

Physiological Response of Wild Dugongs (*Dugong dugon*) to Out-of-Water Sampling for Health Assessment

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Abstract

The dugong (*Dugong dugon*) is a vulnerable marine mammal with large populations living in urban Queensland waters. A mark-recapture program for wild dugongs has been ongoing in southern Queensland since 2001. This program has involved capture and in-water sampling of more than 700 dugongs where animals have been held at the water surface for 5 min to be gene-tagged, measured, and biopsied. In 2008, this program expanded to examine more comprehensively body condition, reproductive status, and the health of wild dugongs in Moreton Bay. Using Sea World's research vessel, captured dugongs were lifted onto a boat and sampled out-of-water to obtain accurate body weights and morphometrics, collect blood and urine samples for baseline health parameters and hormone profiles, and ultrasound females for pregnancy status. In all, 30 dugongs, including two pregnant females, were sampled over 10 d and restrained on deck for up to 55 min each while biological data were collected. Each of the dugongs had their basic temperature-heart rate-respiration (THR) monitored throughout their period of handling, following protocols developed for the West Indian manatee (*Trichechus manatus*). This paper reports on the physiological response of captured dugongs during this out-of-water operation as indicated by their vital signs and the suitability of the manatee monitoring protocols to this related sirenian species. A recommendation is made that the range of vital signs of these wild dugongs be used as benchmark criteria of normal parameters for other studies that intend to sample dugongs out-of-water.

Key Words: dugong, *Dugong dugon*, capture, sampling, vital signs, heart rate, oral temperature, respiration, health assessment, manatee, sirenian

Introduction

The dugong is a vulnerable marine mammal whose populations have undergone significant declines along the urban coast of Queensland, Australia (Marsh et al., 2002). Southern Queensland has one of the fastest growing human populations in Australia, and each year, several dugong mortalities occur as a direct result of water-based activities (e.g., boating and netting). However, there have also been a significant number of unexplained mortalities of dugongs in southern Queensland. It is difficult to assign cause of death or even to assess body condition of dugongs at death because no information is available regarding baseline health parameters of wild dugongs. Medically examining wild animals in their natural habitat is critical to understanding not only the health of individual animals, but for providing an indication of the overall health of a population and the ecosystem (Moore, 2008). It is becoming increasingly apparent that health assessment is an important tool for conservation and management of wild sirenians and their habitats (Bonde et al., 2004; Bossart, 2006), particularly as their coastal habitats are impacted by increasingly large human populations.

Recently, a mark-recapture program has been established for wild dugongs in urban southern Queensland, Australia. This program gene-tags individual dugongs and thus obtains biological information including morphometrics and reproductive status with a view to compiling life history information for dugongs in the region (Lanyon et al., 2002). The population in this area is significant with close to 1,000 dugongs using Moreton Bay alone (Lanyon, 2003) of which more than 800 individuals of both sexes and all body sizes have been tagged. The ongoing nature of this program allows collection of longitudinal data at the level of the individual animal (e.g., growth, reproductive rates, and health) and also population trends.

All previous sampling of dugongs in this program has been conducted in the least intrusive manner possible because of initial concerns about the possible predisposition of dugongs to capture stress myopathy (Anderson, 1981; Marsh & Anderson, 1983). In other programs, a small number of dugongs have died soon after capture using nets (Heinsohn et al., 1976; Marsh et al., 2002) or after they were removed from the water (F. Carrick, pers. comm., May 2008). To mitigate the possibility of mortality associated with capture, dugongs have been sampled in-water during the current mark-recapture program—that is, each animal has been restrained in the water at the surface for 4 to 6 min after a brief pursuit of ≤ 10 min (Lanyon et al., 2002, 2006b). This in-water approach has been thought to be less intrusive to wild dugongs compared to beaching the animal, and it is also more cost-effective in terms of the number of personnel and support vessels required to maximise sampling rate. However, a major drawback to in-water sampling is that it is impossible to collect some physiological samples, including blood and urine, which are critical to a detailed health assessment. Dugongs restrained at the water surface do not remain sufficiently still to allow collection of a blood sample uncontaminated by seawater, and urine is excreted directly into the water. Recent expansion of this tagging program has presented an opportunity for detailed health assessment of wild dugongs by removing animals from the water. The related West Indian manatee (*Trichechus manatus*) has been assessed for health and body condition during routine out-of-water captures over several years now, and a series of successful sampling and monitoring protocols has been developed (e.g., Wong, 2008; Stamper & Bonde, in press).

The aim of the current study was to sample wild dugongs from urban Moreton Bay out-of-water to collect data on health and body condition through clinical and endocrine analyses of blood, urine, tears, mucus, and other media difficult to collect through regular in-water sampling. Ultrasound examination was used to assess reproductive status of females. This paper reports on the physiological response of wild dugongs to out-of-water sampling with a focus on the monitoring of vital signs (i.e., body temperature, heart rate, and respiratory rate). We followed the same protocols for monitoring vital signs as for the West Indian manatee (Bonde et al., 2004; Bossart, 2006; Wong, 2008; Stamper & Bonde, in press). The applicability of this monitoring regime to this species is discussed. The results from the analyses of haematology, blood cytology, serum biochemistry, and endocrinology will be reported elsewhere as part of a larger comprehensive study evaluating the health status of dugongs in southern Queensland, Australia.

