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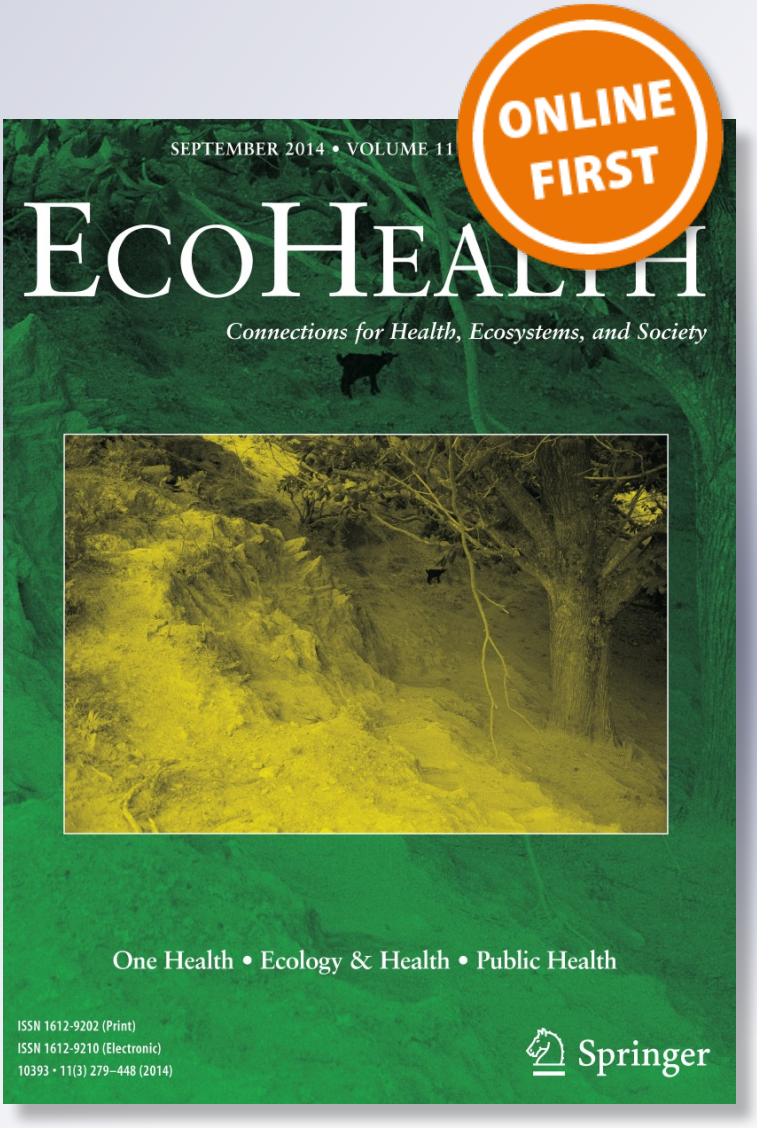

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Original Contribution

Clinical and Pathological Findings in Green Turtles (*Chelonia mydas*) from Gladstone, Queensland: Investigations of a Stranding Epidemic

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Abstract: An investigation into the health of green turtles was undertaken near Gladstone, Queensland, in response to a dramatic increase in stranding numbers in the first half of 2011. A total of 56 live turtles were subject to clinical examination and blood sampling for routine blood profiles, and 12 deceased turtles underwent a thorough necropsy examination. This population of green turtles was found to be in poor body condition and a range of infectious and non-infectious conditions were identified in the unhealthy turtles, including hepato-renal insufficiency (up to 81%, 27/33 based on clinical pathology), cachexia (92%, 11/12), parasitism (75%, 9/12), cardiopulmonary anomalies (42%, 5/12), gastroenteritis (25%, 3/12), masses (25%, 3/12) and mechanical impediments (17%, 2/12 based on necropsy). Overall, there was no evidence to indicate a unifying disease as a primary cause of the mass mortality. Recent adverse weather events, historic regional contamination and nearby industrial activities are discussed as potential causative factors.

Keywords: green turtle, health, reptile, disease, mortality, gladstone

Introduction

Green turtles (*Chelonia mydas*) are a marine reptile found through tropical and subtropical waters, including coastal areas of Queensland, Australia (Seminoff 2004; Limpus 2008). This species is listed as vulnerable in Queensland and Australia and is protected under Australian federal (*Environmental Protection and Biodiversity Conservation Act 1999*) and Queensland state (*Nature Conservation Act 1992*) legislation. Threats to eastern Australian green turtle populations are largely related to human activities, such as harvesting of sea turtles, entrapment and entanglement in fishing gear, boat strike, and habitat degradation of feeding grounds. However, disease from natural causes and secondary to human activities are also important factors (Limpus 2008). As part of efforts to protect marine turtles, the Queensland Government maintains a database (StrandNet, http://www.ehp.qld.gov.au/wildlife/caring-for-wildlife/strandnet-reports.html) that records sick, dead and injured sea turtles and other marine wildlife that strand along the Queensland coastline, which has been active since 1996 (Biddle and Limpus 2011). This data provides the

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Figure 1. Port Curtis and the city of Gladstone, Queensland, Australia. The main study area for this investigation was focussed at the Boyne River Estuary at Tannum Sands, 26 km south-east of Gladstone (oblong). Two stranded turtles from within Port Curtis also underwent necropsy examination (stars). The crosses indicate sites of port development activities in 2011. (http://maps.google.com.au/maps?hl=en-GB&rlz=1T4DAAU_en-GBAU267AU267&q=gladstone+queensland&um=1&ie=UTF-8&sa=X&ei=RIsEUfKrGOiPiAfh5oH4BA&ved=0CAsQ_AUoAA, accessed 27/1/2013).

empirical rationale for determining local conservation strategies for sea turtles and other marine wildlife, as well as being a source of useful comparative information for turtle health and debility elsewhere.

