5. METHODS

5.1 Water Sampling

Water samples were collected with Niskin bottles through the full depth of the water column or upper 300 m at deep stations seaward of the shelfbreak (2-10 sampling depths per hydrocast). Sampling depths were spaced evenly throughout the water column or mixed layer. The Niskin bottles were acid-soaked (ca. 1% AR grade HCl) prior to each cruise and stored with unused sample water between hydrocasts. At most stations, vertical profiles of salinity and temperature were recorded with a conductivity/temperature/depth (CTD) instrument (either the AIMS constructed CTD or an EG&G SCTD). The CTDs were calibrated each cruise against discrete *in situ* temperature measurements made with reversing thermometers and salinity samples collected on the same CTD cast or a hydrocast immediately following the CTD cast. In the event of a CTD malfunction, water temperatures were measured with reversing thermometers (two protected and one unprotected per Niskin bottle) and discrete water samples were collected for salinity measurements. Discrete salinity measurements were made ashore with a Plessey 6230N salinometer calibrated against IAPSO standard seawater.

5.2 Underwater and Diurnal Light Measurements

Subsurface irradiance profiles were measured at a number of stations with a Biospherical QSP-200 underwater scalar (4 π) irradiance sensor. Surface irradiance was measured concurrently with a QSR-240 reference sensor. The underwater sensor was lowered in 1 to 5 m steps with surface and subsurface irradiance and wire angle being recorded at each depth stop. To the extent possible, light readings were not taken when clouds or haze obscured the sun. Underwater light values are presented as the percent of surface irradiance.

Diurnal time series of surface irradiance were measured on days when phytoplankton productivity measurements were made. Between 1983 and 1985, light readings were taken at half-hourly intervals by a remote weather station located at Rib Reef (18° 24'S 146° 53'E), somewhat to the south of the Tully box. The weather station was fitted with a LICOR LS-90 (2 π) quantum sensor. Daily fluxes were calculated by trapezoidal integration. For the 1990 Cairns box productivity measurements, the analog output from the ship-based surface reference sensor (Biospherical QSR-240) were digitized at one second intervals from 0400 to 2000 local time by a JED data logger (JED Microprocessors P/L, Boronia, Vic.). Individual readings were averaged over one minute intervals and stored in the logger until downloaded to a microcomputer each day. The 4 π light collecting sphere of the Biospherical QSR-240 quantum sensor is relatively insensitive to ship motion. The sensor was empirically calibrated at various natural sun angles on a clear day against a vertically pointing LICOR LS-190 quantum sensor (2 π geometry).

5.3 Zooplankton Sampling

Water column zooplankton stocks were sampled at most hydrographic stations with duplicate near-bottom to surface vertical net hauls. The net (0.5 m diameter, 73 µm mesh net) was equipped with a Rigosha flow meter to estimate the volume of water filtered. Zooplankton samples collected on individual net tows were split once with a Folsom splitter. One half of the sample was then filtered onto a pre-weighed disk of 73 µm mesh netting. The other split was preserved in formalin for archiving and later microscopic examination if warranted. The filtered disk of zooplankton was frozen until further processing could take place.

Filtered zooplankton biomass samples were dried for several days at 60°C. The zooplankton and netting disk were weighed together and the zooplankton dry weight calculated by

difference. The carbon and nitrogen composition (as % of dry weight) was determined for a number of samples collected over several years (Figure 11) with a LECO CHN analyzer standardized against AR grade EDTA.

5.4 Nutrient Subsampling

Water samples were collected using single (unreplicated) casts at individual sites. Shortly after collection of the water samples, subsamples of seawater were dispensed from each Niskin bottle into a new or acid-soaked 60-ml plastic syringe for dissolved nutrient analyses. The water was then filtered through a Sartorious Minisart-N® cellulose acetate filter cartridge (0.45 µm pore diameter) directly into replicate (2X for inorganic nutrients plus 2X for dissolved organic nutrients, if collected) acid-washed plastic test tubes or plastic scintillation vials with screw cap closures. In most cases, one filter cartridge could be used for all samples at a single station without significant clogging. Approximately 10 ml of seawater was flushed through the filter before subsamples were dispensed into the sample tubes. The tubes/vials were frozen immediately in a clean laboratory freezer. Care was taken not to over-fill sample tubes/vials or to tip them while they were being frozen.