Materials and Methods

Capture and Restraint

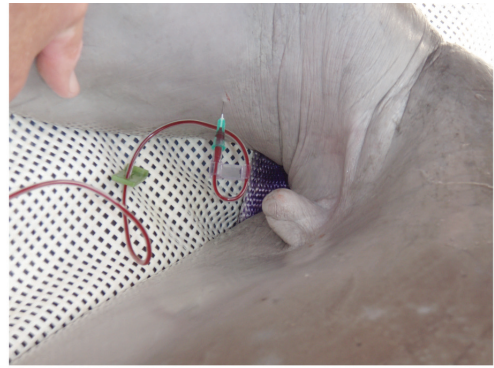
Wild dugongs were captured opportunistically over the shallow seagrass banks in eastern Moreton Bay (latitudes $27^{\circ} 20.09'$ to $27^{\circ} 24.87'$ S; longitudes $153^{\circ} 21.26'$ to $153^{\circ} 23.84'$ E; water depths 1.6 to 3 m) using the dugong rodeo technique (Lanyon et al., 2006b) as part of a long-term mark-recapture program (Lanyon et al., 2002). Thirty dugongs of both sexes and assorted size classes were sampled over the periods 19 to 23 May 2008 ($n = 13$) and 11 to 17 May 2009 ($n = 17$): 15 adults (> 260 -cm body length), nine subadults (241 to 260 cm), and six juveniles (≤ 240 cm). Upon capture, each dugong was evaluated for general disposition and body appearance, tagged (following Lanyon et al., 2002, 2006b), and measured in-water. Morphometrics included total body length measured in a straight line from snout to fluke notch; fluke width (Heinsohn, 1981); and girths at each of peduncle, anus, umbilicus (maximum girth), and axilla. The dugong was manoeuvred into a large, custom-made PVC polyester mesh stretcher (MakMax Aust.) that was 4 m long with 1 m sides and ends that folded up and could be secured with broad Velcro® straps to contain the animal. The woven fabric used in the stretcher was strong, lightweight, non-slip, and drained readily. The stretcher with dugong was slung between two inflatable boats 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 (Figure 1a).

Vital Signs

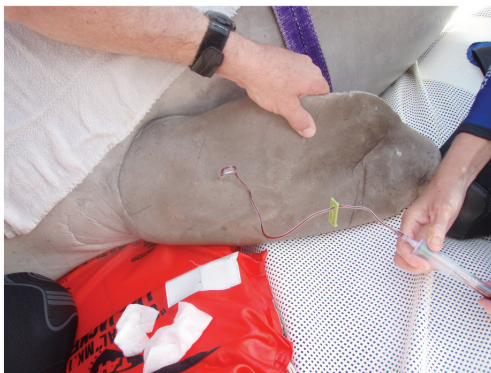
Monitoring of respiratory rate commenced immediately upon pursuit and prior to capture of each dugong. Continuous monitoring of the dugong's vital signs to determine physiological state—that is, temperature, heart rate, and respiration (THR) as per Wong (2008)—commenced immediately after the dugong was placed on deck. This protocol followed that developed for West Indian manatees to facilitate a species comparison. Oral temperature was recorded at 5-min intervals via a temperature probe placed laterally along the mandible past the posterior mandibular molar (Figure 1a). Normal oral temperature in manatees closely approximates core body temperature (Gallivan et al., 1983; Wong et al., 2007) and can be beneficial for determining sudden temperature changes during observation. 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 17 dugongs, a doppler



A.



B.



C.



D.

Figure 1. Out-of-water sampling of wild dugongs: A. Dugong with oral temperature probe in left buccal (mouth) cavity and doppler heart detector deployed under sternum to measure resting heart rate; B. Blood sampling from medial surface of left pectoral fin of adult female dugong. Note large axillary nipple; C. Blood sampling from lateral surface of left pectoral fin; and D. Plastic Frisbee® placed under urinogenital opening to collect urine, and portable image ultrasound transducer used to assess pregnancy status of adult female dugong.

foetal heart detector with a 2-Mhz probe (Model PD1+, Ultrasound Technologies Ltd.) was used with the transducer placed under the sternum (Figure 1a). It was originally anticipated that measurements would be made at 5-min intervals simultaneously with oral temperature, however, since the logistics of obtaining a good heart rate reading with the stethoscope required cessation of all other on-board activities, this was not possible at such a frequency. More heart rate readings were obtained per animal when the doppler unit was employed. The number of respirations in each 5-min period was recorded with the aid of a stopwatch. THR for dugongs was compared to field reference ranges for Florida manatees (Wong et al., 2007; Wong, 2008).

Blood Collection and Processing

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 arterio-venous plexus accessed via the palmar medial (more commonly) (Figure 1b) or lateral surface (Figure 1c) 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 anticoagulants and supportive

media following the protocol used for West Indian manatees (Stamper & Bonde, in press). A total of 60 to 100 ml of blood was collected from each dugong. Blood samples were centrifuged immediately after collection to separate plasma and serum components from red blood cells.

Ultrasound Pregnancy Testing

An image ultrasound machine (GE Logiqbook) was used to scan the abdomen of each female dugong for evidence of pregnancy (Figure 1d). 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.

Other Samples

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 this sample was frozen. Urine 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 (Figure 1d). Mouth contents were collected from the buccal cavity between the horny pads. Tears were sampled from each eye onto sterile eye spears. A sample of mucus from the exhaled blow of the dugong was collected by holding an inverted sterile vial over the nares as the dugong exhaled deeply. 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.

Results

Capture and Restraint

General appearance and disposition of all 30 dugongs upon capture suggested that they were apparently healthy animals and suitable candidates for out-of-water sampling. Dugongs were evaluated in terms of body girth, condition of epidermis, and absence of external signs of disease or fresh injury.

Restraint of dugongs in water for initial sampling and tagging followed previously established protocols (Lanyon et al., 2002, 2006b). During out-of-water sampling, none of the 30 sampled

dugongs attempted to move on the mattress; however, as a precautionary measure, dugongs were gently and firmly restrained at critical times, including during the needle stick for blood collection (i.e., two to three researchers placed their body weight firmly against the main trunk and fluke of the dugong for short periods of < 1 min).

