MATERIALS AND METHODS

Sample Collection

The standard sampling procedure within each station, or location (note that station and location will be used interchangeably), was to randomly select three sites and to collect replicate samples from each site. The number of replicate samples varied between sediments and biota, between species and, sometimes, between locations and sites. Sample collection for sediments and biota as indicator organisms or species was conducted during two seasons in the Torres Strait: one at the end of the dry or trade-wind season (pre-monsoon) and the other following the monsoon season (post-monsoon). Protocols followed during sampling are detailed in the accompanying report (Gladstone (ed.) 1993).

Sediments

The sampling stations or locations from where sediment samples were collected are presented in Figure 1. The locations of sampling stations, in coordinates, were pre-determined and their positions identified on site using Global Positioning System (GPS) navigation. Each sampling station was represented by an area defined as a square placed centrally over the station coordinates. Each side of the square had a length of 200 metres. Sampling sites were similarly located using GPS navigation. Pre-monsoon sampling was carried out over the period 15-25 September, 1991 while post-monsoon sampling was carried out over the period 4-13 March, 1992.

Sediment samples were collected using a number of techniques. These included a specially modified Smith-McIntyre grab made from high grade stainless steel, polypropylene hand-corers and a gravity corer. The aim was to determine the most effective means of sampling sediments and ensuring that the fine surface material was not lost. The gravity corer proved not to be successful in the sometimes relatively shallow water and sediments of varying levels of consolidation, and its use was abandoned. Both grab and diver hand-core samples were collected from three locations (S10,S2 and S5). As sampling at a single station often required several hours, replicate samples were necessarily collected over sometimes quite considerable variation in current strength and direction. Station means therefore represent the integration of values over a significant portion of a flood-ebb tidal cycle. Two locations, S10 and S2, were sampled using both techniques on two occasions during the post-monsoon season