Gladstone (23.8489°S, 151.2631°E) is a major port city on the Queensland coast, built around Port Curtis (Fig. 1), and is located at the southern end of the Great Barrier Reef Marine Park. The port is partly enclosed on the eastern side by Curtis and Facing Islands, and receives water from river catchments of the Fitzroy, Calliope and Boyne Rivers (Fig. 1). Port Curtis and the surrounding region supports a number of industries, including processing and transport of aluminium, magnesium, coal and various petroleum products, a local fishing industry, tourism, a coal-fired power station and agricultural activities. Recent projects to expand port facilities commenced on May 20th, 2011, with dredging and land reclamation activities being undertaken for the construction of multiple liquefied natural gas processing and export terminals in the Western Basin area of the port (Gladstone Ports Corporation 2011). The port also supports a wide range of coastal marine habitats, including rocky and coral reefs, tidal and sub-tidal sea grass meadows, mangroves and soft-bottom habitats, which provide habitat for a number of threatened species such as the green, loggerhead (*Caretta caretta*), hawksbill (*Eretmochelys imbricata*), olive ridley (*Lepidochelys olivacea*) and flatback (*Natator depressus*) turtles and dugong (*Dugong dugon*).

Following a period of prolonged drought, a strong La Niña event on the south-western Pacific Ocean with associated cyclone activity brought heavy, prolonged rainfall to most of coastal eastern Queensland during December 2010 and January 2011, producing the wettest summer on record for Queensland (BOM 2012). This resulted in extreme flooding of a number of rivers along the Queensland coast, including the Fitzroy, Calliope and Boyne Rivers, and had significant impacts on the local coastal environment, contributing to reductions in biomass of sea grass beds in the Gladstone region through increased turbidity and settling of silt on sea grass meadows (McCormack et al. 2013). Losses were more extreme at sites from the inner Port and Rodds Bay than the outer Port. These sea grass beds are important feeding grounds for resident populations of green turtles and dugongs.

The year 2011 also saw a dramatic increase in the number of stranded marine turtles along the Queensland coast (Fig. 2; data extracted from StrandNet), with reported stranding numbers in the Rockhampton region (23°S latitude block which includes Port Curtis) almost six times higher than in previous years, identifying this region as one of four 'hotspots' for marine herbivore (green sea turtle and dugong) stranding in 2011 (DEHP 2011). The vast majority of these strandings for both Gladstone and all of Queensland were of green turtles (Meager and Limpus 2012). The other three hotspots for Queensland in 2011 were in the region of Townsville (19°S latitude), Hervey Bay (25°S latitude) and Brisbane (27°S latitude). The unusual weather patterns of 2011 had effect on each of the hotspots. Townsville received outflow of the Burdekin River and was in the footprint of Cyclone Yasi with major flooding outflow and coastal habitat damage. Gladstone received outflow of the Fitzroy River, the largest catchment in eastern Australia that experienced record level flooding. Hervey Bay received record level flooding with outflow from two large rivers (the Burnett and Mary). Brisbane received record level flooding from the Brisbane, Pine, and Logan Rivers into Moreton Bay. These four latitudinal blocks correspond to the outflow from all the major catchments in eastern Queensland. All four sites have several characteristics in common which may predispose their resident green turtle populations to stranding. Each has an embayment supporting a resident green turtle population;

each significant catchment system flushed large volumes of freshwater into the embayment; each has high human populations, industry and agricultural lands that utilise the river systems; and each site, along with many others up the Queensland coastline, had significant losses of sea grass fields reported. Gladstone is of further significance as the associated river outflows receive groundwater from extensive underground coal deposits and associated mining within the catchments with likely chronic elevation of potentially toxic compounds in the upstream systems and as sediment in the embayment. Gladstone offers the only site of the four hotspots to have comprehensive animal stranding, health and necropsy data as well as environmental data. Therefore it is the focal point of this paper.

At the request of the Queensland Department of Environment and Resource Management [Q-DERM, now called Queensland Department of Environment and Heritage Protection (DEHP)], an investigation into the health of green turtles in and around Gladstone was undertaken in early July 2011 to determine if disease was contributing to the unusually high level of mortality. This investigation at this location was prompted by the escalating numbers of stranding in April 2011 after December–January's flooding and immediate loss of sea grasses. In addition, dredging of the port for new shipping channels associated with mining development on the northern aspect of the bay began on 21 May (approximately 6 weeks prior to this investigation) and the bund wall around a land reclamation site in the Western Basin at the Port of Gladstone was completed on 21 July 2011 and the pumping of dredge spoil into this reclamation area began on 6th September. The bund wall was found to be leaking sometime between July and September (Diggles and Diggles 2014; Pattison-Sowden 2014) and actions commenced to reduce sediment leakage from the area.

This paper describes the results of health assessment of 56 green turtles and necropsy results of 12 green turtles from the coastline immediately around Gladstone, and discusses the role of disease and environmental causative factors as contributors to the increased number of stranded turtles observed in this region in 2011. Similar detailed information is not available for the other hotspots and therefore have not been included as part of this analysis.