Sampling Times

Total sampling time for individual dugongs ranged from 45 to 97 min (Table 1). This included pursuit time prior to capture, initial in-water sampling to tag and measure the animal, transportation of the dugong in a stretcher from the capture boat to the research vessel, out-of-water sampling, and then hoisting and weighing in the stretcher prior to release.

Pursuit times ranged from 2.5 to 10 min, which followed the usual protocol of no pursuit extending past 10 min (Lanyon et al., 2006b). In-water sampling times ranged from 3.3 to 9.3 min; this included taking measurements, tagging, and some biopsy in addition to manoeuvring the animal into the stretcher. The duration of the stretcher transport phase (3.5 to 32.6 min) was dependent on the distance of the capture location to *RV Sea World One*. This large vessel drew ~2 m water, so it was positioned in deep waters just off the edge of the shallow seagrass banks as close as possible to the capture site. All dugongs, except ID 08687, were orientated in the stretcher tail-first. This was the preferred position because the animal's breathing was unaffected by oncoming waves as the transport boats moved forward. When positioned head-first, transport was necessarily slower to avoid wash over the dugong's head which might impede breathing.

Dugongs were held on deck for periods ranging from 27 to 55 min, except the first dugong of each annual field trip; these were held for 62 and 65 min while procedures were established (Table 1). The major determining factor for time on deck was the time required to collect blood. For 13 dugongs, blood was collected with one needle stick only (taking 11.6 ± 1.0 min; mean \pm SE) and eight dugongs received two needle sticks (13.6 ± 1.0 min). The other nine dugongs required further sticks to achieve the target volume of blood, but blood collection was completed within 25 min. After sampling, the dugong was suspended in the stretcher to measure body weight, and then the stretcher was swivelled off the main deck and lowered into the water for release, a procedure taking 2 to 9 min.

Dugong Morphometrics

Body length (BL), measured in a straight line from snout to fluke notch, was used as the standard body

Table 1. Time (hours:minutes:seconds) for each phase of the sampling operation for wild-caught dugongs in Moreton Bay; total sampling time includes pursuit time prior to capture, in-water sampling, stretcher transport, out-of-water sampling, and weighing prior to release.

Dugong ID	Time (h:m:s)					
	Total sampling	Pursuit	In-water sampling	Stretcher transport	Out-of-water sampling	Weigh & release
08686	1:37:00	0:05:00	0:07:51	0:17:56	1:02:03	0:04:10
08687	1:30:00	0:04:58	0:04:08	0:30:09	0:44:35	0:06:10
08688	1:01:40	0:03:56	0:04:04	0:09:11	0:39:39	0:04:50
08689	1:24:00	0:04:00	0:04:09	0:24:10	0:46:21	0:05:20
08690	1:07:00	0:03:40	0:06:00	0:15:15	0:36:45	0:05:20
08691	1:24:03	0:05:00	0:04:00	0:32:43	0:39:17	0:03:03
08692	1:13:00	0:03:22	0:04:38	0:27:43	0:30:17	0:07:00
08693	1:09:00	0:04:00	0:05:01	0:10:15	0:40:44	0:09:00
08694	1:03:20	0:05:00	0:04:35	0:11:25	0:34:00	0:08:20
08695	0:57:54	0:03:13	0:05:31	0:09:56	0:33:20	0:05:54
08696	1:05:00	0:03:15	0:03:30	0:12:29	0:41:06	0:04:40
08697	1:04:50	0:03:32	0:04:28	0:11:55	0:40:25	0:04:30
08698	1:04:00	0:04:43	0:05:57	0:10:29	0:37:46	0:05:05
09872	1:36:37	0:05:09	0:07:19	0:18:36	1:05:33	0:05:38
09873	0:59:14	0:04:17	0:05:44	0:11:59	0:37:14	0:04:23
09874	1:19:13	0:05:37	0:09:18	0:08:45	0:55:33	0:05:44
09875	0:56:02	0:02:51	0:07:54	0:09:28	0:35:49	0:04:39
09876	1:14:41	0:09:50	0:05:37	0:18:57	0:40:17	0:05:01
09877	0:55:28	0:04:56	0:05:55	0:13:23	0:31:14	0:06:36
09878	0:58:08	0:02:32	0:06:38	0:06:03	0:42:55	0:04:40
09879	0:57:11	0:03:21	0:05:25	0:15:29	0:32:56	0:03:34
09880	0:56:19	0:07:23	0:08:29	0:03:38	0:36:49	0:02:16
09881	1:12:02	0:03:26	0:04:37	0:13:15	0:50:44	0:05:00
09882	0:50:02	0:06:04	0:06:44	0:03:34	0:33:40	0:02:11
09890	0:49:08	0:03:15	0:06:45	0:12:12	0:26:56	0:02:11
09891	1:11:54	0:09:08	0:08:34	0:05:14	0:48:58	0:03:38
09892	0:54:10	0:03:12	0:08:46	0:10:52	0:31:20	0:01:51
09895	1:02:39	0:03:00	0:05:34	0:11:49	0:42:16	0:03:11
09896	0:45:49	0:06:47	0:06:15	0:04:17	0:28:30	0:02:33
09897	1:03:24	0:10:00	0:04:53	0:06:28	0:42:03	0:03:24

size measure for dugongs (Spain & Heinsohn, 1975; Lanyon et al., 2002). The average of three measures was used as the final in-water BL measure. Since these measurements were taken while the dugong was moving (submerging and then surfacing at intervals to breathe), some error was anticipated. Consequently, out-of-water BL measures were also taken, and since these measurements were taken by the same personnel following strict protocols and on a stationary dugong, these were accurate and precise with zero error in each case. The discrepancy between the in-water vs out-of-water BL measures was less than $\pm 5\%$ error, expressed as a percentage of the out-of-water BL (Table 2) for all but one dugong; mean % error of in-water BL measures was -0.4% . Thus, the in-water BL measure, as is routinely used, was considered an excellent reflection of true BL,

accurately assigning dugongs to 20-cm interval size classes. Further, the in-water BL measure was closest to straight rather than a curvilinear out-of-water BL measure (Table 2).

