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Short Note

Three Skin Sampling Methods for Molecular Characterisation of Free-Ranging Dugong (*Dugong dugon*) Populations

Janet M. Lanyon,[1] Helen L. Sneath,[1] and Trevor Long[2]

[1]*School of Biological Sciences, The University of Queensland, St. Lucia, Qld 4072, Australia; E-mail: j.lanyon@uq.edu.au*
[2]*Sea World, P.O. Box 190, Surfers Paradise, Qld 4217, Australia*

The dugong (*Dugong dugon*) is a vulnerable marine mammal (International Union for the Conservation of Nature [IUCN], 2010) whose populations have undergone significant declines throughout the Indo-Pacific region, particularly over the past century (Marsh et al., 2002). Collecting information on dugong populations has always presented a challenge because of their fully aquatic lifestyle, cryptic nature, and often remote and murky water habitats (Lanyon et al., 2002, 2006). Regional-scale information regarding dugong population distribution, size, and trends has been obtained through standardised aerial surveys (Marsh et al., 1993, 1996), while biological and life history information has been gathered through opportunistic analysis of carcasses (Marsh, 1995; Marsh & Kwan, 2008). With increasing threats to dugong populations globally (Marsh et al., 2002), there is a need for information on the extent, dynamics, connectivity, relatedness, and resilience of local wild populations.

Conservation genetics has been used to identify breeding populations and quantify genetic diversity in threatened and vulnerable species, including dugongs (McDonald et al., 2001). Microsatellite markers developed for dugongs have the capacity to generate DNA genotypes that can reliably and unambiguously discriminate individuals (Broderick et al., 2007; McHale et al., 2008; Kellogg-Hunter et al., 2010). These multilocus genotypes can be used to "gene-tag" individuals for movement and mark-recapture population studies of free-ranging dugongs (Lanyon et al., 2002) if sufficient proportions of focal populations can be sampled.

The genetic sampling of live, fully aquatic mammals has been challenging, but several methods have been developed successfully. The collection of sloughed skin or faeces from the water column (e.g., Amos et al., 1990; Parsons, 2001) is perceived as "less invasive" and has been used to amplify mtDNA control region and sex. However genetically profiling individuals using these techniques presents difficulties due to low quantity/poor quality DNA obtained that may result in unreliable results or scoring errors (Taberlet & Waits, 1998; Morin et al., 2010). Alternatively, the collection of tissue samples with remote biopsy darting (i.e., veterinary capture rifle or crossbow (Lambertsen, 1987; Krützen et al., 2002) has overcome this problem, providing tissue samples that yield enough DNA for most analyses, including individual genotyping.

The Florida manatee (*Trichechus manatus latirostris*) is unusual in its docility such that free-ranging individuals can often be approached so closely by swimmers in water or from land or boat that dermotome samples from the trailing edge of the fluke (R. K. Bonde, pers. comm., April 2006) or small dorsal biopsy cores (Carney et al., 2007) can be removed for genetic marking without restraint of the animal. For hundreds of free-ranging dugongs in Moreton Bay, southern Queensland, microsatellite DNA profiles have been obtained from skin sampled after capture (Lanyon et al., 2002). Capture of dugongs requires relatively clear water and a sustained and skilled research effort to obtain sample sizes sufficient for population studies (Lanyon et al., 2002, 2006). On the other hand, non-invasive faecal sampling of dugongs has resulted in the collection of mitochondrial DNA (Tikel et al., 1996) but not nuclear (microsatellite) DNA, possibly due to the low abundance of sloughed cells and very active degradation in the hindgut. Biopsy sampling of dugongs using a crossbow has not been successful because of poor penetration of the thick dermis (R. W. Slade, pers. comm., 1998), and biopsy darts have not yet been trialled.

We describe here a method for sampling skin from live, free-ranging dugongs through approach without capture. Although capture for skin biopsy is usually successful for dugongs, there are times when capture is precluded because of physical (e.g., murky water, small area of shallow banks),

behavioural (e.g., dugongs close to deep water, dugongs not fleeing), ethical (e.g., not wishing to catch cow-neonate pairs), or other factors (e.g., unavailability of experienced capture team) (Lanyon et al., 2006). In these cases, an alternative method is required that still has the capacity to allow researchers to collect a sample suitable to genetically characterise a large proportion of a dugong population. The skin-scraping method described here is less disruptive to dugongs than capture, provides the capacity to sample more animals per sampling period, and results in skin samples with a high yield of extractable nuclear DNA. This method is anticipated to be most useful for research programs operating on limited resources or when dugongs occur in remote or murky water habitats.