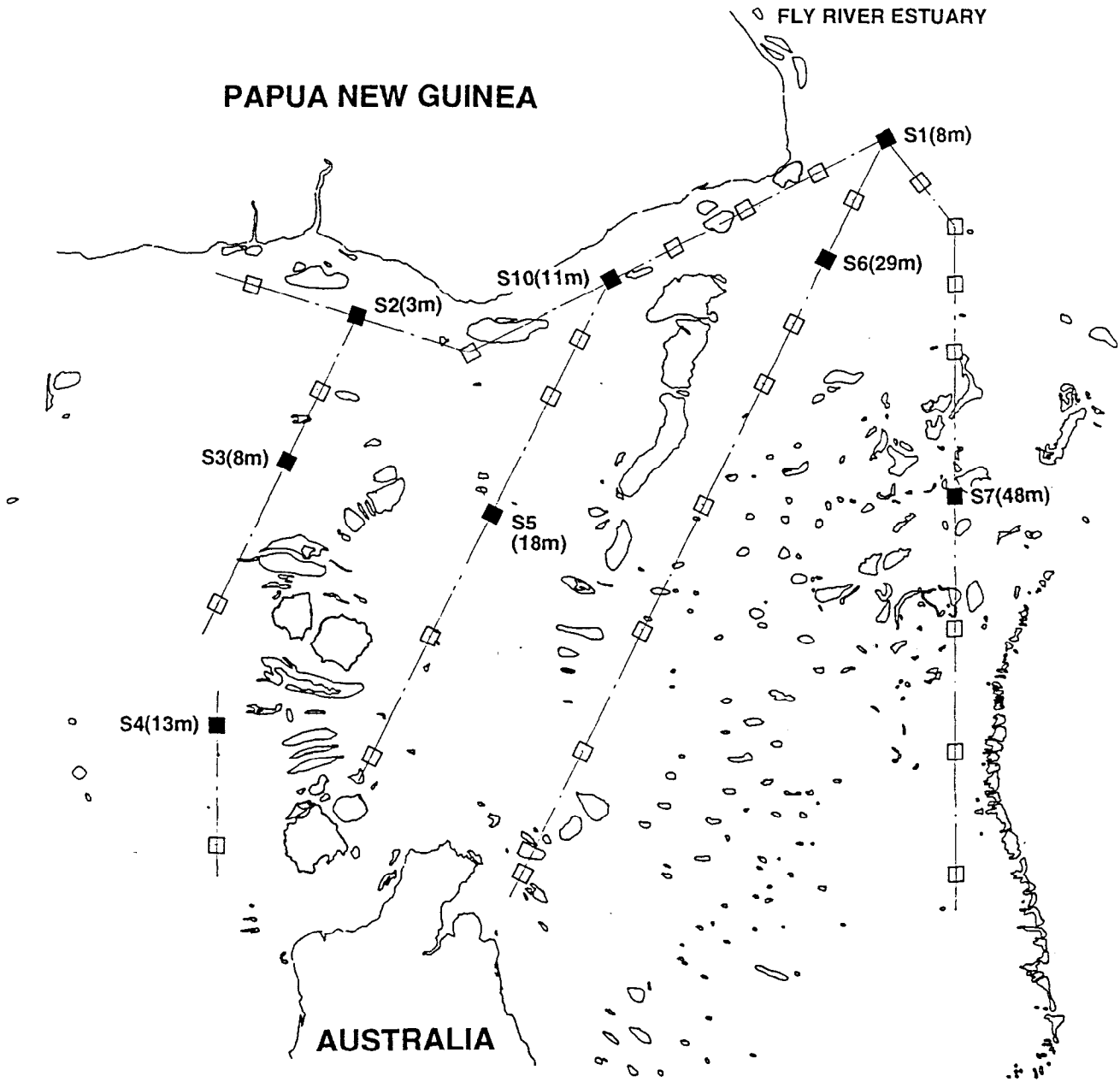

Figure 1. Map of the Torres Strait and northern Great Barrier Reef identifying the location of sampling stations at which pilot study sediment samples were collected. The approximate water depth (in metres) is indicated in parentheses. The conceptual design of the scientific programme is illustrated by a series of transects. Scale: 1 cm = 18 km.

separated by five and six days respectively. These samples therefore represent variation over a spring-neap tidal cycle. On one occasion, the sampling area of station S10 was also increased to 2000 x 2000 metres.

Sediment sampling protocols and laboratory protocols are detailed in respectively. Gladstone et al (1993) and Waite and Szymczak (1991). Excess water from the grab was allowed to drain before the sample was deposited on a plastic container. The sample was then transfered to a laminar flow hood where the surface sediment of each grab sample, to a depth of approximately 5 cm, was sub-sampled using the hand-corers. The sub-samples from each grab were then combined to give a single replicate sample. The hand-core samples were collected by divers using SCUBA equipment. Two hand-core samples of the surface 5 cm of sediment were combined within a laminar flow hood to provide a single replicate sample. All sediment samples were then stored in acid washed plastic containers and stored within a freezer on board the M.V. Western Venturer.

Biota

The sampling stations or locations from where biota as indicator species were collected are presented in Figure 2. No species were found at all sampling locations as the stations correspond to a wide range of quite different habitats, from coral reefs surrounded by clear oceanic waters to coastal mangroves. The locations of sampling stations were pre-determined only in a general sense (i.e. to the level of reef or island). Preliminary surveys of the biota indicated where individual species could be found (if at all) in relative abundance. Pre-monsoon sampling was carried out over the period 8-27 October, 1991 while post-monsoon sampling was carried out over the period 8 April to 1 May, 1992. A listing of the species and locations from where samples as indicator species were collected during the two seasons is presented in Table 1. Some samples were not analysed for this report because of either insufficient funds for analysis or because difficulties in collecting and processing made them unsuitable for future indicators (eg. *H. ovalis*) A summary of each species suitability is presented in Gladstone (ed.) (1933).

Raine Is samples were collected by the TSBS, for the Raine Is Corporation and the Queensland Department of Primary Industries. Data used in this report from these samples can be found in Barry and Rayment (1992).