Body weight of sampled dugongs ranged from 217 to 307 kg for juveniles (≤ 240 -cm out-of-water straight BL), 334 to 424 kg for subadults (241- to 260-cm BL), and 435 to 568.5 kg for adults (> 260 -cm BL). BL to body weight relationship was examined for both male and female wild dugongs. There was no difference between males and females so all dugongs were pooled. The regression of BL to weight was highly significant ($F_{1,29} = 188.76$; $p < 0.001$) with a second order polynomial equation of $y = -0.009x^2 + 8.2163x - 1,126$; $\text{adj. } R^2 = 0.866$ (Figure 2).

Two of the five adult females sampled in 2008 were identified as pregnant after abdominal

Table 2. Body weight (kg) and body length (BL) measures (cm) of 30 wild-caught dugongs of both sexes; BL was measured in a straight line from snout to fluke notch while the dugong was restrained at the water surface (in-water) and on deck (out-of-water). Curved BL was also measured out-of water to clarify error of the routine in-water BL measure. In all cases, the mean of three measures is reported. Error inherent in the in-water BL measure is reported as a percentage of the more precise out-of-water measure.

Dugong ID	Sex	In-water mean straight BL (cm)	Out-of-water straight BL (cm)	Out-of-water curved BL (cm)	% error of in-water BL measure	Body weight (kg)
08686	male	248.0	244	259	2.0	329.0
08687	female	243.0	242	253	0.5	325.0
08688	female	247.0	255	271	-3.0	394.5
08689	female	282.0	287	296	-2.0	498.0
08690	female	272.5	278	285	-2.0	484.5
08691	female	281.0	281	295	0.0	559.0
08692	male	271.0	272	282	-0.5	441.0
08693	male	270.0	278	292	-3.0	457.5
08694	male	213.5	216	227	-1.0	275.5
08695	female	245.0	241	254	2.0	300.5
08696	female	216.5	216	228	0.0	217.0
08697	female	296.0	300	316	-1.0	568.5
08698	male	232.5	235	246	-1.0	260.0
09872	female	257.0	262	275	-2.0	435.0
09873	male	220.0	225	243	-2.0	307.0
09874	female	265.0	257	271	3.0	387.5
09875	female	266.0	267	281	-0.5	435.0
09876	female	286.0	285	299	0.5	435.0
09877	female	305.0	309	320.5	-1.0	489.0
09878	female	246.0	231	242	7.0	300.0
09879	female	267.0	280	291	-5.0	445.0
09880	male	238.0	238	253	0.0	299.0
09881	male	291.0	292	314	-0.5	512.0
09882	female	248.0	257	269	-3.0	334.0
09890	female	257.0	258	276	-0.5	403.5
09891	female	262.0	259	278	1.0	424.0
09892	male	257.0	253	269	2.0	372.0
09895	female	290.0	288	309	1.0	536.0
09896	male	--	280	292	--	461.0
09897	female	--	280	297	--	487.0

ultrasound examination, and in each case a medium to late-term foetus was identified. Endocrine analysis also supported pregnancy of these two females, with higher faecal progesterone levels (690 to 1,506 ng/g) compared to 57 to 85 ng/g progesterone in the other females sampled in 2008 and 2009, where levels > 400 ng/g indicate pregnancy (Burgess & Lanyon, unpub. data). The vital signs (THR) of these confirmed pregnant dugongs were not significantly different than those of other adult dugongs so are not considered separately hereafter.

Monitoring of Vital Signs

Oral Temperature—The surface water/air temperatures in Moreton Bay at the times of sampling were

19° to 19.5° C (mean 19.1° C) in 2008 and 21.5° to 22.3° C (mean 22° C) in 2009. Since annual differences in water temperature had a highly significant effect on oral temperature (single factor ANOVA: $F_{1,218} = 88.87$; $p < 0.001$), sampled dugongs were considered in terms of two separate annual cohorts. In 2008, oral temperature of individual dugongs as they were initially brought onto deck ranged from 24° to 30.5° C. Final oral temperatures of dugongs just prior to release (and after periods of 30 to 50 min) ranged from 25° to 33.3° C. Mean oral temperature of all dugongs over the out-of-water period ranged from 25.2° to 32° C (mean \pm SE 29.4° C \pm 0.58). In 2009, oral temperature of individual dugongs as they were initially brought onto deck ranged from 26.9° - 32.5° C. Final oral