Dugongs were sampled at three sites that lie along a 600-km coastal strip of southern Queensland, Australia: Moreton Bay (MB), Great Sandy Straits (GSS), and Shoalwater Bay (SB). Each of these sites supports a significant dugong population, which has been accorded some degree of protection through regulation of activities perceived to be threatening (e.g., netting, trawling, and boating). The most southern site, MB (27.4° S), is situated at the subtropical limit of the dugong, supports a resident population of close to 1,000 dugongs in often clear water (Lanyon, 2003) and has been the site of a mark-recapture program since 2000-2001 (Lanyon et al., 2002). The GSS region (25.8° S) lies 300 km further north and consists of a 1 to 10 km wide waterway that extends for 60 km between mainland Australia and Fraser Island. GSS is made up of a complex of shallow channels, inlets, sandbanks, and mud islands with mostly turbid water conditions throughout. This region is less developed than MB with a few fishing towns dotted along its western shore. An aerial survey of the combined Hervey Bay-GSS region in 1988 suggested that it supported the largest single population of dugongs on the Queensland coast (2,206 ± 420) (Preen & Marsh, 1995). Shoalwater Bay (22.3° S), in the southern Great Barrier Reef (GBR) region, is another 300 km north and is an Australian Defense Force training zone, remote from human settlement and with restricted human activity (Great Barrier Reef Marine Park Authority [GBRMPA], 1997). Population estimates for dugongs in the SB area are thought to have declined from 765 ± 161 to 406 ± 78 over the period 1987 to 1994 (GBRMPA, 1997). Shoalwater Bay has muddy substrate, a large tidal range, and, consequently, high water turbidity.

Dugongs were sampled in the GSS region in each of 4 y (2006 to 2009 inclusive), and in SB during one pilot trip in 2008. Dugongs have been captured and sampled in MB each year since 1997 but were first sampled via skin biopsy without capture in March 2002. Dugong sampling was typically conducted in fair weather conditions (i.e., no rain, winds ≤ 15 to 20 kts, cloud cover < 6 oktas), and at times that minimised glare off the water (i.e., not close to dawn or dusk when the angle of the sun to the water was low). Sampling trips were timed to coincide with the daytime high and following ebb tides when dugongs were most likely to be foraging over shallow subtidal or intertidal seagrass beds.

On the day prior to commencement of most sampling trips, aerial surveys were flown in a high-winged aircraft at an altitude of ~300 m to record positions of groups of dugongs onto a GPS and, hence, reduce search time in boats prior to sampling. Flight paths were designed to cover the major dugong foraging areas as identified from previous aerial surveys, but these also included areas of potential dugong habitat. On the water, dugongs were sampled opportunistically as encountered during boat transects across the GPS locations identified during the aerial survey. During the search phase, sampling boats were driven at nonplaning speeds to comply with marine park regulations that aim to reduce the risk of collision with dugongs and sea turtles.

Since sampling of dugongs involves approach and then pursuit, a sampling vessel must be capable of some acceleration, a speed of 20 to 25 kts, and be manoeuvrable. A 15-hp motor is considered the minimal requirement in order to be able to match pace with a rapidly swimming dugong for skin biopsy sampling. Semi-rigid-hulled inflatable boats of various sizes (2.4 to 5.8 m length) and with engine sizes ranging from 15 to 110 hp have been used. Further, use of a vessel with low inflatable sides to enable the sampler(s) to lean comfortably across the bow of the boat to collect samples is highly recommended. In GSS, two small sampling boats were run concurrently to maximise the sampling rate of dugongs. Each boat team consisted of at least two people (i.e., a boat driver and sampler/data recorder). When extra personnel were available, they functioned as an extra sampler or data recorder/photographer.

