Sources of Carbon Supporting Benthic Fauna of Roebuck Bay & Eighty Mile Beach

Short term collaborative investigation program by:

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1 Objectives

Roebuck Bay and Eighty-mile Beach are nationally (ANCA, 1996) and internationally recognised (Ramsar) as important locations for shorebirds (or ‘waders’) providing a key migratory stopover in spring and autumn. These areas also provide a home for up to 150,000 birds in the non-breeding season. They are two of approximately 20 locations worldwide characterised by soft bottom intertidal mudflats supporting large numbers of migratory shorebirds, and are the foremost internationally important sites for shorebirds in the Asia-Pacific flyway system (Pepping *et al*., 1999). The ability of Roebuck Bay and Eighty-mile Beach to support large numbers of shorebirds, and to facilitate their annual migration to and from these beaches (travelling 30,000 kms per year at 70 km per hr over 18 days continuous flight) appears to relate to the particularly abundant and diverse food source. From feeding behaviour and dietary studies of the shorebirds, this food source comprises the benthic meiofauna resident in the extensive tidal mudflats.

Surveys of the benthic invertebrate fauna of the tidal mudflats of Roebuck Bay and Eighty-mile Beach have indeed revealed a very diverse and abundant fauna (Pepping *et al.* 1999, Piersma *et al*., 2000, Piersma *et al*. 2002). Pepping *et al.* (1999) recorded 161 taxa from quantitative samples from Roebuck Bay, with another 30 taxa recorded opportunistically. In was estimated that the samples could yield in excess of 500 species if identified to the lowest possible level. Piersma *et al*. (2000) recorded about 112 taxa from Eighty-mile Beach in October 1999, with taxonomic resolution ranging from species, family to phyla. Again, considerably more taxa would be recorded if sampls wre etaken to species level. Piersma *et al*. (2002) recorded 205 taxa from quantitative samples from Roebuck Bay, with at least 60 additional taxa recorded opportunistically, and with approx 50 taxa not recorded previously. Given that the level of taxonomic resolution has been fairly broad, the potential biodiversity of Roebuck Bay and Eighty-mile Beach runs into many hundreds of invertebrate species. This high biodiversity, and high abundance of benthic invertebrates in the mud provides a very rich food source for resident and migratory shorebirds.

As well as being important locations for shorebirds, Roebuck Bay and Eighty-mile Beach hold important values for the Traditional Owners, providing artisanal food items, including various shell fish, mudcrabs and fish species, with both areas featuring in Dreamtime stories.

Inappropriate use of each area not only could impact on the shorebird values of these Ramar sites, but could also affect the cultural use and values of the beaches. The intertidal fauna appears to be a critical attribute of the mud flats. Current information on the biology of the benthic fauna of Roebuck Bay and Eighty-mile Beach is largely descriptive and little is know of the relationships amongst species nor their energy requirements.

The township of Broome is a growing centre for tourism with associated pressures ever expanding (i.e. visitation to the bay, 4x4 access to the beach, boat and beach fishing in the bay, hovercraft tours, general boating activities with associated flotsam and jetsom). There are also a range of other industries developing in the area with the potential to impact on the bay (i.e. irrigated cotton using inland groundwaters, pearl fishing, port activities etc). Similarly, Eighty-mile Beach is susceptible to growing pressures (4x4 access to the beach, boat and beach fishing, and irrigated cotton using inland groundwaters) with the potential to impact the mudflats. In order to refine the ability to predict future effects of these activities on the ecological health of the mudflats (and therefore the viability of each area to continue to support shorebird populations in the long term) it is necessary to acquire process-based biological information, in particular, an understanding of the food web of the benthic fauna mudflats. Without an understanding of the main energy sources (*viz*. carbon) supporting the food web of the benthic fauna of the tidal flats it is not possible to properly manage these systems.

Studies of the food webs of tropical estuarine systems in which there are mangroves have identified a range of possible carbon sources driving the food webs, including mangroves, marine plankton, estuarine micro-algae, seagrasses and marine macro-algae (Boon *et al*. 1997; Loneragan *et al*. 1997). The relative proportion of each carbon source varies depending on the system in question (Rodelli *et al*., 1984; Newell, *et al*., 1995, Primavera, 1996, Hogarth, 1999).