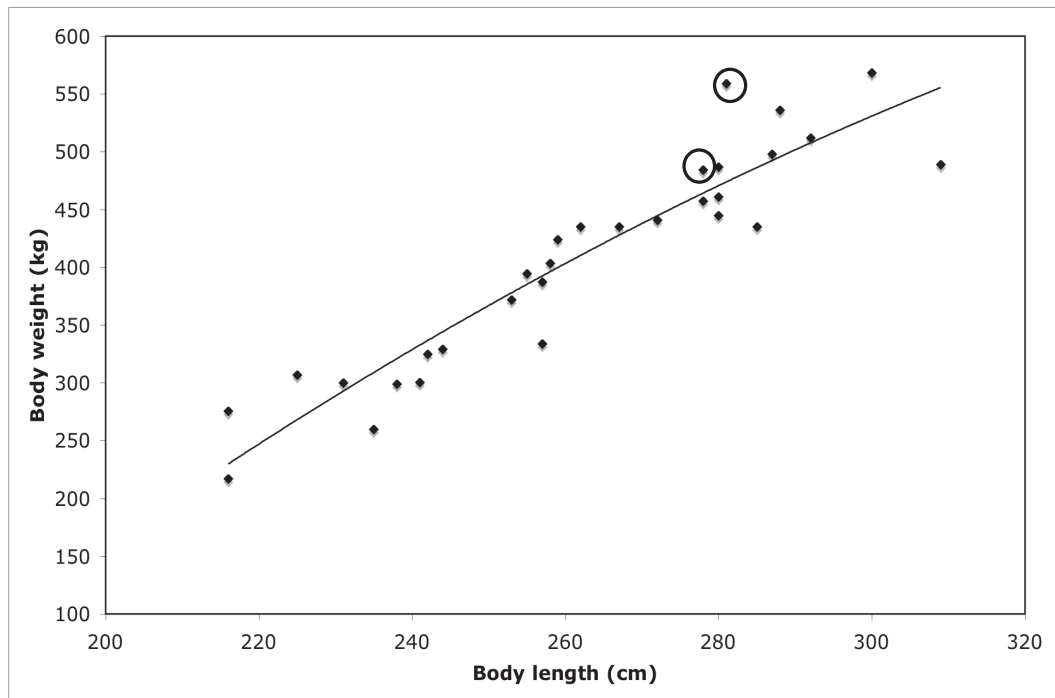


Figure 2. Body weight (kg) against body length (cm) for 30 wild-caught male and female dugongs; dugongs confirmed as pregnant by ultrasound are circled. Fitted trendline is a second order polynomial: $y = -0.009x^2 + 8.2163x - 1,126$; adj. $R^2 = 0.866$.

temperatures of dugongs just prior to release (and after periods of 27 to 55 min) ranged from 25.9° to 34.2° C.

For each dugong, except for the first sampled (08686), there was an increase in oral temperature (0.5° to 6° C in 2008, 1.2° to 5.5° C in 2009) with time held out of water (Figure 3). However, there was no direct relationship between magnitude of temperature increase and total time on deck. This increase occurred despite efforts to keep body temperature down through shading the animal from direct sunlight, application of wet towels over the dugong's body, and periodic dousing of the animal's entire trunk with fresh seawater. The widely fluctuating oral temperature of the first dugong sampled probably reflected poor positioning of the probe and is not considered further.

Oral temperatures for individual dugongs (both years combined) ranged from 24° to 34.2° C (mean 30.7° C \pm 0.36), and these are low compared to those measured in West Indian manatees (Figure 3).

Heart Rate—Heart rate (beats per minute [bpm]) was measured one to four times for each dugong during the first sampling period when only a stethoscope was available, but two to six times using the Doppler device. Heart rate

readings ranged from 40 to 96 bpm ($n = 30$) and varied markedly between individual dugongs, with most having a mean heart rate between 60 and 85 bpm (Figure 4). Although there was no obvious relationship between mean heart rate and size of dugong, heart rate of non-adult animals (< 260-cm BL) appeared to be more variable than adults based on greater variance (Figure 4).

Respiration—The interval between successive breaths taken by dugongs varied with phase of the sampling operation and whether the dugong was in or out of water. There were marked differences in breathing intervals of dugongs dependent on sampling phase (Figure 5a). While dugongs were actively pursued, intervals between breaths ranged from 10 to 169 s with a mean breathing interval of 69.7 ± 2.8 s ($n = 30$ dugongs). These breathing intervals fell within the 10 to 210 s range recorded for 412 dugongs during previous pursuits prior to capture (Lanyon et al., 2006b). The respiration rate reported for free-ranging dugongs is one breath every 60 to 120 s while feeding in shallow water, with longer submergence times of 120 to 740 s when travelling or in deeper waters (Anderson & Birtles, 1978; Chilvers et al., 2004).

Dugongs breathed significantly more frequently and regularly (mean breathing interval 11.3 ± 0.35 s,

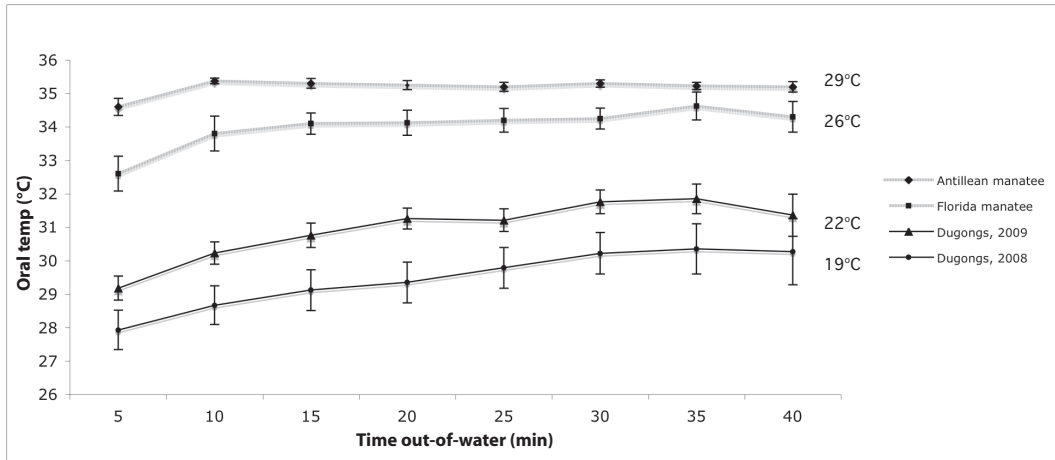


Figure 3. Mean (\pm SE) oral temperatures ($^{\circ}$ C) of 30 wild-caught dugongs against time out-of-water (min) ($n = 13$ in 2008; $n = 17$ in 2009) compared to oral temperatures of the two subspecies of West Indian manatees: Florida manatees ($n = 21$) and Antillean manatees ($n = 26$); mean surface water temperatures ($^{\circ}$ C) during sampling of each group of sirenians are shown on the graph. Manatee data were sourced from Wong et al. (2007).

