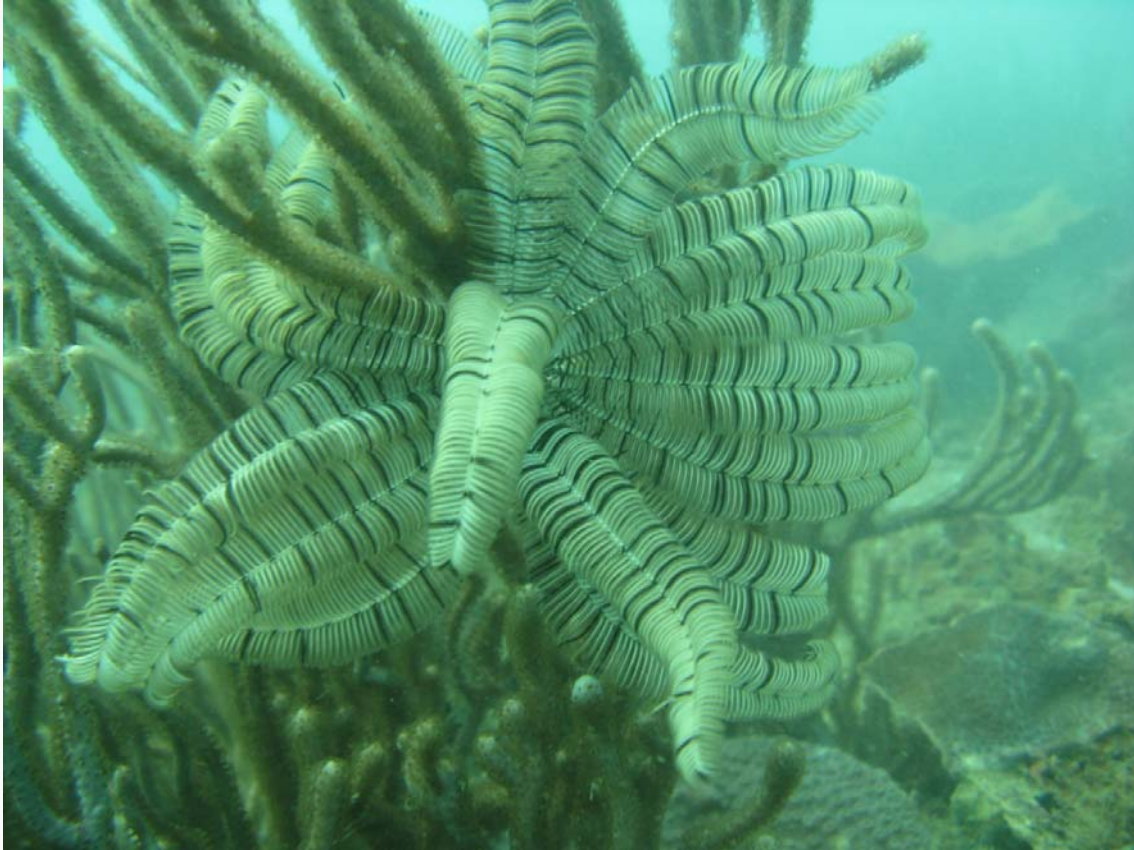


SKM

OUTER HARBOUR DEVELOPMENT, PORT HEDLAND

STABLE ISOTOPE PRELIMINARY STUDY



Prepared by Pendoley Environmental Pty Ltd

For SKM

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Revision 0



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Marine Conservation
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1 INTRODUCTION

1.1 Context and Scope of Work

BHP Billiton Iron Ore operates a port in the Port Hedland region of Western Australia. The current port operations consist of processing, stockpiling and ship loading facilities at Nelson Point and Finucane Island (referred to as the Inner Harbour), located on opposite sides of the Port Hedland Harbour. The marine construction activities including dredging and the development of a new jetty/wharf structure, berths and ship-loading infrastructure have the potential to impact on foraging habitat for turtles within the Port Hedland region.