Skin samples were collected from dugongs of both sexes and all size classes (including neonates). Individuals on the periphery of dugong herds were preferentially targeted for sampling in the first instance to minimize disturbance to the herd. Each focal individual was slowly and stealthily tracked by boat, usually for less than 2 min, until it was positioned close to the boat's starboard bow. The objective was to remain in close continual contact with the dugong so that it could be sampled when it surfaced to breathe. Once the driver of the boat commenced close approach, the

sampler moved into position at the starboard bow. In most cases, the dugong's response was to accelerate underwater upon approach by the boat and to increase dive duration; however, some large adult dugongs appeared unconcerned and continued to forage until the boat was alongside. If a dugong accelerated away from the vessel, the boat driver endeavoured to keep behind and to one side of the dugong, matching its speed while tracking. Then, as the dugong slowed just prior to ascent, this was a cue for the driver to move forward and make final adjustments in positioning the boat to facilitate sampling. The sampler stood ready throughout the pursuit and kept the driver informed of the dugong's position relative to the boat, with an outstretched arm pointing in the animal's direction. All crew wore polarized sunglasses to aid in tracking the animal below the water surface.

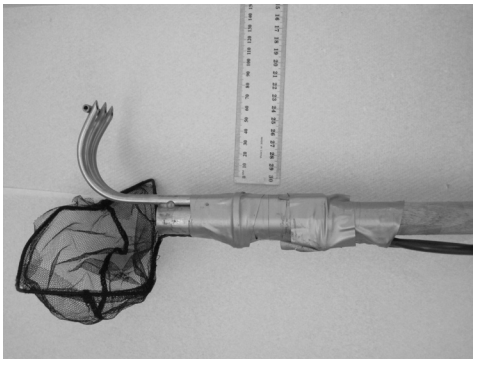
Locating and then tracking a dugong in very murky water was challenging. The visual signs of a dugong's presence included the dugong's head and/or dorsum breaking the water surface, or even an exhaled blow. Muddy plumes often indicated positions of feeding dugongs, though it should be noted that dugongs were usually situated at the ends and sometimes several metres in advance of these trackways. During pursuit, if direct visual contact was lost with a dugong, clues to facilitate resighting included smooth flukeprint(s) on the water surface caused by the beat of a tail or turbulent swirls as mud was thrown often resulting in a muddy underwater trail (particularly when water was shallower than 1.3 m). With practice, it was possible to anticipate where dugongs were likely to surface given these trackway signs and a dugong's propensity to travel in straight lines and usually towards the closest deeper water. Auditory aids included small splashes or exhaled blows. Tracking cow-calf pairs in murky waters was usually facilitated by the calves' light colouration and their habits of slipstreaming above the cow closer to the water surface and surfacing more frequently.

During pursuit, respiration rates of each dugong were monitored. The aim was to sample the dugong as soon as possible after commencement of pursuit (at first breath); however, sometimes dugongs took more than one breath before the boat was positioned for successful sampling. Individual dugongs were pursued for no longer than 10 min. In the case of cow-neonate calf pairs, both animals were biopsied within a maximum combined pursuit time of 5 min to limit intrusion on the pair. Both dugongs were sampled simultaneously as they surfaced by two samplers, each with a biopsy device, or sampled one after the other using two separate devices.

During this study, three biopsy implements to sample skin from dugongs without capture were trialled. The first device was a stainless steel, three-pronged scraper head mounted on a 25 mm diameter × 1.8 m wooden pole with a collection net mounted behind (Figure 1a), hereafter referred to as a *pole-scraper*. Each of the three curved, hollow, and sharpened prongs (50 mm diameter, spaced 10 mm apart) was designed to scrape and hold a small strip of epidermis (2 to 5 mm wide and of variable length up to 120 mm long). Each prong had an 8 mm drainage hole drilled into its topside 40 mm behind the scrape end to facilitate retrieval of the sample using forceps. The prongs were arranged at slightly different angles so that during deployment only one of these would directly contact the dugong's curved dorsum depending on orientation of the sampler to the animal. The pole-scraper could be deployed by a sampler standing in or leaning across the bow (usually starboard side) and was designed to maximise reach of the implement towards the dugong, which could be up to 2 m away. In the event that the skin sample was not retained within a prong, it was usually washed into the 120 × 100 mm gauze hand-net mounted behind the scraper head.

