1.0	0	0	0	1.6	0	4.2	0	0	0.0 (0.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Other	Detritus	65.6	55.9	46.5	68.8	49.2	60.0	62.5	21.1	50.0	8.5 (12.4)	3.7 (5.9)	2.3 (3.8)	4.1 (4.5)	1.5 (3.2)	5.3 (6.9)	1.7 (2.7)	0.3 (0.9)	5.5 (14.1)
	Amphipod	21.9	8.5	4.7	25.0	6.6	0	16.7	10.5	0	0.7 (2.3)	0.0 (0.2)	0.1 (0.5)	0.3 (0.9)	0.1 (0.2)	0.0 (0.0)	0.1 (0.3)	0.0 (0.2)	0.0 (0.0)
	Ozobranchidae	0	0	0	0	1.6	0	4.2	0	0	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Plastic	Macro-plastic	3.1	1.7	2.3	12.5	8.2	0	0	0	0	0.0 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

<sup>1</sup>PB: Pelican Banks, Boy: mouth of Boyne River; FI: Facing Is., Quo: Quoin Is., ST: South Trees, Nar: The Narrows, WI: Wiggins Is., WC: Wild Cattle; CH: Chinaman Is.

Table 6.3. The index of relative importance (IRI) of identified species observed in the oesophageal lavage samples of foraging Green turtles (n=329) from sample locations within Port Curtis, Queensland.

Grouping	Food Item	% IRI by location <sup>1</sup>								
		PB n=96	Boy n=59	FI n=43	Quo n=16	ST n=61	Nar n=5	WI n=24	WC n=19	Ch n=6
<b>Mangrove</b>	<i>Rhizophora stylosa</i>	0.308	0	47.6	0	0	20.5	0.211	0	0
<b>Seagrass</b>	<i>Zostera muelleri capricorni</i>	69.1	37.3	0.863	0.228	74.7	0	0.006	33.3	0.965
	<i>Halophila ovalis</i>	10.7	0.191	0.155	18.3	0.432	0	0	0.038	67.7
	<i>Halophila pinnifolia</i>	6.13	19.5	0.032	0.135	21.3	0.073	0.004	66.6	0
	<i>Cymodoce rotundata</i>	0.113	0.453	0	0	0	0	0	0	0
<b>Phylum Rhodophyta</b>	<i>Herposiphona secunda</i>	0.007	0.257	0	1.89	0	0	0	0	0
	<i>Gracilaria</i> sp.	0.642	21.0	0.033	0.284	0.043	0	0.006	0	0
	<i>Hypnea</i> sp.	4.43	20.1	0.022	2.86	1.59	0	1.31	0.003	0.519
	<i>Audouinella</i> sp.	0	0.136	0	0	0	0	0	0	0
	<i>Sarconema</i> sp.	0.021	0.008	0	0	0	0	0	0	0
	<i>Catenella nipae</i>	0.085	0	36.2	57.6	0.168	57.2	1.25	0	10.1
	<i>Polysiphonia</i> sp.	0.165	0.005	0.041	7.76	0.513	3.75	2.25	0.005	3.46
	<i>Chondria</i> sp.	0.027	0	0	0	0	0	0	0	0

Table 6.3. Continued.

Grouping	Food Item	% IRI by location <sup>1</sup>								
		PB n=96	Boy n=59	FI n=43	Quo n=16	ST n=61	Nar n=5	WI n=24	WC n=19	Ch n=6
Phylum Rhodophyta	<i>Laurencia</i> sp.	0.123	1.03	0.016	0	0	0		0	0
	<i>Bostrychia tenella</i>	0	0	13.7	0.116	0	3.39	0	0	0.739
	<i>Amansia glomerata</i>	0.036	0.025	0	0	0	0	0	0	0
	unidentified Sp. 1	0	0.061	0	0	0	0	0	0	6.29
	unidentified Sp. 2	0	0	0	4.34	0	0		0	0
	unidentified Sp. 3	0.003	0	0	0	0	0	0	0	0
Phylum Chlorophyta	<i>Ulva lactuca</i>	0.0	0.016	0.035	0	0	0	0	0	0
	<i>Ulva polyclada</i>	0.014	0	0	0.031	0.236	10.6	93.9	0	0
	<i>Codium</i> sp.	0.005	0	0	0	0	0	0	0	0
Phylum Ochrophyta	<i>Sargassum flavicans</i>	0.013	0.01	0	0	0	0	0	0	0
Animal	Porifera	0	0	0	0	0	0	0	0	0
	Mollusca	0	0.002	0	0	0.007	0	0	0.003	0
	Cnidaria	0.003	0	0.027	0	0	0	0	0	0
	Crustacea	0	0	0	0	0	0	0	0	0
Other	Detritus (shell)	7.81	3.60	1.27	6.35	1.02	4.50	1.05	0.062	9.22
	Amphipod	0.196	0.005	0.007	0.101	0.005	0	0.008	0.003	0
	Ozobranchidae	0	0	0	0	0	0	0	0	0
Plastic	Macro-plastic	0	0	0	0	0	0	0	0	0

<sup>1</sup>PB: Pelican Banks, Boy: mouth of Boyne River; FI: Facing Is., Quo: Quoin Is., ST: South Trees, Nar: The Narrows, WI: Wiggins Is., WC: Wild Cattle; CH: Chinaman Is.

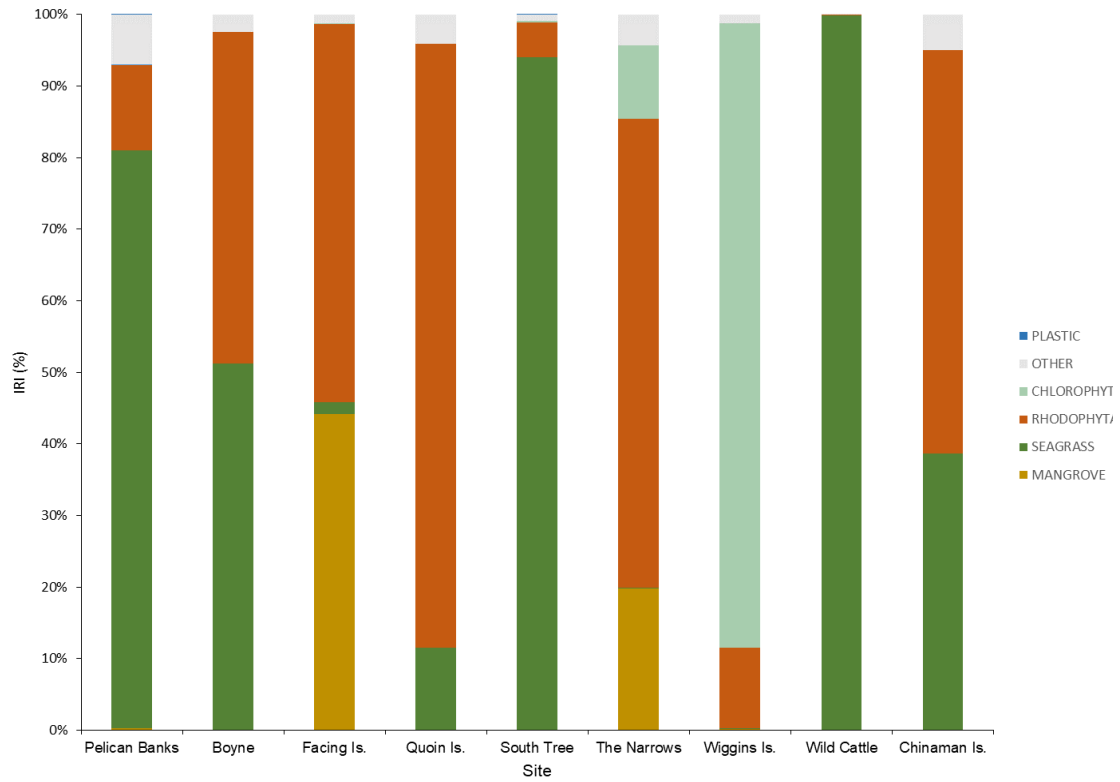


Figure 6.1. The index of relative importance (IRI) of ingested species grouped within six forage categories observed in the oesophageal lavage samples of foraging Green turtles (n=329) from different habitats within Port Curtis, Queensland.





Table 6.4. The number of ingested species and index of relative importance (IRI) of species grouped within seven forage categories observed in the oesophageal lavage samples of foraging Green turtles (n=329) from different locations within Port Curtis, Queensland.

Food items	No. of species by location <sup>1</sup>									IRI % by location <sup>1</sup>								
	PB n=87	Boy n=51	FI n=37	Quo n=7	ST n=53	Nar n=4	WI n=15	WC n=18	Ch n=6	PB n=87	Boy n=51	FI n=37	Quo n=7	ST n=53	Nar n=4	WI n=15	WC n=18	Ch n=6
<b>Mangrove</b>	1		1			1	1			0.3	0.0	44.2	0.0	0.0	19.8	0.2	0.0	0.0
<b>Seagrass</b>	4	4	3	3	3	1	2	3	2	80.7	51.2	1.6	11.5	94.0	0.1	0.0	99.9	38.7
<b>Phylum Rhodophyta</b>	11	10	6	7	4	3	4	2	5	12.0	46.4	52.9	84.4	4.9	65.6	11.3	0.0	56.3
<b>Phylum Chlorophyta</b>	3	1	1	1	1	1	1	0	0	0.0	0.0	0.0	0.0	0.2	10.2	87.3	0.0	0.0
<b>Phylum Ochrophyta</b>	1	1	0	0	0	0	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Animal</b>	4	1	1	0	2	0	0	1	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Other</b>	3	2	2	2	3	1	2	2	1	7.0	2.4	1.2	4.1	0.9	4.3	1.2	0.1	5.0
<b>Plastic</b>	1	0	0	0	1	0	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

<sup>1</sup>PB: Pelican Banks, Boy: mouth of Boyne River; FI: Facing Is., Quo: Quoin Is., ST: South Trees, Nar: The Narrows, WI: Wiggins Is., WC: Wild Cattle; CH: Chinaman Is.

Table 6.5. The number of ingested species and index of relative importance (IRI) of species grouped within eight forage categories observed in the oesophageal lavage samples of foraging Green turtles (n=329) from different habitats within Port Curtis, Queensland.

Food items	No. of species by habitat <sup>1</sup>				IRI % by habitat <sup>1</sup>			
	BE n=50	BS n=171	M n=40	RR n=16	BE n=50	BS n=171	M n=40	RR n=16
<b>Mangrove</b>	1	1	1	0	0.0	0.8	27.6	0.0
<b>Seagrass</b>	4	4	3	3	55.6	81.3	17.3	40.0
<b>Phylum Rhodophyta</b>	11	13	7	8	40.4	13.3	52.9	53.3
<b>Phylum Chlorophyta</b>	2	3	1	1	1.5	0.7	0.0	1.1
<b>Phylum Ochrophyta</b>	1	1	0	0	0.0	0.0	0.0	0.0
<b>Animal</b>	1	4	1	0	0.0	0.0	0.0	0.0
<b>Other</b>	2	3	1	2	2.5	3.8	2.2	5.6
<b>Plastic</b>	1	1	1	0	0.0	0.0	0.0	0.0

<sup>1</sup>BE: bays or estuaries with shallow soft sediments; BS: bays or estuaries with seagrass, M: mangrove, RR: rocky reef

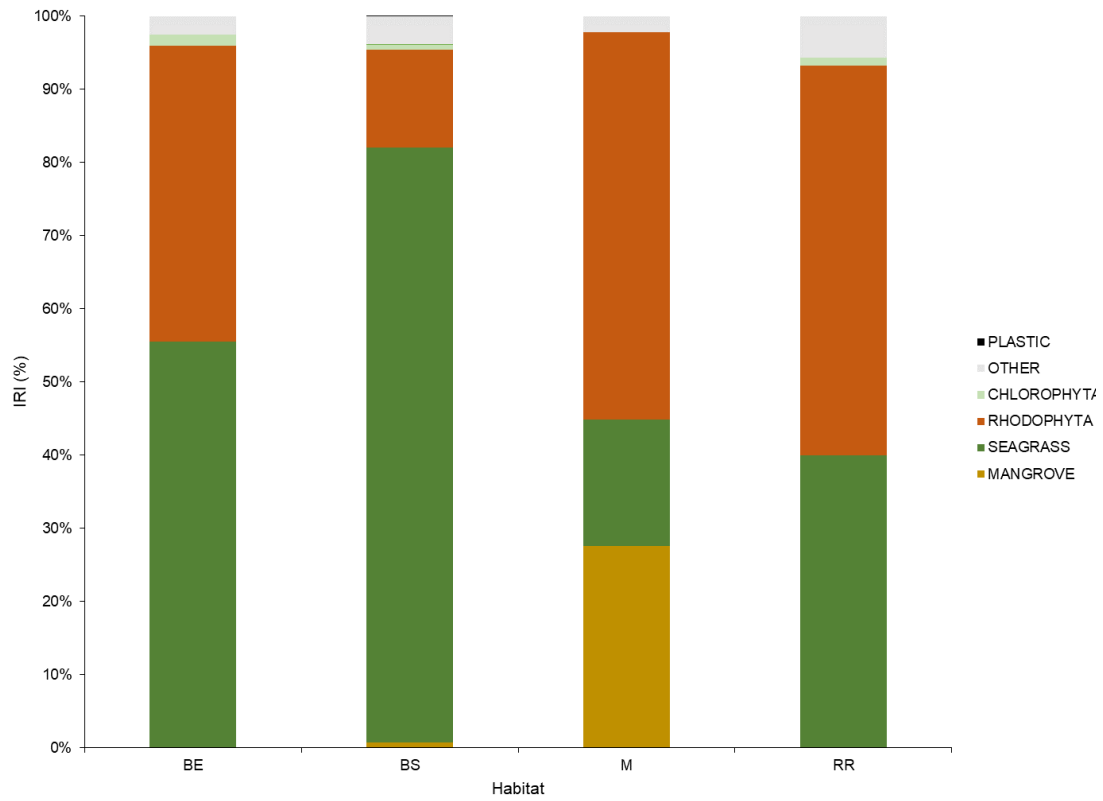


Figure 6.2. The of relative importance (IRI) of species grouped within six forage categories observed in the oesophageal lavage samples of foraging Green turtles (n=329) from different habitats within Port Curtis, Queensland.

Habitats are BE: bays or estuaries with shallow soft sediments; BS: bays or estuaries with seagrass, M: mangrove, RR: rocky reef.

Table 6.6. Index of relative importance (IRI) of ingested food items from the opportunistic repetitive oesophageal lavage samples from 15 foraging Green turtles within Port Curtis, Queensland. Values in bold are those greater than 10%.

Date	Tag #	Sex	Age Class	Local	Habitat	<i>Rhizophora stylosa</i>	<i>Zostera muelleri capricorni</i>	<i>Halophyila ovalis</i>	<i>Halophila pinnifolia</i>	<i>Hypnea</i> sp.	<i>Polysiphonia</i> sp.	<i>Catenella nipae</i>	<i>Bostrychia tenella</i>	<i>Sargassum</i> sp.	Mollusca	Amphipod	Detritus
28/09/2017	QA45574	F	J	Boyne	BE	0.00	0.01	0.00	<b>0.97</b>	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00
12/04/2018				Boyne	BE	0.00	0.00	0.00	<b>0.86</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
07/11/2017	QA61403	F	J	PB	M	0.09	<b>0.17</b>	0.04	0.00	0.00	0.00	<b>0.60</b>	0.00	0.00	0.00	0.00	0.10
19/06/2018				PB	BS	0.09	<b>0.72</b>	0.01	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
28/06/2017	QA61558	F	A	PB	BS	0.00	<b>0.57</b>	0.00	0.08	0.00	<b>0.23</b>	0.00	0.00	0.04	0.00	0.01	0.07
24/05/2019				PB	BS	0.00	<b>0.88</b>	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.02
18/06/2018	QA84236	F	J	ST	BS	0.00	<b>0.44</b>	0.00	<b>0.56</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
06/10/2018				ST	BE	0.00	<b>1.00</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22/06/2018	QA84254	F	J	Boyne	BE	0.00	0.00	0.00	0.00	<b>1.00</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25/05/2019				Boyne	BE	0.00	0.00	<b>0.60</b>	0.00	0.00	<b>0.39</b>	0.00	0.00	0.00	0.00	0.00	0.00
06/10/2018	QA86110	F	J	ST	BS	0.00	<b>0.73</b>	0.00	<b>0.28</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
01/07/2019				ST	BE	0.00	<b>0.77</b>	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
05/10/2018	QA86124	F	J	ST	BS	0.00	<b>0.98</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
15/09/2019				ST	BS	0.00	<b>0.92</b>	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
20/06/2018	QA86208	F	SA	ST	BS	0.00	<b>0.97</b>	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
20/05/2019				ST	BS	0.00	<b>0.95</b>	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00
05/10/2018	QA87020	F	J	ST	BS	0.00	<b>0.94</b>	0.02	0.03	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
04/07/2019				ST	BS	0.00	<b>0.92</b>	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00

Table 6.6. Continued.

Date	Tag #	Sex	Age Class	Local	Habitat	<i>Rhizophera stylosa</i>	<i>Zostera muelleri capricorni</i>	<i>Halophyila ovalis</i>	<i>Halophila pinnifolia</i>	<i>Hypnea</i> sp.	<i>Polysiphonia</i> sp.	<i>Catenella nipae</i>	<i>Bostrychia tenella</i>	<i>Sargassum</i> sp.	Mollusca	Amphipod	Detritus
10/04/2019	QA87051	M	J	ST	M	0.00	0.01	0.00	<b>0.98</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
22/05/2019				ST	RR	0.00	<b>0.14</b>	0.00	<b>0.18</b>	0.00	<b>0.68</b>	0.00	0.00	0.00	0.00	0.00	0.00
10/04/2019	QA87052	F	J	ST	M	0.00	0.00	0.00	0.09	<b>0.91</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15/09/2019				ST	BE	0.00	0.00	0.00	0.00	<b>0.99</b>	0.01	0.00	0.00	0.00	0.00	0.00	0.00
09/04/2019	QA87139	F	J	FI	M	0.00	0.00	0.00	0.00	0.00	0.00	<b>0.50</b>	<b>0.49</b>	0.00	0.00	0.00	0.01
17/09/2019				FI	BS	<b>0.97</b>	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00
10/04/2019	QA87178	F	J	ST	M	0.00	<b>0.17</b>	0.00	<b>0.79</b>	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.01
15/09/2019				ST	BS	0.00	<b>1.00</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12/04/2019	QA87192	F	J	ST	BS	0.00	0.00	0.00	<b>1.00</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
04/07/2019				ST	BS	0.00	<b>1.00</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22/05/2019	QA87214	F	J	ST	RR	0.00	0.05	<b>0.19</b>	0.00	0.00	<b>0.12</b>	<b>0.62</b>	0.00	0.00	0.00	0.00	0.03
15/08/2019				ST	BS	0.00	<b>1.00</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

## Introduction

Port Curtis receives outflow from the Calliope and Boyne Rivers, as well as some outflow from the Fitzroy Catchment, via The Narrows. These outflows can carry with them land-based sediment, nutrients, and chemical contaminants into the port, and nearby Great Barrier Reef lagoon (Angel *et al.*, 2010; Jones *et al.*, 2019; Schaffelke *et al.*, 2012). In addition, coal, liquid natural gas (LNG), and grain export, the alumina smelter (bauxite import and alumina export), the power station, tourism to the Great Barrier Reef, large and small vessel transport, and diverse light industry all potentially introduce chemical contaminants into the port. The Green turtle population within Port Curtis is therefore potentially exposed to these land- and port activity-based chemicals. However, the extent to which chemicals are present, their accumulation characteristics in Green turtles, and potential health effects, remain poorly understood, at both temporal and spatial scales.

The first study on chemical contamination of Green sea turtles foraging in Port Curtis was conducted from January 2011 to February 2012, by Gaus *et al.* (2012), in response to higher than usual mortality rates of Green turtles, and other marine wildlife, in this area. In that study, blood samples from 40 Green turtles captured in the Boyne River estuary were analysed for a suite of organic and inorganic contaminants. The measured contaminants were then evaluated against concentrations known to have health effects in a range of vertebrate species. Most organic contaminants (e.g. pesticides, flame retardants, organotins, perfluorinated compounds) and some elements (Al, Fe, Mn and Zn) were considered to be of 'relatively low concern' (at concentrations that were low compared to other sea turtle and vertebrate populations, and where no adverse health effects have been reported in vertebrates). Some organics (e.g. polychlorinated dibenzo-p-dioxins, dibenzofurans and dioxin-like polychlorinated biphenyls) and elements (Ag, Cu, Cr, Mo and Pb) were considered to be 'possibly of concern' (at concentrations in the upper range of other sea turtle and vertebrate populations, and where effects have been reported in vertebrates following short term exposure). Seven elements (As, Cd, Co, Hg, Ni, Se and V) were considered to be 'of concern' (at concentrations clearly higher than other sea turtle and vertebrate populations, and where acute health effects have been reported across different vertebrate taxa). It was clear from these results that analysis of contaminants, and elements in particular, over larger temporal and spatial scales was warranted, to more comprehensively assess the impacts of chemicals in this Green turtle population.

In the years following 2011, a number of programs in Port Curtis continued to collect blood samples from foraging Green turtles. These programs include the "Integrated Study of the Gladstone Marine System", from 2012-2014 (Babcock *et al.*, 2015), and the current project, "Increasing the Understanding of the Green Turtle Population in Port Curtis", from 2016-2019. These programs continued to collect Green turtle samples from the Boyne River estuary, but also included other foraging sites within Port Curtis, including the Pelican Banks, Facing Island, Wiggins Island, South Trees and Wild Cattle Island (Fig 1.1, Chapter 1). Blood samples collected within these programs were archived for a range of analyses, and were included in a number of studies, including the results presented in this chapter. Over

this sampling period (2011-2019), there have been a number of major flooding events in Port Curtis (e.g. 2013, 2017), as well as expansion of port infrastructure, and port activities such as capital and maintenance dredging, and increased boating traffic. These natural and anthropogenic processes can increase contaminant concentrations, bioavailability, and toxicity of chemicals in coastal areas, and introduce a greater quantity and diversity of chemicals, including legacy chemicals and contaminants of emerging concern (Beale *et al.*, 2017; de Freitas *et al.*, 2019).

Blood samples (n=15) from Green turtles captured on the Pelican Banks in 2016, were included in a recent publication that used chemical analysis (to measure element concentrations) and cell-based bioassays (to indicate presence and effects of organic contaminants) to compare the toxicity of Port Curtis, Moreton Bay and Hervey Bay foraging Green turtle populations (Finlayson *et al.*, 2021). This study identified that the mean concentrations of elements such as Co, Sb, Mo, Mn, As and Se were above the reference intervals established by Villa *et al.* (2017) from a clinically healthy Green turtle population in a remote foraging ground, considered to be relatively free from typical coastal chemical inputs. In particular, Co was 13.3 times higher in Port Curtis Green turtles compared to Moreton Bay turtles, although similar to Hervey Bay Green turtles. Most other element concentrations were similar between these three sites, and the cell-based bioassay results indicated that the Pelican Banks Green turtles may be less impacted by organic contaminants, compared to Moreton Bay and Hervey Bay turtles. However, it is important to note that this study included Green turtles from the Pelican Banks only. With such a diversity of land- and port activity-based sources of contamination within Port Curtis, more in depth investigations into the spatial variability of Green turtle toxicology in this area was warranted.