However, there is a lack of information regarding the utilisation patterns and feeding ranges of the marine turtles within the Port Hedland region. This is primarily due to the lack of systematic biological surveys in the region. Therefore, there is a need to understand which food resources are important to foraging turtles in the region in order to determine the potential impacts of the Outer Harbour Development on these habitats.

Foraging habitat surveys typically involve either aerial surveys or the physical capture and handling of turtles in the water. These methods can be limited by a number of factors including weather conditions, large costs that can be involved and the health and safety issues associated with in-water capture of turtles. Stable isotope analysis may be a more useful, cost effective and safer method to identify the food sources turtles are feeding on, and therefore which areas are important as foraging habitat for turtles.

1.2 Objectives

This preliminary study's objective was to identify whether stable isotope signatures would be a useful method for identifying food sources of marine turtles in the region. If this was possible, then the study aimed to investigate whether or not turtles were foraging in the region, and what habitats may be considered important for foraging turtles in the Port Hedland region.

1.3 Stable Isotopes as a Tool

Stable isotope studies are becoming increasingly used in dietary and trophic studies within marine ecosystems. This is because unlike conventional methods such as gut and faecal sampling, which give a snapshot of an animal's diet, stable isotope studies allow inferences to be drawn about an animal's complete diet over an extended time period (Arthur *et al.* 2008; Wallace *et al.* 2006). The principle behind using stable isotopes in dietary studies is that "you are what you eat"; the elements in an animal's tissues will be a composite of the prey items it has consumed. During biological process the ratio of heavy to light isotopes of an element (the stable isotope signature) may change, but it does so in a predictable way, so that it is possible to backtrack, and determine the isotope signature of its prey items. By collecting a range of possible prey items from hypothesised foraging grounds, it is possible to match these isotope signatures and therefore infer the likely prey items of an animal.

Two isotopes commonly used in dietary studies are carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$). The two isotopes have differing enrichment factors and spatial patterning and hence have different utilities in

dietary studies. $\delta^{13}\text{C}$ ratios are enriched only slightly in animal tissues, which means they are not good indicators of trophic levels, but are useful in describing carbon sources. $\delta^{13}\text{C}$ ratios tend to become more negative when moving from neritic to oceanic environments, and also when moving to higher latitudes. This spatial patterning can allow inferences about the location of prey items to be determined, as the $\delta^{13}\text{C}$ signature of an animal should approximate that of its prey. In contrast, because of the greater enrichment level of $\delta^{15}\text{N}$ signatures, they are useful in determining the trophic level of animals. The $\delta^{15}\text{N}$ ratio in endothermic animal tissues tends to be between 3 and 5% higher than that of their prey (Seminoff *et al.* 2009). Recent studies have found this enrichment level to be lower in marine turtles, ranging from 0 to 3% in hard shelled species. The enrichment levels of both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ vary depending on the tissue type. Enrichment of $\delta^{13}\text{C}$ is negligible or negative in white blood, red blood and blood plasma, but up to 2.5% in turtle skin tissue. Enrichment of $\delta^{15}\text{N}$ is highest in plasma but most consistent in skin tissue, where it ranges from approximately 1.5 to 2.5%. Muscle tissue of loggerhead turtles (*Caretta caretta*) has been found to have $\delta^{15}\text{N}$ signatures approximately 1% higher than skin tissue and $\delta^{13}\text{C}$ signatures approximately 1.5% more negative than skin (equating to approximately 1.5% less enrichment) (Revelles *et al.* 2007).

2 METHODS

2.1 Sampling

2.1.1 Turtle Samples

Flatback and green turtles were identified during an earlier desktop review and field surveys as the species' present within the Port Hedland region (Pendoley Environmental, 2009a) and therefore most likely to be impacted by the proposed Outer Harbour Development. Samples from these species were collected from the region between 8 and 12 December 2009. A total of nine flatback and two green turtle tissue samples were collected from the Port Hedland region (**Table 1**).