The second device was a *biopsy punch* consisting of a stainless steel base cylinder (25 mm diameter × 100 mm long) mounted onto a 1.5-m wooden pole, and with a 6 mm diameter × 40 mm long stainless steel biopsy head welded alongside. The distal edge of the biopsy head was sharpened to penetrate epidermis, and the interior was fitted with a series of three small internal backward-facing barbed hooks arranged around the internal perimeter (Figure 1b). This device was deployed by a sampler standing at the bow using a downward thrusting or stabbing motion when the dugong's dorsum was either above or just below the water surface. The resultant biopsy sample was a small (5 mm diameter) core of surface epidermis and underlying fibrous dermis to a depth of 8 mm, which was retained by the internal barbs and removed with forceps.

The third device was a *hand-scraper* that has been used previously to obtain skin samples during dugong captures (Lanyon et al., 2002). This device consisted of a stainless steel cylinder (25 mm diameter × 100 mm long, 1.5 mm wall thickness) with a single grater-type tooth (8 mm wide, 8 mm long, with 4 mm gap height) set centrally into a 45° angled closed end (Figure 1c). The other end of the cylinder was open but sealed during operation with a piece of fabric (cloth or tape) held in place with a stout rubber band. During sampling, the scraper was held in the palm of the hand, anchored by a wrist-strap attached to the cylinder. The cylinder end with the grater tooth was drawn firmly along the dugong's dorsum to scrape a short strip of epidermis



A.

B.

C.

Figure 1. Skin biopsy implements used to sample the dorsal epidermis of dugongs: A. *Pole-scraper:* head with three prongs mounted on wooden pole with collecting hand-net taped to pole; B. *Biopsy punch:* biopsy head mounted to side of stainless steel base cylinder and pole; and C. *Hand-scraper:* 100-mm long stainless steel cylinder with single grater tooth and wrist strap.

(Figure 2a). The sampler was careful to target an area of the dorsum that was clear of barnacles or heavy scar tissue. A skin sample of 3 to 4 mm wide and up to 80 mm long was retained within the device. The skin sample was removed from the grater tooth using forceps or from the device after removing the fabric end and gently agitating the device in a container of clean seawater. It has been proven that the hand-scraper is the superior of the three devices trialled here.

A total of 672 skin samples were obtained by biopsy without capture from wild dugongs in three locations up until November 2009. These include 266 skin samples from MB, 377 from the GSS region over 19 d, and 29 from SB in 2.5 d (Table 1). Skin biopsy samples were successfully collected by each of the three biopsy devices tested during this study: 209 samples by pole-scraper, 19 by biopsy punch, and 444 by hand-scraper (Table 1). Each epidermal sample, including those of dimensions only ~1 mm^2, yielded sufficiently good quality mitochondrial and nuclear DNA suitable for gene-tagging individual dugongs.

The sampling devices varied in their ease of use, success in obtaining and securing a skin sample, and in the behavioural responses elicited. The pole-scraper was the first and only device used for biopsy without capture in MB from 2002 through 2006. Between one and 37 dugongs were sampled by pole-scraper in each of these years because most dugongs were captured at this site. Since sampling with a pole-scraper was sporadic and opportunistic during these years, success rates of the device cannot be calculated over this period. Sampling (= pursuit) times for successful pole-scrapes in MB ranged from 1 to 9 min, mean time 2.8 ± 0.2 (s.e.) min, and modal time 1 min (Table 1). In contrast, all sampling in the murky GSS and SB sites has been through skin biopsy without capture. In the GSS region, the pole-scraper was used in the first 2 of 4 y of sampling; it was not used in SB. Attempts to collect skin using the pole-scraper were not always successful. In GSS in 2006, 51 dugongs were successfully sampled out of 86 pursuits (i.e., 59% success rate). The 35 unsuccessful pursuits were mostly related to dugongs either escaping into deep or murky water or not being within reach of the pole-scraper when surfacing. For 20% of these, the sample was not properly secured by the device. Sampling times