Finlayson *et al.* (2021) also identified that blood element concentrations in Port Curtis Green turtles have generally not changed, relative to the elements reported in 2011 by Gaus *et al.* (2012), with the exception of silver and mercury, which were not included in the former study. However, it is important to note that Gaus *et al.* (2012) sampled Green turtles from the Boyne River estuary while Finlayson *et al.* (2021) sampled turtles from the Pelican Banks, so these temporal comparisons must be treated with caution. Investigating temporal trends in the contamination of Green turtle populations is challenging, as large variances in blood contaminants across the population are often observed. As illustrated by Gaus *et al.* (2012) and Finlayson *et al.* (2021), blood element concentrations can vary significantly between individuals of the same population, sometimes by more than an order of magnitude. This is likely due to the high fidelity of Green turtles to narrow foraging locations (Shimada *et al.*, 2020; Siegwalt *et al.*, 2020), resulting in variable chemical exposure within the same population, depending on where individual turtles specifically forage. This potentially large variation in Green turtle contaminant levels within sites makes it difficult to detect temporal changes. However, this can be overcome, to some extent, by sampling the same individuals over time. Ideally, investigations into the temporal variability in element contamination of foraging Green turtle populations should therefore focus on sampling recaptured turtles.

Investigating spatial and temporal trends of chemical contamination in sea turtles is of little value if not placed in a toxicological context, from which to evaluate and monitor health risks. Very little is known about the effects of chemicals in sea turtles, primarily due to the logistical and ethical constraints of conducting traditional toxicity tests on large, long-lived,

endangered species (Finlayson *et al.*, 2016). However, the establishment of Green turtle blood element concentration reference intervals (Villa *et al.*, 2017), now allows researchers and managers to consider element concentrations of Green turtles in the context of a clinically healthy Green turtle population. In addition, the establishment of sea turtle cell cultures and development of cell-based toxicity assays has recently provided a rapid and ethical approach to understanding the effects of chemicals in sea turtles (Finlayson *et al.*, 2019a; Finlayson *et al.*, 2019b; c). These bioassays can be used to assess the toxicity of individual chemicals, and the effect concentrations can then be used to conduct more accurate risk assessments of the impacts of particular chemicals on sea turtle health. They can also be used to assess the relative toxicity of different populations and sub-populations of sea turtles (Finlayson *et al.*, 2021; Finlayson *et al.*, 2020).

The primary aim of this chapter was to assess the temporal and spatial accumulation of elements in Green turtles foraging in Port Curtis, and to provide an assessment on the impact this may be having on Green turtle health, using reference intervals, supported by new toxicological information generated from cell-based bioassays. To achieve this aim we analysed elements in blood collected from free-ranging Green turtles captured and recaptured between 2011 and 2018 from the Boyne River estuary, Pelican Banks and Facing Island. There were sufficient recaptures in 2013, 2014, 2016, 2017 and 2018 to investigate the temporal changes in element concentrations over this period, and in 2017 and 2018 only, for spatial comparisons. To place these Green turtle blood element concentrations in a toxicological and health perspective, we categorised each element into two categories, 'of concern' and 'relatively low concern', in relation to deviations from recently established reference intervals. Overall, this study presents the longest known temporal analysis of element concentrations in recaptured sea turtles and provides information for managers on the temporal and spatial trends in element concentrations in Port Curtis Green turtles.

## Methods

### Turtle capture

Green turtles were captured within Port Curtis from 2011 to 2018, using both rodeo and netting methods described in Chapter 1 and Gaus *et al.* (2012). Turtles used in this study were captured at three sites: the Boyne River estuary (n=19), Pelican Banks (n=16), and Facing Island (n=2). All turtles were captured in accordance with the standard practices approved under the Department of Agriculture and Fisheries (DAF) Animal Experimentation Ethics Committee: Queensland Turtle Conservation (QTC) Project SA 2018-11-660, 661, 662, 663, 664. The use of nets for the capture of turtles was in accordance with DAF General Fisheries Permit 191182, issued to EHP/DES.

### Blood collection

Blood (10 mL) was taken from the dorsal cervical sinus using methods described by Owens and Ruiz (1980). Blood was collected using sterile 18G needles and disposable syringes and added to sodium heparinised BD Vacutainers® immediately after collection. Blood was then stored at -20°C until analysis. All blood samples were collected using methods detailed in DAF permit ENV/13/15/AEC, administered by the Griffith University Animal Ethics Committee.



## Element analysis

Sample preparation, acid digestion, and quality control followed previously validated methods for Green turtle whole blood (Villa *et al.*, 2017). Briefly, whole blood was homogenised using acid rinsed polytetrafluoroethylene boiling chips (Sigma-Aldrich; Z243558-1EA, Merck Pty Ltd), and a benchtop vortex (MO BIO Vortex Genie® 2). The homogenate (1 mL) was aliquoted to an acid rinsed 10 mL polyethylene tube, and 1 mL concentrated HNO<sub>3</sub> (67-70% w/w Baseline, Seastar Chemicals, Canada) was added, and rested overnight at room temperature. The tubes were then gradually heated to a maximum of 89°C in a heating block (approx. 2 h), with the subsequent addition of 1 mL H<sub>2</sub>O<sub>2</sub> (30% w/w Baseline, Seastar Chemicals, Canada), and an additional hour of heating until fully digested.

Quality assurance and control samples were prepared and treated identically to the blood samples: method blanks (n=6) composed of 1 mL Milli-Q water; two randomly selected whole blood samples spiked with calibration solution (see below for composition) to final injected concentrations of 10 µg/L and 100 µg/L; and a Seronorm 1 (Sero, Norway) whole blood certified reference material (CRM). All digests were filtered using disposable syringes and hydrophilic polyvinylidene fluoride filters (0.45 µm Millex-HV, Merck Pty Ltd. Australia), and diluted (20-fold) with Milli-Q water prior to analysis for trace elements via ICP-MS (Agilent 7900). The instrument was calibrated using a commercially available standard mixtures that included arsenic (As), barium (Ba), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), nickel (Ni), lead (Pb), antimony (Sb), selenium (Se), thorium (Th), titanium (Ti), uranium (U), vanadium (V), and zinc (Zn). Internal standards (bismuth (Bi), indium (In), scandium (Sc), terbium (Tb) and yttrium (Y)) were added online, via a mixing tee, to a final concentration of approximately 100 µg/L for each element. Calibration, blanks, and sample data were evaluated using Agilent's ChemStation software (ICP-MS MassHunter, version 4.3). All concentrations were reported as µg/L in whole blood.

Method detection limits (MDLs) were defined as three times the standard deviation of the method blanks. Spike and Certified Reference Material (CRM) recovery results were also calculated for each run. To investigate potential impacts of long-term storage of samples on elemental concentrations, select samples from earlier years were re-processed and analysed for comparison against results from previous years, performed on different instruments. Since the samples were analysed over multiple runs, spanning many years, the MDLs, and spike and CRM recoveries varied slightly between runs. Nevertheless, within run spike, CRM recoveries, and repeat analyses across years, produced results that were consistent, and within acceptable limits, for the purposes of this study, with MDLs maintained at suitably low levels for all elements.

## Temporal trends in element concentrations

To assess the temporal changes in element concentrations in the Green turtles foraging in Port Curtis, the concentration of elements for which there are reference intervals were plotted against year of sample collection. Samples from recaptured turtles were obtained opportunistically, with higher success rates occurring at those locations where the mark-and-capture efforts were highest and most consistent over time. Concentration data for turtles captured at the Pelican Banks and Facing Island were pooled for these analyses and plotted

separately to turtles from the Boyne River estuary. Only element concentrations that were above the detection limit on all recaptures of the same turtle were included. Data points from the same recaptured turtle were joined with a line so that the change in blood element concentrations within individuals over time could be observed. Temporal patterns were established for each element, and these data were used to model general trends in Green turtle blood element concentrations over the eight-year sampling period (2011-2018). These general trends were then discussed in the context of rainfall events and human activities in Port Curtis over this period. Rainfall data for the Calliope catchment between January 2010 and December 2018 were extracted from the Water Monitoring Information Portal (Department of Natural Resources, Mines and Energy).

#### Spatial comparisons of element concentrations

Blood element concentrations in the Green turtles foraging at the Boyne River estuary, Pelican Banks and Facing Island were also spatially compared. Element concentrations for these three sites were plotted separately for each year of sample collection (2011, 2103, 2104, 2016, 2017, 2018), and differences between locations were assessed using the Kruskal-Wallis test function in R version 4.0.1, via the ggpubr package (v0.4.0), with significant differences evaluated at a p-value of 0.50.

#### Results and discussion

A total of 77 blood samples were collected and analysed, from 37 individual Green turtles captured in at least two different years throughout the study period (2011-2018). Of these samples, 39 (from 19 turtles) were from the Boyne River estuary, 33 (from 16 turtles) from the Pelican Banks and four (from two turtles) from Facing Island. One turtle was captured and sampled on three separate occasions (Pelican banks, 2016, 2017 and 2018), and one on four separate occasions (Boyne River estuary; 2014, 2016, 2017, 2018). All turtles were recaptured at the same site where each was originally captured, further supporting satellite telemetry and tag-recapture data that illustrates strong fidelity of Port Curtis Green turtles to narrow foraging ranges.

#### Temporal variation

Sixteen elements (As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Ti, V and Zn) were regularly detected, and measured at concentrations consistently above the reference intervals (RIs) established by Villa *et al.* (2017). These elements are discussed in more detail below and presented in Figures 7.1 to 7.16. The remaining six elements (Ca, K, Mg, Na, Th, U) had very few blood concentrations above the detection limit, and/or did not have published RIs, and were not included in further analysis and interpretation.

Generally, the Boyne River estuary and Pelican Banks turtles followed similar temporal trends in their blood element concentrations. There were also some temporal trends in concentrations that were consistent for groups of elements. Specifically, five of the elements (As, Cd, Cu, Fe, Se) were above the RIs in 2011 and decreased to within the RIs in 2014, where they remained until increasing rapidly to well above the RIs again in 2017, and then either stayed high, or showed signs of decreasing slightly, in 2018. Five elements (Ba, Pb, Ti, V, Zn) showed a similar spike in 2017/18, with the difference of starting within the reference intervals in 2011. Antimony (Sb) could also be considered to follow this pattern, but was mostly detected in 2016-2018, with the exception of two turtles captured in 2013 and

2014. Three elements (Mn, Mo, Ni) were within the RIs in 2011 and increased to above RIs in 2014, before returning to within RIs. Chromium (Cr) showed a similar pattern to Mo, Mn and Ni, with the difference of not increasing to above RIs in 2017-2018. Cobalt (Co) showed a similar temporal pattern to Mn, Mo and Ni, but was above the RIs at all times.

Notwithstanding the comparisons against the RIs, and combining Pelican Banks and Boyne River estuary turtles, the temporal trends in blood trace element concentrations in Port Curtis Green turtles followed two broad patterns (Figure 7.17): 1) Co, Cr, Mn, Mo, Ni - concentrations were generally low in 2011, followed by a spike in the concentrations in 2013/14, a return to low concentrations in 2016, and another spike (except Cr) in 2017/18, although there are signs of concentrations decreasing from 2017 to 2018 in some individuals; and 2) As, Ba, Cd, Cu, Fe, Pb, Sb, Se, Ti, V, Zn - concentrations were generally low in 2011, and remained low until a spike in 2017/18, although, again, signs of concentrations decreasing from 2017 to 2018 in some individuals.

These general trends indicate that the exposure of Port Curtis Green turtles to trace elements has changed over this eight-year sampling period. It is not known what has caused these changes to exposure, although the following rainfall patterns and port activity suggest that climatic and/or anthropogenic activities could be involved. Rainfall data collected from the Calliope River station 132001A at Castlehope (Figure 7.18) indicate larger than average wet season rainfall events at the end of 2010 (associated with Tropical Cyclone *Yasi*) early 2013 (associated with Tropical Cyclone *Oswald*), and early 2017 (associated with Tropical Cyclone *Debbie*). While no Green turtles were captured in 2012 following Cyclone *Oswald*, the high concentrations of many elements in turtles captured in 2011 and 2017/2018, may be associated with the rainfall events of Tropical Cyclones *Tasha* and *Debbie*, respectively. It is well known that large rainfall events can increase inputs of trace elements from land-based sources, and coastal flooding events can increase resuspension of benthic coastal sediment, and their associated contaminants (e.g. Coates-Marnane *et al.*, 2016).

There were also peaks in turtle blood Co, Cr, Mn, Mo, and Ni concentrations in 2014. While 2014 was a period not associated with very high rainfall, relative to the aforementioned rainfall events, it was the year following one of the highest level flooding events in the region within the previous 100 years. There may also be port developments and activities, such as maintenance and capital dredging, and boating traffic that contributed to these changes in exposure of Green turtles to trace elements. It is well established that these types of activities can result in the resuspension of sediments and their associated contaminants, and the introduction of new elements (de Freitas *et al.*, 2019). The spatial analysis (see next section) provides further evidence that port activities may be having an impact on the element concentrations observed in Port Curtis Green turtles. However, further investigations into potential links between port activities and environmental contaminant levels are warranted.

In interpreting these data, it is also important to consider that essential elements, in general, are taken up and regulated more efficiently than non-essential elements. In addition, the residence time of any element in blood is expected to be greatly influenced by species-specific metabolic needs, and detoxification strategies, as well as overall health and individual variation. To date there is a paucity of data for the toxicokinetics and

toxicodynamics of trace elements in reptiles. Thus, an observed change in elemental blood concentration from above RI limits back to within RI limits (as observed for some elements) does not necessarily indicate that the risk to turtle health has been reduced, as blood is only a snapshot in time of an active metabolic pathway. Low concentrations of many elements were observed between 2011 and 2016, with signs of additional elements decreasing in 2018, following the 2017 rainfall event. It is likely that, although blood concentrations have reduced in these periods of suspected low exposure conditions, these elements are bioaccumulating in other tissues (liver, kidney, brain, etc), where they can elicit toxic effects, particularly chronic.

# Arsenic

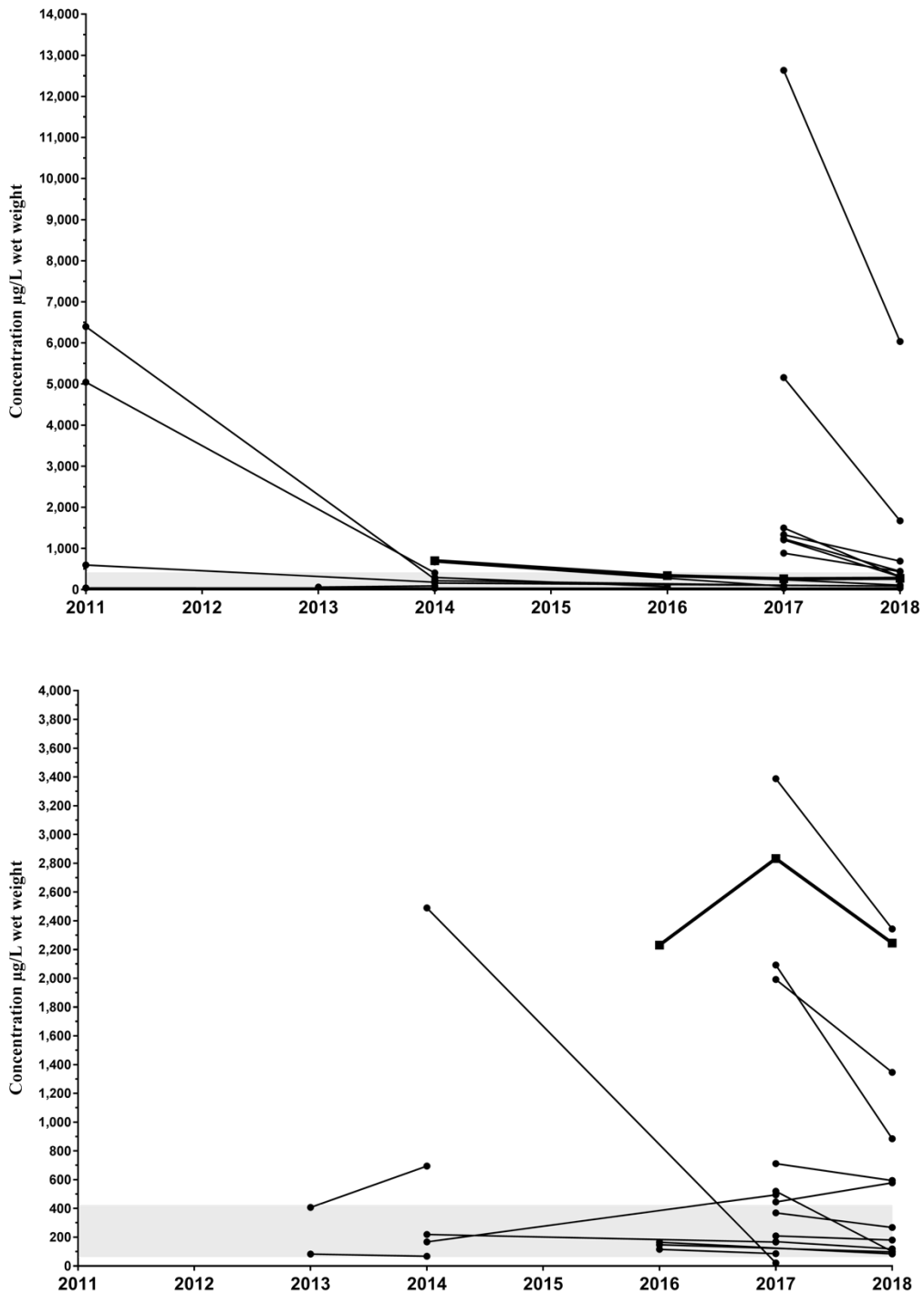


Figure 7.1. Arsenic (As) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary (top panel) and the Pelican Banks (bottom panel) between 2011 and 2018.

Data from the same turtle are connected with a line. The turtles captured three (Pelican Banks) and four (Boyne) times are represented with a different symbol (filled square) and bold line. Grey bars illustrate the reference interval established by Villa *et al.* (2017).

## Barium

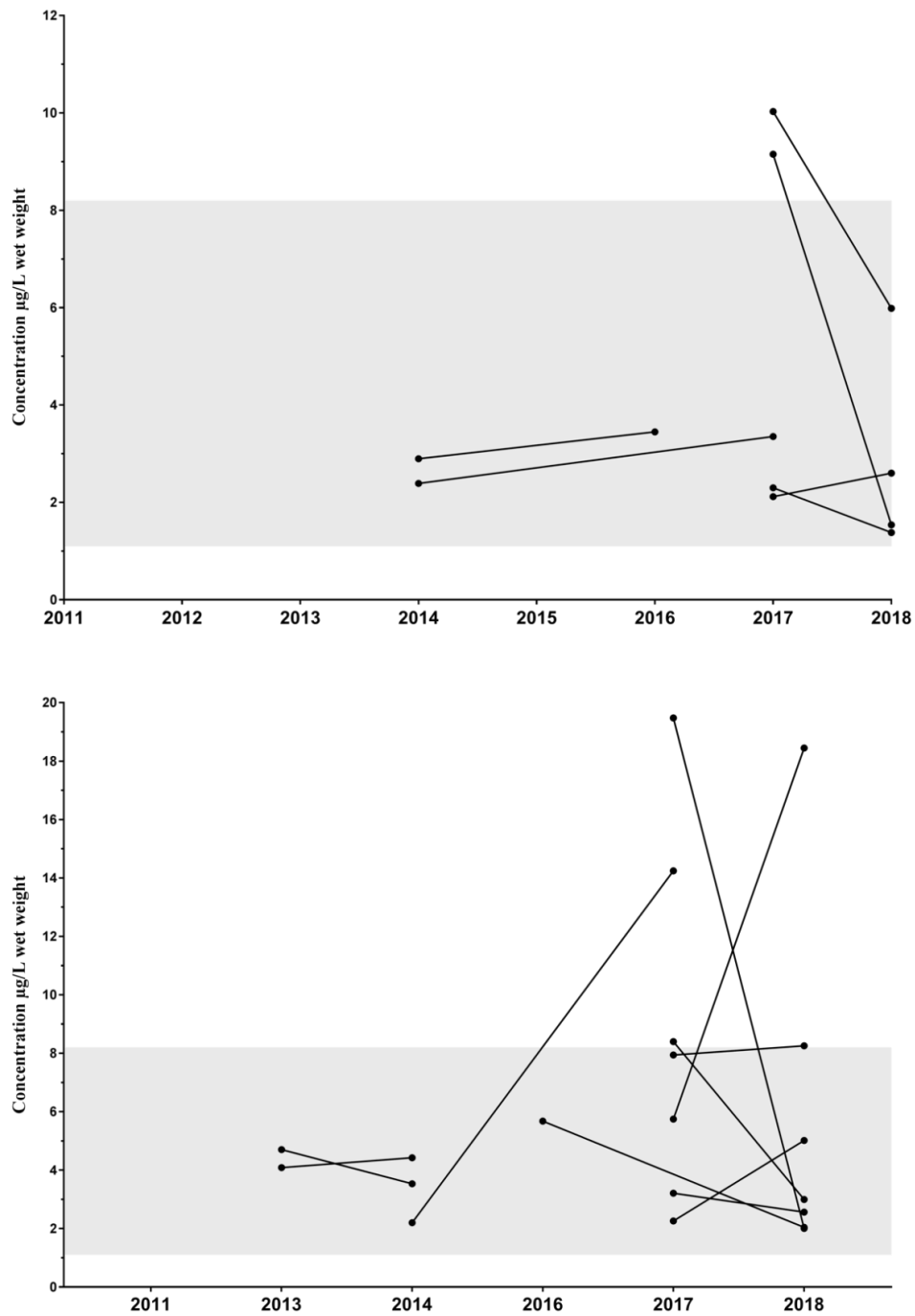


Figure 7.2. Barium (Ba) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary (top panel) and the Pelican Banks (bottom panel) between 2011 and 2018.

Data from the same turtle are connected with a line. Grey bars illustrate the reference interval established by Villa *et al.* (2017).