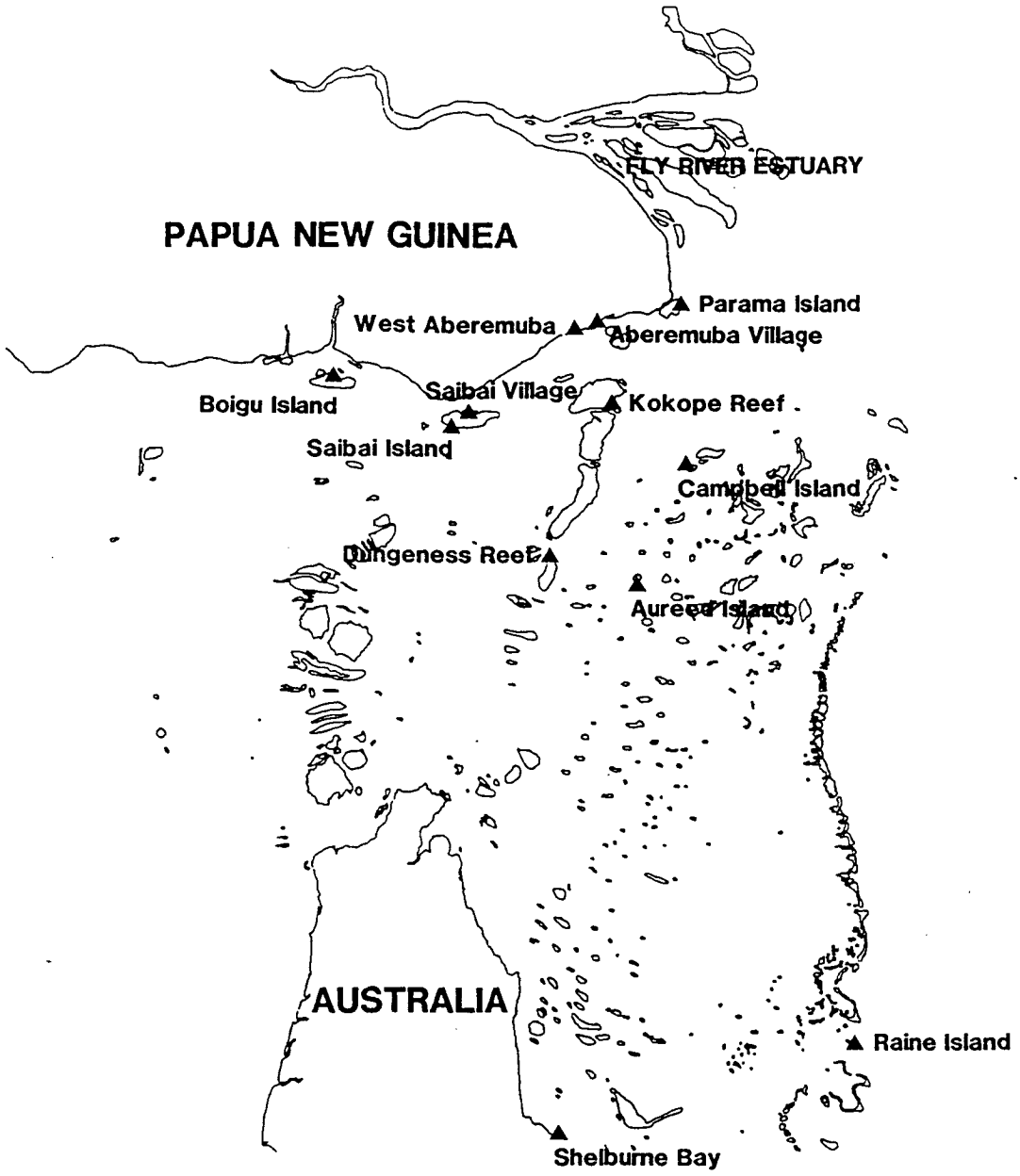

Figure 2. Map of the Torres Strait and northern Great Barrier Reef identifying the location of sampling stations at which pilot study indicator species were collected. Scale: 1 cm = 28 km.

Table 1. Listing of the species and locations from where samples as indicator species were collected during the pre- and post-monsoon seasons.