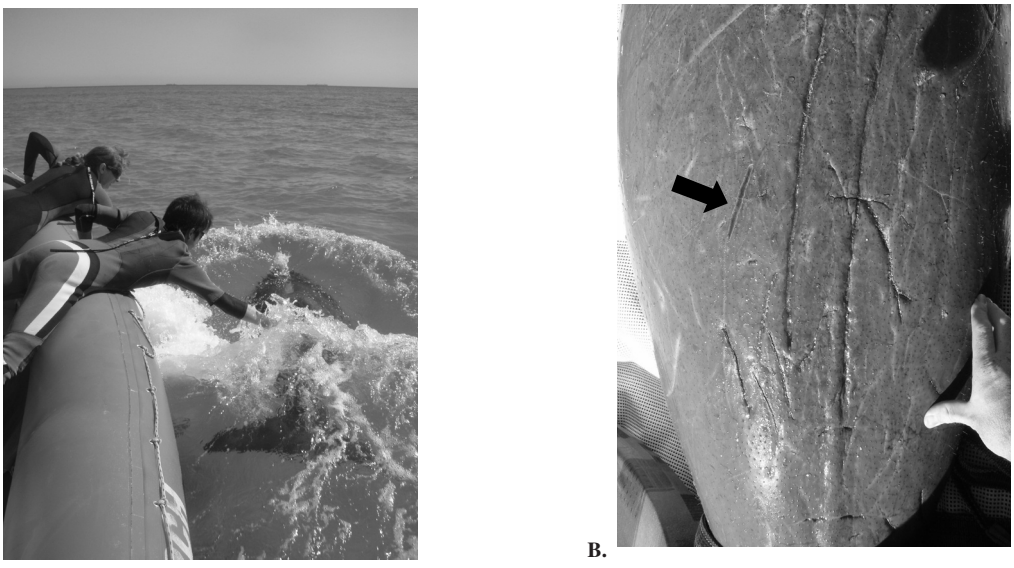


Figure 2. Epidermal dorsal scraping on free-ranging dugongs: A. Deployment of the hand-scraper device from the right bow of sampling boat; and B. The dorsum of a live, free-ranging adult dugong showing typical scarring patterns caused by erupted tusks of conspecifics and a fresh scar caused by the hand-scraper (arrowed).

by pole-scraper in GSS were similar to those in MB and ranged from 1 to 8 min, with a mean time of 2.8 ± 0.2 min and modal time of 2 min (n = 51). In 2007, 32 dugongs were successfully sampled out of 40 pursuits in GSS (i.e., 80% success rate), which reflected better boat positioning, improved pole technique, and shorter sampling times (i.e., 1 to 7 min, mean 2.4 ± 0.16 min [n = 60], modal time 2 min; Table 1). Within each year there was significant variation in the success rate (45 to 100%) of different teams of samplers at GSS, and this was related to the experience and proficiency of both the boat driver and sampler. In particular, a good pole-scrape technique involved firm pressure of the device applied to the dorsum and a careful upward sweeping motion of the pole after taking the sample in order to break contact of the skin strip with the dorsum and retain the sample on the device. If the dugong's dorsum was underwater at the time of sampling, the skin sample was likely to be washed away.

The biopsy punch was developed as a device to sample dugongs both at and below the water surface. Theoretically, if the dugong could be sampled immediately upon close approach instead of waiting for it to surface, sampling efficiency could be improved. The biopsy punch was trialled in 2007 with 19 dugongs successfully sampled—17 in GSS and two in MB. More than 50% of these dugongs were entirely underwater alongside the boat when sampled; however, for 70% of the dugongs sampled, it took multiple attempts to obtain a biopsy core. Consequently, these sampling times were longer: from 1 to 4 min with a mode of 3 min (Table 1). In some cases, poor accuracy of aim contributed to increased sampling time; but in several cases, the biopsy punch failed to extract a skin plug on the first or second sampling attempt and this appeared to be related to the size of the dugong, and presumably to the toughness of its dermis, as well as to the strength/body mass of the sampler. Of more concern, however, was the dugong's response to use of this device compared to responses elicited by the two scrapers (see next page).