Flatback turtle tissues collected were all related to nesting (non-resident) turtles and included:

- Muscle tissue biopsy samples from four turtles nesting at Cemetery Beach (three of these turtles were also satellite tracked following nesting as part of another study);
- Four eggshells collected from Downes Island and Paradise Beach; and
- One hatchling collected from the Port Hedland region.

Green turtle scutes (from dead turtles) were collected from North Turtle Island and Paradise Beach and may be representative of resident and/or non-resident turtles.

Table 1: Collection sites and number of turtle samples collected from the Port Hedland area.

Collection location	Species	Life stage	Sample type
Cemetery Beach	Flatback	Nesting female	Muscle
	Flatback	Nesting female	Muscle
	Flatback	Nesting female	Muscle
	Flatback	Nesting female	Muscle
	Flatback	Hatchling	Tissue
Downes Island	Flatback	Egg	Shell
	Flatback	Egg	Shell
Paradise Beach North	Flatback	Egg	Shell
	Flatback	Egg	Shell
	Green	Adult	Scute
North Turtle Island	Green	Adult	Scute

2.1.2 Prey Items

A range of potential prey items were collected from the Port Hedland region by the SKM marine team between July and September 2009. These included soft corals (including sea whips), algae, sponges, jellyfish and seagrass. The samples were collected from the water quality/coral health monitoring sites used for the proposed Outer Harbour Development marine monitoring programme, ranging from three to 33 km offshore (**Table 2**). Although an effort was made to collect at least one prey type from each sampling site, this was not possible due to time restrictions. A map showing the location of each of the collection sites can be found in **Appendix 1**.

Table 2: Collection sites and number of potential prey item samples collected from the Port Hedland area.

Collection Location	Environment	Approx. distance from the mainland (km)	Sample Type				
			Soft Coral	Algae	Sponge	Jellyfish	Seagrass
Weerde Reef	Inshore	3	2	2	1	-	-
Weerde Island	Inshore	3	-	-	-	-	1
Cape Thouin	Mid-shore	10	2	2	2	-	-
Minilya Bank	Mid-shore	16	-	-	-	1	-
Little Turtle Island	Offshore	19	2	-	1	-	-
Cornelisse Shoal	Offshore	33	2	-	2	1	-
Coxon Shoal	Offshore	28	3	1	2	-	-

2.2 Sample Preparation

All samples were rinsed with distilled water in the field and frozen until processing in the laboratory. Samples were again cleaned in the laboratory and a representative sub-sample was chosen for analysis. Soft coral, sea whip, sponges, eggshell and scute samples were treated with HCl to dissolve carbonates. These samples were rinsed again in distilled water.

All samples were then dried in at 60 °C and ground to a fine powder. Approximately 10-30 mg were analysed for stable isotope signatures.

2.3 Stable Isotope Analysis

Samples were analysed for Nitrogen and Carbon content (%), $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, using an Automated Nitrogen Carbon Analyzer-Mass Spectrometer consisting of a 20/20 mass spectrometer connected with an ANCA-S1 preparation system (Europa Scientific Ltd., Crewe, UK). Multi-points normalisation was used in order to reduce raw values to the international scale (Paul *et al.* 2007). Normalisation was completed on the basis of international standards provided by IAEA: $\delta^{13}\text{C}$ - NBS22, USGS24, USGS40; and for $\delta^{15}\text{N}$ - N1, N2, USGS40 and laboratory standards. The external error of analyses (1 std dev) was not more than 0.15 ‰ for $\delta^{13}\text{C}$ and 0.30 ‰ for $\delta^{15}\text{N}$.

3 RESULTS

3.1 Nitrogen Sources

$\delta^{15}\text{N}$ ratios are generally enriched $\sim 0\text{-}3\%$ with each trophic level. Flatback turtle tissue had the highest $\delta^{15}\text{N}$ signature, averaging approximately 12.5% . The different tissues sampled from flatback turtles exhibited similar ratios. Jellyfish had a $\delta^{15}\text{N}$ signature of 10.6% , approximately 2% lower than flatback turtle tissue, suggesting jellyfish form part of the flatback turtle diet. Green turtles had a lower $\delta^{15}\text{N}$ signature (10.3%) compared with flatback turtles, indicating they feed on prey items from lower trophic levels. Sponges had a $\delta^{15}\text{N}$ signature of 7.6% , approximately 3% lower than green turtle tissue, suggesting that sponges are prey items for green turtles. Seagrasses and algae had the lowest $\delta^{15}\text{N}$ signatures at 1.18 and 2.8% respectively (**Figure 1**).