Species	Location (pre-monsoon)	Location (post-monsoon)
Thalassia hemprichii	Campbell Is, Dungeness Rf	Campbell Is, Dungeness Rf, Kokope Rf, Aureed Is
Thalassodendron ciliatum	Dungeness Rf, Kokope Rf	Dungeness Rf, Kokope Rf
Tridacna crocea	Aureed Is, Campbell Is,Dungeness Rf, Kokope Rf	Aureed Is, Campbell Is, Dungeness Rf, Kokope Rf
Tridacna maxima	Campbell Is, Raine Is	Aureed Is, Campbell Is
Pinctata margaritifera	Aureed Is, Campbell Is	Aureed Is, Kokope Rf, Campbell Is, Dungeness Rf
Hyotissa hyotis	Aureed Is, Kokope Rf	Aureed Is, Kokope Rf, Campbell Is, Dungeness Rf
Chama plinthota	Aureed Is,	Aureed Is, Kokope Rf, Campbell Is, Dungeness Rf
Trochus niloticus	Aureed Is, Dungeness Rf Kokope Rf, Raine Is	Aureed Is, Kokope Rf, Campbell Is, Dungeness Rf
Strombus luhuanus	Aureed Is, Campbell Is	Aureed Is, Campbell Is
Stichopus chloronotus	Aureed Is, Kokope Rf	Aureed Is, Kokope Rf, Campbell Is, Dungeness Rf
Lutjanus carponotatus	Aureed Is	Aureed Is, Kokope Rf, Cam pbell Is, Dungeness Rf
Polymesoda erosa	Boigu Is, Saibai Is, Shelburne Bay	Boigu Is, Saibai Is, Parama Is,West Aberemuba, Aberemuba Village
Halophila ovalis		Kokope Rf, Dungeness Rf

The bivalve molluscs *T. crocea*, *T. maxima*, *P. margaritifera*, *H. hyotis* and *C. plinthota* all burrow into or are firmly attached to the reef substratum and mostly needed to be dislodged using a hammer and chisel. The soft tissue of each of these species is protected by shell and was not damaged or touched in any way during the removal process. The reef fish *L. carponotatus* was collected either by line fishing (pre-monsoon sampling) or spearfishing (post-monsoon sampling). In the latter case, the stainless steel spears and tips were cleaned regularly with nitric acid and any rust removed. Fish were speared such that the viscera, eye/otolith area or tail muscle were not damaged. All other species were collected without resort to the use of metal collection devises.

Individual specimens were immediately washed in clean seawater to remove surface material, double bagged, labelled and stored in insulated containers with sealed plastic ice bricks. On return to the research vessel (M.V. Western Venturer or R.V. Sunbird) the shells were scrubbed in de-ionized water with a nylon brush, measured with plastic

callipers, individually double bagged and labelled. Only samples of *P. erosa* were frozen while the remaining species samples were maintained at approximately 4°C. Biota collecting protocols and laboratory protocols are detailed in respectively Evans-Illidge et al (1993) and Rayment and Murphy (1991.)

The sampling stations or locations from where biota as community fishery samples were collected are presented in Figure 3. A listing of the scientific, common and Islander names of the species collected is presented in Table 2. Samples were collected by community members using normal fishing techniques over a period of nine months from June, 1991. Samples were subsequently washed in clean seawater, double bagged, labelled and frozen. In most cases the fishes were envicerated before being bagged.