The hand-scraper was first deployed by a boat-based sampler in 2007 but has been used for routine sampling of dorsal skin during captures in MB since 1997. Hand-scraping dugongs from the boat requires more accurate positioning of the dugong very close to the bow of the boat so that the dugong surfaces within arm's reach of the sampler. The sampler must apply firm downward pressure against the exposed dorsum and finish the scrape manoeuvre with a slight upward wrist rotation to secure the sample before the animal submerges. Collection of the skin sample is facilitated by the dugong's forward swimming momentum against the scraper device. Sampling success rates for hand-scraping were consistently high at 88 to 94% (and up to 100% success rate for individual trips in MB), with a modal sampling time of 2 min, which was comparable to using the other devices (Table 1). Moreover, the number of dugongs that could be sampled in a single 4-h (high to ebb tide) period by a single 2-man team

was as high as 58 (GSS in 2009), with 150 dugongs sampled over 3.5 d in water of moderate to high turbidity. If boat positioning was good, the majority of dugongs could be sampled at their first surfacing event. In a few cases, the skin was lost after sampling due to it exiting the device through the grater tooth (if the device was submerged) or through a small drainage hole opposite the grater tooth. To prevent such loss, sample the exposed dorsum only (recommended), cover the drainage hole with a forefinger, or manufacture the device without this hole.

Dugongs' responses to skin sampling varied depending on the device used. Adult males and very old female dugongs have erupted and emergent tusks (Marsh, 1980), which are used against conspecifics during agonistic displays, and presumably during courtship (Anderson & Birtles, 1978; Preen, 1989). As a result, almost all wild dugongs, including large calves, have characteristic dorsal scarring that consists of tusk rake marks. This scarring varies from light and irregular scars (Figure 2b) to heavy and prominent coalesced scars or *whiteback*. Since tusk injury by conspecifics is common, possibly even inflicted on a seasonal basis, methods of sampling via dorsal scraping more closely mimic natural injury patterns compared to the biopsy punch. Hand-scraping elicited fewer and more moderate behavioural responses by dugongs than biopsy punch and pole-scraping. In most cases, there was minimal behavioural response to contact of the hand-scraper along the dorsum. Most dugongs continued travelling at the same speed, and only a few accelerated slightly. In a few cases, dugongs continued to forage seemingly uninterrupted. In contrast, on the few occasions when the hand-scraper contacted the flanks of a dugong, the reaction was immediate, with the dugong either turning away from the sampler or splashing the flukes. This suggests that the skin on the flanks may be more sensitive than on the dorsum. In some other marine vertebrates in which the dorsum is attacked by conspecifics (e.g., blue sharks) (Pratt & Castro, 1990), the dorsal epidermis tends to be thicker; this has not been investigated in the dugong. The mark left after either hand- or pole-scraping was superficial compared to natural tusk rake injuries sustained by wild dugongs (Figure 2b).

In contrast to the hand-scraper, successful operation of the biopsy punch required significant downward pressure to be exerted against the dugong's dorsum. In more than 50% of cases, dugongs responded strongly by changing direction, producing a tail splash, or accelerating away underwater. The pole-scraper elicited a similar escape response in some cases, and this appeared to be directly related to the amount of downward pressure exerted by the sampler. In a few cases, adult dugongs that had been sampled via pole-scraper kicked out at the sampler or boat with their tail flukes. More rarely, a few large, often heavily scarred (and presumably older) adults fluke-slapped the boat soon after approach and even before collection of the skin sample, a reaction to the boat approach rather than to sampling. Although we did not deliberately track dugongs after sampling, preferring to limit our intrusion, we have noticed that disturbance caused by skin scraping appears to be short-lived. Dugongs that were visually tracked after sampling usually returned to the same area, rejoined their herd, and/

Table 1. Number of dugongs sampled and success rate using each of the three biopsy devices: pole-scraper, biopsy punch, and hand-scraper; sampling locations include Moreton Bay (MB), Great Sandy Straits (GSS), and Shoalwater Bay (SB). Sampling success rate % refers to the proportion of biopsy samples that were successfully obtained from a total number of attempts. Sampling times (min) include the range, mean ± s.e., and modal time (mode) taken from the start of the pursuit to successful collection of a skin sample.

