Australian Marine Mammal Centre
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PROJECT TITLE: Determining baseline health and disease parameters for wild dugongs in urban and non-urban waters of northern Australia.

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OBJECTIVES

1. Determine what a healthy dugong is, by developing baseline clinical (health and disease) and biological (body condition) parameters for dugongs in non-urban Torres Strait.
2. Conduct detailed post-mortem examinations of dugong carcasses in urban south-east Queensland to establish cause of death and look for any emergent diseases or other health issues.
3. Examine clinical and biological parameters of wild dugongs in urban south-east Queensland (Moreton Bay) and compare their health and disease status to dugongs in non-urban areas and dugongs found dead in the same urban areas.

PROJECT SUMMARY

Dugong populations are significantly declining along the urban coastlines of northern Australia and can only sustain low levels of mortality. To initiate protection policies, major threats including human-associated and natural disease must be clearly defined. At present, a significant number of dugong mortalities remain undiagnosed. This project involves a unique collaboration between veterinary specialists and biologists to: (i) identify baseline health and disease parameters by necropsy of healthy dugongs harvested for food in non-urban Torres Strait and, (2) use these benchmark parameters to inform and develop methods for post-mortem diagnosis of causes of death and assessment of health status in urban Moreton Bay. Queensland.

Health assessments have been conducted on 66 live dugongs (of all ages and both sexes) in Moreton Bay during four field trips conducted over the period 2008-2011. This includes one fieldtrip after the major Queensland floods of Dec 2010-Jan 2011. Thirteen dugongs were also examined from the Burrum Heads region of Hervey Bay in June 2011. Biological and clinical samples including blood, urine, mucus, faeces and skin have been collected from each animal for haematological, bacteriological, endocrine, cytological, genetic and toxicological analysis (as appropriate). Blood haematology, serum biochemistry and trace element analysis have been completed and the results are currently being interpreted. Endocrine analysis of serum and faeces has been completed and three publications are in prep. Microbial and disease screening work, and toxicology is on-going.

Retrospective analysis of histopathological specimens collected from each of 40 dugong carcasses recovered from Moreton Bay and necropsied over the period 1997- 2007 is being conducted. Cause of death and baseline pathology of > 20 cases has already been conducted by a postgraduate student, with validation of histopathology findings by one of the PIs (Patterson-Kane). Biological information regarding diet, age, reproductive state, genetics has been determined for each case. This retrospective review analysis will continue. Tissues collected from each of these previous necropsies have undergone heavy metal analysis and a publication is currently in prep. Further toxicological analyses (e.g. POPs) will be conducted when further funds become available.

During this current project 2008-2011, >25 dugong carcasses have been recovered from the Moreton Bay area and necropsied. Full necropsy reports including gross morphology, histo-pathology findings and likely cause of death have been submitted to DERM's Marine Stranding Database. Biological information including body size, age (through tusk age), reproductive state (endocrine analysis), diet (ingesta analysis), population of origin (genetics) and resight history (if the dugong was a tagged individual) has been compiled for each carcass. These data will be added to the on-going dugong mortality database, and will form part of a major review document for dugong deaths in south-east Queensland. In addition, tissues have been collected from all the major organ systems for histo-pathology, disease screening, parasitology, microbiology and toxicology. Necropsy of dugongs in southern Qld will continue throughout 2010-2011.

Necropsy of dugongs harvested for food by indigenous people in Torres Strait was conducted during a one-month field trip to Mabuiag Island in April 2011.

COMMENTS ON OVERALL PROGRESS TO DATE

Excellent progress has now been made on each of the three objectives (see below for details) and against the revised workplan of Nov 2010 (Appendix 1.). Since we were granted a project extension through to early 2012, the following advances have been made:

- New investigators have joined the research team in place of Patterson-Kane and Trott. Dr Lucy Woolford, University of Adelaide, joined as the replacement clinical and anatomical pathologist, and Drs Di Ouwerkerk, Animal Sciences DEEDI and Gabriel Milinovich, University of Queensland, as microbiologists.

- In April 2011, a one-month field trip was conducted to Mabuiag Island, Torres Strait. A team of 3 biologists (Lanyon, Burgess, Mingramm), 2 microbiologists (Ouwerkerk and Milinovich) and a clinical & anatomical pathologist (Woolford) worked closely with local rangers, community elders and the Torres Strait Regional Authority to sample freshly harvested dugongs, for health.

- In May-June 2011, full health assessments were conducted on a total of 29 dugongs in Moreton and Hervey Bays.