# Cadmium

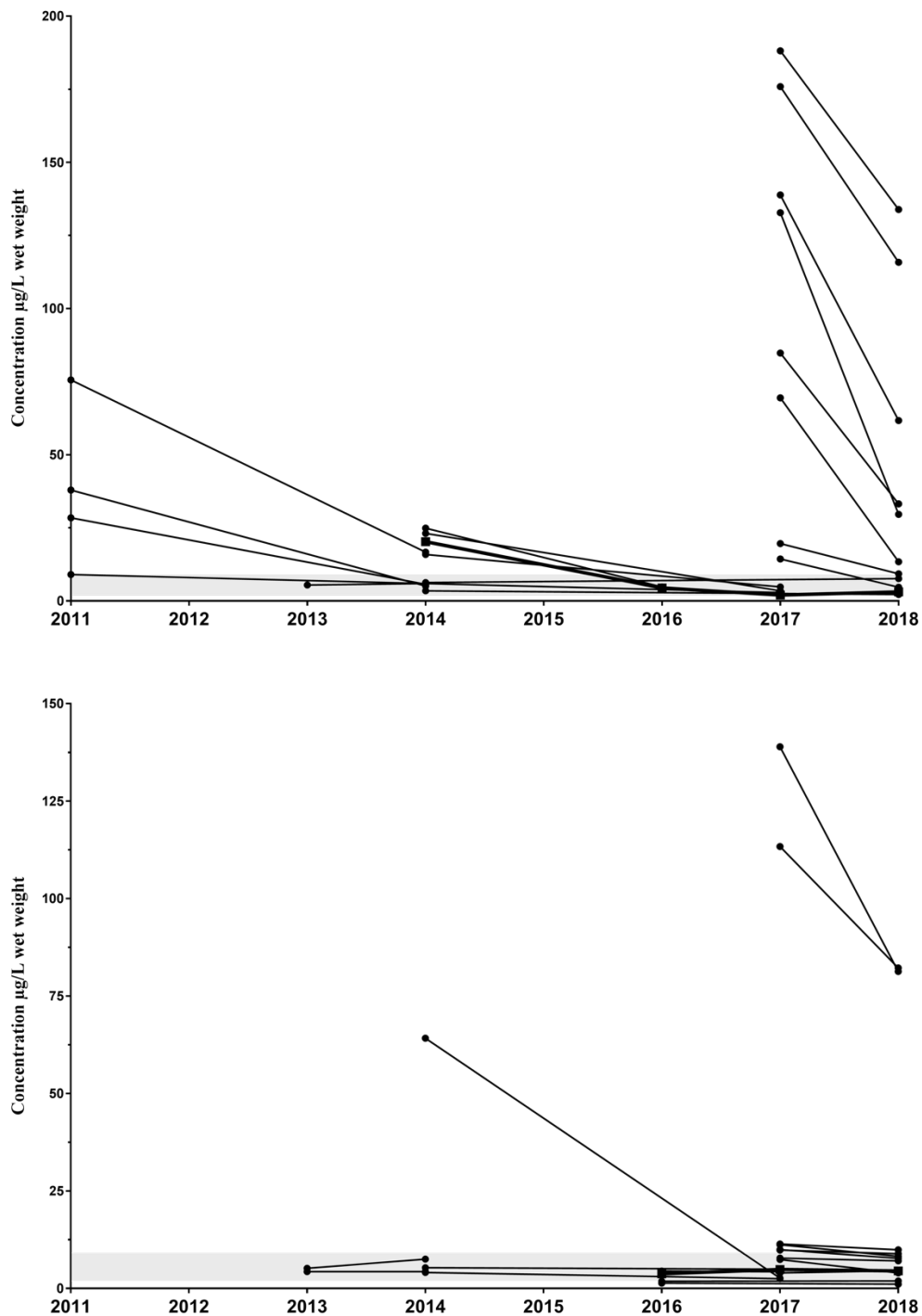


Figure 7.3. Cadmium (Cd) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary (top panel) and the Pelican Banks (bottom panel) between 2011 and 2018.

Data from the same turtle are connected with a line. The turtles captured three (Pelican Banks) and four (Boyne) times are represented with a different symbol (filled square) and bold line. Grey bars illustrate the reference interval established by Villa et al. (2017).

# Cobalt

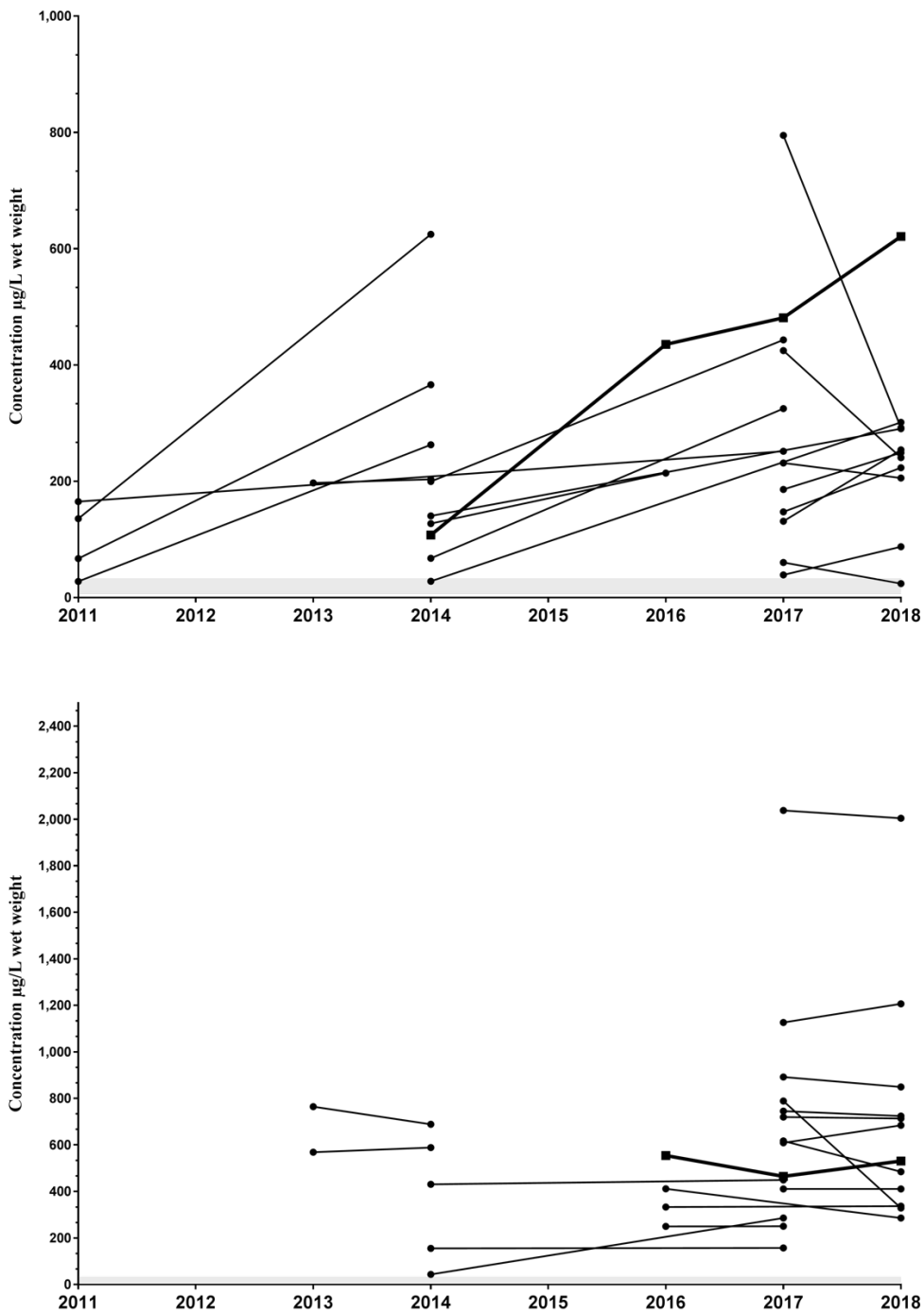


Figure 7.4. Cobalt (Co) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary (top panel) and the Pelican Banks (bottom panel) between 2011 and 2018.

Data from the same turtle are connected with a line. The turtles captured three (Pelican Banks) and four (Boyne) times are represented with a different symbol (filled square) and bold line. Grey bars illustrate the reference interval established by Villa et al. (2017).



# Chromium

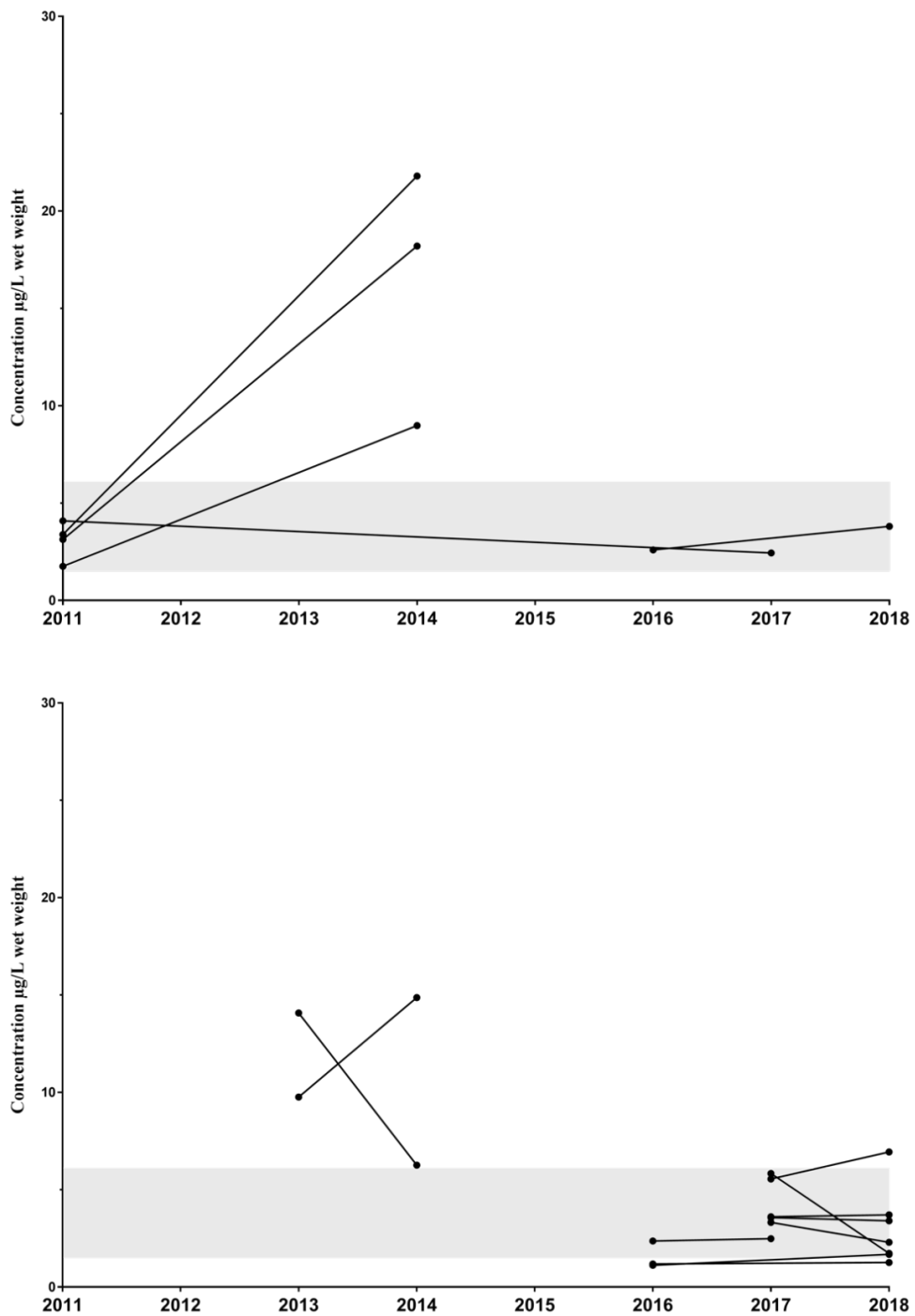


Figure 7.5. Chromium (Cr) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary (top panel) and the Pelican Banks (bottom panel) between 2011 and 2018.

Data from the same turtle are connected with a line. Grey bars illustrate the reference interval established by Villa *et al.* (2017).

# Copper

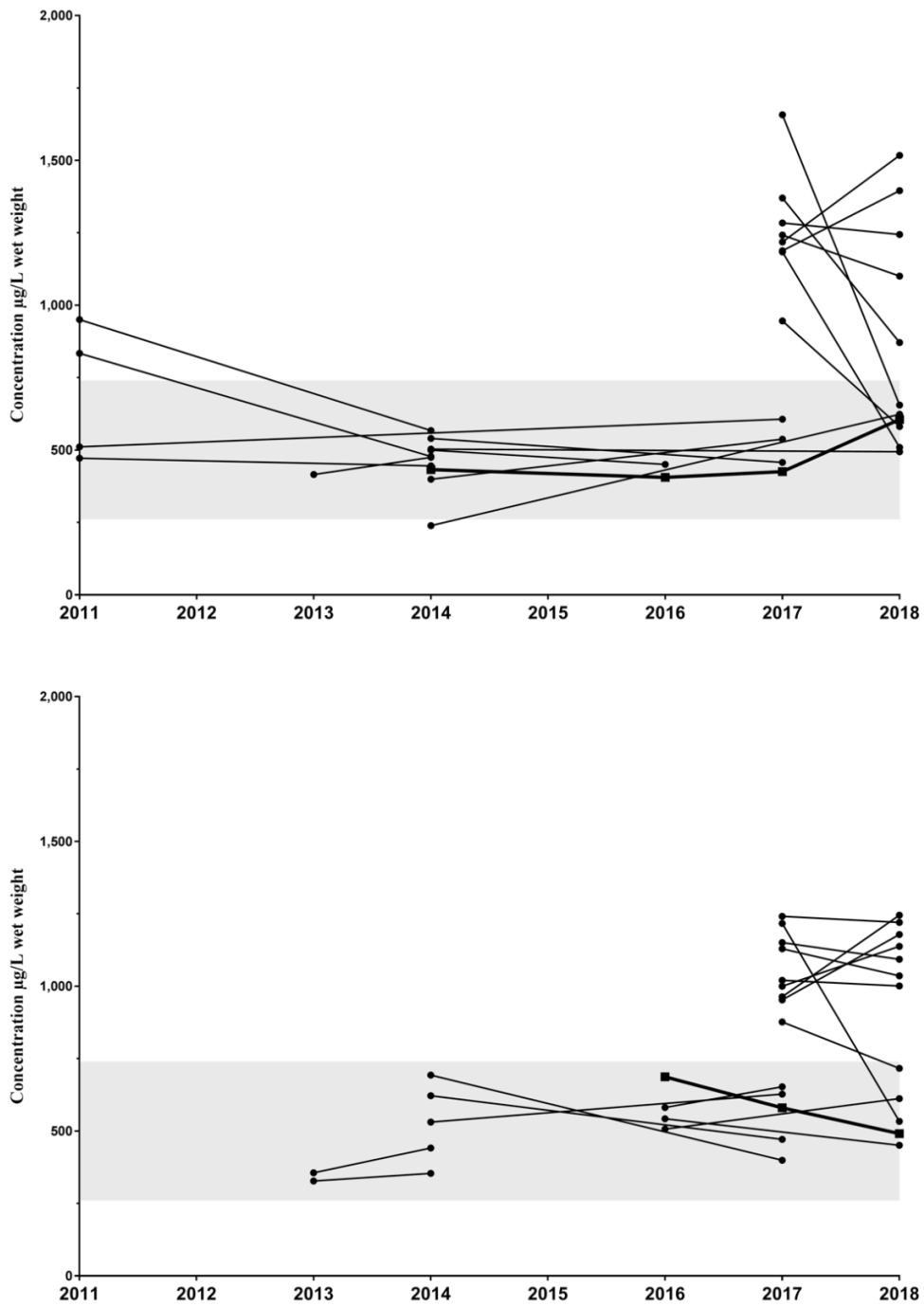


Figure 7.6. Copper (Cu) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary (top panel) and the Pelican Banks (bottom panel) between 2011 and 2018.

Data from the same turtle are connected with a line. The turtles captured three (Pelican Banks) and four (Boyne) times are represented with a different symbol (filled square) and bold line. Grey bars illustrate the reference interval established by Villa *et al.* (2017).

# Iron

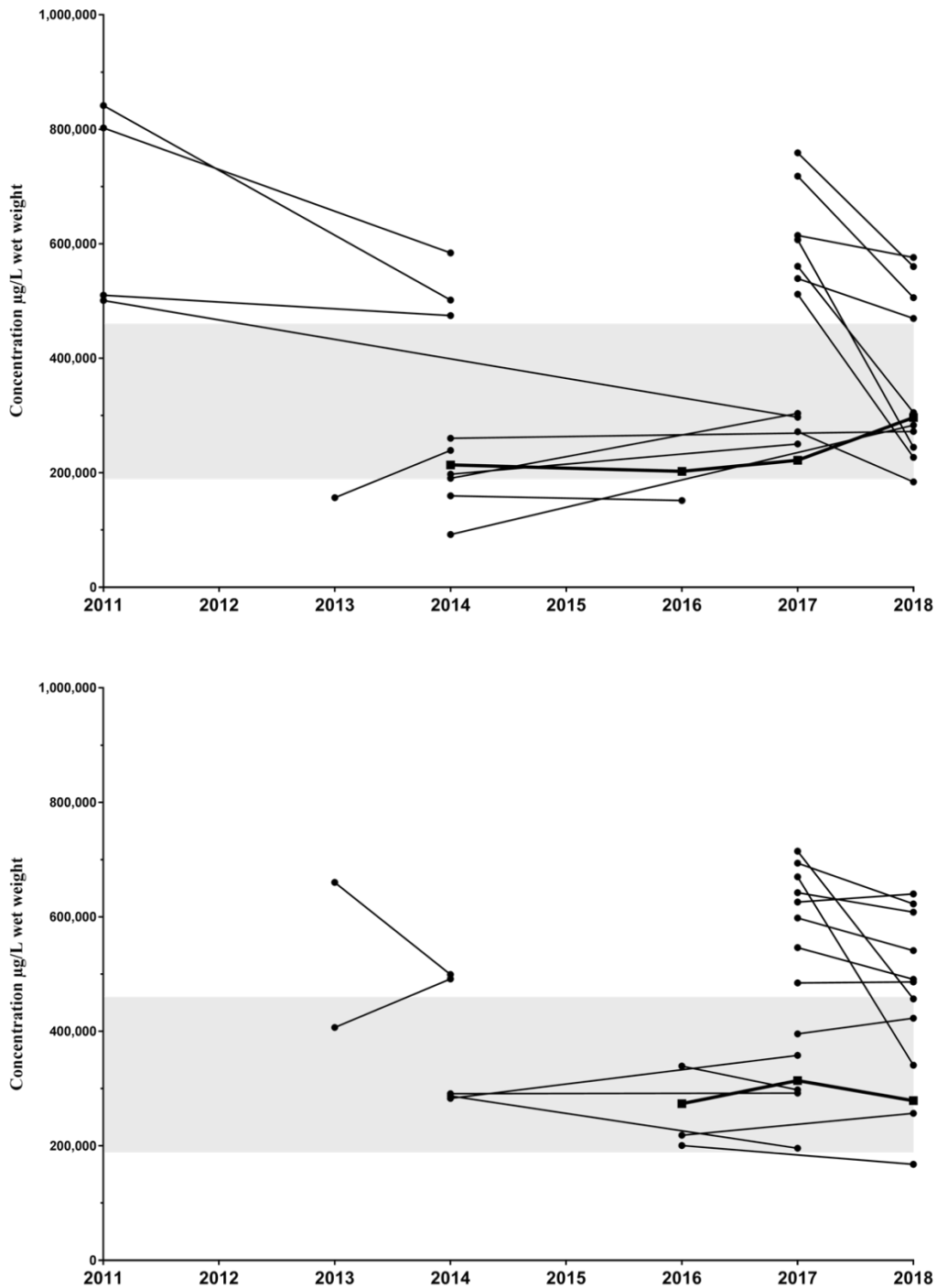


Figure 7.7. Iron (Fe) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary (top panel) and the Pelican Banks (bottom panel) between 2011 and 2018.

Data from the same turtle are connected with a line. The turtles captured three (Pelican Banks) and four (Boyne) times are represented with a different symbol (filled square) and bold line. Grey bars illustrate the reference interval established by Villa *et al.* (2017).

## Manganese

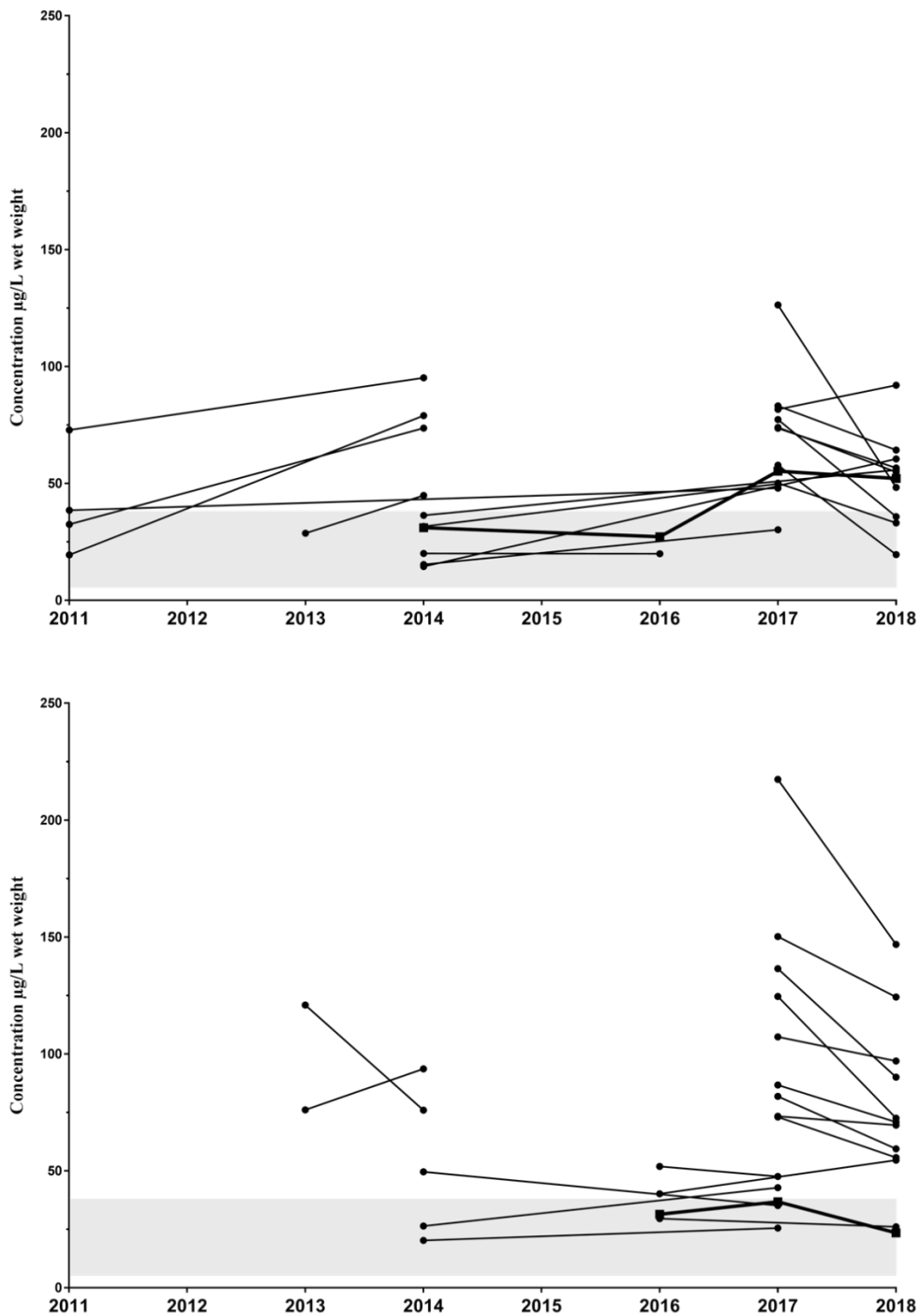


Figure 7.8. Manganese (Mn) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary (top panel) and the Pelican Banks (bottom panel) between 2011 and 2018.

Data from the same turtle are connected with a line. The turtles captured three (Pelican Banks) and four (Boyne) times are represented with a different symbol (filled square) and bold line. Grey bars illustrate the reference interval established by Villa *et al.* (2017).