Sampling device	Location & year	# dugongs sampled	Success rate %	Sampling times (min) Range	Sampling times (min) Mean ± s.e.	Mode (min)
Pole-scraper	MB 2002-2006	126	--	1-9	2.8 ± 0.2	1
	GSS 2006	51	59	1-8	2.8 ± 0.2	2
	GSS 2007	32	80	1-7	2.4 ± 0.2	2
Biopsy punch	MB 2006	2	100	--	--	--
	GSS 2007	17	59	1-4	2.7 ± 0.2	3
Hand-scraper	GSS 2007	12	94	--	--	--
	SB 2007	29	88	1-8	3.5 ± 0.4	2
	GSS 2008	115	94	1-8	2.4 ± 0.1	2
	GSS 2009	150	93	1-9	2.5 ± 0.1	2
	MB 2007-2009	138	93	1-10	3.4 ± 0.4	2

or resumed feeding within a few minutes, suggesting that such sampling caused no long-term nor adverse effects. Furthermore, injury scars from dorsal scrapes were not apparent in those dugongs that were recaptured later.

Once the skin sample was collected, each component of each sampling device was thoroughly rinsed in fresh seawater and then in 70 to 100% ethanol to avoid contamination of the device between dugongs. Skin samples were placed in salt-saturated DMSO solution and frozen (for nuclear and mitochondrial DNA analysis). Nuclear DNA was extracted and analysed for identity and sex against 26 microsatellite loci (Broderick et al., 2007) and two sex primers (McHale et al., 2008) that we have developed specifically for dugongs. Individual genotyping requires good quality and quantity of DNA to avoid allele dropout and/or null alleles that may result in unreliable identifications or scoring errors. Each epidermal skin sample yielded sufficiently high quantities of extractable nuclear DNA, suggesting that the hypodermis does not need to be sampled for genetic material in the dugong. To compare DNA yield, 16 samples of skin collected by each of the three biopsy methods in the GSS in 2006-2007 were randomly selected, and the extracted nuclear DNA concentrations were quantified. There were no differences in mean concentrations of nuclear DNA in skin sampled by the three methods (single factor ANOVA: $F_{2, 47} = 1.76$; $p = 0.18$): the biopsy punch yielded 408 ± 77 ng/µl nuclear DNA (mean ± s.e.), range 916 ng/µl; the pole-scraper yielded 266 ± 70 ng/µl, range 1,027; and the hand-scraper yielded 254 ± 41 ng/µl, range 569. In contrast, in Florida manatees, a single trial of our hand-scrape device did not appear to yield adequate amounts of nuclear DNA (R. K. Bonde, pers. comm., April 2009), suggesting that the skin of the manatee may require a different sampling approach (e.g., Carney et al., 2007).

In addition to the skin sample, additional biological information was collected for each dugong. Body length (cm) was estimated visually, based on prior experience in estimating and then measuring real body length of dugongs in MB. Although dugongs could be sexed by molecular markers using a skin sample, they were assigned a "visual" sex if they rolled laterally during pursuit and presented their genital area for inspection (rarely) or were accompanied by a calf, indicating a possible female. Very occasionally, dugongs defecated during pursuit, and the floating faecal sample was retrieved for later endocrine analysis, yielding information on sex, maturity, and reproductive status (Burgess et al., 2009). Any obvious or unusual body injury, scarring, or pathology was recorded, and it was photographed if personnel were available.

This study has demonstrated that it is possible to obtain large numbers of samples for genetic analysis from free-ranging dugongs using techniques that are rapid, inexpensive, and cause minimal disturbance to the animals. Of the three biopsy implements trialled here, the simple hand-scraper was superior in terms of success rate, short sampling time, ease of use, and minor effect on the behaviour of the dugong. Epidermal samples obtained through scraping of the dorsum yielded good quality nuclear DNA that was sufficient for genetic analysis. One of the greatest difficulties with this method is that approach to the dugong must be sufficiently close to contact the epidermis. However, this becomes easier with good boat technique and practice in stealthy approach. It is also important to optimise visibility of the tracked animals by choosing fair weather days and times when lighting conditions are best. For example, we have found that in the frequently murky waters of SB, light penetrance of the water and, hence, sampling are facilitated when a high tide falls close to noon. In summary, then, it is suggested that when genetic/molecular information is sufficient to answer a research question, biopsy sampling of dugongs may be preferable to capture. This method will be useful to researchers who wish to examine population structure through genetics; sample a large proportion of the population; or work in areas that are challenging because of their remote location, narrow seagrass banks, or murky waters.

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