Materials and Methods

Study Site and Animal Selection

Investigations to assess the clinical health of green turtles were undertaken over a 3 day period (9th–11th July, 2011) in the Boyne River Estuary at Tannum Sands, Queensland, approximately 26 km south-east of Gladstone and at the entry to Port Curtis (Fig. 1) in response to an elevated concentration of green turtle strandings at this estuary. Live

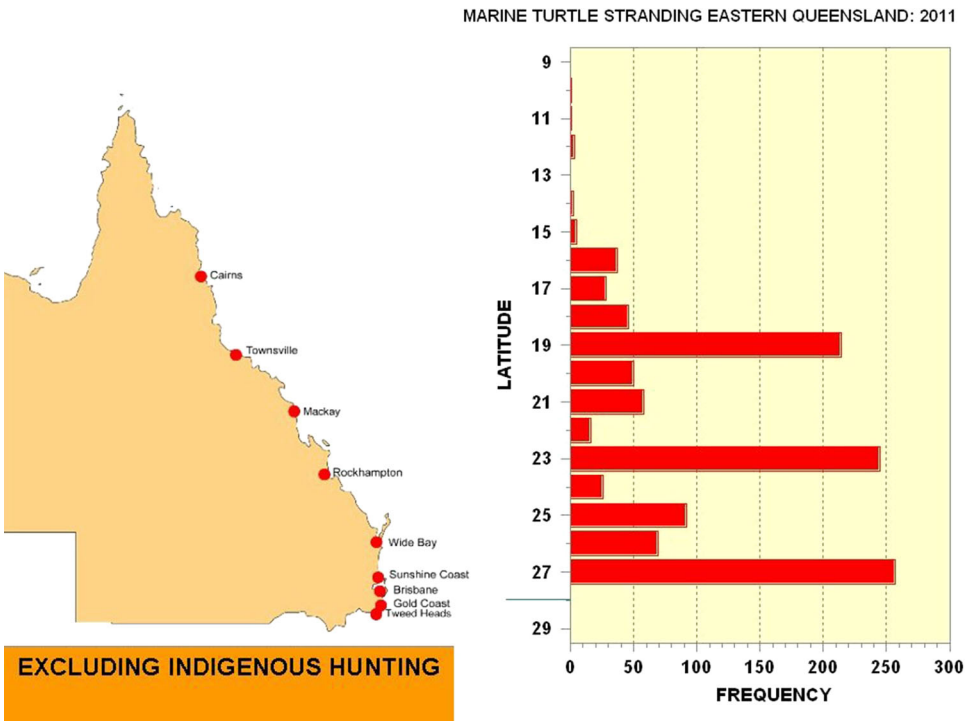

Figure 2. Marine turtle stranding by 1° latitudinal blocks along the eastern Queensland coast for 2011. Gladstone (Port Curtis) is located within the Latitude block 23°S, which also encompasses Keppel Bay, Port Alma and Northern Rodds Bay.

green turtles (n = 53) were collected from the intertidal and sub-tidal waters at the mouth of the Boyne River and underwent health assessment and blood sampling. Turtles basking on the intertidal flats were hand collected at low tides by day and night (n = 42) (Limpus et al. 2005b) and 11 were collected in shallow sub-tidal waters via turtle rodeo techniques (Limpus 1978). Subsequent to this exercise, a further three live turtles were collected by Gladstone Ports Corporation (GPC) on 23rd July and 16th August, 2011, as part of on-going port development works, approximately 30 km from the Boyne Estuary. These turtles also underwent health assessment and blood sampling by staff from DERM and GPC prior to their return to the wild and the results were included with the original 53 live turtle samples.

Deceased turtles (n = 12) were submitted to the School of Veterinary Sciences, The University of Queensland (Gatton campus) for comprehensive necropsy examination. All but two turtles submitted for necropsy examination were collected from the Boyne River estuary. Five turtles were caught as live animals during the clinical examination exercise in July 2011. These turtles were in extremely poor body condition and were assessed as having a poor prognosis for survival upon return to the wild. They were humanely euthanized by a registered veterinarian with intravenous Lethabarb (sodium pentobarbitone 325 mg/mL) delivered at greater than 100 mg/kg turtle bodyweight. Five turtles were found deceased around the mouth of the Boyne River by DERM staff, between 26th April to 24th August 2011, and were processed according to DERM standard protocols (http://www.ehp.qld.gov.au/wildlife/caring-for-wildlife/pdfs/op-wl-mpk-marine-wildlife-strandings-110610.pdf, accessed November 25, 2011). The remaining two turtles submitted for necropsy examination were collected as stranded carcasses at South Trees Inlet and Quoin Island, within Port Curtis, 10 and 16 km, respectively, from the main study site (Fig. 1). These turtles were found on 23rd August and 19th September, 2011.

This study was conducted under permit from the Animal Ethics Committee of The University of Queensland (Permit SVS/037/11/ARC/DERM/AUSTZOO) and was operating under permits (Scientific Purposes Permit WISP09021911 and Marine Parks Permits QS2011/CVL1414) from Q-DERM.