- Mr Arthur Wong has been accepted as a PhD candidate in Biological Sciences at The University of Queensland, under the supervision of Lanyon & Woolford. Mr Wong will replace Ms Alison Gillespie as a postgraduate student. Mr Wong is currently trying to secure a scholarship and further research funds to continue and expand the current project, to examine blood haematology and biochemistry and conduct immune studies including lymphocyte proliferation on wild dugongs. At this stage we are hopeful that he will commence in January 2012.

- Several manuscripts have already resulted from this project and are either published or have been accepted for publication. A number of other publications are in preparation and well advanced.

Recent publications resulting from this work funded by AMMC:

- Lanyon JM, Sneath HL, Long T (2011) Evaluation of exertion and capture stress in serum of wild dugongs, *Dugong dugon*. *Journal of Zoo and Wildlife Medicine, In press*
- Lanyon JM, Sneath HL & Long T (2010) Three skin sampling methods for molecular characterisation of free-ranging dugong populations. *Aquatic Mammals* 36 (3): 298-306.
- Lanyon JM, Sneath HL, Long T & Bonde RK (2010) Physiological response of wild dugongs (*Dugong dugon*) to out-of-water sampling for health assessment. *Aquatic Mammals* 36 (1): 46-58.
- Owen H, Gillespie A, Burgess EA & Lanyon JM (2010) Small intestinal volvulus in a free-ranging female dugong (*Dugong dugon*). *Aust. Vet J*. 89 (7): 276-278.

Publications in preparation from this work funded by AMMC:

- Lanyon JM & Sneath HL (2011) Heavy metal concentrations in the blood and body tissues of free-ranging dugongs and dugong carcasses from an urban coastal environment. *In prep.*
- Lanyon JM, Sneath HL, Long T, Burgess EA & Woolford L. (2011) Changes in serum biochemical indicators of body condition in wild dugongs, Dugong dugon after a catastrophic inshore flood event. *In prep.*
- Woolford L and Lanyon JM (2011) Normal organ histology and background pathology in wild caught dugongs from the Torres Strait. *In prep.*

PROJECT OBJECTIVES

Objective 1: Determine what a healthy dugong is, by developing baseline clinical (health and disease) and biological (body condition) parameters for dugongs in non-urban Torres Strait.

METHODS

A one-month fieldtrip to conduct necropsy of wild harvested dugongs in Torres Strait was conducted over the period 8 April – 6 May 2011. Six dugongs, which had been hunted for consumption by humans, were sampled from Mabuiag Island, a small island in the Bellevue Island group lying approximately 100 km north of Thursday Island in the north-western Torres Strait (9° 57'S, 142° 11'E). This region represents a relatively pristine marine environment, largely devoid of anthropogenic impacts, apart from traditional hunting.The dugong population in this region represents the largest in the world with a population estimate of >25,000 individuals (Marsh *et al.* 2007). Necropsies conducted on each of the hunted dugong carcasses occurred following traditional hunts carried out by the indigenous people of the region. The research team was notified by local rangers, the hunters or other members of the Mabuiag Island community of 6 of the 8 dugongs harvested during this period. Fewer dugongs than anticipated were harvested and sampled due to unforeseen circumstances, i.e., the non-availability of boat fuel (the barge from Cairns did not deliver fuel for three consecutive weeks) and then serious damage to the island's main fuel pump which meant that the hunters were unable to obtain fuel over a prolonged period.

Sampling and analysis

Traditional hunting is an exclusively male activity in which dugongs are pursued by small power boat until exhausted (> 10 minutes), harpooned with a detachable 3-pronged head (wap), secured by rope to a boat and then drowned until dead. Individual pursuit times for

each dugong were not available but were likely to be greater than 10 mins (T. Whap, pers. comm.); however the approximate times of capture were recorded. Hunting typically occurred during the night between 2:00 and 4:00 h. Dead dugongs were towed to shore where they remained for several hours prior to the butchering process.

Post-mortem sampling of various tissues and blood was opportunistic and depended on factors such as time since death, the condition of the animal, its size and the access given to the researchers by the hunters. Necropsies were conducted *in situ*, coinciding with the butchering process, and typically occurred around five hours after death and before the hottest part of the day. Body morphometrics (body length, fluke width, and girth at each of peduncle, anal, umbilical and axilla) as well as sex and body condition (defined on a 5-point scale) were recorded for each carcass. Tissue samples were collected from each carcass and included (where possible) skin, muscle, blubber and all major organs and organ systems including liver, kidney, spleen, lungs, gall bladder, gastrointestinal tract, urogenital tract, brain, pituitary, lymph nodes (see Appendix 2.) for histopathology and blood and muscle enzyme analysis. Gut contents from all sections along the tract were collected for dietary, microbial and parasitological analyses. Tusks were collected for ageing when possible.