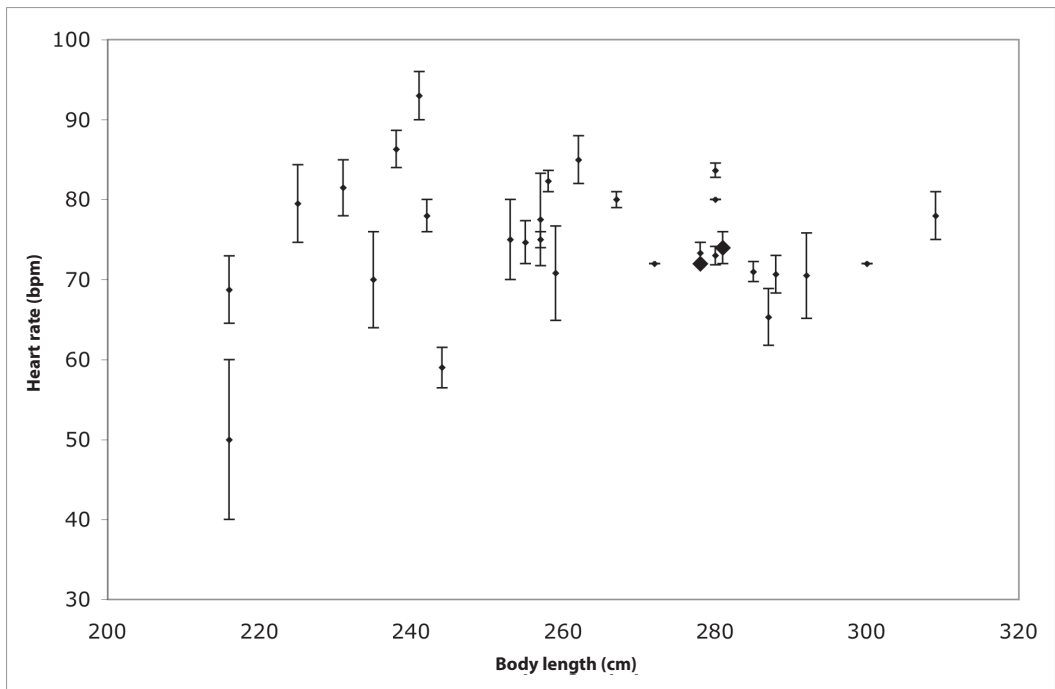
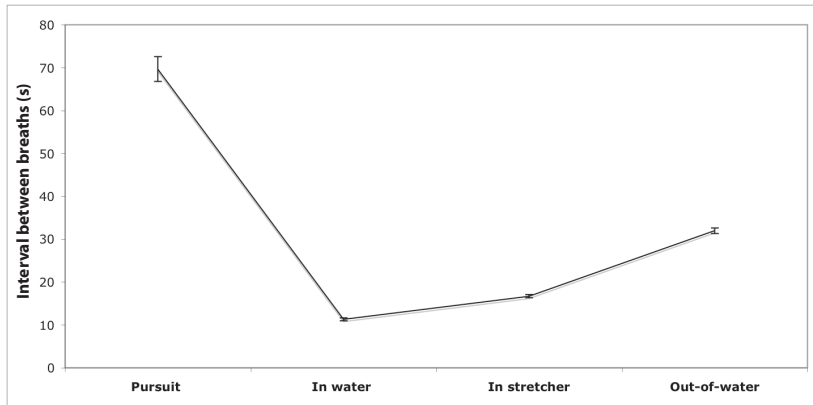


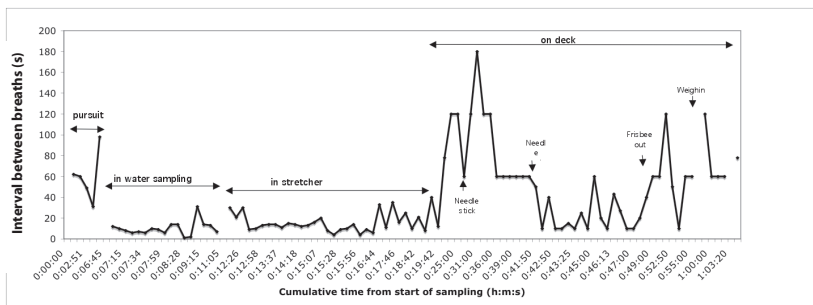
Figure 4. Mean (\pm SE) heart rates (beats per minute [bpm]) of 30 wild-caught dugongs against body length (cm); the two confirmed pregnant dugongs are marked by large diamonds.

$n = 30$) once they were captured and held in the water (Figure 5a). Respiration rate of some dugongs then dropped briefly when the animal was initially manoeuvred into the stretcher so that overall breathing interval increased slightly during in-water transport (16.7 ± 0.39 s, $n = 30$) (Figures 5a & b).

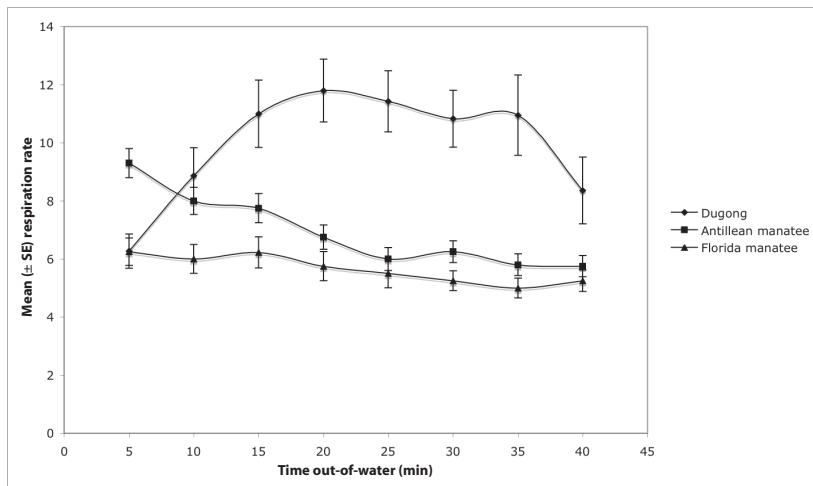
Mean breathing interval for 30 dugongs during the out-of-water phase (including induced and voluntary breaths) was 32 ± 0.64 s (Figure 5a). For all 30 dugongs, removing them from the water caused an immediate depression in respiratory rate with resultant breathing intervals of up to 253 s



A.