Health Assessment of Live Turtles

Live turtles were manually restrained for health assessment, which included assessment of midline curved carapace length (CCL, cm), bodyweight (kg), and carapace barnacle count (Limpus et al. 2005b). Body condition was calculated as per Limpus et al. (2012) and subjectively assessed through examination of concavity of the plastron and muscle mass of the neck and limbs, with turtles being assigned condition scores of good (3), poor (2) and very poor (1) (Table 1). Turtles were also assigned to an age

Table 1. Assessment of Body Condition of Healthy and Unhealthy Green Turtles, Correlating Body Condition Index and Barnacle Count for Each Category.

Body condition	Plastron shape	Muscle mass (limbs and neck)	Average (range) body condition index (log10[wgt] * log10[CCL])	Average barnacle count (range)
Good (3)	At least convex/rounded to flat plastron in inframarginal scutes and caudal to acromion process	Limbs and neck well muscled, no bony protuberances evident	0.62 (0.54–0.78)	17 (0–76)
Poor (2)	Concave plastron in inframarginal scutes and caudal to acromion process	Mild loss of muscle mass with occasional bony protuberance palpable	0.59 (0.55–0.75)	17 (0–59)
Very poor (1)	Very concave plastron in inframarginal scutes and caudal to acromion process, acromion may perforate through plastron	Moderate to severe loss of muscle mass with bony protuberances visible/palpable	0.58 (0.46–0.97)	38 (0–87)

class (small immature, large immature, and mature) according to CCL measurement (Flint et al. 2010b). Turtle gonads were examined via laparoscopic examination to determine gender, maturity and breeding status of turtles for those which the gender had not been previously determined (Limpus et al. 2005b). External injuries were noted and described, as was evidence of external fibropapilloma lesions (Work and Balazs 1999). A blood sample was collected from each turtle for assessment of haematology and blood biochemistry. A maximum volume of 4% bodyweight was collected from each turtle. Blood was collected from the dorsal cervical sinus, using an 18 G 38 mm needle attached to a 10 mL syringe (Wyneken et al. 2006).

Sample Processing

Blood smears for haematology were prepared using fresh blood and a standard wedge technique. These were air-dried and stained using Wrights–Giemsa stain. Remaining blood was placed in a blood tube containing lithium heparin anti-coagulant. After collection of a small sample for analysis of packed cell volume (PCV) and total plasma solids (TPS), the anticoagulated blood sample was centrifuged at 3500×*g* to separate the blood cells and plasma, and the plasma pipetted into 1.5 mL Eppendorf tubes, which were kept frozen at −20°C until analysis. Blood samples collected by Q-DERM and GPC staff were processed in a similar way and kept frozen at −20°C until transported on ice for analysis at The School of Veterinary Sciences, The University of Queensland (Gatton campus).

Pack cell volume, and estimated lymphocytes, heterophils, monocytes, eosinophils and basophils were measured. PCV and TPS were measured using standard techniques. An estimated white blood cell count was determined by examining the stained blood smear under a microscope at 400× magnification and counting the number of white cells in 10 high power fields. This figure was then averaged and multiplied by a factor of two to obtain an estimated white cell count (eWCC × 10^9/L) (Flint et al. 2010a). A differential white cell count was also determined by counting white blood cells under 1000× magnification into different cell groups to obtain a cumulative total of 100 cells, providing a percentage count for each white blood cell class (Flint et al. 2010a).

Biochemical parameters [total bilirubin (TBil), alkaline phosphatase (ALP), aspartate transferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH), uric acid (UA), urea, BUN, creatinine, glucose, calcium, phosphorus, magnesium, sodium, potassium, chloride, total protein (TP), albumin (Alb), and globulin (Glob)] were measured using an Olympus AU4000 automated machine (Olympus Diagnostic Systems, Melville, New York), calibrated according to the manufacturer's instructions.

Necropsy Examination

Necropsy examination was undertaken on turtles that were found deceased or deemed to have a poor prognosis for survival upon return to the wild and therefore humanely euthanized as previously described. A complete necropsy examination was undertaken as per Flint et al. (2009), and included gross and histopathologic examination. A standard set of tissues plus any lesions were collected at gross pathologic examination and placed in 10% buffered neutral formalin for histopathologic examination, which was performed by a registered veterinary pathologist. The pathology was graded by the extent of the lesion and the degree of inflammation. Turtles were also screened for the presence of spirorchiid fluke as part of the necropsy examination. Adult flukes were collected from the heart and aorta using a modification of the techniques described by Platt and Blair (1996) and Stacy et al. (2010). The heart and aorta were collected at necropsy and rinsed with 0.9% saline or 0.2% citrated saline (2 g sodium citrate plus 5 g sodium chloride in 1 L water). The supernatant was filtered using a 250 µm nylon mesh filter to trap adult spirorchiid fluke. The filter was then backwashed using 0.9% saline and the solution was examined in a petri dish under a dissecting microscope. Spirorchiid flukes were identified and collected from the solution using a Pasteur pipette and placed onto a clean glass slide for examination and identification under a compound microscope. Flukes were identified to genus level using a published identification key (Platt 2002). Presence of spirorchiid fluke was also determined at histopathologic examination through observation of adult fluke or eggs in tissue.