## Molybdenum

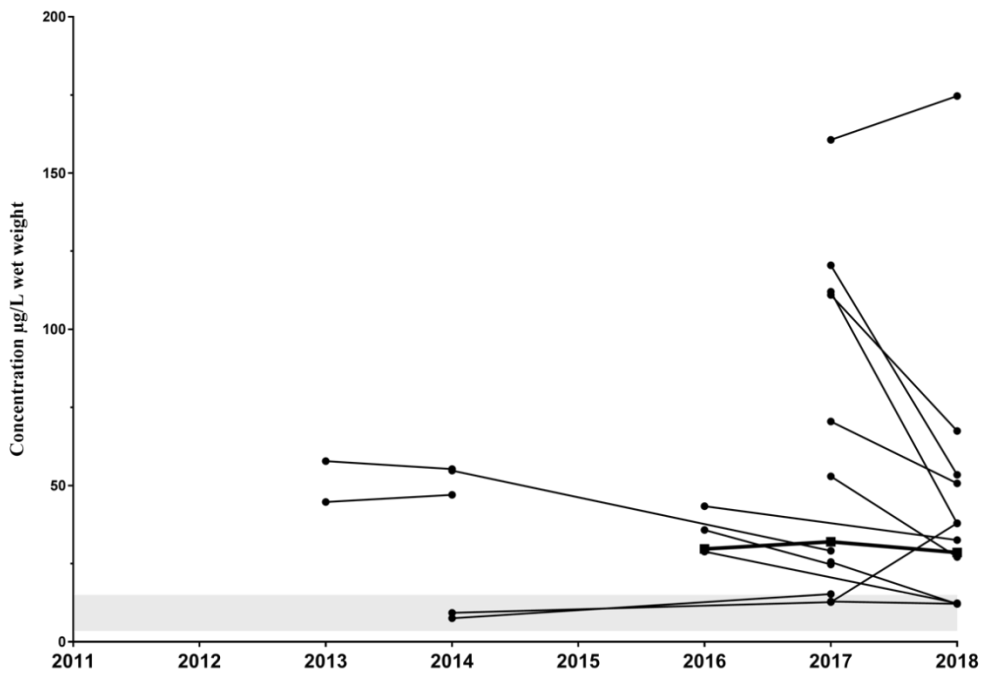
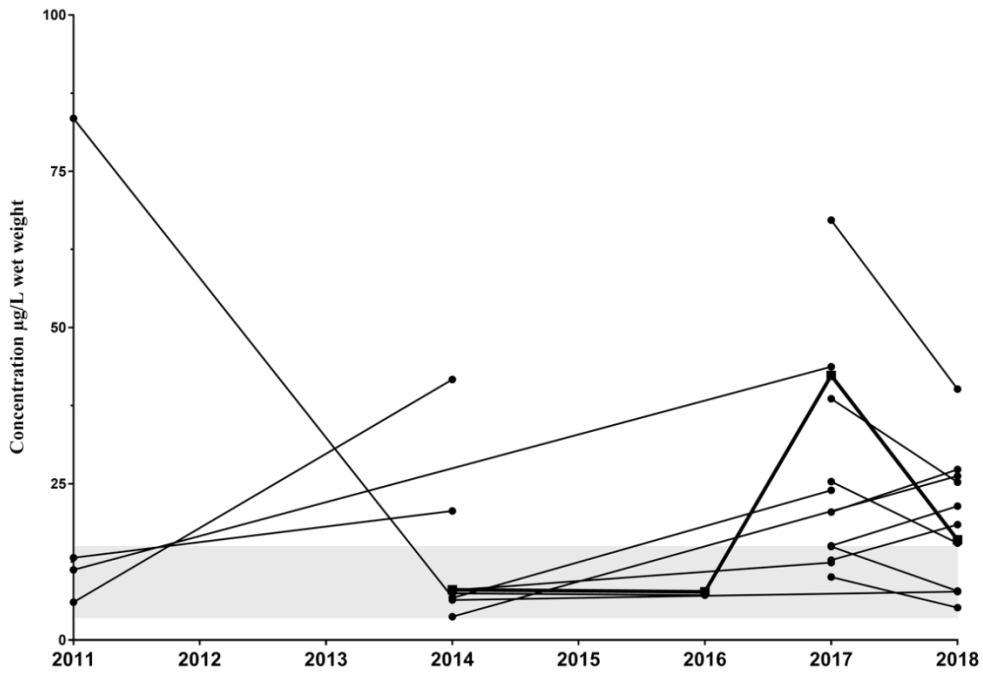


Figure 7.9. Molybdenum (Mo) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary (top panel) and the Pelican Banks (bottom panel) between 2011 and 2018.

Data from the same turtle are connected with a line. The turtles captured three (Pelican Banks) and four (Boyne) times are represented with a different symbol (filled square) and bold line. Grey bars illustrate the reference interval established by Villa *et al.* (2017).

## Nickel

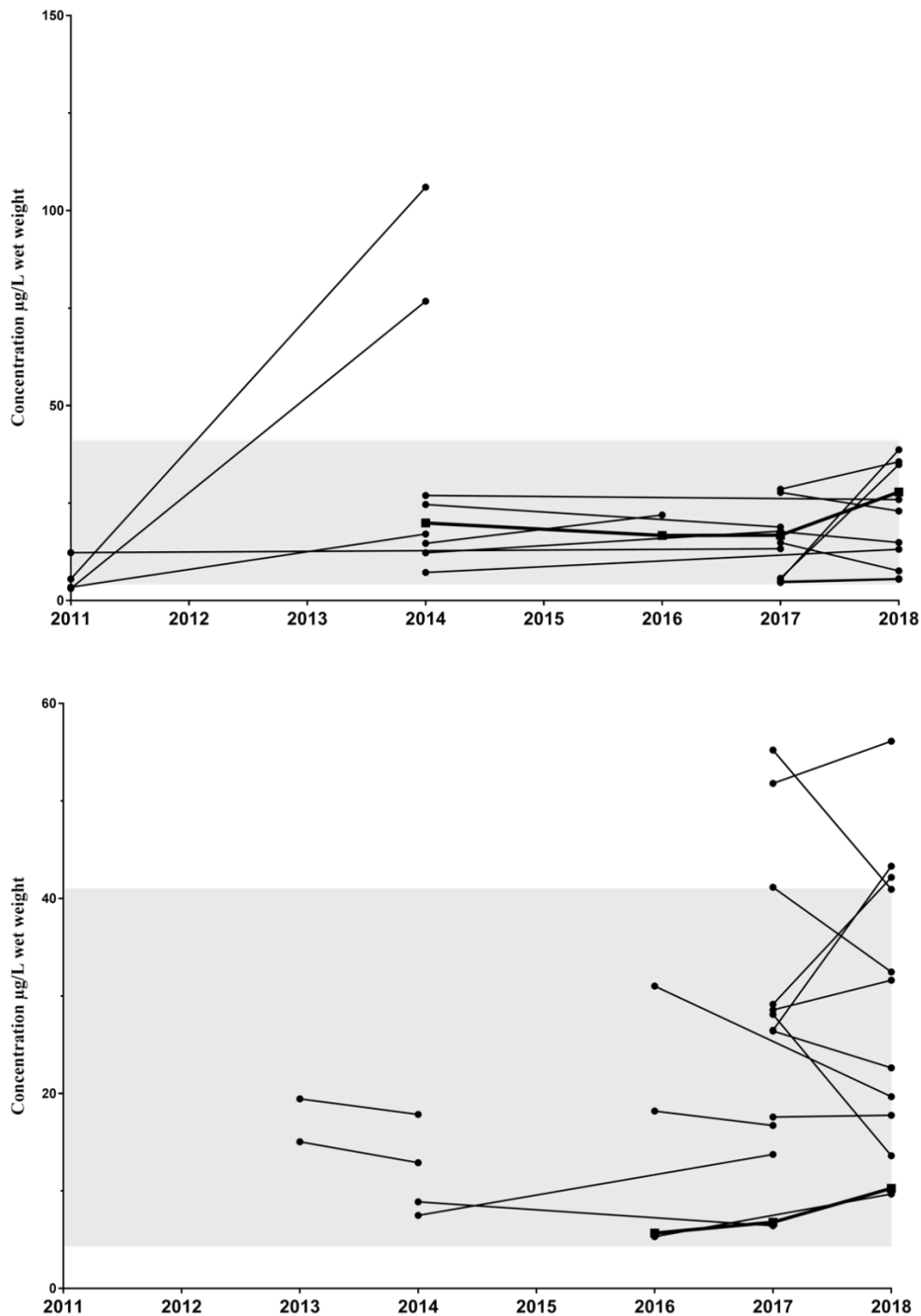


Figure 7.10. Nickel (Ni) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary (top panel) and the Pelican Banks (bottom panel) between 2011 and 2018.

Data from the same turtle are connected with a line. The turtles captured three (Pelican Banks) and four (Boyne) times are represented with a different symbol (filled square) and bold line. Grey bars illustrate the reference interval established by Villa *et al.* (2017).

## Lead

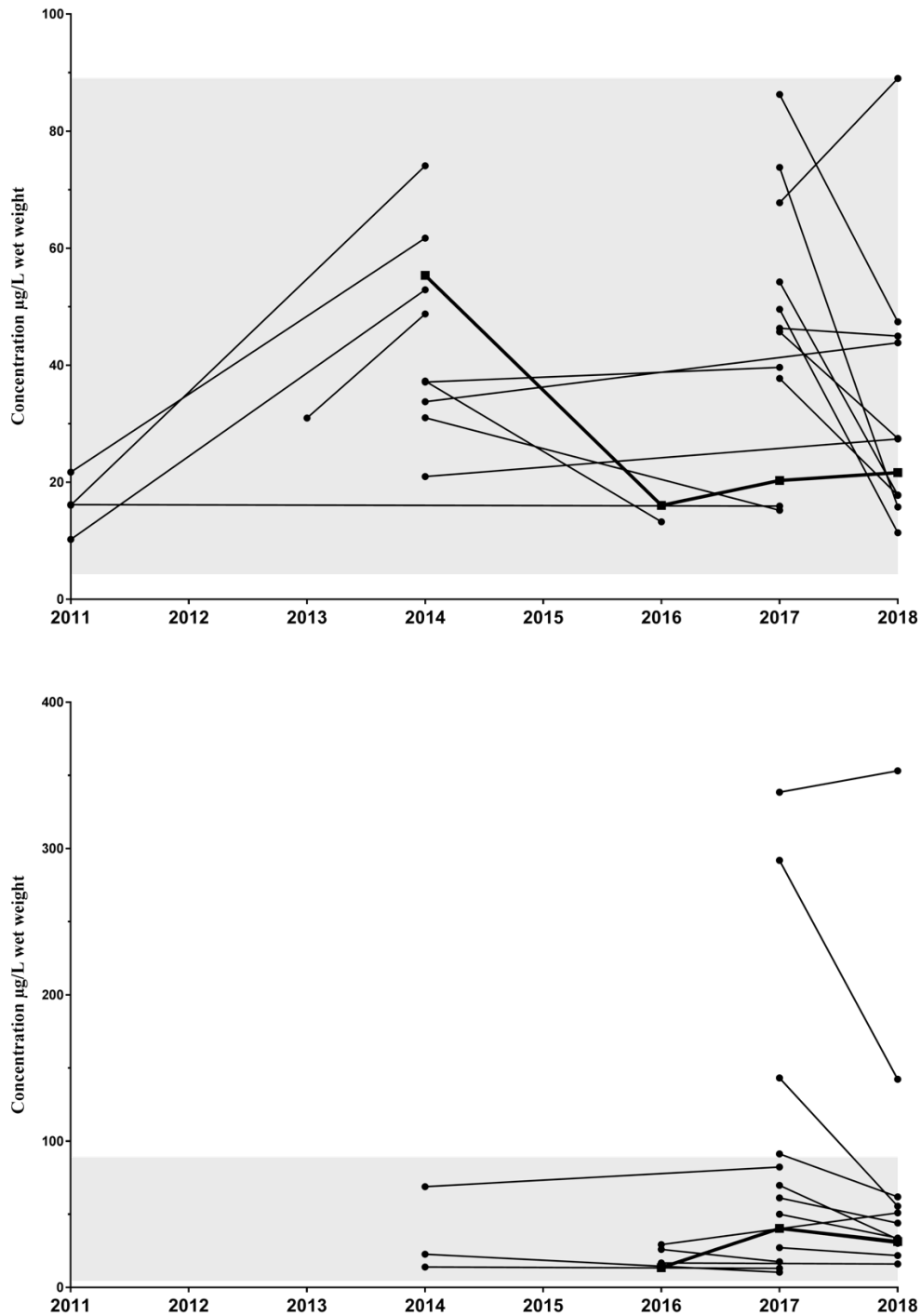


Figure 7.11. Lead (Pb) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary (top panel) and the Pelican Banks (bottom panel) between 2011 and 2018.

Data from the same turtle are connected with a line. The turtles captured three (Pelican Banks) and four (Boyne) times are represented with a different symbol (filled square) and bold line. Grey bars illustrate the reference interval established by Villa *et al.* (2017).

## Antimony

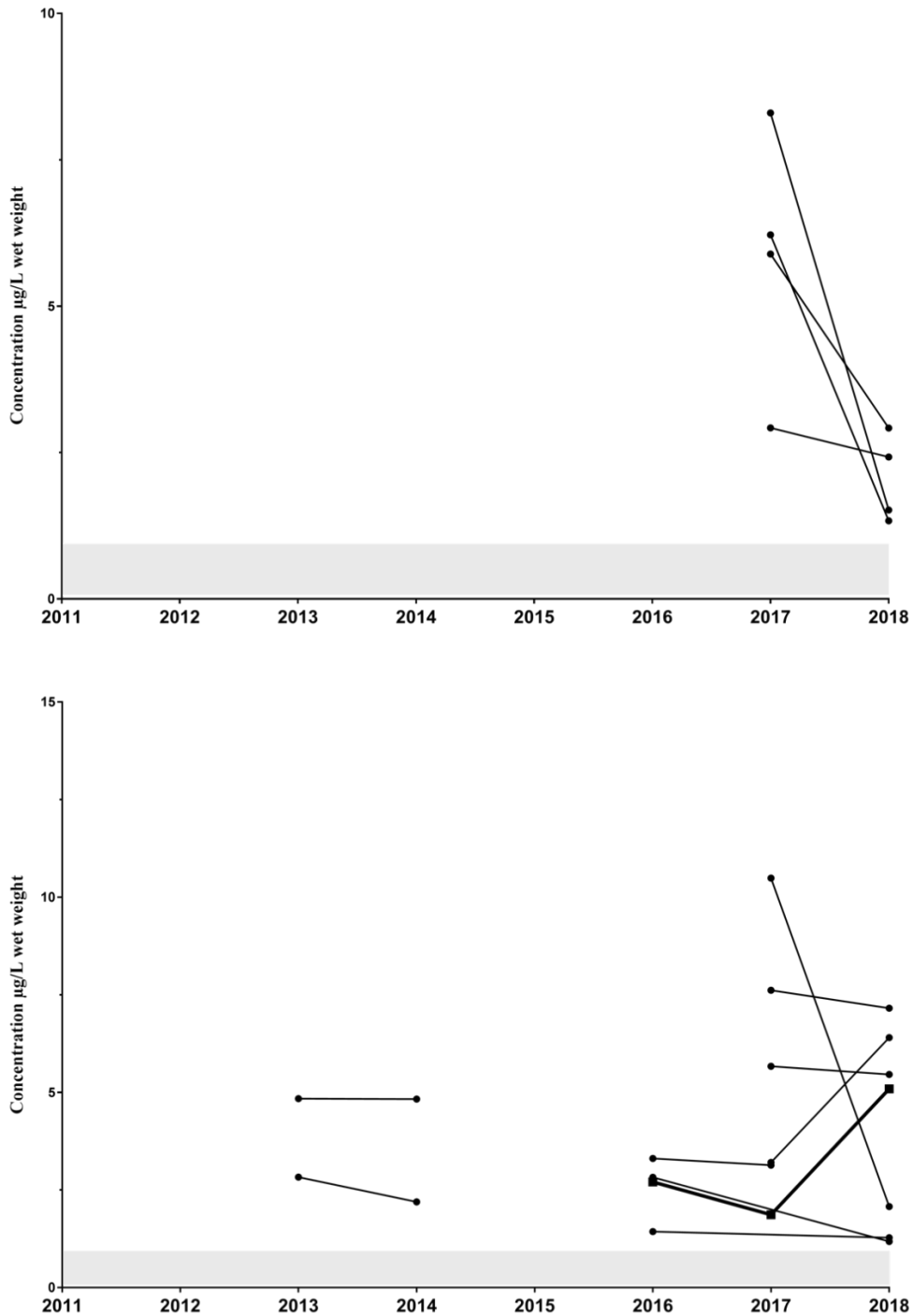




Figure 7.12. Antimony (Sb) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary (top panel) and the Pelican Banks (bottom panel) between 2011 and 2018.

Data from the same turtle are connected with a line. The turtle captured three (Pelican Banks) times is represented with a different symbol (filled square) and bold line. Grey bars illustrate the reference interval established by Villa *et al.* (2017).

## Selenium

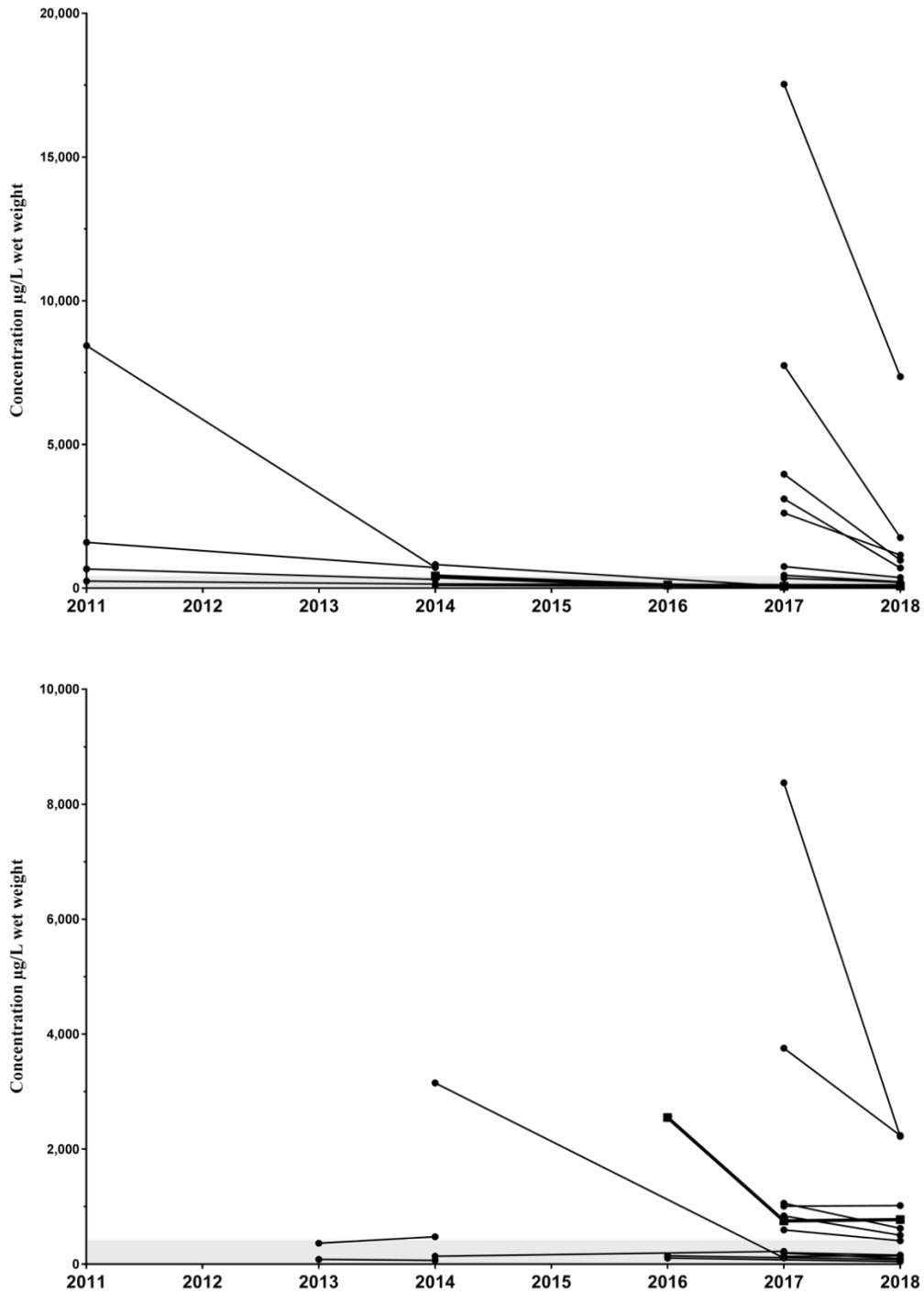


Figure 7.13. Selenium (Se) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary (top panel) and the Pelican Banks (bottom panel) between 2011 and 2018.

Data from the same turtle are connected with a line. The turtles captured three (Pelican Banks) and four (Boyne) times are represented with a different symbol (filled square) and bold line. Grey bars illustrate the reference interval established by Villa *et al.* (2017).

## Titanium

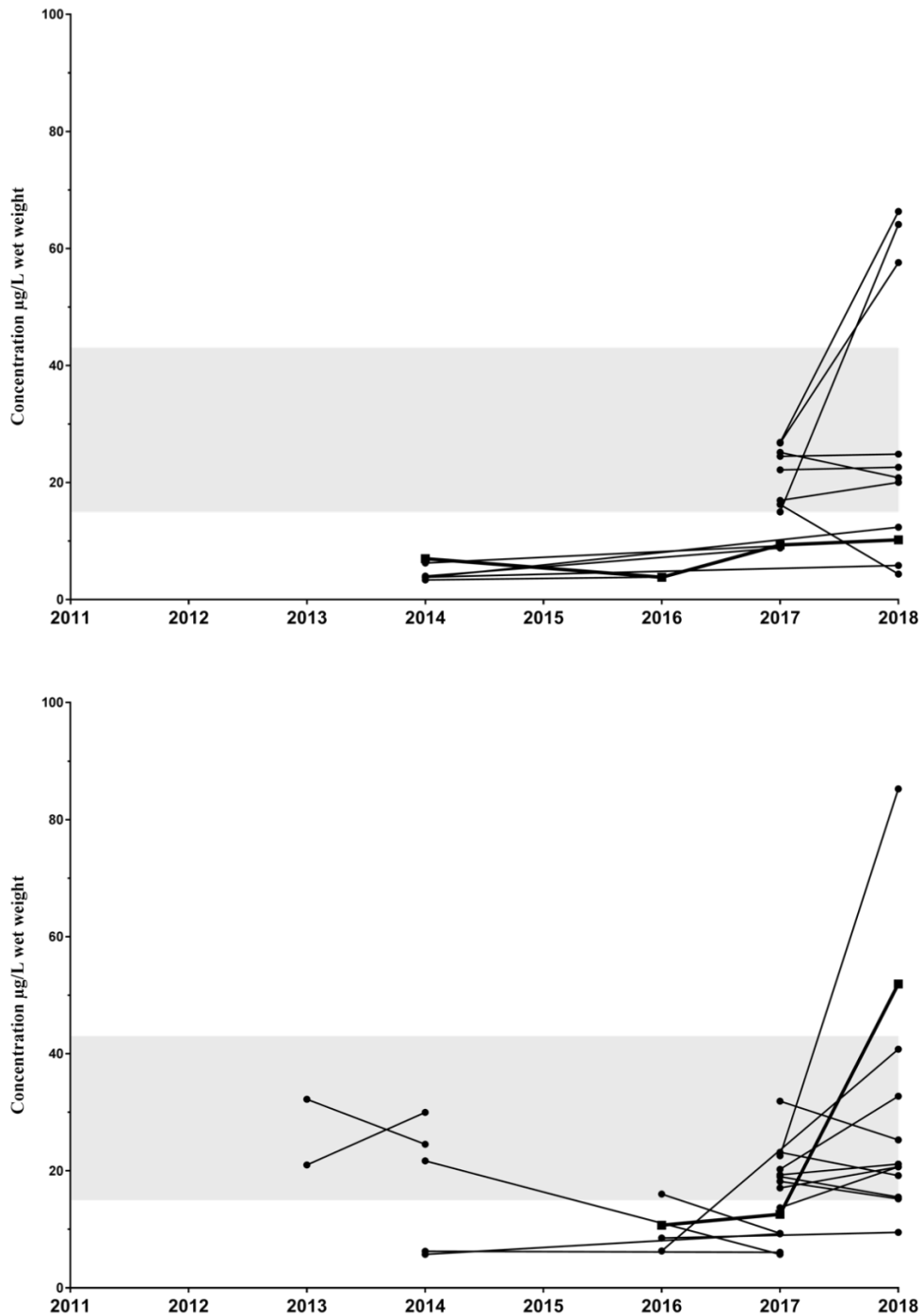


Figure 7.14. Titanium (Ti) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary (top panel) and the Pelican Banks (bottom panel) between 2011 and 2018.