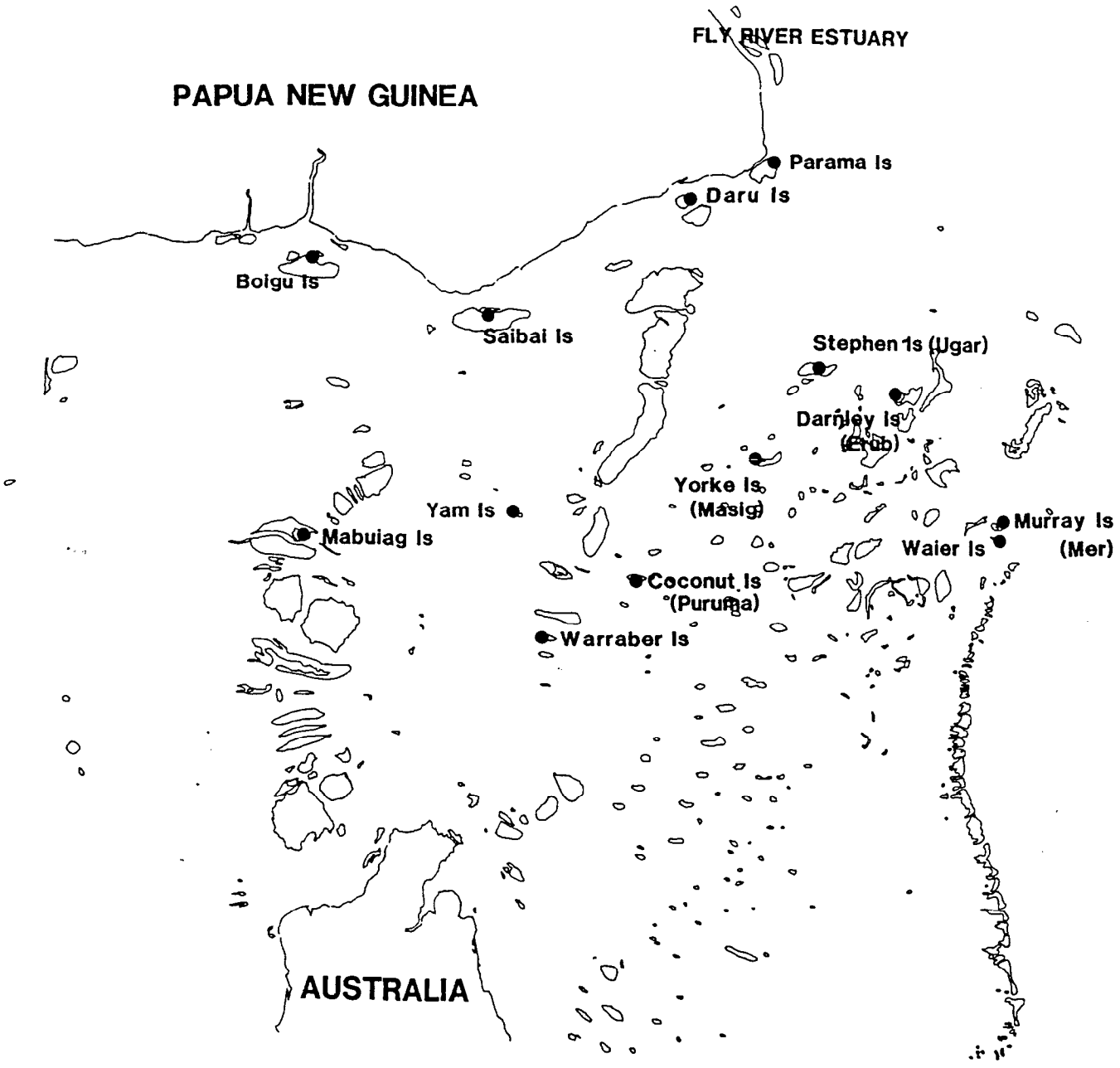

Figure 3. Map of the Torres Strait identifying the islands from which pilot study community fishery species were collected. Scale: 1 cm = 18 km.

Table 2. Listing of the species collected for the community fishery.

Scientific name	Common name	Islander name
Fishes		
Agrioposphyraena barracuda	Barracuda	?
Carangoides fulvogutus	Gold spotted trevally	Duger
Choerodon schoenleinii	Black spot tusk fish	Woona
Epinephelus fasciatus	Footballer cod	?
Epinephelus quoyanus	Longfin rockcod	Garom
Harengula ovalis	Murray Is. sardine	Ari Ari
?	Sardine / hardyhead	Kos
Lates calcarifer	Barramundi	Barramundi
Lethrinus fletus	Grass sweetlip	Snapper
Lutjanus carponotatus	Stripey	Theur, Kauerr, Tanab
Plectropomus leopardus	Coral trout	Withi
Plectropomus maculatus	Barred-cheek coral trout	Withi
Plectorhynchus flavomaculatus	Netted sweetlip	?
Plectorhynchus pictus	Morwong	Peku, Wapa fish
Psammoperca waigiensis	Reef barramundi	Night fish
Scomberomorus commerson	Spanish mackerel	Dubui
Siganus guttatus	Golden lined spinefoot	Erar, Parrsa
Siganus spinus	Black spinefoot	Kibim
Crustaceans		
Panuliris ornatus	Painted crayfish	Kaiar
Panuliris versicolor	Crayfish	Kaiar
Molluscs		
Hippopus hippopus	Horse's hoof clam	Clam shell (?)
Lambis lambis	Spider shell	Arsoorr
Polymesoda erosa	Mangrove cockle	Akul, Eepa
Strombus luhanus	Red-lipped stromb	Kirith
Other		
Chelonia mydas	Green turtle	Waru
Dugong dugon	Dugong	Dhangal

Sample Preparation

Sediments

The frozen samples were transported to a selected analytical laboratory where two representative portions of each sample were separated. One portion was wet sieved and separated into >2 mm, 2000-200 μm, 200-63 μm and <63 μm size fractions. The >2 mm size fraction was removed by wet sieving from the second portion before being dried at 50°C in a stainless steel forced draft oven. Removal of the >2 mm size fraction was achieved using plastic sieves and sea water (pre-monsoon samples) or ultra pure water (post-monsoon samples). In the latter case, the washings were evaporated and included in the <2 mm portion. Samples were subsequently ground to <50 μm particle size using a 'shatter box' grinding mill equipped with a stabilized zirconium grinding head and stored in dry, sealed acid washed plastic containers.