Statistical Analysis

Live turtles were categorised as either healthy or unhealthy according to results of clinical examination. Healthy turtles were considered to be in at least good body condition with no external evidence of injury or illness, including fibropapillomas. All remaining turtles were assessed as unhealthy, and were further categorised according to body condition, which can reflect severity and chronicity of

disease processes in wild animals. Proportions of turtles in each health class were determined with 95% confidence intervals. Descriptive statistics (median, range) were determined for key health indicators (bodyweight, CCL, carapace barnacle count) for comparison with body condition score. These were compared for statistical significance using Kruskal–Wallis testing, with significance determined at $P < 0.05$. Significant results were further examined using two-sample Wilcoxon rank sum test, with significance determined at $P < 0.05$. Statistical comparisons were made using R Commander (Fox 2011).

Blood results from turtles in the healthy group were analysed using Reference Interval (Reference Interval Draft Version, Copyright 2005, University of Cincinnati) (Pesce et al. 2005) to determine the 95% reference range for each parameter. These were compared to previously published reference ranges for this species (Flint et al. 2010a) to determine validity. Once validity was confirmed, results from unhealthy turtles were then compared to the published reference range (Flint et al. 2010a) to determine the proportion of unhealthy turtles with clinically significant abnormal results.

Turtles submitted for necropsy were all from the unhealthy class of animals, having been euthanized as a result of being in poor condition or having stranded and died. Cause of death and prevalent pathological findings were summarised and proportions of turtles affected were determined.

Results

Sample Population

A total of 63 green turtles were sampled for this investigation. Thirty-one turtles (49.2%, 95% CI 36.3–62.1%) were female, 25 turtles (39.7%, 27.6–52.8%) were male. The sex of seven turtles was not determined. The vast majority of turtles were small immature animals (61/63, 96.8%, 89.2–99.1%). There was one adult female turtle (CCL 100.1 cm) and one large immature turtle (CCL 82.5 cm).

Fibropapillomatosis (FP) was evident in 5/63 turtles (7.9%, 3.4–17.3%) examined, with FP scores ranging from A1 to B3 (Work and Balazs 1999).

Health Assessment of Live Turtles

Fifty-six live green turtles underwent health assessment. Key health parameters are summarised in Table 1. Twenty-six turtles were female and 23 were male. Fifty-four turtles were small immature animals, with a CCL ranging from 38.8 to 62.0 cm.

Thirty-five turtles (62.5%, 49.4–73.4%) were classified as unhealthy, and 21 turtles (37.5%, 26.0–50.6%) were considered in good health (good body condition, no evidence of external injury or illness, no evidence of external fibropapilloma). Of those turtles considered unhealthy, 12 (34.3%, 20.8–50.9%) were in poor to very poor body condition, and 19 (54.3%, 38.2–69.5%) were in moderate body condition. Body condition index calculations did not support these categories but demonstrated an overall reduced body condition index for green sea turtles of this region (Table 1). Fibropapillomas were evident in 4/56 turtles (7.1%, 2.8–17.0%). Three turtles positive for FP were otherwise in good body condition and had no other evidence of illness or injury. Only one turtle (2.9%, 0.1–14.9%) presented with evidence of an injury, with loss of a rear limb, which was fully healed and not considered a recent event.

There was a significant difference in the three body condition scores and bodyweight ($P = 0.009$ and $P = 0.01$, respectively, $n = 52$) and the three body condition scores and average total number of barnacles on the carapace (Median 38, 17,17, $P = 0.006$ and 0.008, respectively). Barnacle numbers were significantly higher in turtles in very poor body condition compared to those in poor or good body condition.

Blood samples for haematology and biochemistry were collected from 54 turtles (52 small immature animals, one large immature animal and one adult animal). Results from healthy turtles were the same as those from published reference ranges of healthy green sea turtles in Queensland (Flint et al. 2010a). As these published reference ranges were established using a large dataset ($n = 211$), they were used for comparison of results of blood tests for the unhealthy small immature turtles ($n = 33$) examined in this investigation. 61% (33/54 small immature turtles; 47–64%) of the examined turtles in this investigation were deemed to be unhealthy. The proportions of clinically abnormal (elevated) results are provided in Table 2. Parameters within normal clinical values are not presented.

81% (27/33) of the examined unhealthy turtles exhibited one or more clinical abnormality. Elevated electrolytes, hypoproteinemia, elevated hepato-renal factors, catabolic indicators and markers of acidosis or impaired renal function were the most frequent results.

Table 2. Clinically Significant Haematological and Biochemical Abnormalities in Unhealthy Turtles from Gladstone.

Abnormality	Parameter	Number (n = 33)	Sample proportion (%)
Elevated electrolytes	Cl and Na	11	33
Hypoprotonemia	Alb and Glob	21	64
Elevated hepato-renal factors	TBil, ALP, and AST	7	21
Elevated catabolism factors	CK	8	24
Acidosis	UA and uric acid	3	9
Heterophilia	Heterophils	3	9
Total white cell count	Total white cells	1	3

Few haematologic abnormalities were evident in the unhealthy group, with the main abnormalities being evidence of a mild heterophilia (possible parasitism or bacteraemia), which was found in 3/33 turtles (9.1%, 2–24%) and an elevated total white cell count (possible active acute inflammatory response), which was found in 1/33 turtles (3%, 0–16%) (Table 2).

Necropsy Examination

Necropsy examination was performed on 12 turtles, results of which are summarised in Table 3. Ten of these turtles were found around the Boyne River region, whilst the remaining two turtles were collected from Quoin Island (n = 1) and South Trees Inlet (n = 1), which are within Port Curtis (Fig. 2).