Blood samples were collected as soon as possible and immediately once the internal organs were exposed; blood was collected from the heart, via cardiac puncture, and in some cases from the lungs, using a hypodermic syringe. Whole blood was analysed immediately via Chem 8+ (for serum electrolytes, metabolic wastes and renal indicators) and CG4+ (for pH and blood gas analysis) i-STAT® cartridges, and a series of blood smears was prepared for blood cytology. Blood was also collected into orange top (for heavy metals), grey top fluoroxalate (for lactate), purple-top EDTA (ACTH, aldosterone) and red-top serum (serum biochemistry, blood enzymes, steroid hormones). Tubes were spun at 3000 rpm for 10 mins in a bench centrifuge, and serum or plasma pipetted off. Serum and plasma samples were frozen immediately in a series of 1.0 and 1.5 ml cryovials and stored at -20°C. Frozen separated serum and plasma samples were then stored and transported frozen to IDEXX Laboratories Brisbane for haematological and biochemical analysis. Frozen whole blood was sent to Queensland Health Forensic Laboratory for heavy metal analysis. Steroid hormone analysis was conducted through faecal and serum enzyme immunoassays (EIA) to determine reproductive state (Burgess *et al.* 2011). Faecal glucocorticoid concentrations were also analysed via EIA.

Organ tissue samples and sub-samples of gut contents were fixed in 10% neutral buffered formalin for later histological analysis and small sections of organs were stored frozen for heavy metal, toxicological and enzyme assays. Hindgut contents were further subsampled into Bouin's solution for ciliate analysis, sugar solution for faecal egg floats, and were plated onto various agar media (including SBA, MCA, XLD, MSB) for microbiological culture in on on-site incubator. Samples of six species of seagrass (*Halophila ovalis*, *Halodule uninervis*, *Cymodocea rotundata*, *Syringodium isoetifolium*, *Thalassia hemprichii*, *Enhalus acoroides*) found in the area and many of which form part of the dugong's diet were collected from intertidal beds at Mabuiag Island (south side) and stored frozen for heavy metal analysis.

Preliminary postmortem / health reports were prepared on site and submitted to Mabuiag Island community and to TSRA (Appendix 2.). In addition, presentations to both the Mabuiag Community and TSRA (Thursday Island) were held at both the commencement and completion of the field program. We also hosted a community barbeque. Analyses of samples and interpretation of results are continuing and full reports and scientific publications will be written in collaboration with the Mabuiag Community and TSRA.

Objective 2: Conduct detailed post-mortem examinations of dugong carcasses in urban south-east Queensland to establish cause of death and look for any emergent diseases or other health issues.

METHODS

1. Retrospective analysis of histopathological specimens collected from each of 40 dugong carcasses recovered from Moreton Bay and necropsied over the period 1997- 2007 is being conducted. Histopathology slides from previous cases in Moreton Bay are being re-examined, and disease processes compared with location, body size/age, and sex. Further diagnostic tests are being performed as required, e.g.,immunohistochemistry. Cause of death and baseline pathology of > 25 cases has already been conducted and reports prepared. Biological information including diet, age, reproductive state, genetics has been determined for each case. Tissues collected from each of these previous necropsies (blubber, muscle, liver, kidney) have undergone heavy metal analysis and a publication is currently in prep.

2. Dead dugongs are transported to the School of Veterinary Science by Queensland Parks and Wildlife staff (QPWS). Post-mortem examinations are performed at the University of Queensland Veterinary School; diagnostic data are then provided to Queensland Parks and Wildlife and Queensland Environmental Protection Agency for their annual dugong mortality report and stranding database. During this current project 2008-2011, 25 dugong carcasses have been recovered from the Moreton Bay area and necropsied.

For each dugong carcass, standard body measurements are taken and gross lesions are documented photographically. Standardised samples of all tissues and organs, including those containing gross lesions, are fixed in 10% formalin and processed for histological examination. Serum, and 300g samples of brain, liver, kidney, muscle and blubber are collected and stored at -20^{o}C for toxicological analysis (including heavy metals and algal toxins). These toxicological results will be examined in concert with the haematological and biochemical data to look for indicators of these diseases in these body fluids.