Data from the same turtle are connected with a line. The turtles captured three (Pelican Banks) and four (Boyne) times are represented with a different symbol (filled square) and bold line. Grey bars illustrate the reference interval established by Villa *et al.* (2017).

## Vanadium

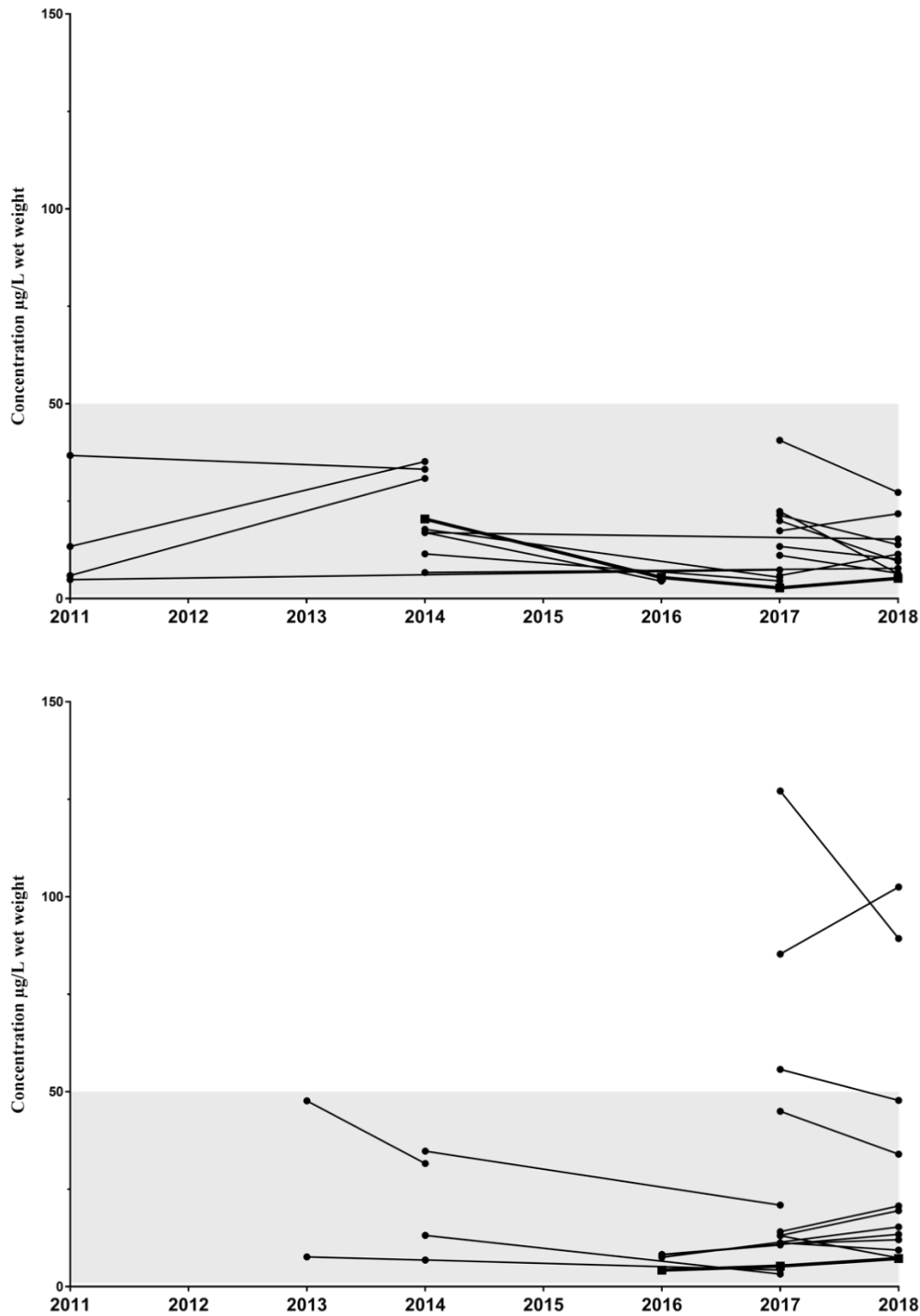


Figure 7.15. Vanadium (V) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary (top panel) and the Pelican Banks (bottom panel) between 2011 and 2018.

Data from the same turtle are connected with a line. The turtles captured three (Pelican Banks) and four (Boyne) times are represented with a different symbol (filled square) and bold line. Grey bars illustrate the reference interval established by Villa *et al.* (2017).

## Zinc

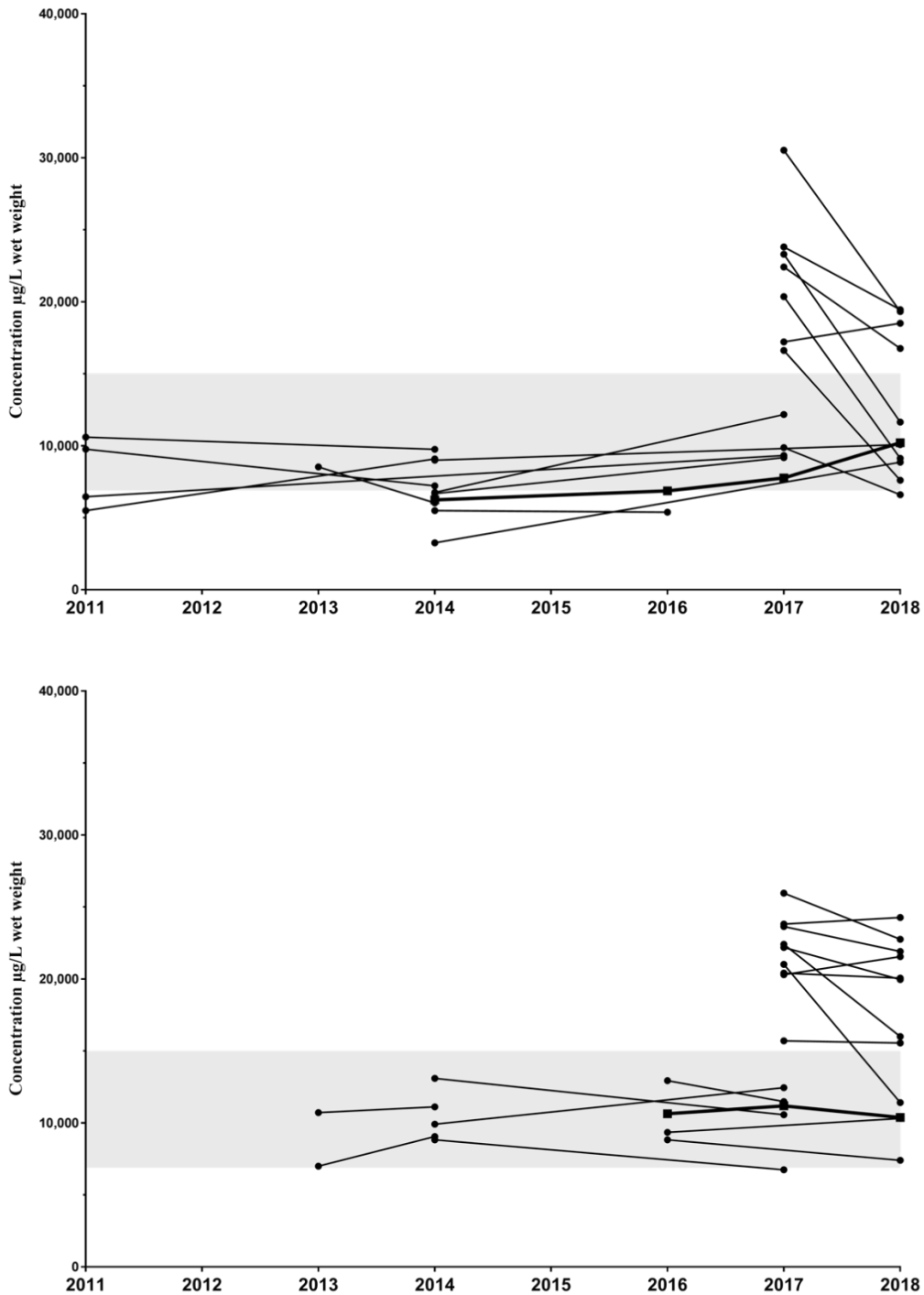


Figure 7.16. Zinc (Zn)\_concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary (top panel) and the Pelican Banks (bottom panel) between 2011 and 2018.

Data from the same turtle are connected with a line. The turtles captured three (Pelican Banks) and four (Boyne) times are represented with a different symbol (filled square) and bold line. Grey bars illustrate the reference interval established by Villa *et al.* (2017).

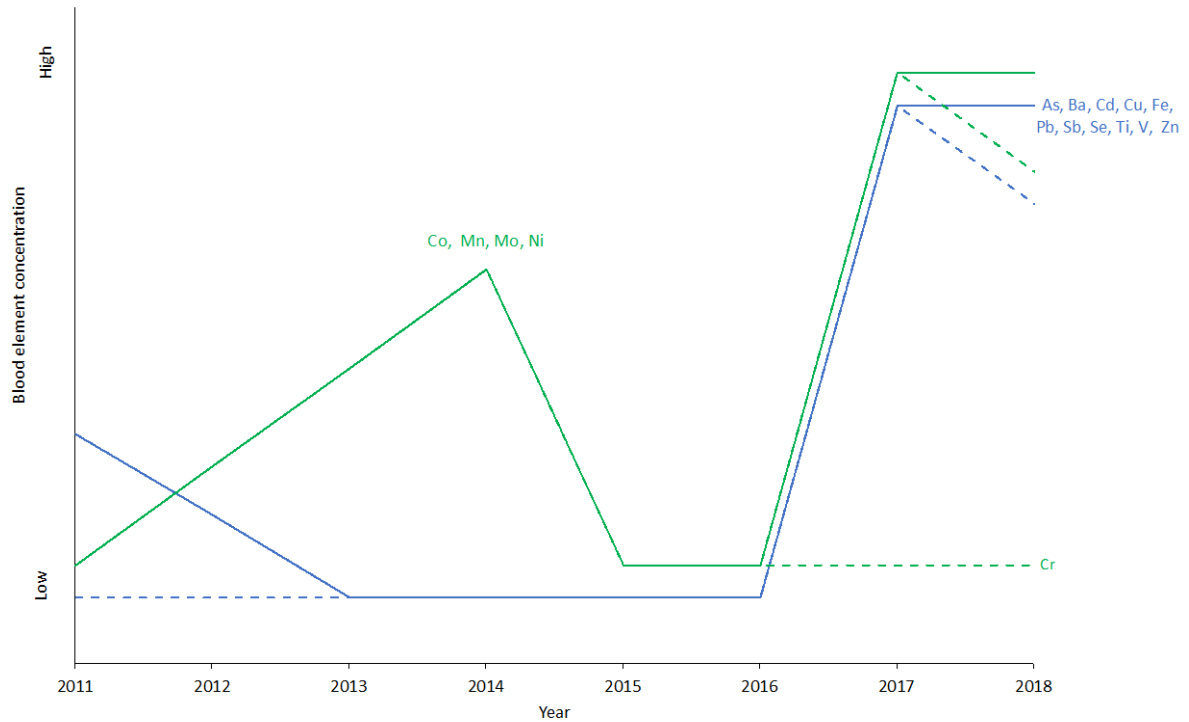


Figure 7.17. The two general temporal trends in blood element concentrations for Green turtles foraging in Port Curtis.

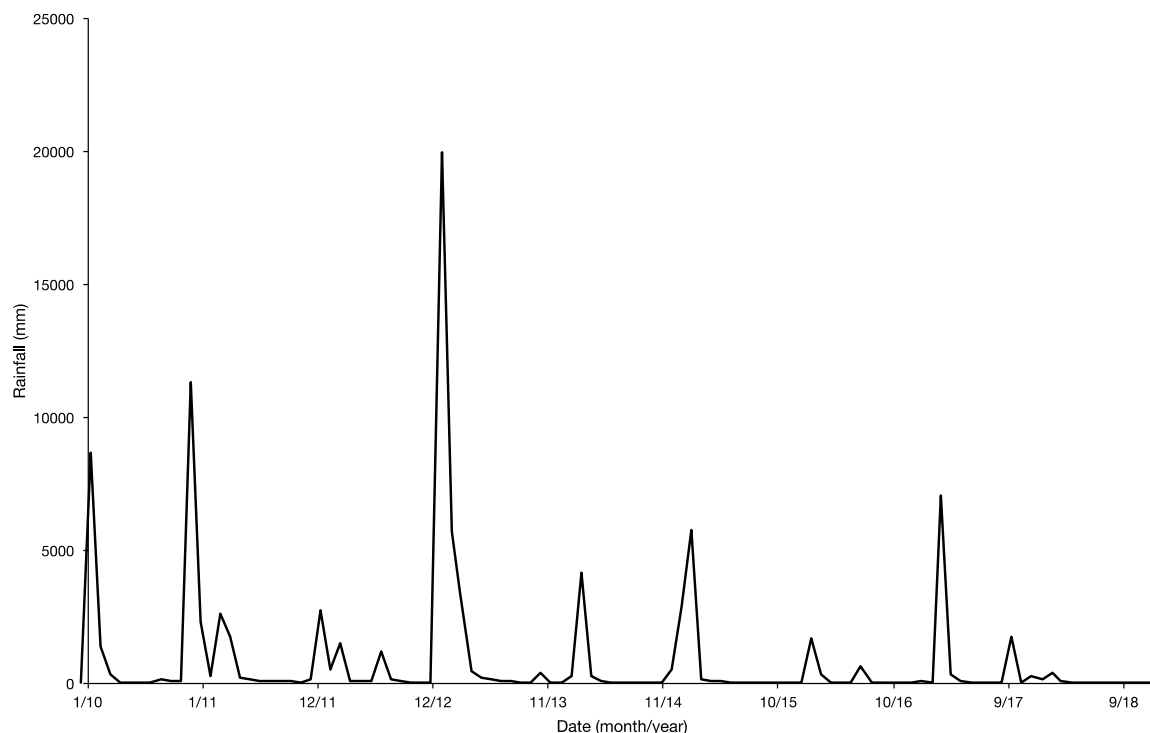


Figure 7.18. Rainfall data collected from the Water Monitoring Information Portal (station 132001A; Calliope River at Castlehope) over the period of the study.

### Spatial variation

Spatial comparisons of recaptured Green turtle blood element concentrations between the Boyne River estuary, Pelican Banks and Facing Island are presented for the same 16 elements that were assessed temporally (As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Ti, V and Zn) (Figures 7.19 to 7.34). Although all years (2011, 2013, 2014, 2016, 2017 and 2018) are presented, only spatial comparisons for 2017 and 2018 were discussed further.

Five elements (As, Cu, Sb, Se, Ti) did not show any spatial differences in 2017 or 2018 and Zn was not spatially different in 2017. Cadmium (Cd) was the only element that was at higher concentrations in the Boyne River estuary Green turtles compared to Pelican Banks and Facing Island (in both 2017 and 2018). The remaining elements were highest in Green turtles from Pelican Banks and/or Facing Island. Pelican Banks and Facing Island turtles often had similar blood element concentrations (e.g. Ba, Co, Cr, Mo, Ni, Zn (2018 only)). However, the two turtles captured near Facing Island often had significantly higher blood element concentrations than both Boyne and Pelican Banks, including Fe (in 2017 only), and Mn, Pb and V (in both 2017 and 2018).

These spatial results indicate that Green turtles foraging adjacent to the Pelican Banks and Facing Island in 2017 and 2018 were generally exposed to higher concentrations of Ba, Co, Cr, Mo, Ni, Zn, and Facing Island turtles, in particular, to Fe, Mn, Pb and V. Again, without detailed information about the geology and port activities within different areas of Port Curtis, the cause of these increased exposures remains undetermined. The interpretation of the

spatial patterns of elevated Green turtle blood element concentrations observed in 2017/18 at Pelican Banks / Facing Island should consider a potential contribution from port activities, such as increased boating traffic and dredging. If the metal concentrations were more driven by rainfall events (increasing the input of land-based pollutants), higher concentrations in the Boyne River estuary turtles would be expected. However, only Cd was higher in the Boyne River estuary Green turtles. The main sources of cadmium in the marine environment are atmospheric loading from metal smelting facilities, and from riverine discharges (Kennish, 1997), which would suggest a land-based or local source of Cd at this site.

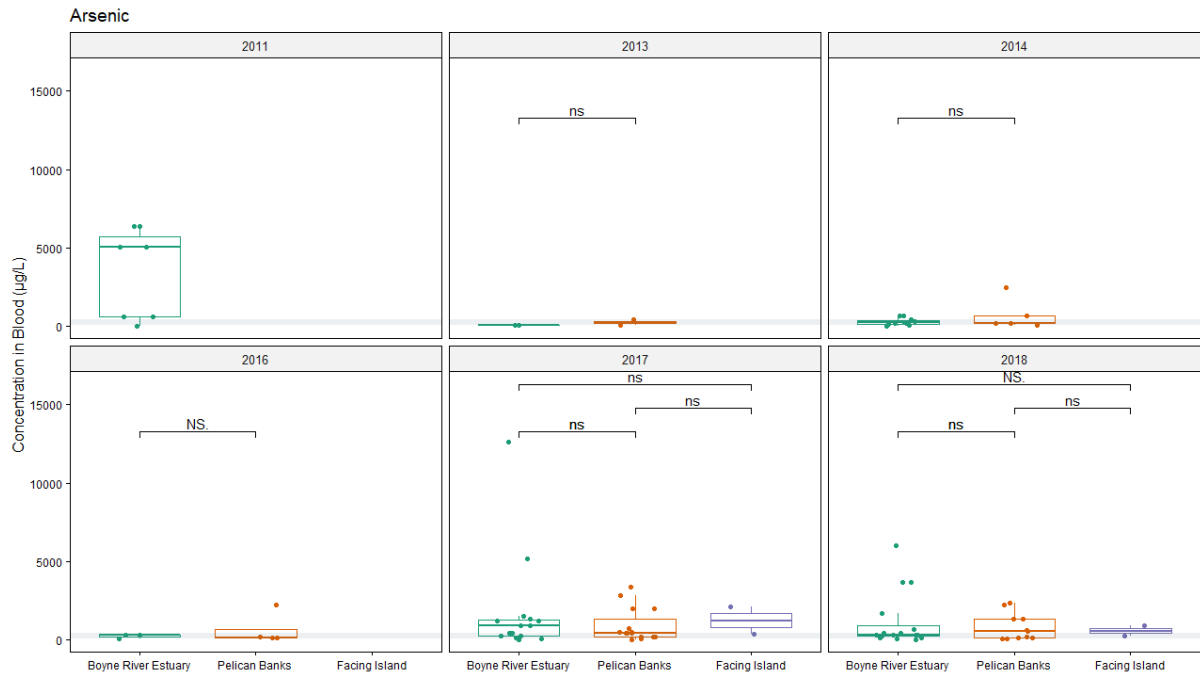


Figure 7.19. Box and whisker plot for arsenic (As) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary, Pelican Banks, and Facing Island between 2011 and 2018.

Grey bars illustrate the reference interval established by Villa *et al.* (2017).

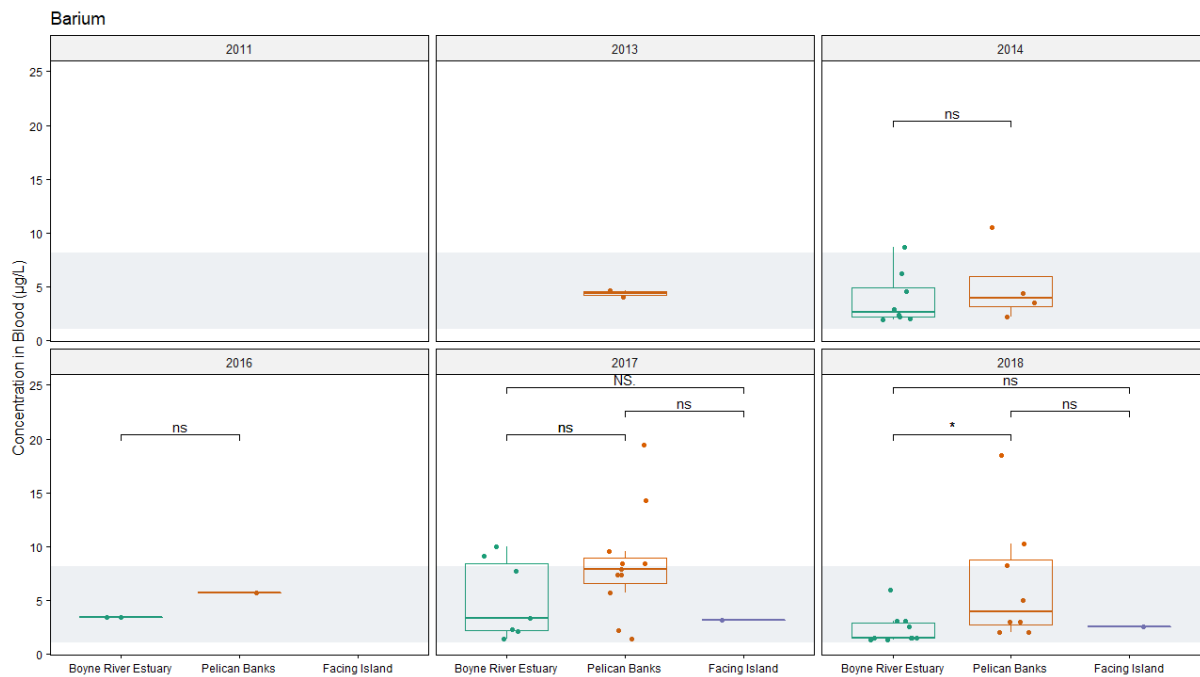


Figure 7.20. Box and whisker plot for barium (Ba) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary, Pelican Banks, and Facing Island between 2011 and 2018.