B.



C.

Figure 5. Respiration rates of wild-caught dugongs during health assessment: A. Mean (\pm SE) interval between successive breaths (s) during respiration by 30 wild-caught dugongs, according to sampling phase (i.e., during pursuit, initial in-water sampling, transport in stretcher, and out-of-water sampling); B. Interval between breaths (s) against cumulative time (min) from start of sampling of a representative dugong (08696) during in-water (pursuit, sampling, stretcher) and out-of-water (on deck) phases. Sampling events that appeared to affect respiration are indicated; and C. Mean (\pm SE) respiration rate (number of breaths/5-min interval) of 30 wild-caught dugongs against time out-of-water (min), compared to respiration rate of the two subspecies of West Indian manatees: Antillean manatees ($n = 26$) and Florida manatees ($n = 21$). Manatee data were sourced from Wong et al. (2007).

(4.2 min) (Figures 5a & b). Six of 13 dugongs and 15 of 17 dugongs, in each of 2008 and 2009, respectively, were encouraged to take their first breath out-of-water by dousing their heads with water and, in some cases in 2008, through concurrent manual stimulation of the snout region (as is used with manatees). Some dugongs required dousing of the head several times during the first few minutes to maintain frequency of respiration. In 2009, more dugongs were artificially encouraged to take their first breaths sooner and then more often over the first few minutes than in 2008. Consequently, the mean out-of-water breathing interval of dugongs in 2009 was 29.8 ± 0.72 s *cf.* 35.2 ± 1.2 s in 2008, and these breathing intervals should be considered as minimum intervals compared to those that might result from unaided breathing.

Respiration rate (expressed as number of breaths/5-min interval) during the out-of-water phase ranged from 1 to 33. However, there were differences in respiration rate between individual dugongs. Sixty percent of dugongs ($n = 18$) had respiration rates that ranged from 3 to 18 breaths/5-min interval; seven had lower respiration rates at 3 to 10 breaths/5 min, and five had higher rates of 6 to 33 breaths/5 min. Mean respiration rate was lowest during the first 5 min out of water, and low compared to manatees at this time (Figure 5c). Respiration rate was affected by the onset of novel procedures, including blood collection and repositioning the dugong (Figure 5b).

Discussion

Thirty dugongs were successfully held out-of-water on deck for periods ranging from 27 to 65 min. These times were comparable to or shorter than the 60 to 90 min for which West Indian manatees are typically held (Bonde, pers. obs., 2008), and it is anticipated that with further experience, particularly with blood collection, dugongs will be held routinely for shorter periods. All dugongs sampled as part of this program appeared to be healthy animals in good body condition with no external signs of morbidity. Sampling was conducted in late autumn (i.e., after the summer seagrass growth period) when nutrient availability is highest but before the drop to winter water temperatures so that we might expect animals to be in good condition at this time of year.

The regression of BL to weight for wild dugongs was highly significant and fit a second order polynomial curve. Spain & Heinsohn (1975) also fitted a polynomial curve to a BL to weight regression for 43 dugongs from north Queensland and elsewhere, but these were all dead animals. The regression curve derived during the current study

was offset to the left of Spain & Heinsohn's curve, indicating that all dugongs in the current study had a significantly larger body weight for BL. This marked discrepancy may reflect one or more of the following: (1) a postmortem weight loss in dead dugongs caused by loss of body fluids and/or tissue decomposition (Spain & Heinsohn, 1975), (2) different weight to body size allometry curves in Moreton Bay dugongs compared with dugongs elsewhere, and (3) dugongs sampled in Moreton Bay were in relatively better body condition.

As far as we are aware, these are the first body weight measures taken of live, wild, apparently healthy dugongs, including two pregnant adults. Further, the largest dugong sampled here had a BL of 309 cm. Since larger dugongs have been measured (i.e., 325 cm in Moreton Bay: Lanyon, unpub. data; and 331 cm in Torres Strait: Bryden et al., 1998), it is likely that some live dugongs exceed 600 kg.

Oral Temperature

The oral temperatures for individual dugongs during out-of-water sampling (range 24° to 34.2° C; mean 30.7° C \pm 0.36) are low compared to those measured in West Indian manatees (Figure 3). In manatees, normal basal body temperature ranges have been reported as 35.5° to 36° C (Murphy, 2003). Oral temperature is considered as an index of body temperature and useful for determining trends in temperature increase or decrease. Researchers sampling manatees in a field situation aim for a steady oral temperature range of between 33.5° and 36.5° C (Wong et al., 2007). Mean oral temperatures of dugongs sampled within each year also fell within a 3° temperature range (28° to 31° C) and followed a similar trend with time on deck as manatees (Figure 3). Normal basal body temperatures for dugongs are unknown at present. The overall lower oral temperatures of dugongs suggest three possibilities. First, the normal oral temperature range for dugongs may be below that of West Indian manatees. Second, measuring oral temperature in dugongs may not reflect basal body temperature in a similar way to manatees. Buccal morphology varies between sirenians with a steeper rostral angle in dugongs, and although we were confident that we were able to place the temperature probe high into the buccal cavity in a position analogous to that in the manatee, the position may not reflect core body temperature in the same way. Third, and more likely, environmental temperature may influence the oral temperature of sirenians (Wong et al., 2007). Surface water temperatures in Moreton Bay at the times of sampling were 19° and 22° C in 2008 and 2009, respectively (with air temperature closely following surface water temperature), and these temperatures were