A veterinary pathologist (PI- Patterson-Kane) reviews all histopathology slides and written reports. Full necropsy reports including gross morphology, histo-pathology findings and likely cause of death have been submitted to DERM's Marine Stranding Database. Biological information including body size, age (through tusk age), reproductive state (endocrine analysis), diet (ingesta analysis), population of origin (genetics) and resight history (if the dugong was a tagged individual) has been compiled for each carcass. These data will be added to the on-going dugong mortality database, and will form part of a major review document for dugong deaths in south-east Queensland.

Objective 3: Examine clinical and biological parameters of wild dugongs in urban south-east Queensland (Moreton Bay) and compare their health and disease status to dugongs in non-urban areas and dugongs found dead in the same urban areas.

METHODS

Wild dugongs were captured opportunistically over the shallow seagrass banks in eastern Moreton Bay (latitudes 27° 20.09' to 27° 24.87' S; longitudes 153° 21.26' to 153° 23.84' E; water depths 1.6 to 3 m) using the dugong rodeo technique (Lanyon et al., 2006) as part of a long-term mark-recapture program (Lanyon et al., 2002). Sixty-six dugongs of both sexes and assorted size classes were sampled in Moreton Bay over the late autumn periods 19-23 May 2008 (n=13), 11-17 May 2009 (n=17) and early winter 11-17 June 2010 (n=20), 28 May- 3 June 2011 (n=16). In 2011, 13 dugongs were sampled for health in the Burrum Heads region of Hervey Bay over the period 5-9 June 2011.

Upon capture, each dugong was evaluated for general disposition and body appearance, physically and gene-tagged (following Lanyon et al., 2002, 2006; 2009), and measured in-water. Morphometrics included total body length measured in a straight line from snout to fluke notch, fluke width, and girths at each of peduncle, anus, umbilicus (maximum girth),

and axilla. Each dugong was manoeuvred into a large, custom-made PVC polyester mesh stretcher (MakMax Aust.) and transported to the examination vessel *RV Sea World One* where it was craned up onto the deck. Once on the deck, dugongs were placed on a padded open-cell foam mattress without physical constraint, for medical examination (Lanyon et al. 2010).

Monitoring of respiratory rate commenced immediately upon pursuit and prior to capture of each dugong. Continuous monitoring of the dugong's vital signs to determine physiological state (i.e., temperature, heart rate, and respiration (THR) as per Wong (2008)), commenced immediately after the dugong was placed on deck (Lanyon et al. 2010). Oral temperature was recorded at 5-min intervals via a temperature probe placed laterally along the mandible past the posterior mandibular molar. Heart rate was measured at irregular intervals during the sampling procedure. For the first 13 dugongs sampled, heart rate was measured via auscultation using a stethoscope placed under the ventral midline at the level of the axilla. For the remaining 37 dugongs, a doppler foetal heart detector with a 2-Mhz probe (Model PD1+, Ultrasound Technologies Ltd.) was used with the transducer placed under the sternum. Each respiration was recorded in real time until the animal was released.

Blood samples were collected as soon as possible after capture to minimise the effects of stress-released hormones on blood composition. Blood was collected from the brachial arteriovenous plexus accessed via the palmar medial (more commonly) or lateral surface of the pectoral flipper at the proximal aspects of the ulna and radius. The flipper was scrubbed thoroughly multiple times (> 5) with betadine solution on cotton gauze, alternating with alcohol and dry gauze, then dried and palpated for the stick site. Blood was drawn slowly using a 21-gauge, 3.8-cm/1.5-inch needle (or 5-cm/2-inch needle in the case of large adults > 270 cm body length, or for a lateral draw) fitted to a 20-cm (14-inch) extension set and Luer® fitted Vacutainer® collar. An initial 5-ml draw-in syringe was used to start the flow, and this sample was used to make multiple blood smears, with the remainder of this sample analysed via i-STAT®. Blood was collected in multiple tubes with a variety of anti-coagulents and supportive media, including orange–top trace element tubes, red-top serum separator tubes, purple top EDTA whole blood tubes, green-top plasma lithium tubes, blue-top citrate tubes, grey-top fluoroxalate tubes. A total of 60 to 100 ml of blood was collected from each dugong. Some blood samples were centrifuged immediately after collection to separate plasma and serum components from red blood cells, and all samples were stored appropriately for analyses.

An image ultrasound machine (GE Logiqbook) was used to scan the abdomen of each female dugong for evidence of pregnancy. Still images and digital video of scans were made of both thorax and abdomen using a 2.5-Mhz probe with focal distance set between 20 and 25 cm.