All turtles that underwent post mortem examination were small immature animals, with eight females and four males. Gross post mortem examination was performed on all 12 turtles. Complete tissue sample sets were collected and submitted for histopathologic examination for 10 turtles; the remaining two turtles had been frozen and thawed prior to necropsy examination, rendering the tissues unsuitable for diagnostic histopathology.

The main pathological finding at necropsy examination was evidence of cachexia (11/12 turtles, 91.7%, 64.6–98.5%), as determined by one or more observation of severe weight loss (below expected weight for size) and loss of body condition, black oedematous internal fat, and gut fill of less than 50% estimated maximum non-pathological capacity. Pathologic changes contributing to death were multisystemic spirorchiidiasis (9/12 turtles), cardiopulmonary disease (5/12), gastroenteritis (3/12), and mechanophysical (2/12). Other unusual findings were suspected neoplastic masses of the central nervous system (2/12) and heart (1/12). These were not considered primary contributors to death (Table 3).

Table 3. Summary of Pathologic Causes of Death and Other Contributing Pathologies of Small Immature Turtles from Gladstone Harbour.

Turtle	Cause of death	Other contributing pathologies
1	Trauma	Fractured jaw, ruptured gut
2	Chronic impediment	Osteo-arthritis
3	Cardiopulmonary disease	Multisystemic spirorchiidiasis
4	Multisystemic spirorchiidiasis	Potential renal disease
5	Multisystemic spirorchiidiasis	Aortic neoplasia
6	Cardiopulmonary disease	Multisystemic spirorchiidiasis, interstitial pneumonia
7	Cardiopulmonary disease	Multisystemic spirorchiidiasis, granulomatous pneumonia
8	Cardiopulmonary disease	Multisystemic spirorchiidiasis, granulomatous pneumonia
9	Bacterial enteritis (ulcerative)	Multisystemic spirorchiidiasis, granulomatous pneumonia
10	Drowning/chronic disease	Interstitial pneumonia, fibropapillomatosis
11	Bacterial enteritis	Multisystemic spirorchiidiasis, meningioma, ulcerative retrobulbar mass
12	Gastroenteritis (ulcerative)	Multisystemic spirorchiidiasis

All turtles that underwent a complete necropsy examination (i.e. gross and histopathology exam) had evidence of multi-organ infection with spirorchiid blood fluke, with varying degrees of associated inflammation. Nine of the 12 turtle with parasite infections were considered to be infected sufficiently to contribute to pathology. Common sites for infection and inflammatory changes included the heart, aorta, gastrointestinal tract, spleen, pancreas, brain, salt glands and lungs. Localised granulomatous vasculitis was found in 6/10 turtles (60%, 95% CI 31.3–81.2%) with accompanying histopathology, and was typically associated with spirorchiid eggs. Inflammation associated with spirorchiid fluke eggs was most severe in one turtle which also had evidence of bacterial gastroenteritis, and may have primarily contributed to the mortality of this turtle. Morphologic identification of adult fluke revealed infection with parasites from the genera *Hapalotrema*, *Learedius*, *Carettacola*, and *Neospirorchis*.

Five of the 12 necropsied turtles indicated cardiopulmonary pathology. One turtle was in fair condition but presumptively diagnosed to have dry-drowned based on necropsy findings and being entangled in a net. It was considered acute as there was surfactant in the airways but no water. It was recorded as having multifocal, lymphocytic interstitial pneumonia and spirorchiid associated pulmonary granulomas. Multifocal, granulomatous pneumonia with intralesional spirorchiids was noted in six additional turtles. Four turtles had mild to moderate, lymphocytic interstitial pneumonia that was considered a non-specific, chronic finding. A bacterial origin was suspected in one case of granulomatous pneumonia as the lesions differed from those typically seen in response to spirorchiids in that they were relatively large, infrequent and had central areas of necrosis. Special stains (Gram, Periodic Acid Schiff, Ziehl-Neelsen) failed to identify a causative agent in this case. In all cases, the pulmonary lesions were considered to be mild to moderate based on the number of inflammatory cells counted per high powered field and the relatively low proportion of tissue affected.

There were three cases of acute or chronic gastroenteritis among the 12 necropsies. Only two had supporting histopathology. One of these cases demonstrated partial thickness intestinal ulceration and repair, indicating some chronicity, in addition to the spirorchiids and an associated inflammatory reaction. In the other case examined histologically, there was acute, partial thickness ulceration of the stomach superimposed on chronic, mild lymphocytic gastritis in addition to the spirorchiids. This case also demonstrated extensive villous blunting in the intestine indicating past exposure to an agent targeting mucosal epithelium; however, this was no longer evident. The bacteria associated with the ulceration, likely Gram negative invaders, were considered secondary due to concurrent pathologies that historically cause mortality. Of these cases, only one had any noted associated liver pathology with moderate numbers of mononuclear (lymphocytes) cells in the periportal connective tissue. Linear foreign body as a causative agent was speculated given the linear nature of the lesions but no evidence of fishing line was present.

Two small immature (CCL = 49.1 and 44.4 cm, respectively) turtles had physical impediments. One had evidence of trauma with intestinal rupture and a fractured mandible, potentially as the result of a linear foreign body, although no foreign bodies were found at necropsy. The other had evidence of significant osteo-arthritis of the shoulders with joint remodelling and restricted movement.

One turtle had an expansile meningeal mass consisting of streams and whirls of spindle shaped cells. The cellular whirls occasionally contained central areas of mineralisation (presumed psammoma bodies). A similar population of cells was present in a retrobulbar mass. A presumptive morphological diagnosis of meningioma was made.