Biota

All biota were transported to the Horn Island Research Station where the initial sample preparation took place. Cleanroom conditions were adhered to and all dissections took place within a certified laminar-flow hood. Dissections were performed using high quality stainless steel instruments on acid washed polypropylene cutting boards. The tissue samples were then placed in acid washed plastic vials, labelled, frozen and transported to Brisbane for freeze-drying and storage prior to analysis.

Leaf material, including a minimal amount of epiphytic algae, constituted the sample for seagrasses. The inclusion of some epiphytic algae was unavoidable in spite of the field collection team members' attempts to avoid plants which were heavily fouled. The kidney of *T. crocea* and *T. maxima* constituted the primary sample, although the adductor muscle and visceral mass were also removed. The Tridacnid clams, in particular, were maintained in a fresh condition in order to avoid contamination of other tissues by the metal-rich kidney during or after freezing. Body muscle tissue constituted the sample for the gastropod molluscs *T. niloticus* and *S. luhuanus*, while muscle tissue was also taken from the tail of the reef fish *L. carponotatus*. However, the liver provided the primary sample for *L. carponotatus*. The whole soft tissue constituted the prepared sample from *P. margaritifera*, *H. hyotis*, *C. plinthota* and *P. erosa*.

Chemical Analysis

Sediments

Dried sediment samples were analysed for a suite of major and trace elements, as well as calcium carbonate and organic carbon content. Samples for aluminium (Al), calcium (Ca), chromium (Cr), iron (Fe), magnesium (Mg), manganese (Mn), nickel (Ni), silica (Si) and zinc (Zn) determination were pelleted using the pressed powder technique and analysed by X-ray fluorescence. Samples for arsenic (As) and selenium (Se) determination were digested using nitric:perchloric:sulphuric acid (13:1:1) and analysed by hydride generation ICP. Mercury (Hg) determination was also by hydride generation ICP while the digestion/extraction procedure involved nitric: hydrochloric acid (6:2) in a 2 hour steam bath. Cadmium (Cd) and lead (Pb) determinations involved a nitric:hydrochloric acid (6:2) digest in a 2 hour steam bath and analysis by graphite furnace AAS, while cobalt (Co) and copper (Cu) determinations involved a nitric:perchloric:sulphuric acid (13:1:1) digest and analysis by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). Standard reference material (BCSS1 - marine sediment) was routinely analysed together with sediment samples and the results maintained within certified values.

Biota

Chemical analysis of most samples was carried out using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for aluminium (Al), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), selenium (Se) and zinc (Zn) concentrations in the tissues of all samples. The concentrations of silver (Ag), strontium (Sr) and uranium (U) were also determined within the kidney of *T. crocea* and the post-monsoon samples of kidney from *T. maxima*. The freeze-dried sample (approx. 0.2 g) was microwave digested using double distilled nitric acid (HNO_3) prior to chemical analysis. Flame and graphite furnace atomic absorption spectrometers (Varian) or ICP-AES were used for *P. margaritifera* samples, but otherwise mainly for some quality assurance samples. The FSIS system for reporting concentration results has been adopted. Standard reference material (DORM-1 - dogfish muscle and TORT-1 - lobster hepatopancreas) was routinely analysed together with biota samples and the results maintained within certified values.

Quality Assurance

All possible precautions were taken in the field and during preparation to avoid contamination of samples. Neoprene diving gloves were worn during collection, while plastic surgical gloves were used to handle any material that was to be analysed directly, or was likely to be taken into the clean room facility on Horn Island, or come in contact with material that was to be analysed. All collecting and measuring equipment was acid washed in 10% nitric acid and thoroughly rinsed with de-ionized water. Sub-samples of the plastic bags that were used had been tested for metal concentrations to ensure that they would not be a source of contamination.