Two measures of total body length (straight and curved snout to fluke notch) were taken for each dugong on deck to check accuracy and precision of in-water measurements. A fresh faecal sample was collected directly from beneath the dugong as it defecated, and part of this sample was frozen and part refrigerated.. Urine, and sometimes semen, was collected by placing a clean plastic Frisbee® beneath the urinogenital opening when the dugong was first brought onto deck and then collecting the contents into a sterile container prior to release of the animal. Mouth contents were collected from the buccal cavity between the horny pads. Tears were sampled from each eye onto sterile eye spears, and nasal mucus onto sterile swaba. A sample of mucus suspended in the exhaled blow of the dugong was collected by holding an inverted sterile vial over the nares as the dugong exhaled. Vaginal mucus and epithelial cells were collected via swab from each female dugong. Each dugong was weighed (to the nearest 0.5 kg) in the suspended stretcher immediately prior to release, and a series of photos were taken of each dugong's body. A short-term waterproof crayon mark was applied to the dorsum to identify and avoid recapture of the same dugong during

the same sample period. Air and surface water temperatures were taken at midday on each sampling day.

Sample analysis

The vital signs of dugongs were monitored during each of the health assessments, and analysis of the 2008-2010 dataset forms the basis of a 2010 publication:

Lanyon JM, Sneath HL, Long T & Bonde RK (2010) Physiological response of wild dugongs (*Dugong dugon*) to *out-of-water* sampling for health assessment. *Aquatic Mammals 36*(1): 46-58.

Basic haematology, blood cytology and biochemical analyses (incl. NEFA, L-lactate) have been completed for whole blood, serum and plasma samples for all 79 dugongs in association with IDEXX Laboratories, Brisbane. Blood analytes were examined for evidence of capture stress myopathy, a condition traditionally thought to affect dugongs at capture. These data form the basis of a 2011 publication:

Lanyon JM, Sneath HL, Long T (2011) Evaluation of exertion and capture stress in serum of wild dugongs, *Dugong dugon*. *Journal of Zoo and Wildlife Medicine, In press*

Biochemical and haematological reference intervals for healthy dugongs in Moreton Bay are being developed. However, the occurrence of a major flood along the Queensland coast over the period Dec 2010- Jan 2011 may have caused dietary and health problems for at least some of the dugongs sampled as part of the 2011 health program. Consequently, it may not be appropriate to include these blood values in a set for reference intervals. This means that complete reference intervals may not be determined until after 2012 sampling, in order to increase sample size of apparently healthy dugongs: further funding will be sought to complete this part of the program. Meanwhile, analyses of blood analytes pre- and post-flood are being conducted and will form the basis of a publication:

Lanyon JM, Sneath HL, Long T, Burgess EA & Woolford L. (2011) Changes in serum biochemical indicators of body condition in wild dugongs, Dugong dugon after a catastrophic inshore flood event. *In prep.*

Analysis of a suite of 20 trace elements/heavy metals were analysed by ICP-OES (Inductively Coupled Plasma- Optical Emission Spectrometry) and ICP-MS (Inductively Coupled Plasma- Mass Spectrometry) on whole blood and serum samples (by Qld Health Laboratories, and Land and Food Science UQ, respectively). These results are being prepared for publication.

Lanyon JM & Sneath HL (2011) Heavy metal concentrations in the blood and body tissues of free-ranging dugongs and dugong carcasses from an urban coastal environment. *In prep.*

Enzyme Immuno-Assays for the reproductive hormones (progesterone, testosterone and oestogen) and for the stress hormones corticosterone and cortisol were conducted on faecal, blood serum and urine samples. Steroid hormone levels have been examined in relation to body morphometrics and reproductive state (e.g. pregnancy confirmed by ultrasound) and at least two papers describing these results are nearing completion and will be submitted this year:

Burgess EA, Lanyon JM, Keeley T and Blyde D (2011) Determining pregnancy in a free-ranging dugong population using fecal progesterone metabolite concentrations and body morphometrics. *In prep.*

Burgess EA, Lanyon JM and Keeley T (2011) Testosterone and tusks: assessing sexual maturity and reproductive patterns in live, free-ranging male dugongs (*Dugong dugon*). *In prep.*

A scoping study to assess relative levels of total (conjugated and unconjugated) steroid hormones (testosterone, progesterone, oestradiol, oestriol, corticosterone) in a range of tissues (faeces, blood, urine, and nasal and vaginal mucus) was conducted using LC-MS/MS technology in association with CIPDD Hub of Organics Qld. Results from this work have been analysed and will soon be publsihed.