Blood results for the five turtles euthanized during the clinical examination exercise revealed borderline to low levels of protein and albumin with associated excessive fluid accumulation noted grossly. One turtle had elevated AST/CK/LDH levels with grossly evident associated loss of muscle tissue. Blood results were otherwise unremarkable for this group.

Discussion

This is the first time an investigation to assess the health of green turtles in the Gladstone region has been undertaken, and is one of the few investigations into causes of mass mortality of sea turtles in Australia (Guinea and Chatto 1992; Gordon et al. 1993). The proportion of turtles considered unhealthy was high, and during this investigation, turtles were more than twice as likely to present in an unhealthy state as compared to recent investigations of green turtle populations from Moreton Bay and Shoalwater Bay (Odds ratio 2.3, 95% CI 1.7–3.0, $P < 0.001$) (Flint et al. 2010a). This latter study was undertaken prior to the devastating floods of 2010–2011, included a site with

minimal anthropogenic activity and provides a comparative baseline for southern Queensland coastline green turtle populations. The vast majority of turtles in the current study consisted of small immature animals, which was similar to the stranding data collected for this region for the year of 2011 (DEHP, unpublished data) and is a reflection of the population structure of green turtles in this region (Limpus and Reed 1985). Turtles from this age class have been found to be more at risk of presenting in poor health compared to other age classes (Flint et al. 2010b) and may be the first animals of a resident population to show the effects of negative environmental influences. Green turtles are the predominant resident species for this region (Limpus and Reed 1985) and, as herbivores, are likely to be the first sea turtle species affected if sea grasses are removed from their foraging areas. The stranding rate for green sea turtles for the Gladstone region increased over 800% in 2011 after the flooding (and almost immediate loss of sea grass) when compared to this region's average stranding rate of recent non-flood years (2005–2010) (Meager and Limpus 2012). Given these factors, the examined animals were a good sentinel representative of the impact of this event and concurrent activities on marine wildlife populations. This phenomenon of an herbivore mass stranding was noted at all four identified hotspots along the Queensland coastline affected by flooding in 2011. Definitive reasons as to why these strandings occurred at the other sites cannot be given due to a lack of in-depth data, but the pattern of environmental stressors, natural and anthropogenic, can be assumed to be significant factors based on the findings of this study.

The most common and consistent finding from results of clinical examination, blood tests and necropsy findings of green turtles in this investigation was cachexia. This is a non-specific finding in sea turtles and may result from a range of health issues, including infectious diseases, malnutrition and toxicoses (Campbell 2006; Wyneken et al. 2006). Our finding of cachexia in clinically unhealthy versus healthy turtles is supported by our clinical presentation but is not supported by application of somatic growth models (Bjorndal et al. 2000; Limpus et al. 2012). However, Limpus et al.'s regression analyses demonstrated that immature green turtles of Gladstone were in poorer body condition when compared with immature green turtles from other Queensland coastal regions in 2011 (Limpus et al. 2012) indicating the local population, even those not succumbing to ill-health, were not performing well.

Cumulative live turtle haematology and serum biochemistry results were strongly indicative of a non-infectious metabolic anomaly, such as hepato-renal insufficiency (elevated TBil, AST and ALT) at the population level (Table 2) (Flint et al. 2010a). From the necropsies, only one animal had lesions of chronic hepatic disease. As such, the metabolic anomalies may be acute.

The only infectious disease consistently found at necropsy of stranded turtles in this study was multi-systemic spirorchiidiasis, which was found in all 10 turtles that underwent histopathological necropsy examination. Studies on the health of green sea turtles in Queensland during the last 25 years have revealed the prevalence of spirorchiid fluke infection to vary between 41 and 98%, with infection contributing to mortality in up to 40% of strandings (Glazebrook and Campbell 1990; Gordon et al. 1998; Flint et al. 2010b). Spirorchiid flukes have been implicated in an outbreak of neurologic disease of loggerhead turtles in Florida, which may have been potentiated by chronic exposure to a toxin (Jacobson et al. 2006).

For all bar one case, cardiopulmonary disease (primarily pneumonia) was most likely the manifestation of multifactorial disease processes potentially exacerbated by environmental stressors, as has been seen in other aquatic species (Halvorsen and Keith 2008). The outlying case was a result of misadventure by dry-drowning in a net. Nets have long been used in this area without detriment to the local turtle population. The cases of gastroenteritis could have been immune-linked but also a mechanical issue due to reduced gut fill, foraging in novel areas exposing the turtle to potential foreign bodies, or toxin exposure. The prevalence of FP for green turtles in Port Curtis was similar to that previously published for sea turtles from Queensland waters (Limpus et al. 1994; Limpus and Miller 1994; Gordon 2005; Flint et al. 2010b). Although FP is commonly reported for sea turtles in some areas of Queensland, to date there is no evidence to implicate FP as a significant cause of mortality of sea turtles in any area of Queensland (Limpus et al. 2005a; Biddle and Limpus 2011). Lesions observed in this study were relatively minor (Work and Balazs 1999). Other masses found were incidental and physical impediments were within expected findings for a normal population (Flint et al. 2010b).

Overall, our findings are suggestive of an insidious, underlying environmental chronic process that predisposed animals to acute secondary and/or opportunistic pathologies when the effects of the flooding and other environmental stressors occurred. Such processes and causative agents are often difficult to identify. Given Gladstone's position at the mouth of a major river system and its long

history as an industrial port, cumulative natural and anthropogenic environmental disturbances may have been significant contributors to this unusual mortality event.