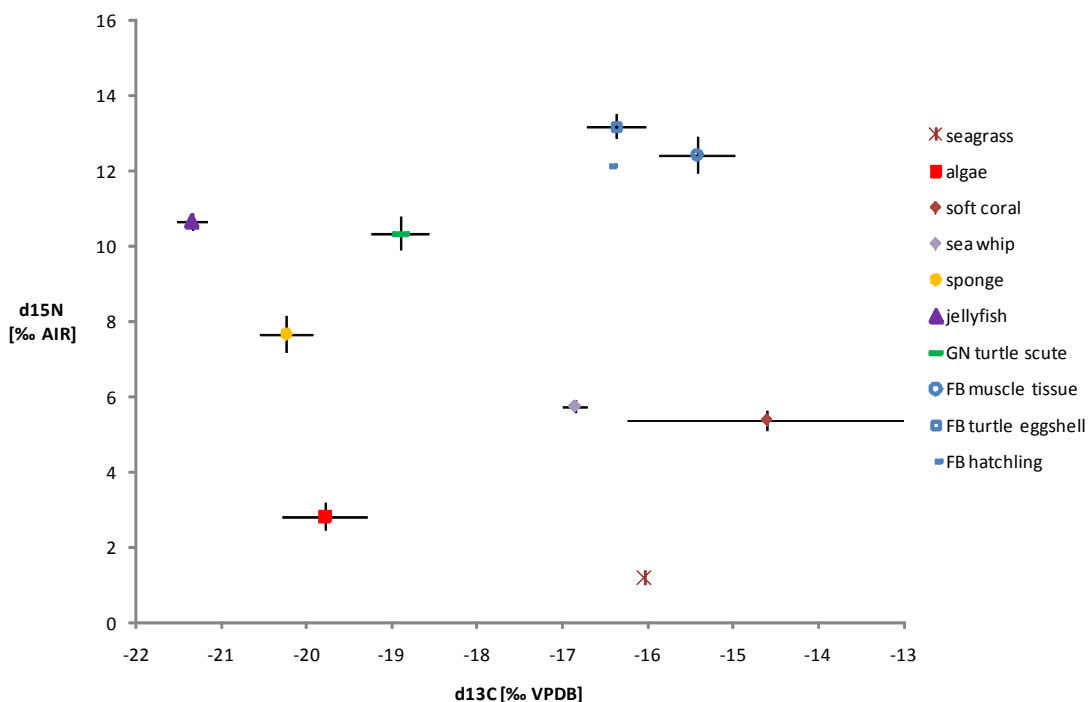


Figure 1: $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ stable isotope signatures (mean \pm SE) of turtle and prey item samples collected from the Port Hedland region, WA.

3.2 Carbon Sources

$\delta^{13}\text{C}$ signatures were lowest (most negative) in jellyfish (-21.3%) and highest in soft corals (-14.6%). The carbon signature of green turtles was between that of algae and seagrasses, but most similar to algae. The carbon signature of flatback turtles overlapped with that of soft corals, and was similar to that of sea whips (**Figure 1**). The $\delta^{13}\text{C}$ ratio of soft corals was highly variable, ranging from -20 to -8.5% .

There was no apparent variation in $\delta^{13}\text{C}$ ratios associated with proximity to shore (**Figure 2**).