Grey bars illustrate the reference interval established by Villa *et al.* (2017).



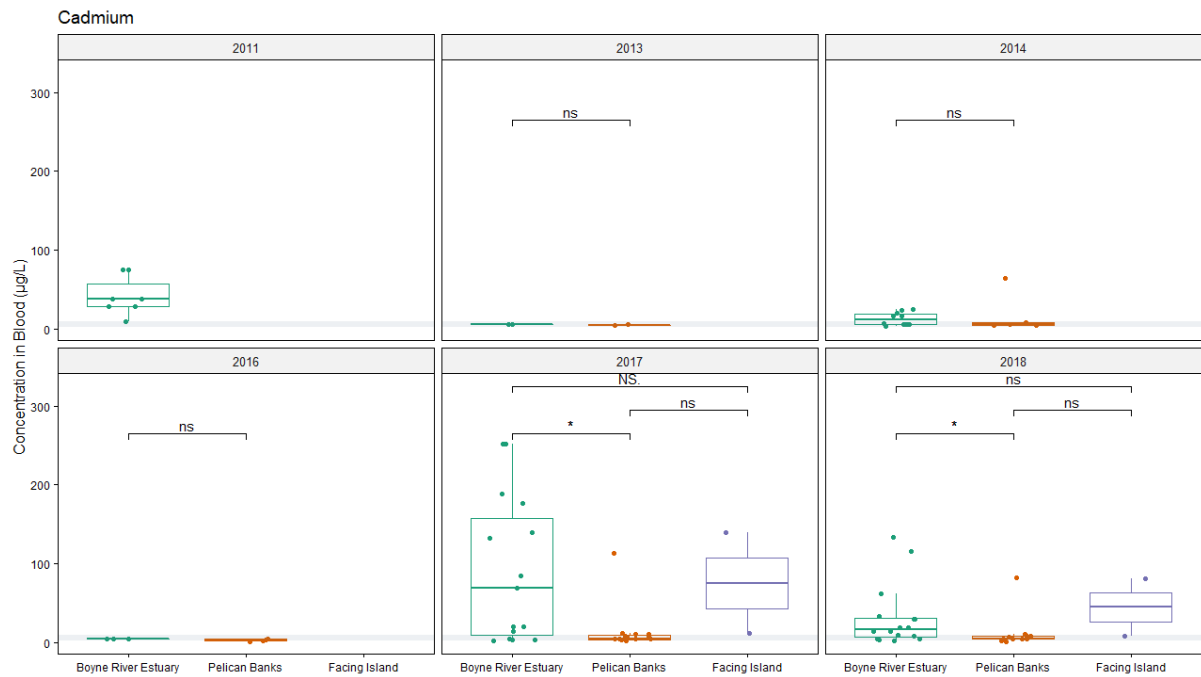


Figure 7.21. Box and whisker plot for cadmium (Cd) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary, Pelican Banks, and Facing Island between 2011 and 2018.

Grey bars illustrate the reference interval established by Villa *et al.* (2017).

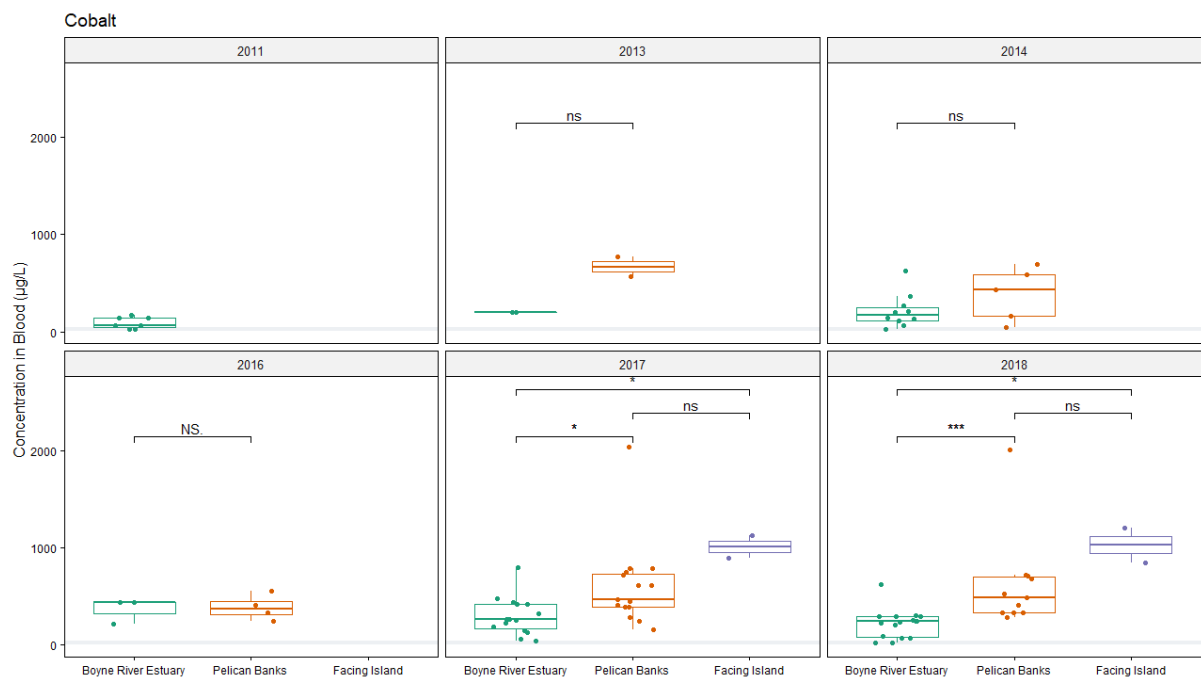


Figure 7.22. Box and whisker plot for cobalt (Co) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary, Pelican Banks, and Facing Island between 2011 and 2018.

Grey bars illustrate the reference interval established by Villa *et al.* (2017).

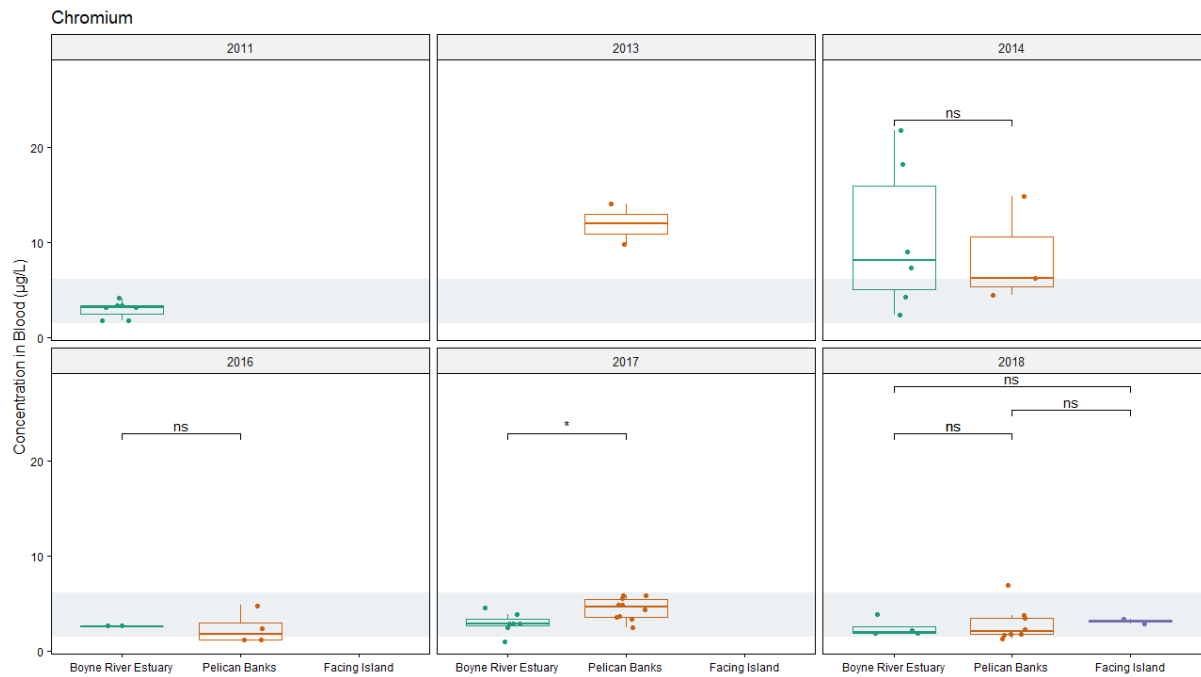


Figure 7.23. Box and whisker plot for chromium (Cr) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary, Pelican Banks, and Facing Island between 2011 and 2018.

Grey bars illustrate the reference interval established by Villa *et al.* (2017).

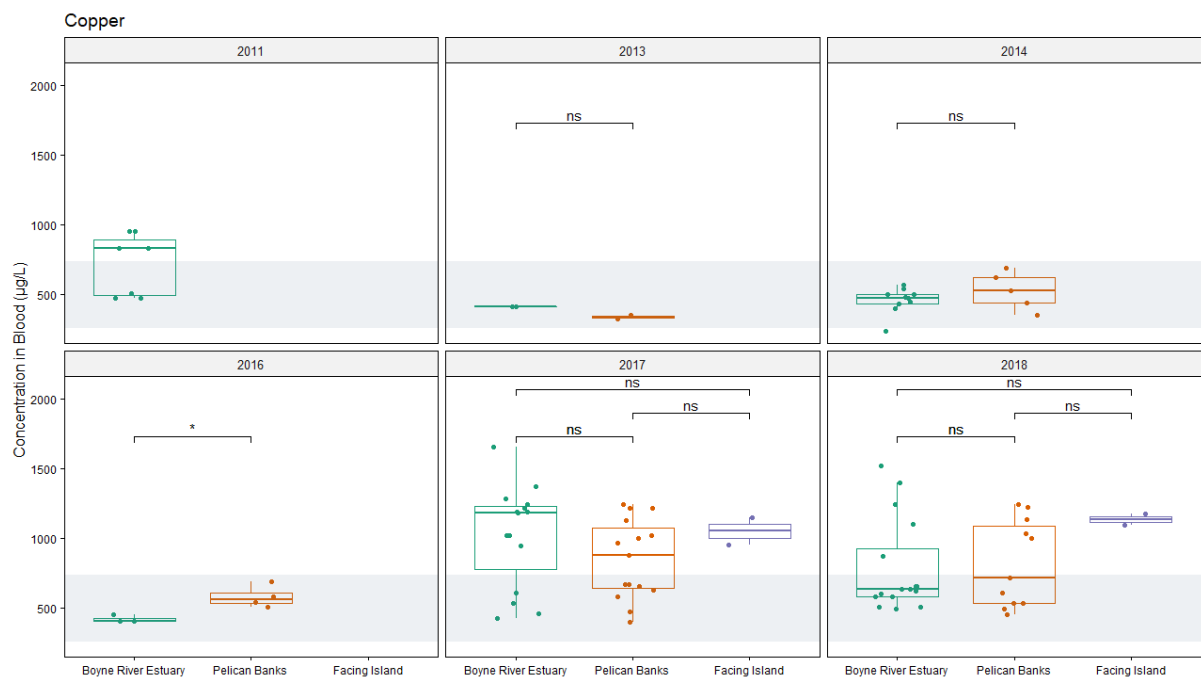


Figure 7.24. Box and whisker plot for copper (Cu) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary, Pelican Banks, and Facing Island between 2011 and 2018.

Grey bars illustrate the reference interval established by Villa *et al.* (2017).

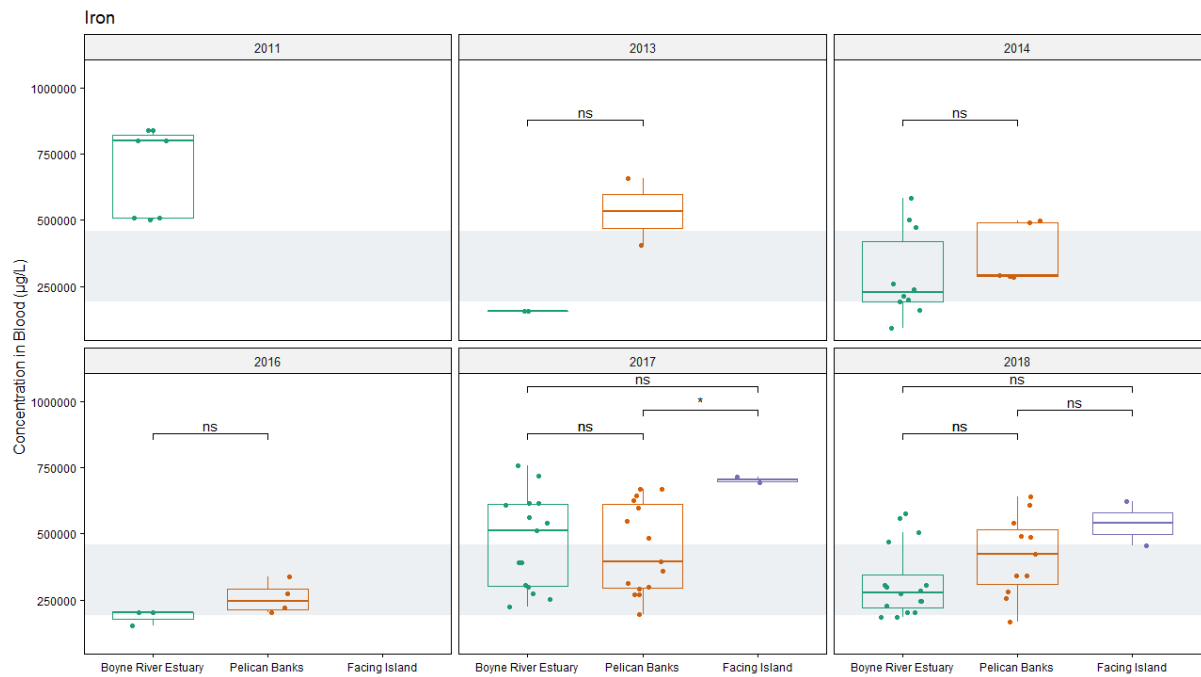


Figure 7.25. Box and whisker plot for iron (Fe) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary, Pelican Banks, and Facing Island between 2011 and 2018.

Grey bars illustrate the reference interval established by Villa *et al.* (2017).

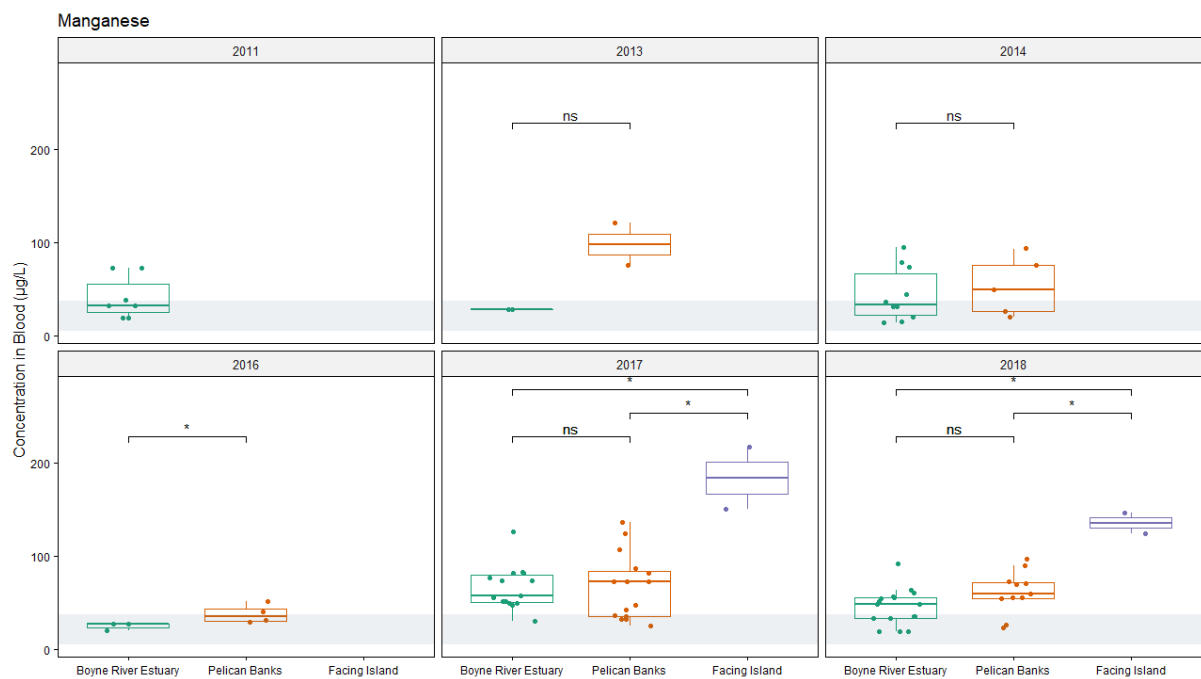


Figure 7.26. Box and whisker plot for manganese (Mn) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary, Pelican Banks, and Facing Island between 2011 and 2018.

Grey bars illustrate the reference interval established by Villa *et al.* (2017).

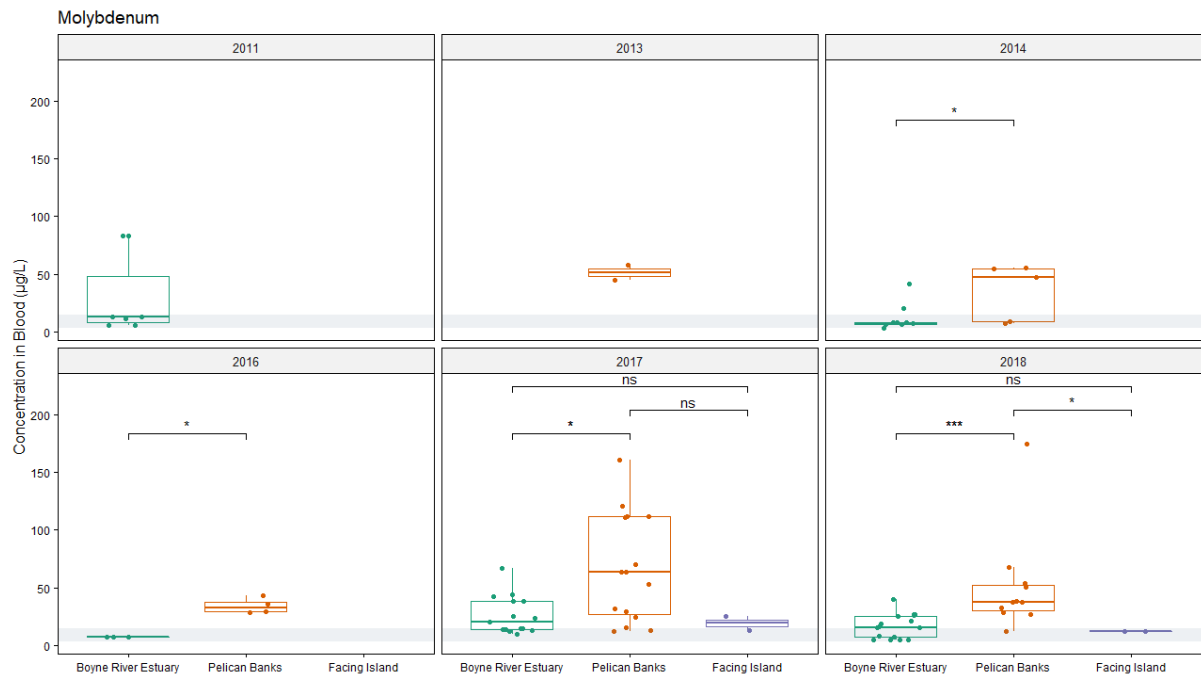


Figure 7.27. Box and whisker plot for molybdenum (Mo) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary, Pelican Banks, and Facing Island between 2011 and 2018. Grey bars illustrate the reference interval established by Villa *et al.* (2017).

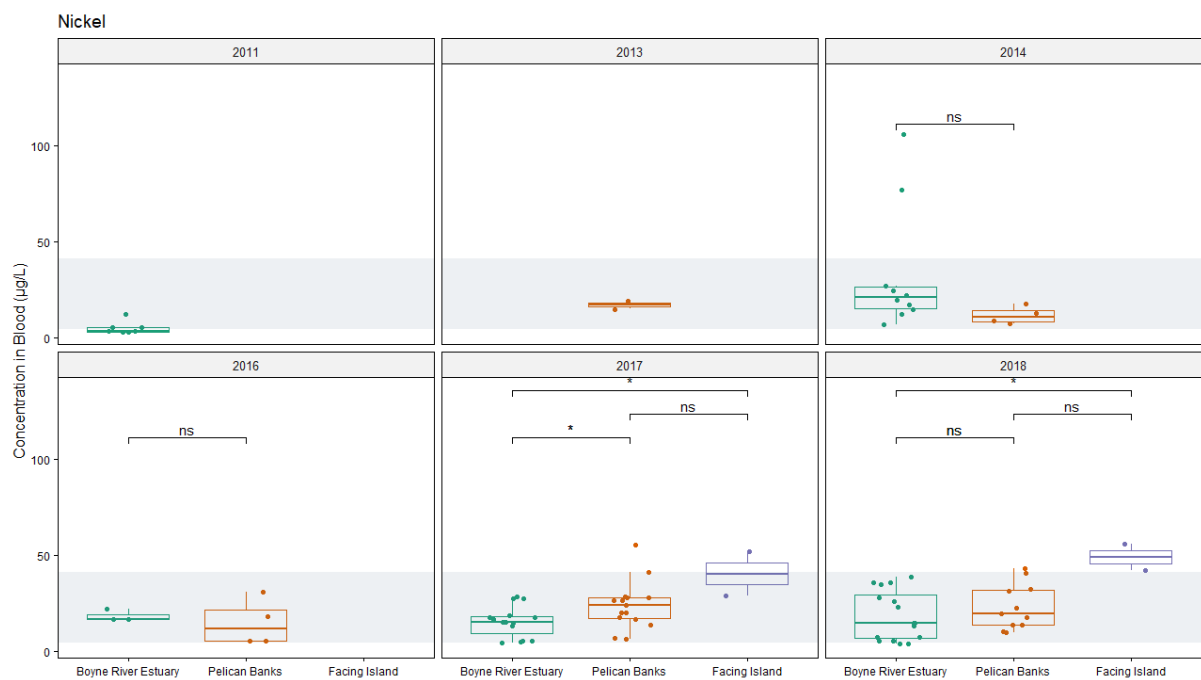


Figure 7.28. Box and whisker plot for nickel (Ni) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary, Pelican Banks, and Facing Island between 2011 and 2018.

Grey bars illustrate the reference interval established by Villa *et al.* (2017).