considerably lower than environmental temperatures for which the manatee data were available (26° and 29° C). Further, the marked differences between oral temperatures of dugongs at the two different ambient temperatures in Moreton Bay at which dugongs were sampled supports this temperature effect. This suggests that oral temperature underestimates body temperature, particularly at lower temperatures, and that the upward trend in oral temperature may indicate warming of the buccal cavity as it approaches body temperature. Based on similar criteria for West Indian manatees and using the same method of oral temperature measurement, the normal range for dugongs is probably between 27° and 30° C at an environmental temperature of 19° C and between 28.5° and 31.5° C at an environmental temperature of 22° C. Based on a possible relationship between oral temperature and water temperature in sirenians, it may be reasonable to speculate that a predictive model could be constructed to determine anticipated oral temperature ranges given ambient temperatures.

Heart Rate

Heart rate ranged from 40 to 96 bpm across all 30 dugongs but varied markedly between individual dugongs, with most having a mean heart rate between 60 and 85 bpm. Heart rates for wild dugongs appeared to be slightly elevated compared to those measured in wild-caught West Indian manatees (i.e., 40 to 70 bpm) (Wong et al., 2007). This may presumably reflect a higher metabolic rate and/or flightier disposition in the dugong compared to manatees (Lanyon et al., 2006a).

Use of the Doppler device to measure heart rate proved superior to the stethoscope for both ease of use and accuracy of reading. ECG monitoring equipment has also proven useful in detecting accurate heart rates during the examination of manatees (Siegal-Willott et al., 2006), and ECG with 3-wire lead placement using suction cups will be trialled on dugongs in the near future.

Respiration

Lifting dugongs from the water caused an immediate and marked depression in respiratory rate in each case. Seventy percent of the dugongs sampled were encouraged to take their first breath out-of-water by dousing the head region (after Wong et al., 2007). In each case, dousing was sufficient to prompt breathing, and the majority of dugongs would then continue to breathe frequently and unassisted. In contrast, manually opening the nares by depressing the nasal valves was not sufficient to prompt breathing. We also took care to position each animal to ensure that they were able to breathe comfortably while out-of-water. The

largest adults, including the two pregnant females, were rolled towards their right sides to alleviate weight on their abdomens, with their heads supported by firm foam cushions (e.g., life jackets) to encourage a straight and unimpeded airway.

It is likely that failure to stimulate breathing upon removal from water may have caused mortalities in previous programs through rapid suffocation of wild dugongs. It is therefore critical to encourage the dugong to breathe immediately to avoid apnea associated with bradycardia. Further, key events caused alterations in the breathing patterns of dugongs during out-of-water sampling. Novel or disruptive events, including needle sticks to sample blood, rolling the animal laterally to place or remove the Frisbee® from beneath the urinogenital aperture, and hoisting to weigh the animal, all caused transitory depression of respiratory rate. In contrast, removal of the needle at the conclusion of blood sampling caused an obvious increase in respiratory rate as did lowering the stretcher back into the water at release.

Respiration rate (number of breaths/5-min interval) during the out-of-water phase ranged from 1 to 33, but most dugongs had rates of 3 to 18 breaths/5-min interval. Mean respiration rate was initially depressed on removal from water and was lower than respiration rate of manatees during a similar phase. The respiration rate of dugongs then increased with time and remained higher and more variable than for manatees (Figure 5c). The normal respiration rate range reported for West Indian manatees on land is 3 to 17 breaths/5-min interval (Walsh & Bossart, 1999; Wong et al., 2007), and the majority of dugongs fell within this range. Hyperventilation in manatees is defined as > 5 breaths/5-min interval occurring for a duration of three consecutive 5-min intervals (Wong et al., 2007). According to this definition, all but one dugong (08694) hyperventilated at some time, and the respiration rate of dugongs was high compared to those measured in manatees. It is possible that this relatively high respiration rate of dugongs is indicative of a difference in metabolic rate or temperament rather than to clinical hyperventilation. Further, some individual dugongs appeared to be more prone to hyperventilation.

Summary and Implications

The techniques used in this program to monitor vital signs of dugongs were the same as those used in health studies of manatees and appeared to be appropriate for obtaining basic physiological data. However, the magnitude of physiological response of dugongs to capture and removal from the water differed from that of manatees. Oral temperature (a proxy for body temperature) was significantly lower than that measured for manatees; however,

low ambient temperatures may have influenced this result. Heart rate and respiratory rate tended to be higher. These species differences may presumably be explained by dugongs having different metabolic rates and temperaments to manatees. The dugong is a faster, more pelagic species, and its rate of metabolic water turnover suggests that it might have a higher metabolic rate than the manatee (Lanyon et al., 2006a).

To date, more than 700 dugongs have been captured and sampled in water with no obvious adverse effects. However, until this study, the response of dugongs to removal from the water was unknown. Of particular concern is the spontaneous depression of respiration by each dugong upon initial removal from water. Immediate stimulation of respiration through simple dousing of the head with water may be a critical factor in successful out-of-water sampling without mortality. Further, restraining the dugong in-water before decking may allow it to freely ventilate for several minutes, thus assisting acclimation to a capture situation. During this study, there was no evidence to suggest that any of the dugongs suffered any adverse effects, as indicated by vital signs, due to capture stress. Similarly, capture myopathy has not been observed in Florida manatees sampled out-of-water (O'Shea et al., 1985; Harr et al., 2008), and the evidence that it occurs in dugongs (based on apparently high serum potassium; Marsh & Anderson, 1983) must be regarded as incomplete at this stage.

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