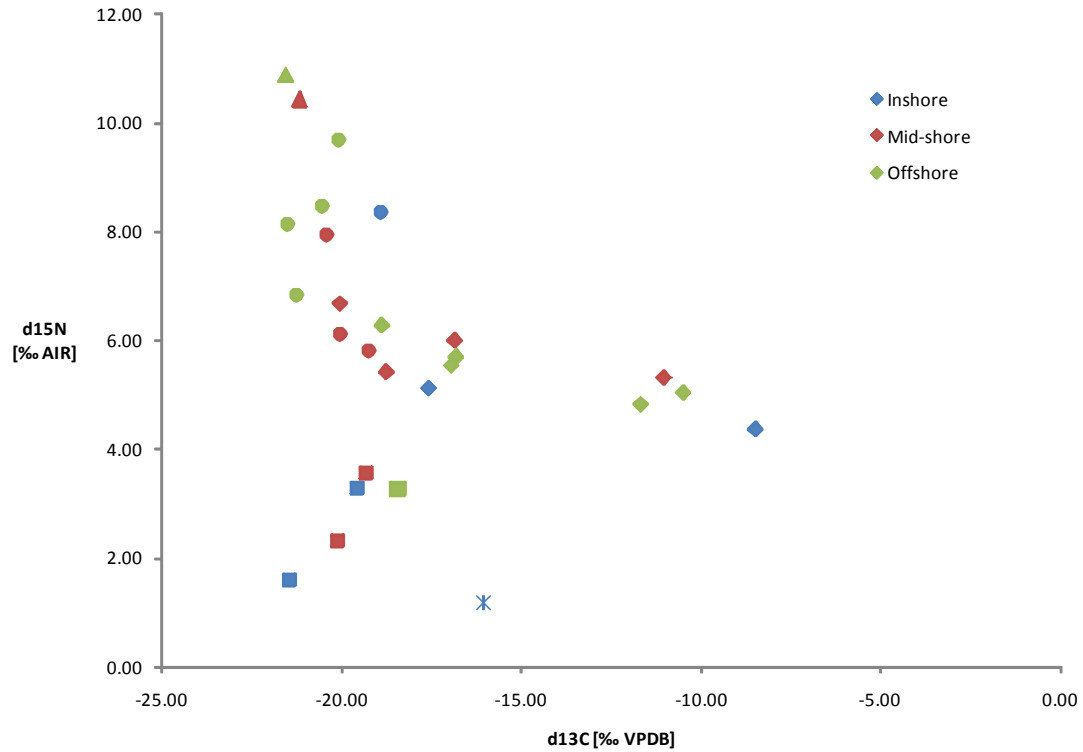


Figure 2: $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ stable isotope signatures of different prey items collected from inshore, mid-shore and offshore sites in the Port Hedland area. The type of prey item is delineated by shape: \blacktriangle Jellyfish, \bullet Sponges, \blacklozenge Soft Corals (including sea whips), \blacksquare Algae and \times Seagrass.

4 DISCUSSION

4.1 Flatback Turtles

The $\delta^{15}\text{N}$ signatures of all flatback turtle tissues were very similar as all tissues were derived from adult females (including the hatchling), which would be expected to feed at a similar trophic level. The $\delta^{15}\text{N}$ signature of flatback turtles was approximately 2 % higher than that of jellyfish, and given that this has been shown to be the average enrichment factor for other hard-shelled turtles (Seminoff *et al.* 2009) it is likely that jellyfish are a large dietary component of these flatbacks. The $\delta^{15}\text{N}$ signature of sea whips and corals were over 6 % lower than flatback turtles, suggesting that these contribute little to the diet of flatback turtles.

In contradiction to this, the carbon signature of flatback turtle tissues was most similar to sea whips and other soft corals and much higher than that of jellyfish, suggesting that sea whips and soft corals are the dominant food sources. These findings have a number of possible explanations:

- Flatback turtles nesting in this region may have travelled from foraging grounds located at great distances from the Port Hedland region. Satellite tracking has shown that many turtles nesting in the Pilbara region forage in habitats in the far north of Western Australia (Pendoley Environmental 2009b; www.seaturtle.org/tracking). Carbon isotopes in the Kimberley region may be higher (less negative) than in the Pilbara due to the depletion of $\delta^{13}\text{C}$ ratios that occurs with higher latitudes in marine ecosystems (Goericke & Fry 1994).
- Flatback turtles are also feeding on other resources, which contributed to their $\delta^{13}\text{C}$ signature.