The objectives of this study are to identify the major carbon sources driving the benthic invertebrate fauna which supports the migratory waders and key artisanal fishery species (fish, shellfish and crabs) of Roebuck Bay and Eighty-mile Beach. By identifying the main carbon sources it will be possible to identify which pathways would be susceptible to potential future land uses/pressures. For example, it may be that mangroves are a critical carbon source supporting much of the food web, therefore heightening the need to protect the narrow mangrove belt fringing Roebuck Bay. Alternatively, marine plankton may be a prime carbon source, reflecting the twice-daily tidal flushing of the mud flats, highlighting the need to minimise impacts on coastal marine plankton/water quality.

The key questions that this study will address are:

- What is the relative importance of the following carbon sources to benthic fauna on the mudflats: marine phyto/zooplankton, mangroves, seagrass, macroalgae or unicellular/filamentous algae on the mud flats?
- Do the same carbon sources drive the benthic invertebrate fauna at Roebuck Bay and Eighty-mile Beach

Stable isotopes of carbon (C) and nitrogen (N) will be used to determine major carbon sources supporting benthic fauna of the mudflats of both areas. These sources will then be related to the availability of each source and potential for impacts of the source from various land uses. The study will assist in focusing on-going management of Roebuck Bay and Eighty-mile Beach to ensure the ecosystems are sustainable, with continuing high ecological health.

2 Background to stable isotope tracing

A fundamental consideration to understanding food webs is to first identify the sources of organic carbon that are *assimilated* by primary and secondary consumers, such as invertebrates and fish. The traditional approach of examining gut contents has a major flaw in that it shows only what is ingested, and not what is assimilated. Easily and rapidly digested items that provide energy may not be obvious/detectable, and items difficult to digest or that were ingested accidentally may appear dominant. Stable isotope tracing techniques have overcome these difficulties (Peterson and Fry 1987, Lajtha and Michener 1994). Most elements of biological importance have at least two stable isotopes, although one form is often far more abundant in natural materials than the other(s). Slight variations in the ratio of these isotopes can occur because of fractionation during chemical and biochemical reactions (e.g. carbon isotopes fractionate during photosynthesis). The technique of stable isotope tracing relies on the precise measurement of these variations in naturally occurring materials.

Stable isotope analysis of carbon has proved particularly effective in the study of aquatic food webs where there are often marked differences in the isotope signatures of the major primary sources (e.g. Rounick and Winterbourn 1982; Peterson and Fry 1987; Rosenfeld and Roff 1992; Boon and Bunn 1994). Although considerable fractionation of carbon isotopes can occur when plants fix CO_2 during photosynthesis, very little change occurs subsequently when organisms eat and assimilate the plant material. The carbon isotope signature of a consumer is determined by diet alone and reflects the signatures of the plant (or plants) consumed: in essence, "*you are what you eat*". In contrast to carbon, stable isotopes of nitrogen show a progressive and predictable fractionation through the food web and can be used to determine trophic position of particular species.

Stable isotope analysis has several advantages over traditional methods for determining the diet of consumers. In particular, the isotope signature of a consumer reflects material assimilated rather than merely ingested. Also, it provides an integration over time based on body tissue turnover rates (i.e. weeks to months), rather than a snapshot of recently ingested food (Peterson and Fry 1987).

3 Study Plan

3.1 Study design and rationale

This section outlines the program for an intensive and comprehensive stable isotope study of the benthic fauna of Roebuck Bay and Eighty-mile Beach.

Sampling will target fish, molluscs and crustacea utilised by shorebirds and for artisanal fisheries in both areas. Knowledge of the food habits of higher consumers will be used to identify likely trophic interactions for key taxa. Examples of food chains to be targeted are presented in Table 1. Samples will be collected of the key benthic invertebrate species and their likely food sources. The need to sample as many different primary sources (e.g. algae, detritus, mangroves, seagrass, terrestrial and aquatic plants etc) as possible cannot be over-emphasised. The most obvious and abundant items are not always the most important in supplying carbon to a system. In many instances there may be an ‘inconspicuous’ source that provides much of the carbon input to a food web (e.g. a thin epiphytic/benthic algal layer with a rapid turn-over, but which is continually grazed and therefore not obvious). It is important that any ‘inconspicuous’ sources are sampled.

Table 1. Examples of trophic chains with different primary carbon sources that are likely part of the food web, and will be sampled as part of the stable isotope study.