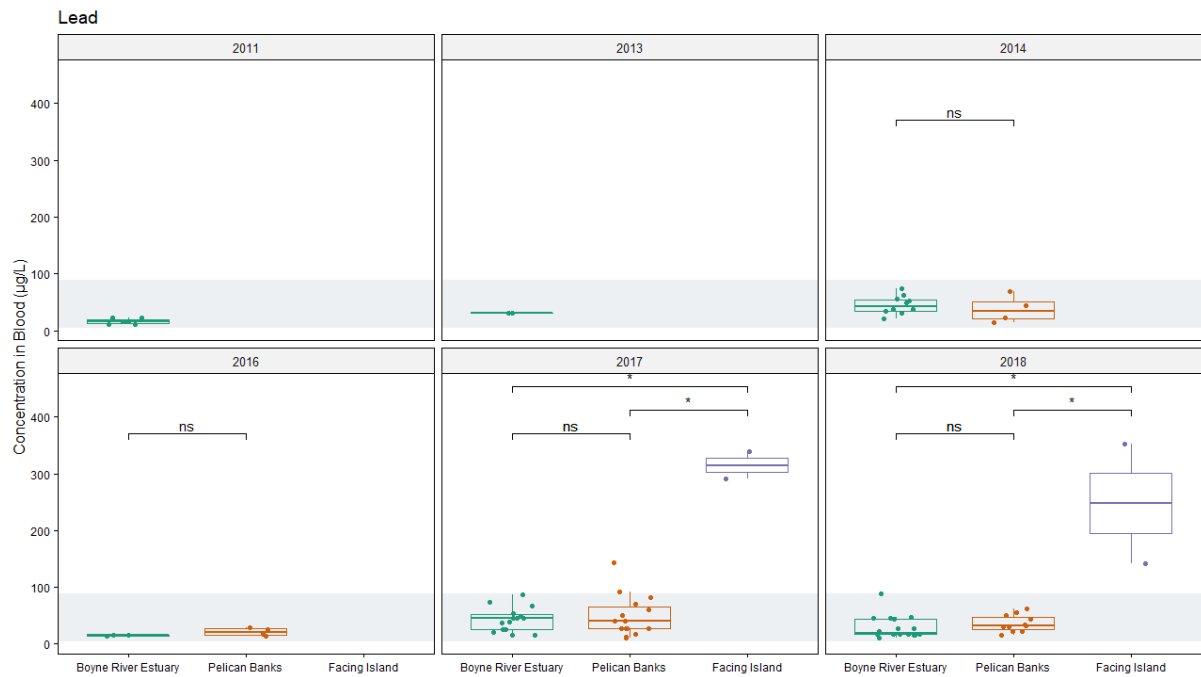


Figure 7.29. Box and whisker plot for lead (Pb) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary, Pelican Banks, and Facing Island between 2011 and 2018.

Grey bars illustrate the reference interval established by Villa *et al.* (2017).

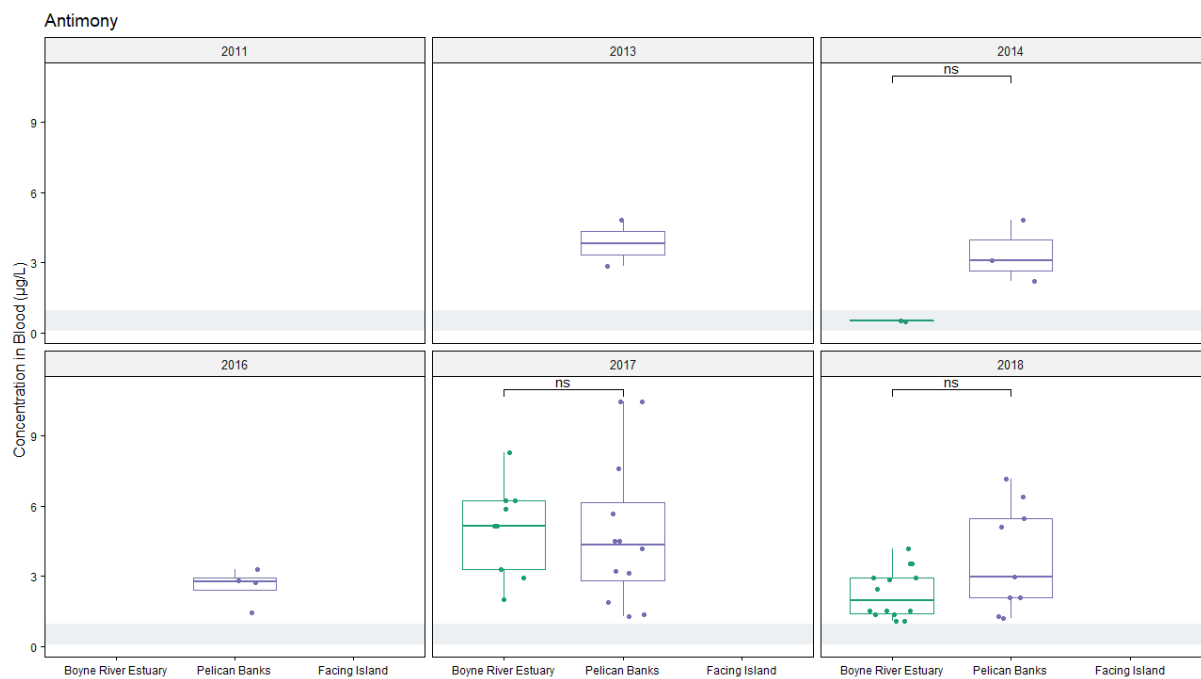


Figure 7.30. Box and whisker plot for antimony (Sb) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary, Pelican Banks, and Facing Island between 2011 and 2018.

Grey bars illustrate the reference interval established by Villa *et al.* (2017).

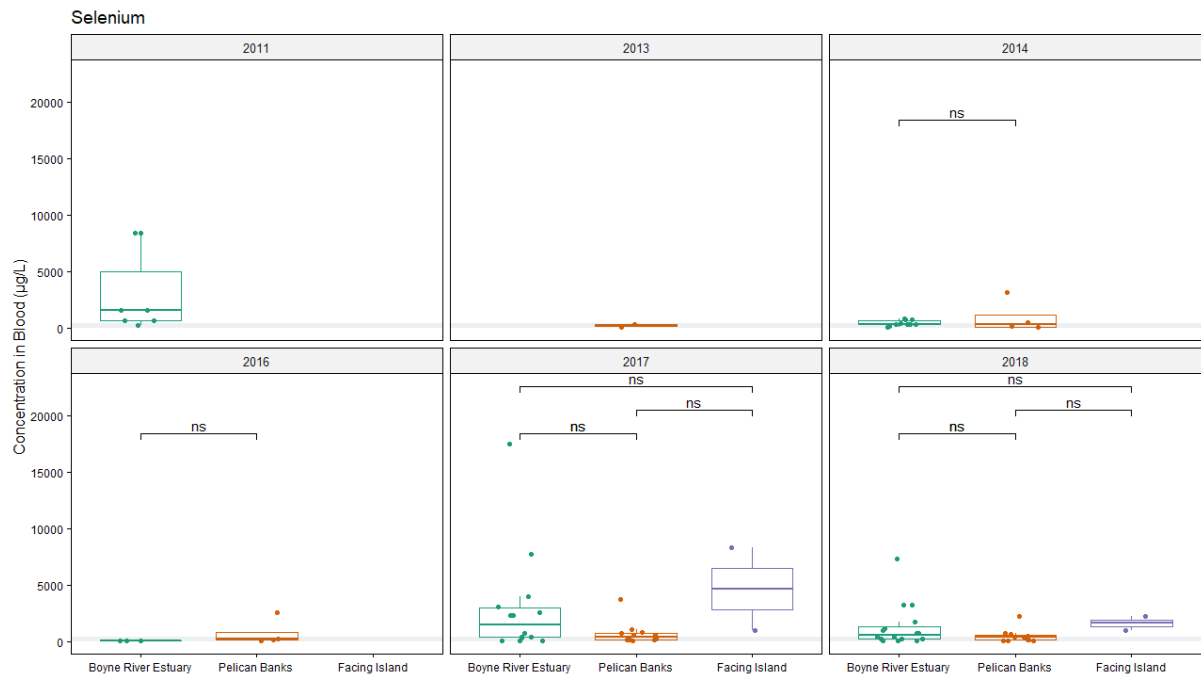


Figure 7.31. Box and whisker plot for selenium (Se) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary, Pelican Banks, and Facing Island between 2011 and 2018.

Grey bars illustrate the reference interval established by Villa *et al.* (2017).

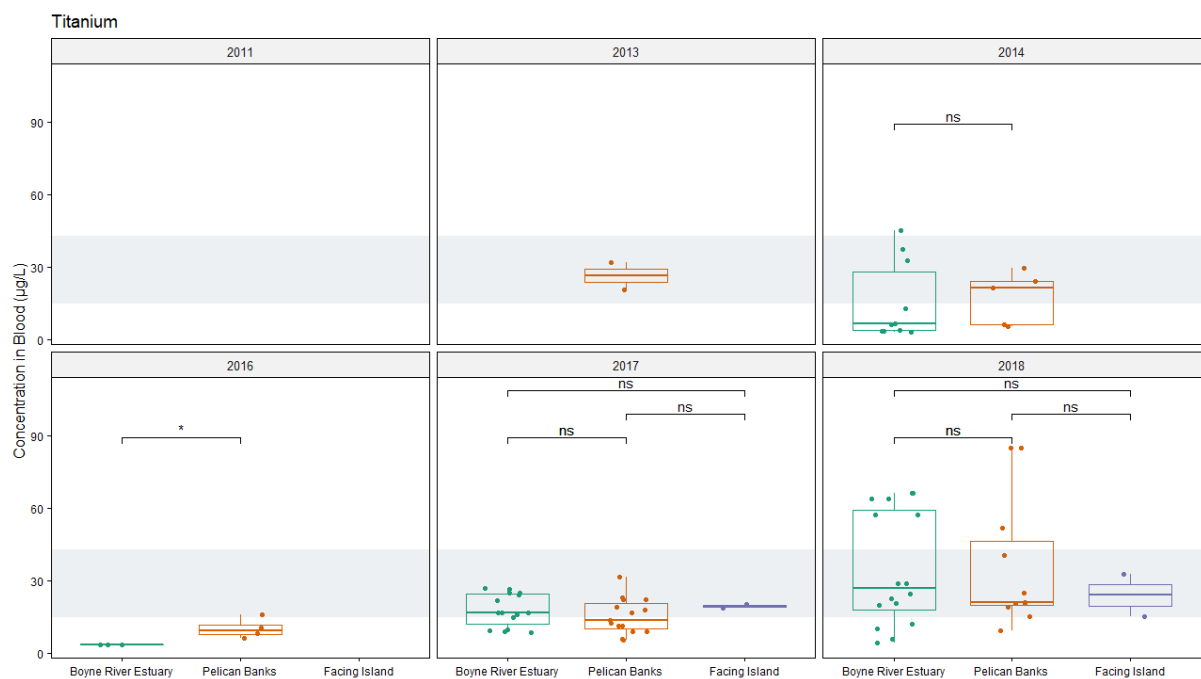


Figure 7.32. Box and whisker plot for titanium (Ti) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary, Pelican Banks and Facing Island between 2011 and 2018.

Grey bars illustrate the reference interval established by Villa *et al.* (2017).

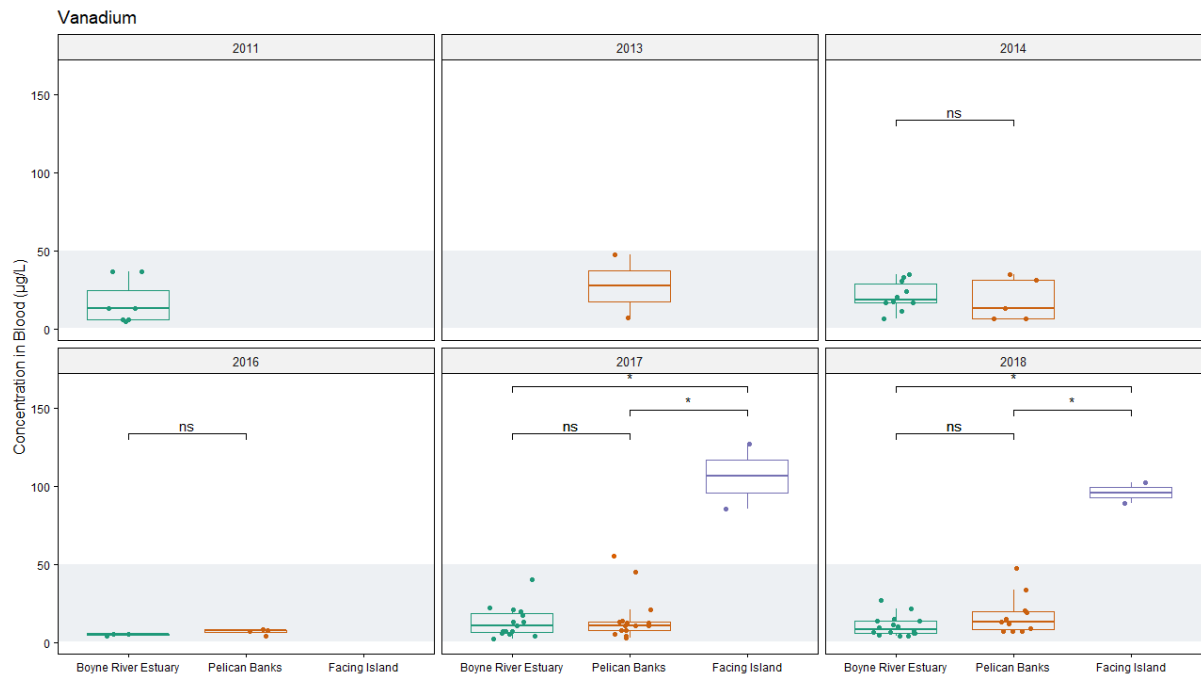


Figure 7.33. Box and whisker plot for vanadium (Va) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary, Pelican Banks and Facing Island between 2011 and 2018.

Grey bars illustrate the reference interval established by Villa *et al.* (2017).

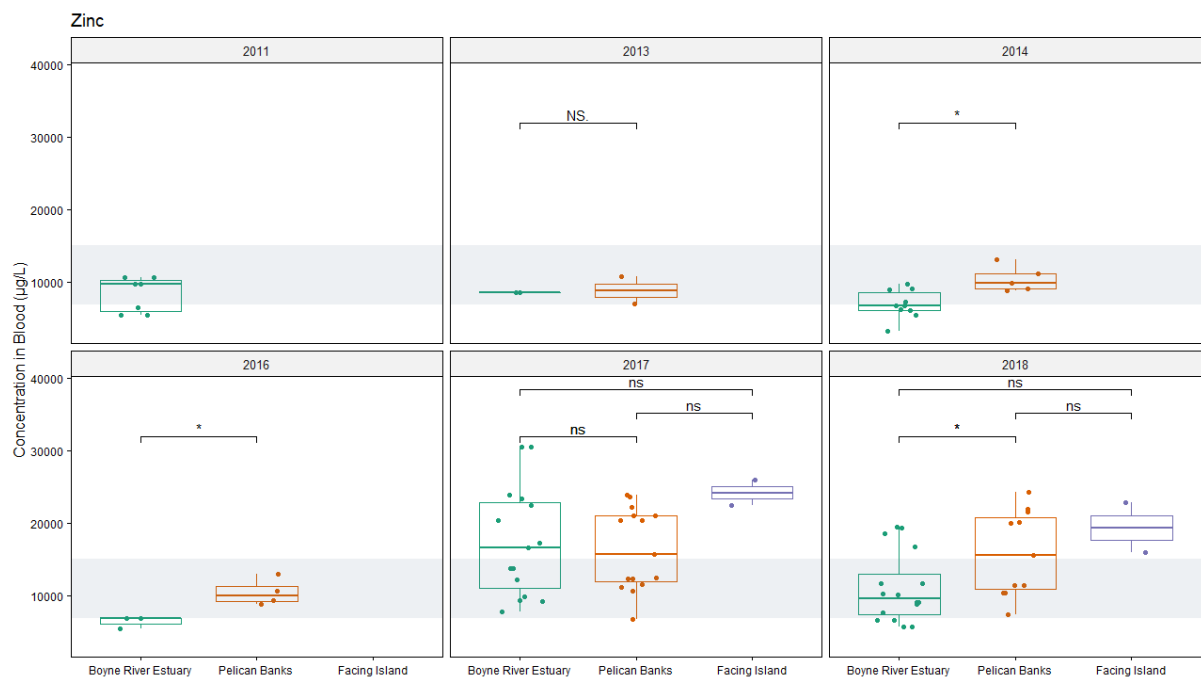


Figure 7.34. Box and whisker plot for zinc (Zn) concentrations in the blood of foraging Green turtles recaptured in the Boyne River estuary, Pelican Banks and Facing Island between 2011 and 2018.

Grey bars illustrate the reference interval established by Villa *et al.* (2017).

## Implications for Green turtle health

Gaus *et al.* (2012) classified elements into three categories relating to their likely impact on the health of Green turtles captured in the Boyne River estuary in 2011: 1) 'of concern' (As, Cd, Co, Hg, Ni, Se, V), 2) 'possible concern' (Ag, Cu, Cr, Mo, Pb), and 3) 'relatively low concern' (Al, Fe, Mn, Zn). However, since then, reference intervals (RIs) for a large number of elements were established for a clinically healthy Green turtle population considered to be remote from chemical inputs (Villa *et al.*, 2017). In much the same way, essential element RIs are important for assessing human health (e.g. iron in blood to identify and manage anaemia). In the absence of a site-specific baseline, prior to any anthropogenic inputs, reference intervals from a population of healthy Green turtles, foraging at a remote location, relatively free from direct coastal inputs, can be used to indicate recent elevated exposure scenarios for both essential and non-essential elements.

For the purposes of this study, we propose to modify the Gaus *et al.* (2012) method of categorising elements found in blood into two categories, based on their relationship to their RIs:

- 1. of concern:** elements that are consistently above the RI, indicating potential chronic elevated exposure, and
- 2. relatively low concern:** elements that are consistently within, or below, the RI.

Of the elements classified 'of concern' in 2012, As, Cd and Se concentrations decreased to within RIs in 2013-2016 (with a couple of exceptions), before increasing to above 2011 concentrations in 2017/18. Cobalt (Co) concentrations remained above RIs throughout the study period (with the peaks in 2014 and 2017/18 described previously), and by 2017/18 were up to more than 10 times higher than the concentrations initially measured in 2011. A stark indication of this pattern can be seen in the Green turtle captured in the Boyne River estuary on four separate occasions over 4 years (2014, 2016, 2017 and 2018), which showed a steady increase in blood Co concentrations from ~100 ug/L (2014) to ~600 ug/L (2018). These results indicate that the risk to Green turtle health from As, Cd, Co and Se have increased since first being identified as chemicals 'of concern' in 2012, with concentrations currently well above RIs. In contrast, Ni and V have only increased slightly over the study period, and with the exception of a couple of turtles, have not increased above RIs. It is unknown what the metabolic response to elevated Ni or V in blood is for Green turtles, nor their capacity to accumulate or detoxify these elements. However, the fact that these elements were considered elements 'of concern' by (Gaus *et al.*, 2012), based on toxicological information, the stability, or slight increase, in the concentrations of Ni and V indicate that they should remain the 'of concern' category in this study.

Of the elements classified as 'possible concern' in 2012, Cu and Pb decreased to, or remained, within the RIs for most of the study period, until 2017/18, when they sharply increased to levels well above the RI, and up to three (Cu) and 15 (Pb) times higher than the 2011 levels. Similarly, Cr increased to up to four times the 2011 levels, and well above the RI in 2013/14, although were generally within the RI for the remainder of the study period. Concentrations of Mo peaked in 2013/14 and again in 2017/18, with a period between within the RIs. In 2017/18, Mo concentrations in some turtles were more than 10 times higher than the levels reported in 2011. Given the increases in the Green turtle blood concentrations of



these four elements in recent years, including regular excursions above the RIs, we recommend that Cu, Pb, Cr and Mo be added to the list of elements 'of concern'.

Of the elements classified as 'relatively low concern' by Gaus *et al.* (2012), Fe concentrations reduced over the study period, although were still above the RI in 2017/18. In contrast, Mn and Zn concentrations increased over the study period, with peaks in 2013/14 (Mn only) and 2017/18 that were well above the RI and were up to nearly 10 (Mn) and six (Zn) times higher in 2017/18, compared to 2011. Based on excursions above the RIs, these results indicate that Fe can remain an element of 'relatively low concern', while Mn and Zn should be elevated to elements 'of concern'.

To support elevating some of the elements to higher categories of concern, recent effects data from cell-based bioassays indicates some of these elements are toxic at relatively low concentrations and, in some cases, at concentrations found in turtle blood in this study. Cell-based bioassays have been performed for several of the elements measured in this study, including Cd, Zn, Cr, Cu, Hg, Co, Pb, As, Mo, Mn, and Sb (Table 7.1). Four different endpoints have been assessed, including cytotoxicity, production of reactive oxygen species (ROS, an indicator of oxidative stress), micronucleus induction (an indicator of genotoxicity), and activity of glutathione-S-transferase (GST, an indicator of detoxification mechanisms and oxidative stress). These assays were performed using a Green turtle cell culture, GT06s-*p*, established by Finlayson *et al.* (2019a). Of the elements of concern, concentrations of Cu in 2017 and 2018 were within the range able to induce effects associated with oxidative stress and genotoxicity. Similarly, concentrations of Co in 2017 and 2018 were within the range that can have genotoxic effects. Cr was the most toxic element in the three bioassays in which it was tested (Table 7.1), although effects were only observed at concentrations above what was detected in turtle blood, with the exception of some individuals from 2014. Cd, As, Mo, Zn, Mn and Pb, were only cytotoxic at concentrations orders of magnitude above what was found in turtle blood. Sb did not illicit an effect in any of the bioassays. It should be noted that Sb was tested at the limits of solubility in the assay media (Finlayson *et al.*, 2020), however, this does not preclude it from exerting toxic effects when accumulated by sea turtles.

As previously mentioned, blood concentrations do not necessarily represent risk to turtle health. Contaminants accumulate in other tissues, such as liver and kidney. Screening risk assessments using tissue concentrations from the literature found significant risk associated with accumulation of some elements in internal tissues, even if blood concentrations did not indicate a risk. In these screening risk assessments, Green turtles from the Boyne estuary were found to be at risk from Cr, Cd and Cu, based on liver or kidney concentrations (Finlayson *et al.*, 2019b, Finlayson *et al.*, 2019c). Since 2011, concentrations of both Cd and Cu have increased in turtle blood from the Boyne estuary, which may indicate a higher level of accumulation in the internal organs, and an increase of associated risk. While the internal concentrations from 2011 came from three turtles euthanised due to extremely poor health, it also highlights the need for toxicokinetic modelling to link blood concentrations, that can be obtained from live turtles, with internal tissue concentrations that require deceased individuals, which can be difficult to obtain.

The elevation of many blood element blood concentrations above reference intervals, supported by both historical and novel cell-based toxicological data, indicate that the exposure of Port Curtis Green turtles to trace elements may be impacting their health. This could potentially manifest at the population level as measures such as poorer body condition, slower growth rates and impaired reproductive performance. Assessment of population parameters in Chapter 5 indicated that Port Curtis Green turtles had poorest body condition in 2018 (corresponding to a period of high trace element exposure presented in this chapter), and lower body condition compared to Moreton Bay turtles, although higher than Shoalwater Bay turtles. In addition, the size-specific somatic growth rate of the Port Curtis Green turtle population was much slower than other major sGBR Green turtle foraging populations, up to 1.8, 2.5 and 2.7 slower than Shoalwater Bay, Moreton Bay, and Clack Reef populations, respectively. Finally, with respect to reproductive performance, Port Curtis Green turtles commence breeding at a smaller size compared to Moreton Bay and Heron-Wistari Reef populations (although similar to Shoalwater Bay). However, it is important to note that this may not necessarily reflect different ages at first breeding, as the sites with larger size at first breeding also had faster growth rates. Overall, these observations indicate that further investigations into the mechanistic links between high element exposure and reductions in body condition, growth and reproductive performance are warranted.