In summary, results of this study indicate that jellyfish and soft corals are likely components of the flatback turtle diet, consistent with previous reports which have reported soft corals (such as sea pens and sea whips) and jellyfish as predominant food items (Bjorndal 1997). This study could not provide conclusive evidence of whether flatback turtles actually forage in the Port Hedland area, as the isotopic signatures from the flatback tissues collected reflect the integration of dietary resources consumed on foraging grounds before migration to the nesting beaches at Port Hedland. To determine this, ideally turtle tissue samples would comprise of those from resident turtles as well as breeding females. In addition, a wider range of invertebrate prey items (including sea pens, sea feathers, sea whips, molluscs, bryozoans and jellyfish) would be required, and samples collected from a number of the hypothesised foraging grounds.

4.2 Green Turtles

The $\delta^{13}\text{C}$ signature of the green turtle scutes was between that of seagrass and algae, which is consistent with them having a diet comprised of these groups. The $\delta^{15}\text{N}$ signature of the green turtle scutes was higher than would be expected than if they fed only on algae and seagrasses. The $\delta^{15}\text{N}$ signature of green turtles was 10.3 % which is 7.5% greater than that of the algae and 8.5% greater than that of the seagrass sample. Previous studies have found the $\delta^{15}\text{N}$ tissue concentration of green turtles to be approximately 0.22 to 2.92 % greater than their food source (Seminoff 2006). This implies that green turtles also feed on higher order organisms, as well as primary producers.

This greater than expected enrichment of $\delta^{15}\text{N}$ has been found by other stable isotope studies of green turtles, and is thought to be due to the incidental consumption of animal items such as fish eggs and invertebrates, however it is possible that green turtles also selectively consume animal items (Hatase *et al.* 2006, Godley *et al.* 2008). Studies of gut contents of green turtles from the Indian Ocean are limited and have reported a diet predominantly of seagrasses and algae, however green turtles in the eastern Pacific have a much more carnivorous diet, consuming jellyfish, polychaetes, fish and molluscs (Bjorndal 1997). It is possible that the green turtles, from which the samples in this study came, were foraging on biota present in this area, based on the carbon and nitrogen signatures.

4.3 Conclusions

The results of this preliminary study indicate that stable isotopes are a useful tool for determining food sources of turtles and therefore, inferring potential foraging areas within the Port Hedland region.