Queensland experienced extreme environmental conditions in the time preceding this event as a result of one of the strongest La Niña events on record for the east coast of Australia 5 months prior to the mass mortality event reported (BOM 2012). This extreme weather caused extensive flooding in many parts of Queensland, including Gladstone, and resulted in dramatic changes to water quality, salinity and silting along the coast (DERM 2011a). Water temperatures remained stable outside of seasonal variation (seatemperature.org 2014), with temperatures remaining well above 15°C for the 12 months prior to this investigation (DERM 2011b). Gladstone historically has water temperatures that remain above a minimum of 19°C even during the coldest month of July (seatemperature.org 2014). This suggests cold stunning, which typically occurs when water temperatures rapidly fall to below 12°C, as reported for Florida turtles (Wyneken et al. 2006) which has similar temperature parameters and variations and turtle species as Queensland (Limpus 1997; Owen et al. 2013), and is a common causative factor in sea turtle mass mortalities in the North Atlantic Ocean, is unlikely to have contributed to this mortality event. However, the other effects of flooding are highly likely to have negatively impacted Gladstone's green sea turtles.

Flooding may have significantly impacted sea turtle species by scouring rivers and estuaries and/or negatively affecting sea grass pastures. The floods that occurred in 2011 comprised record level water heights. The coastal and upstream regions of Gladstone have long established coal mining, metal smelters and agricultural cropping and pasture usage. Such enterprises are known sources of trace metals and various other contaminants that have likely entered the marine system. While contaminants have not been monitored comprehensively before and during the stranding event, flooding associated physical disturbances and physico-chemical changes in soil, sediments and water have been shown to facilitate mobilisation of metals from catchments and their release from sediment stores (Schulz-Zunkel and Kreuger 2009; Weber et al. 2009). Similarly, considerable releases and increased bioavailability of metals and metalloids or other contaminants buried in sediments can be associated with anthropogenic disturbances such as dredging (Shipley et al. 2009). Gladstone Harbour has a 100 year history as one of the busiest ports in Australia as a multi-commodity point of export, and is subject to on-going maintenance, upgrade and harbour remodelling. Sea grasses are proficient accumulators of metals, sequestering them from both the water and sediment environment in which they grow (Besar et al. 2008), and present an important source of dietary exposure to turtles (Komoroske et al. 2012). It has also been demonstrated that sea grass metal concentrations can increase rapidly in response to metal inputs and increased bioavailability via rainfall events or localised developments (Prange and Dennison 2000). It is thus conceivable that turtles experienced elevated contaminant exposure via these events and pathways. In combination with their deteriorating health and nutritional status and the associated effects on contaminant mobilisation, detoxification and elimination pathways during a period of low food abundance (Eisler 2007), this may well have contributed to the poor body condition, suspected immunosuppression and hepato-renal pathologies seen in this study.

Exposure to toxins was investigated in parallel with this study as a three tiered analytical screening of blood and tissue samples from the same green turtles (Gaus et al. 2012). Gaus et al. (2012) found a range of metals (arsenic, cadmium, cobalt, mercury, nickel, selenium, and vanadium) were present at levels clearly above those reported for green turtles and other marine megafauna species from other locations, and were near or above acute tissue based effect concentrations reported across various vertebrate taxa. While the lack of exposure baselines and toxicological reference data for reptiles present ultimate limitations for the interpretation of metal concentrations in green turtles, these findings are of concern and may have directly impacted the health of the population. Other contaminants were also present in green turtle blood and tissues at concentrations that may be of concern regarding chronic exposure regimes (polychlorinated dibenzo-*p*-dioxins and dibenzofurans, dioxin-like polychlorinated biphenyls, silver, copper, chromium, molybdenum, and lead), and although unlikely to result in acute effects, may have lowered the ability of the turtles to cope with the short-term and potentially cumulative stressors associated with higher metal exposure and other events leading up to the mass stranding in 2011.

Much of the clinical pathologies (e.g. hepato-renal) and necropsy (e.g. spirorchiidiasis and cardio-pulmonary) findings reported in this study could be linked to one or more of these contaminants. The sources of contaminants in the above study was not assessed, however, there are a range of activities in the Gladstone region that may have

contributed to this both acutely and chronically, including a variety of agricultural and industrial practices. The potential for exposure may also have been a direct result of or exacerbated by extreme environmental conditions or industrial activities causing localised disturbances.

Although the insidious chronic stressors causing mobilisation of toxins from stored fats due to protracted catabolism may contribute to some elevated parameters seen, many of the metals examined in Gaus et al's (2012) study do not readily bioaccumulate and have a rapid blood clearance rate. Therefore, the reported toxins likely increased rapidly prior to sampling and, at least to some degree, contributed to pathologies seen in this study. The series of events and presentation of the examined animals are highly indicative of an acute on chronic condition that resulted in the 2011 unusual mortality event.

Natural and anthropogenic cumulative stressors very likely caused the mass stranding seen in the Gladstone green sea turtles, and potentially in the other hotspots along the Queensland coastline, 5 months after catastrophic flooding in 2011. Given natural phenomena cannot be controlled and are becoming more prevalent, it is important that anthropogenic factors are appropriately monitored and minimised in regions where industry and marine life interface.

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