1.	Mangrove Litter ↓ Herbivorous crab ↓ Predator
2.	Algae/epiphyton on mud ↓ Molluscan grazer ↓ Predator
3.	Mangrove detritus ↓ Prawns ↓ Predator
4.	Phytoplankton ↓ Zooplankton ↓ Filterfeeders

Therefore, as many elements of the food web as possible will be sampled. Samples will include, where present, benthic algae, phytoplankton, epiphyton, seagrass, mangroves, other dominant riparian tree/shrub species, detritus, zooplankton, a range of key species representative of the benthic invertebrate fauna, including molluscs, crustacea and fish species covering different suspected trophic levels and with different dietary sources and including those taxa used in the artisanal fishery.

Based on literature values of recent studies in Australian estuarine habitats (Boon *et al*., 1997; Loneragan *et al*., 1997), it is proposed that a maximum of 5 samples (replicates) of each source will be taken from each area. Where necessary, samples of species may be stratified into different size classes (juvenile, sub-adult and adult) to take into account possible changes in diet with increasing age (*viz*. size; often neglected in stable isotope studies, may show a dependence of larval/juvenile stages on a specific carbon source, loss of which may be very detrimental). Each size class will be replicated (n = 5). Using this approach, sampling will not exceed 500 individual samples for C and N isotope analysis from Roebuck Bay and Eighty-mile Beach (total = 1000).

3.2 Artisanal food items

Both Roebuck Bay and Eighty-mile Beach are in daily use by Traditional Owners. Traditional owners inherently possess a great knowledge of the ecology of the systems, understanding when and where specific species of subsistence value may be collected. Species such as mud crabs, ‘cockles’, various other bivalves and gastropods play an important role in the culture and food resource of Aboriginal people using the areas. Traditional Owners will be engaged to assist will the collection of subsistence food items, so that food web models may be constructed to directly address these important resource.

3.3 Seasonality

Loneragan *et al*. (1997) sampled a tropical estuary in the Gulf of Carpentaria (Northern Australia), where there is very marked seasonality, with 80-90% of annual rainfall occurring in the summer wet season. The study demonstrated seasonal ‘changes’ in carbon signature of penaeid prawns. This was interpreted as a wet season-induced movement of juvenile prawns from mangrove creeks into coastal seagrass beds, giving a combined seagrass/mangrove signature in samples from the seagrass beds. Seasonality is not expected to be a significant issue at Roebuck Bay and Eighty-mile Beach since the benthic fauna is relatively sessile and neither areas is significantly affected by riverine flows.

3.4 Sample collection

All sampling is to be coordinated by AW Storey, in association with logistical support from G. Pearson and under taxonomic direction from M. Lavaleye. Sampling must be well coordinated, using standardised approaches because:

a.) it is important to collect as many sources as possible - especially the "inconspicuous” sources that may not be obvious to the inexperienced, but are often very important to the food web. Without samples of these sources it may not be possible to identify primary carbon sources and interpret energy flow,

b.) it is vital that clean and "pure" samples of different sources are obtained. For example, a mixed sample of algae and detritus will not be informative,

c.) proper handling, identification, labelling and storage of the samples is critical, and finally,.

d.) taxonomic expertise is necessary to identify and isolate different benthic taxa, providing sufficient biomass (several mg dry mass) for subsequent analysis.

Mangroves will be sampled by hand-picking new-growth leaves, herbivorous crabs and grazing molluscs will be collected by hand, mangrove detritus will be isolated by elutriation of mangrove surficial sediments. Tissues (dorso-lateral muscle) of fish (e.g. mudskippers/gobies, fish larvae) will be dissected under clean-room conditions from fish caught in nets and/or by hand. Penaeid prawns will be collected by nets. Different taxa of benthic invertebrates will be collected from grab samples. Benthic filamentous algae will be removed from surficial sediments under low-power binocular microscope or by separation on a light gradient. Size-specific plankton nets will be used for separating phyto- and zooplankton (e.g. 53 μm and 110 μm nets). Depuration of gut contents of whole animals to be analysed (e.g. grazing molluscs and/or crabs) is recommended to avoid contamination.

All samples will be frozen on site, and transported to the University of Western Australia for pre-processing (sample drying, grinding, and weighing) and subsequent isotopic analysis using standard mass spectrometric techniques, which utilise calibration against internationally-accepted isotope ratio standards.