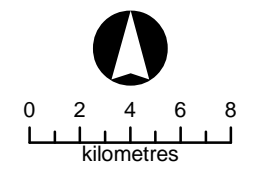
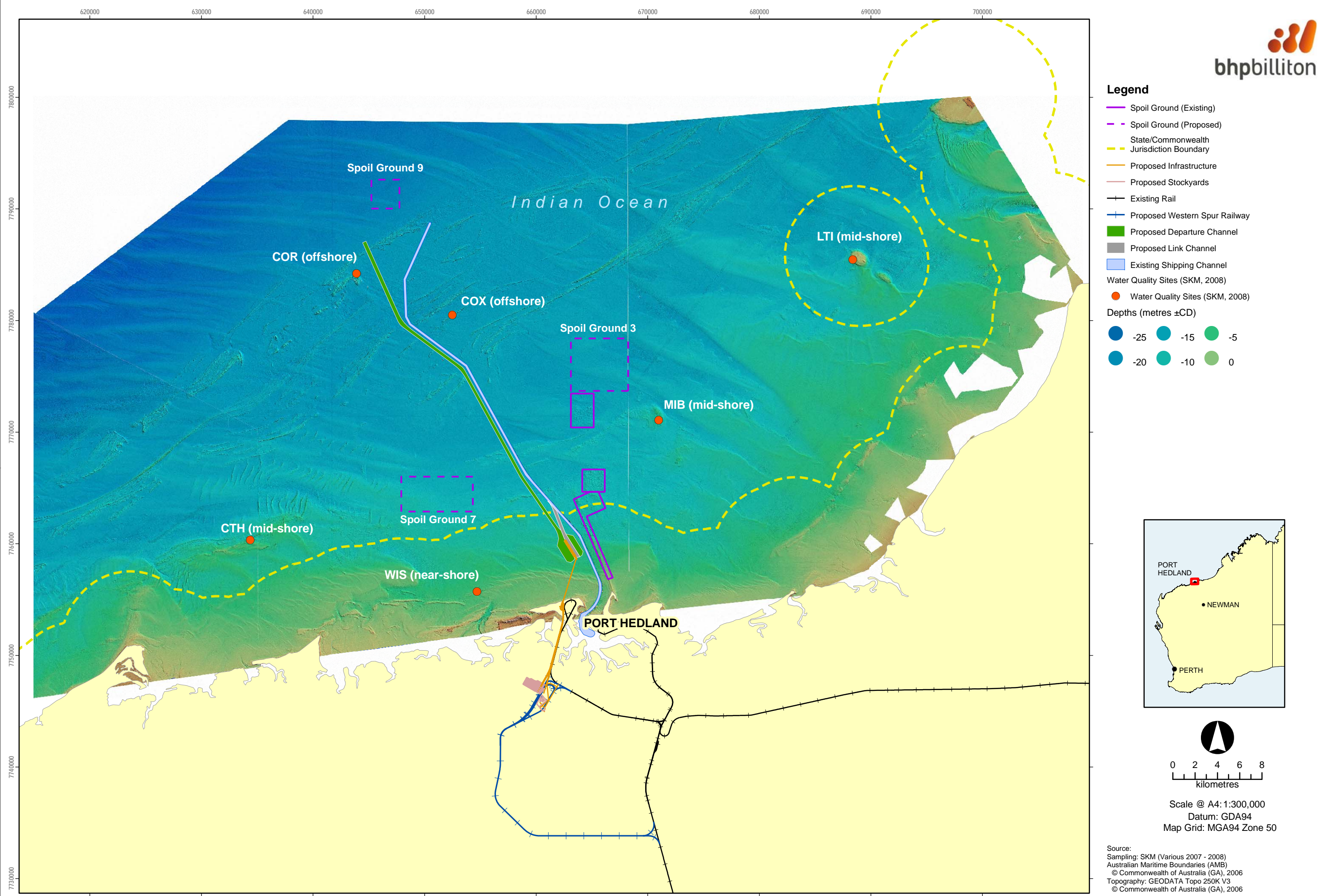
The diet of flatback and green turtles within the Port Hedland area is similar to that previously reported for these species elsewhere in their range. Areas of soft coral habitat are considered potential foraging grounds for flatback turtles from other nesting populations that may forage in the Port Hedland area. Seagrass and algal habitat within the Port Hedland area is considered important foraging habitat for green turtles. Sponge habitat may also be important green turtle foraging habitat, however, it is unknown whether green turtles are selectively feeding on or incidentally ingesting sponges while grazing on algae and seagrasses.

If greater insights into the foraging grounds of turtles using the Port Hedland area are required it is recommended that the study is expanded to include more turtle samples from different tissues, prey samples from a broader geographical range and from a greater number of groups. This may enable geographical patterns in $\delta^{13}\text{C}$ ratios to be established for each of the prey groups, and therefore allow inferences to be drawn about the most-likely carbon sources (and hence foraging grounds) for each of the turtle samples.

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Appendix 1: Prey sample collection sites



Scale @ A4: 1:300,000
 Datum: GDA94
 Map Grid: MGA94 Zone 50

Source:
 Sampling: SKM (Various 2007 - 2008)
 Australian Maritime Boundaries (AMB)
 © Commonwealth of Australia (GA), 2006
 Topography: GEODATA Topo 250K V3
 © Commonwealth of Australia (GA), 2006

Figure 2.2 Location of water quality monitoring sites offshore from Port Hedland

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