Table 7.1. Summary of published 50% effect concentration ( $EC_{50}$ ) values for cytotoxicity, induction ratios of 2 ( $IR_2$ ) values for reactive oxygen species (ROS) production and micronucleus induction, and concentration required to exceed the detection limit ( $EC_{DL}$ ) for (GST activity established using Green turtle skin cell toxicity bioassays (GT06s-p). Cytotoxicity data averaged from Finlayson *et al.* (2019a), Finlayson *et al.* (2019b), and Finlayson *et al.* (2020). NT: not tested.

Contaminant	Mean $\pm$ SD $EC_{50}$ values (mg/L)	Mean $\pm$ SD $IR_2$ values (mg/L)		Mean $\pm$ SD $EC_{DL}$ values (mg/L)
	Cytotoxicity	ROS production	Micronucleus induction	GST activity
$Cd^{2+}$	5.5 $\pm$ 0.48	>2.8	>2.8	NT
$Zn^{2+}$	96 $\pm$ 59	>24	>24	NT
$Cr^{6+}$	0.75 $\pm$ 0.58	0.03	0.01	NT
$Cu^{2+}$	23 $\pm$ 1.5	0.22	0.76	12
$Hg^{2+}$	2.8	0.12	0.10	NT
$Co^{2+}$	230	1.7	0.65	140
$Pb^{2+}$	>830	>190	73	NT
$As^{5+}$	530	NT	NT	>270
$Mo^{6+}$	9200	NT	NT	>1300

Mn <sup>2+</sup>	>150	NT	NT	50
Sb <sup>5+</sup>	>2.1	NT	NT	>0.21

## Conclusions

The Green turtle capture, mark recapture program (CMR) in Port Curtis from 2011 to 2019, and subsequent trace element analysis of blood samples, has been successful in providing an indication of temporal and spatial trends in element exposure in the Port Curtis Green turtle population, via non-lethal biomonitoring (blood samples). Both temporal and spatial differences in exposure of Port Curtis Green turtles to trace elements were observed, with further investigations in the potential links to climatic conditions and anthropogenic activity in the port warranted. The observed concentrations also supported the Gaus *et al.* (2012) assertion that some elements are at levels that are a concern to Green turtle health, and that elements generally pose an increasing threat to Green turtles in this region over time, with more elements considered of concern now than in 2011.

The potential impacts of trace metals on Green turtle health were supported by new toxicological information, generated from novel and ethical techniques that have provided species-specific tools for placing the biomonitoring results in an appropriate health-risk context. These tools were developed using cell cultures established from Queensland Green turtles, providing additional rigor to the toxicological assessments. Further support of links between trace element exposure and impaired population health was observed in the reduced body condition, growth rates and reproductive performance of the Port Curtis Green turtle population reported in Chapter 5, warranting further investigations into the possible mechanistic links between these.

The Green turtle monitoring program in Port Curtis is uniquely suited for biomonitoring now that reference intervals are available, and longitudinal data exists for this site, both temporally and spatially. These data have strong regional implications for industrial and urban ports, and coastal areas where Green turtles forage. Future program design should include additional non-lethal biomonitoring matrices (e.g. of the keratin scutes), which can provide more time integrated indications of temporal changes in trace element exposure.

Learnings from this long-term monitoring program should be broadly disseminated to serve as an example of what can be achieved through joint-ventures and long-term resource and funding commitments. We recommend a biomonitoring and toxicological scientific advisory board be established to review and evaluate future biomonitoring at Port Curtis and to interpret the results using the latest tools.

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## CHAPTER 8

### GENERAL DISCUSSION OF THE GREEN TURTLE FORAGING POPULATION IN PORT CURTIS

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With 3423 recorded sightings of Green turtles, including 1576 captures of 1232 individual Green turtles within Port Curtis during the four years of the contracted study, the Green turtles is now recognized as the dominant, prolific turtle species inhabiting the intertidal flats, mangrove forests and adjacent shallow subtidal waters of Port Curtis. The study was not designed to assess the abundance and distribution of the other species of marine turtles (Loggerhead, Flatback, Hawksbill and Olive Ridley turtles) which occur primarily in the deeper subtidal waters of the Port.

The CMR analysis was only able to provide an estimate of the size of the resident foraging population of Green turtles at two study sites, Pelican Banks and off the Boyne Estuary, with a combined 4-year mean population size of 1170 (95% credibility interval 1154-1186). When considering the unquantified abundance of Green turtles observed at the other study sites, it is concluded that many thousands of Green turtles forage throughout Port Curtis (Chapter 5). Given the relatively small size of Port Curtis compared to other Green turtle study sites such as Moreton Bay and Shoalwater Bay, the Port Curtis study was commenced with the assumption that individual Green turtles would occupy relatively substantial areas of the Port. The capture-mark-recapture (CMR) flipper tagging studies (Chapter 2) and the satellite telemetry studies (Chapter 4) have shown that this is not the case. These studies have shown that individual Green turtles show strong fidelity to localized small areas of the Port waters with separate areas of the Port supporting relatively independent sub-populations.

The CMR flipper tagging studies (Chapter 2) and the population genetics studies (Chapter 3) have identified that Port Curtis supports a foraging population of Green turtles from the sGBR genetic stock that breeds in the southern Great Barrier Reef region. The genetic studies identified that a significant proportion of Port Curtis foraging population also was derived from genetic stocks that bred in the central and eastern Coral Sea regions (Coral Sea and northwest New Caledonian stocks).

The uniform sampling of the Green turtle foraging population of Port Curtis was hampered by the natural regularly occurring high turbidity of waters throughout the Port that limited the suitability of the turtle rodeo method for capturing turtles. The strength of tidal currents also restricted where the use of nets could be effectively used to capture turtles. However, this study identified that foraging Green turtles aggregated primarily at five sites within Port Curtis (Chapter 2). These sites were characterised by being adjacent to outflows from rivers and creeks or, in the case of the Pelican Banks, with outflow from the Port where there is regular reversal of strong tidal currents and associated settlement of sediments to form wide shallow flats supporting seagrass and algal pastures. The majority of the Green turtles within Port Curtis forage over the intertidal and subtidal flats adjacent to outside and inside of the outflow areas of the estuaries of Colosseum Creek, Boyne River, South Trees Inlet, Calliope River and the entrance to the Port between Curtis and Facing Islands. This latter area includes the Pelican Banks.

The study failed to locate any area with a concentration of foraging Green turtles within the turbid waters of the western Basin or at the southern end of The Narrows except in the vicinity of Wiggins Island.

The telemetry studies (Chapter 4) quantified habitat use by defining individual “home ranges” within 95% utilisation distributions (UDs) for 72 tracked individuals over the short period of up to 13 months of tracking,. The average home range area for the 72 tracked individuals was 19.9 km<sup>2</sup> (range = 1-115 km<sup>2</sup>, median=11.4 km<sup>2</sup>). The telemetry studies also demonstrated that turtles sampled at one study site within Port Curtis are unlikely to represent habitat use of turtles in the wider port. Thus, future turtle studies and sampling should be targeted directly to areas of interest for monitoring and management. This is a challenging issue to address within Port Curtis where many areas such as dredged channels that are characterised by deep or turbid water where turtle capture by turtle rodeo or blocking nets is unreliable. However the satellite telemetry studies detected little overlap between Green turtle foraging ranges and dredged channels. Therefore it can be concluded that there will be little overlap of the movement of large industrial vessels and tugs and the foraging habitat of the Green turtles. This applied even when the turtles were foraging within only hundreds of meters of Port infrastructure and a dredged channel as occurs at South Trees. In contrast, the home range summaries indicate that collision with high speed recreational vessels may pose a threat to turtles foraging in shallow intertidal waters such as on the Pelican Banks, South Trees and off the Boyne River mouth (Chapter 2).

The telemetry tracking showed that the proportion of turtles using multiple non-contiguous areas as part of their home range, rather than remaining in one area, appeared somewhat higher in years in which Port Curtis experienced major flooding events from the Fitzroy and adjacent outflows (2013 and 2017), though the difference was not statistically significant. While adult turtles temporarily will leave their foraging home range during breeding migrations, there is some indication that turtles also are capable of adjusting their space-use in response to environmental disturbance or resource availability.

The size range of Green turtles foraging within Port Curtis is now well defined (Chapter 2):

- Very small immature turtles recruit to residency in Port Curtis with a mean CCL = 43.2 cm, with no detectable differences in the size at which they recruited with respect to gender or year of recruitment.
- Adult female Green turtles in Port Curtis are larger on average than adult males
- There was no detectable difference in size of either the adult females or males across the seven years of study.
- Adult females within Port Curtis commence breeding at a relatively small mean CCL = 99.2 cm.

Given recent concerns regarding climate change and feminisation of turtle populations, the gender of the Green turtles foraging in Port Curtis was examined using data gathered across nine years of study, 2011-2019, sampled from all study sites within the Port (Chapter 2):

- The sex ratio of Green turtles varied across the age classes within Port Curtis:
  - adults had approximately equal proportions of female and males (51% females; 1.03:1 ratio);

- large immatures, small immatures and recently recruited very small immature turtles showed an overall increased proportion of females in the younger age classes (to 64% female; 1.77:1 ratio).
- A comparison of sex ratios within multiple foraging areas dominated by Green turtles from the sGBR stock identified that:
  - Lower female biased adult sex ratios are associated with sampling sites in close proximity to the focal courtship and nesting region for the sGBR genetic stock;
  - Female biased adult sex ratio is highest at foraging areas 3° or more in latitude away from the core breeding area for the sGBR stock
- The sex ratio of small immature Green turtles from sGBR stock dominated foraging areas has fluctuated mostly within the range of 0.6 to 0.8 female across recent decades but with no obvious tendency towards increasing feminisation within the stock.

Each captured turtle was visually examined to identify external evidence of reduced health (Chapter 2). This external examination of the turtles in Port Curtis identified only a low incidence of compromised health among the turtles captured:

- From anthropogenic sources:
  - Turtles fractured from almost entirely interactions with outboard powered vessel = 3.2%;
  - Entangled in fishing gear and marine debris = 0.7%;
- From presumed disease and/ internal health issues:
  - Partly or extremely emaciated turtles = 7.9%;
  - Fibropapillomatosis = 3.6%. The low incidence of Fibropapillomatosis as recorded by the presence and severity of external tumours in Port Curtis (3.6%) is at the lower range of the incidence of tumoured turtles in coastal bays in Queensland and slightly higher than that recorded at Heron-Wistari Reefs on the outer margin of the GBR. As previously recorded for Moreton Bay and Shoalwater Bay, Green turtles in Port Curtis displayed a capacity for recovery from Fibropapillomatosis.

Fibropapillomatosis is not considered to represent a significant threat to the foraging Green turtle population in Port Curtis.

The health of the Port Curtis population as a whole was explored via analyses of a number of population parameters and comparing these with the population performance at other foraging areas dominated by the sGBR Green turtle genetic stock (Chapter 5).

The Fulton body condition index (BCI) was calculated using body mass (kg) and curved carapace length (CCL) (cm) for 1068 Green turtles of known age class, sex and habitat sampled in the Port Curtis region from 2016-2019 (Chapter 5).

- There was a broad range in the BCI within size classes for all foraging sites examined within Port Curtis
  - There was no difference in BCI between sexes for the juvenile and subadult turtles;



- Adult females had a greater BCI compared to adult males.
- Spatially, BCI was significantly higher for Green turtles sampled in the Western Basin than elsewhere in the Port, followed by turtles at South Trees.
- While there was only limited temporal variation in estimated BCI over the 4 yr period, there appeared to be a lower mean BCI in 2018 than in the other years within Port Curtis. Whether the lower BCI for Port Curtis in 2018 was a consequence of the localised high flood level in Gladstone in early 2017 was not investigated.
- When compared with other Green turtle foraging populations dominated by the sGBR genetic stock, Port Curtis displayed an intermediate BCI across all three age classes relative to Moreton Bay (highest age class-specific BCI) and Shoalwater Bay (lowest).

Somatic growth was calculated from 177 growth rate estimates derived from the capture-mark-recapture profiles for 164 uniquely tagged Green turtles sampled in the Port Curtis region and captured from 2010 through 2019 with a minimum of eight months between successive captures (Chapter 5).

- The Green turtles resident in foraging habitats of Port Curtis grew more slowly at any given size or age compared with turtles in other foraging areas dominated by the sGBR stock in Moreton Bay, Heron-Wistari Reefs and Shoalwater Bay.
  - Green turtles living in Port Curtis and Shoalwater Bay commence breeding at a smaller size, CCL = 99.2 cm, than those living in Heron-Wistari Reefs and Moreton Bay.

Poor growth performance and associated small size at commencement of breeding might reflect suboptimal foraging habitats in Port Curtis.

It has been established that the size of annual breeding populations for Green turtles throughout eastern Australia and extending into southeast Asia fluctuates in response to the El Nino-Southern Oscillation (ENSO) climate fluctuations:

- In eastern Australia, there is approximately an 18 month lag between the climate signal and the proportion of the breeding female population coming ashore on beaches for nesting.
- There is strong synchrony in fluctuations in annual breeding numbers across widely spatially separated sGBR and nGBR genetic stocks.
- There is strong synchrony in annual fluctuations in Green turtle breeding numbers at nesting beaches supporting the sGBR genetic stock.
- There is strong synchrony in annual fluctuations in the proportion of adult female and male Green turtles preparing for breeding from dispersed foraging areas and the size of the annual breeding population at Heron Island, the primary index beach for monitoring breeding within the sGBR genetic stock.

Green turtles from numerous widely dispersed foraging areas including Port Curtis migrate each year to breed on the sGBR Capricorn-Bunker cays. Heron Island within the Capricorn Group is the primary index site for monitoring trends in the size of the annual sGBR Green turtle breeding population. The annual fluctuations in the Green turtle breeding population at Heron Island was used as the index of the annual fluctuations for the combined breeding

populations across the entire foraging range for the sGBR genetic stock. The long term breeding census data from the Heron Island nesting population and from Shoalwater Bay and Moreton Bay as representative foraging populations within the sGBR genetic stock are derived from the DES Queensland Turtle Conservation Data Base. These data formed the basis for comparison of the variations in annual breeding rates of Green turtles foraging within Port Curtis with those of the more widely distributed foraging population for the sGBR genetic stock.

- The annual fluctuations in adult male breeding rates recorded in Port Curtis show comparable synchrony with the previous records of male Green turtles foraging in Shoalwater Bay and Moreton Bay and the female breeding rate recorded at Heron Island.
- There has been a marked lack of synchrony of fluctuations in adult female breeding rate within Port Curtis during the three year period 2017-2019 and the approximately synchronous fluctuations in annual breeding rates previously recorded for females foraging at Shoalwater Bay and Moreton Bay and nesting at Heron Island.
  - The adult female foraging population in Port Curtis has displayed anomalously low breeding rates during 2017 -2019 relative to other monitored populations within the sGBR stock. No such anomalous breeding rate is evident for the adult male population within Port Curtis during the same period.

Recruitment has not been a well estimated demographic parameter for the Port Curtis Green turtle foraging population using the current capture-mark-recapture-based sampling program (Chapter 5). However, using identification of recently recruited juvenile Green turtles from the open ocean, which have shifted from a pelagic life history phase to benthic foraging phase within Port Curtis, the proportion of new recruits to the small immature age class of the population occurred at the rate of 0.14 per annum during 2016-2019 (Chapter 2). Similarly, using visual examination of gonads of adult females via laparoscopy to identify females in vitellogenesis for their first breeding season, the rate of recruitment of new breeding females into the adult female foraging population of Port Curtis during 2016-2019 was very low at 0.10 per annum (Chapter 2).

The diet of Green turtles foraging in Port Curtis was defined using analysis of gastric lavage samples from 329 turtles captured across nine study areas within the Port during 2015 – 2019: Pelican Banks, Chinaman Island, Facing Island, Quoin Island, The Narrows, Wiggins Island, the mouth of the Boyne River, South Trees, and Wild Cattle Island (Chapter 6). The Green turtles in the Port were primarily vegetarian in diet, foraging on a wide range of vegetation taxa. Their diet varied temporally and spatially within the Port depending on when the samples were taken.

- Vegetation that dominated the ingested items included 1 species of mangrove, 4 species of seagrass, 14 species of red algae (Phylum Rhodophyta), 3 species of green algae (Phylum Chlorophyta), 1 species of brown algae (Phylum Ochrophyta). Four animal taxa that appeared to be intentionally eaten included Porifera, Mollusca, Cnidaria, and Crustacea). Items that may have been unintentionally eaten included detritus, amphipods, Ozobranchid leeches and plastic debris.
- Food items with a high (>50%) frequency of occurrence at more than one site were the seagrasses *Zostera muelleri*, *Halodule pinnifolia*, *Halophila ovalis*, the red algae *Catenella nipae* and *Bostrychia tenella* and red mangrove (*Rhizophora stylosa*).

- The diet of the Green turtles varied strongly across the sampling sites within Port Curtis. When food items were grouped into higher-level taxonomic forage categories:
  - There was a strong predominance of seagrass in turtle diets at Pelican Banks, South Trees, and off Wild Cattle Island.
  - Red algae were the dominant food items off Quoin Island.
  - Green algae were the primary food ingested adjacent to Wiggins Island.
  - Turtles at the mouth of the Boyne River primarily ate red algae and seagrass.
  - Turtles along the western side of Facing Island ate a diet of red algae and mangroves.
- Fifteen Green turtles were sampled for diet at multiple times during the study:
  - All were recaptured at the same sites as their original captures;
  - The interval between successive sampling events ranged from 6 wk to 23 mth.
  - Based on IRI analyses, eight of the 15 turtles had different predominant food items across the two sampling periods.
    - Of those, five turtles switched between eating seagrass and algae and the other three changed between two species of seagrass.
- Ingested plastic debris generally occurred at a low incidence, with a frequency of occurrence ranging from 0% at multiple sites to 12.5% off Quoin Island.
  - Types of ingested debris included plastic fibres, fishing line, and flat hard and soft plastic fragments.
  - All ingested plastic fragments were small, less than 0.5 cm in length, and occurred at an insignificant volume.

The ecotoxicology components of these studies (Chapter 7) present the longest known temporal analysis of element concentrations in sequentially sampled sea turtles. The study describes temporal and spatial trends in element concentrations in Port Curtis Green turtles. A total of 77 blood samples were collected and analysed, from 37 individual Green turtles captured in at least two different years throughout the study period, 2011-2018. The assessment of the temporal and spatial accumulation of metals in Green turtles foraging in Port Curtis and the impact this may be having on Green turtle health used previously established reference intervals (RIs), supported by new toxicological information generated from cell-based bioassays.

- All turtles were recaptured at the same site where each was originally captured.
- Sixteen elements (As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Ti, V and Zn) were regularly detected, and measured at concentrations consistently above the RIs.
  - Six elements (Ca, K, Mg, Na, Th, U) had very few blood concentrations above the detection limit, and/or did not have published RIs, and were not included in further analysis and interpretation.

No unidirectional trend in metal concentrations across the study period was detected. The temporal trends in blood trace element concentrations in Port Curtis Green turtles followed two broad patterns:

- Co, Cr, Mn, Mo, Ni - concentrations were generally low in 2011, followed by a spike in the concentrations in 2013/14, a return to low concentrations in 2016, and another spike (except Cr) in 2017/18, although there are signs of concentrations decreasing from 2017 to 2018 in some individuals;

- As, Ba, Cd, Cu, Fe, Pb, Sb, Se, Ti, V, Zn - concentrations were generally low in 2011, and remained low until a spike in 2017/18, although, again, with signs of concentrations decreasing from 2017 to 2018 in some individuals.

These general trends indicate that the exposure of Port Curtis Green turtles to trace elements has changed over this eight-year sampling period. It is not known what has caused these changes to exposure, although extreme rainfall events (with associated high level flooding) and port activity suggest that climatic and/or anthropogenic activities could be involved.

In interpreting these data, it is necessary to consider that essential elements, in general, are taken up and regulated more efficiently than non-essential elements. In addition, the residence time of any element in blood is expected to be influenced by species-specific metabolic needs and detoxification strategies, as well as overall health and individual variation. To date there is a paucity of data for the toxicokinetics and toxicodynamics of trace elements in reptiles. Thus, an observed change in elemental blood concentration from above RI limits back to within RI limits (as observed for some elements) does not necessarily indicate that the risk to turtle health has been reduced, as blood is only a snapshot in time of an active metabolic pathway.

Low concentrations of many elements were observed between 2011 and 2016, with signs of additional elements decreasing in 2018, following the 2017 rainfall event. It is likely that, although blood concentrations have reduced in these periods of suspected low exposure conditions, these elements could be bioaccumulating in other tissues not sampled in the present study (liver, kidney, brain, etc), where they can elicit toxic effects, particularly chronic effects.

Collectively these diverse studies that have been conducted across nine years in Port Curtis have demonstrated that a number of significant parameters with respect to the population dynamics and population health of the foraging Green turtle population of Port Curtis vary in response to temporal and spatial variability in the habitats, climate and probably port management.

Localised studies confined to small areas of Port Curtis and/or short term studies of just a few years will not provide a definitive description of the population dynamics and health of the resident Green turtle foraging population for the Port as a whole.

In particular, it is now apparent that longitudinal studies that span years before and years following major perturbations within the Port are required to unambiguously identify the consequences of such events as major dredging programs or major floods. With Green turtle foraging aggregations occurring at the areas of river/creek outflows, attention should be paid to whether the structural changes to habitat associated with dredging or flooding events are the result of erosion or sediment deposition, the extent to which the changes in marine vegetation are in response to turbidity and/or salinity changes. With individual Green turtles tending to adhere to spatially confined areas, with few turtles switching between sites as part of their short-term foraging range, inferences from localised diet and ecotoxicology studies are most likely to be indicative of microhabitat conditions within Port Curtis, rather than the wider Port as a whole.

With Port Curtis being within the distributary of the Fitzroy River, draining the largest river catchment in coastal eastern Australia and receiving discharge from two small local rivers (Boyne and Calliope Rivers) attention must be paid to the environmental consequences of major flooding events. The present study is identifying that extreme flooding events may be the primary drivers with respect to Green turtle habitat use, diet, body condition, breeding rates and spikes in metal loads within turtle blood. Separating the effects of sediment and chemical discharge from the rivers from those associated with resuspension of sediment and chemicals caused by habitat disturbance within Port Curtis is confounded when major floods and major dredging events occur in close proximity, as occurred in 2011.

The elevated levels and temporal and spatial changes of metal toxins recorded in Green turtles during this study need to be investigated in response to temporal and spatial changes in habitat conditions resulting from major perturbations within Port Curtis. However the temporal and spatial changes of chemicals within Port Curtis also should be considered in parallel with the continually increasing impacts to the Port habitat and turtle populations that will result from:

- the growing urban development surrounding the Port,
- increasing industrial development surrounding the Port that encompasses more than the Port infrastructure for unloading and loading vessels of the Gladstone region,
- increasing industrial shipping and recreational vessel traffic within the Port.

These have the potential for increasing the chemical input to Port Curtis waters.