4 Budget

Item	Unit	no. units	cost / unit	net cost
Field Component				
Airfare to Broome (rtn) AWS, ML & GP	flight	3	$850.00	$2,550.00
Airfare Netherlands to Perth - ML	flight	1	$2,500.00	$2,500.00
Traditional Owners - assist with collecting	days	20	$200.00	$4,000.00
Vehicle hire (4x4)	weekly hire	3	$526.00	$1578.00
Fuel for vehicle (~ 3000 km @ 7 km/l)	litres	430	$1.20	$516.00
Hovercraft hire	monthly hire	1	$5,000.00	$5,000.00
Excess baggage/freight	volume	1	$600.00	$600.00
Salary - ML	monthly rate	3	$5,000.00	$15,000.00
Salary - GP	Daily rate	20	$215.00	$4,300.00
Accommodation – Broome Bird Observ.	Daily rate per person	60	$25.00	$1,500.00
Accommodation - in Perth - ML	daily rate	6	$150.00	$900.00
Food etc in Broome	Daily Rate	60	$30.00	$1,800.00
				$40,244.00
Sample processing				
C & N sample prep. – clean/dry/grind/wt	Per sample rate	1000	$20.00	$20,000.00
C & N isotope analysis - Mass spec.	Per sample rate	1000	$30.00	$30,000.00
Incidentals (bags, collecting equipment)	General	1	$250.00	$250.00
				$50,250.00
Reporting				
Data processing, analysis and report prep.	AW Storey – 20% FTE Senior Lecturer	0.2	$76,000.00	$15,200.00
Data entry	daily rate	3	$300.00	$900.00
Report production (copy, plates, binding)	misc	1	$300.00	$300.00
				$16,400.00
	Total (excl GST)			**$106,894.00**
	GST			$10,689.40
	Total (incl GST)			**$117,583.40**

AWS = Andrew Storey, GP = Grant Pearson, ML = Marc Lavaleye

5 Reporting

The data from the study will be interpreted in terms of carbon and nitrogen signatures of primary producers, and primary, secondary and higher consumers identifying dominant energy sources, and trophic relationships for selected/available species of benthos, fish, crustacea and molluscs. These data will be integrated with any existing food web information. Replication will allow comparisons of food web structure, carbon sources and trophic position for individual species.

Once results for all samples have been collated, the possible contributions of different sources (i.e. riparian vegetation, mangroves, seagrass, estuarine algae and marine plankton) to the assimilated carbon in primary consumers will be determined using a simple mixing model (Loneragan *et al*., 1997):

$$P_A = (\delta^{13}C_{consumer} - f - \delta^{13}C_{sourceB}) / (\delta^{13}C_{sourceA} - \delta^{13}C_{sourceB})$$

where P_A = proportion of source A; and f = isotopic fractionation (‰). In all cases, an isotopic fractionation (f) of 0.2 will be used at each trophic level (see France, 1996). The mixing model calculates the contribution of each primary source assuming only two sources are contributing to the isotopic signature of a consumer. By using different end points, the range of possible contributions of different primary sources can be estimated. The likely contributions of primary consumers to the assimilated carbon of secondary consumers will also be determined using this approach.

Stable nitrogen isotope values will be used to determine the trophic position of consumers. These approaches will be applied specifically to sources of artisanal value to determine the importance of different primary sources to these food items.

Other analyses to be performed will include comparison of the food web structure of Roebuck Bay to Eighty-mile Beach. This will be undertaken using analysis of variance (ANOVA) to test for differences in delC signature of sources (producers and consumers) that occur in both estuaries to detect significant shifts that may be related to tailings disposal. Food webs will be constructed for each system.

Within individual species, changes in carbon and nitrogen signatures with body size will be investigated using ANOVA and regression analysis to look for possible dietary (viz. carbon source) switches with development. This is particularly important in identifying early life stages that may be reliant on a specific source, especially if the source is particularly susceptible to mine impacts.

Finally, critical links in the food chains supporting species that represent most value to the artisanal fishery (i.e. those that are most abundant and most easily caught) will be identified and discussed.

6 Schedule

The following timetable is proposed:

Activity	Date
Complete field work	???
Complete C & N analysis	???
Submit main study final report	???

The exact details of the program schedule will be finalised following discussions with participants.

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