Participating laboratories all took part in the 1991 and 1992 U.S. National Oceanic and Atmospheric Administration (NOAA) National Status and Trends Quality Assurance Programme. Within that programme, the laboratories demonstrated that they had the ability to produce accurate and repeatable analytical results for a wide range of trace metals. The analysis and reporting of results from standard reference materials was carried out on a regular basis. In addition, approximately 10% of samples were sub-sampled and sent to a second and sometimes third laboratory to ensure that the results were reliable. These results (not presented) showed good agreement.

Statistical Analyses

Preliminary analysis of the data showed that the variances were not always homogeneous. A natural logarithmic transformation was therefore applied to all concentration data ($\log_e$(concentration+1)) prior to statistical analysis to ensure that the variances were made homogeneous and normally distributed.

A nested (hierarchical) Analysis of Variance (ANOVA) was used to assess differences in metal concentrations among locations (sampling stations) for the three indicator species. Where significant differences existed among three or more locations from where samples were collected, Tukey's HSD test was used to determine which sampling stations were different from each other.

The concentrations of some metals (e.g. Cd and Hg) in certain samples were, on occasions, found to be below the analytical detection limit. In this situation, use of the detection limit for the actual concentration would clearly bias the results upward. A more realistic approach, and one which has been widely adopted (e.g. Burdon-Jones & Denton, 1984a; Ward et al., 1986; NHMRC, 1991), is to assume that the actual value

lies midway between the detection limit and zero. It is this approach which was taken for the present data analyses.

Normalization procedures

Many trace metals are primarily associated with particle surfaces and differences in concentrations between locations, or even sites within locations, may result simply from differences in the particle size distribution of the sediment samples. Two approaches to the normalization of trace metals in sediments are frequently used: granulometric and geochemical methods. Both are investigated in this study. NOAA (1988) used the former method and normalized concentration data by dividing the raw concentration by the fraction by weight of sediment particles (from the same location) less than 64 mm. NOAA note that this is equivalent to assuming that there are no trace metals associated with the coarser sediment size fractions. Clearly this is not strictly true and the method biases the concentrations upwards where the coarse fraction is very large. Consequently, NOAA do not use this method to compare locations where the sediment contains less than 20 percent fine-grained material.

Geochemical normalization is thought to be better than granulometric methods because it compensates for the mineralogical as well as granular variablility in trace metal concentrations in sediments (Loring, 1991). Aluminium concentration has been widely used as a geochemical method to normalize many, but not all, trace metal concentrations with respect to granular variability because it is a major constituent of the fine-grained aluminosilicates with which trace metals are associated in tropical waters (e.g. Windom et al., 1989; Loring, 1991; Zubir, 1992). This technique also suffers from a similar bias to the granulometric method where aluminium concentrations (i.e. the fine-grained sediment fraction) are very low, as the denominator is then small relative to the numerator. Loring (1991) identifies the following requirements for effective geochemical normalization: (1) significant granular variations should occur between sediment samples; (2) a statistically significant relationship should exist between (a) the normalizer and grain size distribution; and (b) metal content and the normalizing element; and (3) it should be possible to provide accurate and precise analysis of the metal of interest and normalizing element.

Power analysis

Of the metals for which concentration data are available, six have been selected as being of particular importance. These are arsenic (As), cadmium (Cd), copper (Cu), mercury

(Hg), selenium (Se) and zinc (Zn). Arsenic, cadmium and selenium were all found to be in high concentrations in a range of food items that are consumed by Torres Strait Islanders (Dight, 1992 and reported here), while copper, mercury and zinc are all known to be associated with Fly River discharge or mining operations within the catchment (Baker, 1991; Eagle & Higgins, 1991; Ross, 1991).

The power of the sampling program to detect a specified increase in metal concentration was derived from formulae provided by Cohen (1988) and Mapstone (pers. comm.) for medium variability of a range of means. For any particular trace metal, the range of means was the difference between the maximum and minimum mean value taken from the various sampling stations. Within population standard deviation was estimated from the sites Means Square value from the ANOVA results of the pilot study data. An adjusted n value (n') was calculated from the formula in Cohen (1988) for fixed main effects in factorial designs. Power estimates are based on a range of means for four sampling stations.

The selected number of sites for each species was based on the time taken to collect and process samples, and the likely availability of the species of interest. For example, it would be possible for two teams of three persons to collect and process samples of *T. crocea* from a maximum of eight sites in one day. A greater number of sites would also be likely to result in some overlap among sites.