A pilot study to investigate levels of ovarian reserve biomarkers (AMH and Inhibin-B) has being conducted on serum from adult and subadult dugongs as a prelude to a broader collaborative project examining fertility in wild dugongs (see 2011 application for AMMC funding).

Urine was examined grossly and microscopically for sediment cytology, e.g., sperm, casts, crystals, lipid and contaminant materials. Levels of blood, protein, leucocytes, glucose, ketones, nitrite, bilirubin, and urobilinogen were measured using a commercially available dipstick (Multistix 10SG, Siemens Healthcare Diagnostics, Tarrytown NY); and urine specific gravity was measured using a refractometer (Reichert Veterinary Refractometer, Cambridge Instruments INC, Buffalo, NY).

Fresh live sperm were collected from adult male dugongs during this study. Consequently, studies examining sperm morphology, motility, cryo-preservation and ultrastructure are being conducted in collaboration with reproductive biologists from Taronga Western Plains Zoo and Adelaide University.

Studies of the microbial ecology of the hindgut of the dugong (based on DGGE analyses of faecal samples) are underway. This is the first part of a broader study to examine the microbes of sirenians. The first publication has been submitted:

Eigeland KA, Lanyon JM, Trott DJ, Ouwerkerk D, Blanshard W, Milinovich G, Merson, S and Klieve AV (2011) Bacterial community structure in the hindgut of wild and captive dugongs. FEMS Microbiol. Ecol. *Submitted*.

In-water blood collection

During this study we have also commenced trials to collect blood from dugongs during routine tagging as part of our on-going mark-recapture program in Moreton Bay (Lanyon et al. 2002, 2006). If successful, this new method of blood collection will allow us to collect blood biochemical and haematological data on large numbers of dugongs without the need for out-of-water sampling, i.e., in addition to those dugongs sampled during health assessments (Lanyon et al. 2010).

Wild dugongs will be captured in Moreton Bay, south-east Queensland using the dugong rodeo technique. Up to one hundred apparently healthy dugongs (50 of each sex) will be sampled *in-water* over the next two year period. Upon capture, each dugong will be evaluated for general body condition, sexed, measured, photographed and tagged (Lanyon et al. 2002, 2006, 2010). A further routine tag method, whereby a small tissue sample is clipped from the trailing edge of the tail fluke (‘a cookie’), results in minor bleeding. Following clipping of the tail fluke, the tail will be elevated and between 250-500µl of uncontaminated blood will be collected. All blood collection equipment will be held free of the water. The small wound will be allowed to bleed freely for 30 sec and blood will be collected directly into pediatric tubes using capillary action SAFE-T-FILL® tubes (Ram Scientific). Direct smears will be made immediately from the capillary tube, air dried and fixed in 100% methanol.
Whole blood will be analysed using a portable i-STAT® blood analyser.

REFERENCES

Lanyon, J.M., Sneath, H.L., Kirkwood, J.M. & Slade, R.W. (2002). Establishing a mark-recapture program for dugongs in Moreton Bay, south-east Queensland. *Australian Mammalogy 24 (1),* 51-56.

Lanyon, J.M., Slade, R.W., Sneath, H.L., Broderick, D., Kirkwood, J.M., Limpus, D., Limpus, C.J., Jessop, T. 2006. A method for capturing dugongs (*Dugong dugon*) in open water. *Aquatic Mammals*, 32, 196-201.

Lanyon JM, Sneath HL, Long T & Bonde RK (2010) Physiological response of wild dugongs (*Dugong dugon*) to *out-of-water* sampling for health assessment. *Aquatic Mammals 36*(1): 46-58.

Marsh H.D., Hodgson A., Lawler I., Grech A. & Delean S. (2007). Condition, status and trends and projected futures of the dugong in the Northern Great Barrier Reef and Torres Strait; including identification and evaluation of the key threats and evaluation of available management options to improve its status. In: *Marine and Tropical Sciences Research Facility Report Series.* Reef and Rainforest Research Centre Limited, Cairns

Wong, A. W. (2008). Monitoring oral temperature, heart rate, and respiration rate of field-captured Florida and Antillean manatees (*Trichechus manatus latirostris and T. m. manatus*). (Unpublished masters dissertation). University of Florida, College of Veterinary Medicine, Gainesville, Florida, USA. Appendix F.