Duplicate 250 ml subsamples of water from each sampling depth were filtered onto precombusted (> 400°C overnight) Whatman GF/F filters (25 mm diameter, for particulate nitrogen (PN) and phosphorus (PP) analyses. The filters for each analysis were folded and stored frozen in envelopes of pre-combusted aluminium foil. The stated pore size for GF/F filters is 0.7 µm (Grasshoff, 1983), but in practice, they behave in a manner similar to membrane filters with 0.45 µm pores (Furnas, unpubl. data).

Duplicate 100 ml subsamples of water from each sampling depth were filtered onto Whatman GF/F glass fiber filters for chlorophyll analyses (Parsons et al., 1984). At a smaller number of stations additional 100 ml aliquots were filtered onto polycarbonate membrane filters (25 mm diameter, 10 and 2 µm pore diameter - Nuclepore) to assess the contribution of pico (< 2 µm size fraction) and nanoplankton (2-10 µm size fraction) to total community chlorophyll. Small amounts (a few drops) of $MgCO_3$ suspension were added to the water samples during filtration and the filters were stored frozen in aluminium foil packets until analysed.

From November 1989 onward, duplicate 1-litre subsamples of water were filtered onto pre-weighed Nuclepore filters (47 mm diameter, 0.4 µm pore diameter) for gravimetric suspended solids determinations. The filters were sucked dry, but not rinsed with deionized water, and stored in pre-combusted scintillation vials until processed further. The mass of salt (mean = 0.23 mg l^{-1} equivalent) associated with the small amount of seawater remaining on the filters was estimated by covering clean filters with small volumes (2-3 ml) of filtered seawater, then sucking them dry and processing them in parallel with sample filters.

5.5 River Sampling

Dissolved and particulate nutrient samples were collected periodically from most of the major rivers (Herbert, Murray, Tully, So. Johnstone, No. Johnstone, Russell, Mulgrave, Barron) along the north Queensland coast which discharge directly into or immediately downstream of the two study boxes. No water samples were collected from the Daintree River. All rivers were sampled at least four (4) times per year from 1988 onward, and some, particularly the Herbert, Tully and So. Johnstone, at frequencies up to several times per day during flood events by individuals (A. Mitchell, H. Sturmey) or through cooperative arrangements with government agencies (Queensland Department of Primary Industries, Bureau of Sugar Experiment Stations (BSES), Cairns City Council). Water samples were generally collected at mid-channel from road bridges that crossed the river above the level of saltwater intrusion. Samples were taken

near the surface with 1-2 litre weighted glass or plastic bottles that filled slowly as the sampler sank. Water samples were processed immediately in the field, where possible, or at nearby laboratory facilities soon after collection. Storage and processing temperatures were not greatly different from *in situ* temperatures. Dissolved nutrient samples were processed in a manner similar to the seawater nutrient samples. Because of the increased particle loads in the rivers, smaller volumes (25-100 ml) were filtered for PN, PP and suspended solids determinations. Only a small number of chlorophyll samples were collected. Processed river nutrient samples were stored in local laboratory freezers or in chests of dry ice until returned to AIMS for analysis. All water subsamples were taken in duplicate. Replicate samples were collected on a number of occasions to assess the level of variability associated with sampling and sample processing. Samples were also collected on north- and southward legs of sampling trips (1-2 days separation) to assess day-to-day variation in nutrient concentrations.

On four occasions during 1987-88, more detailed longitudinal sampling was carried out in the estuary of the Murray River. The intent was to establish: 1) lateral variability in nutrient concentration and speciation across sections of the lower river, 2) vertical variability within the water column and 3) changes in chemical speciation occurring within the estuarine sections of north Queensland rivers. On these occasions, water samples were collected with an acid-washed Niskin bottle from an inflatable boat.

5.6 Rainwater Sampling

Fifty-one (51) rainwater samples were collected over a four year period (1987-91). Most were collected at AIMS (Cape Cleveland), but samples were also taken at sea in the central GBR and northern Coral Sea. Rainwater samples were collected in either an acid-washed PVC cylinder fitted with a drain spout or a polyethylene collector made from a 2.5 litre reagent bottle (previously containing AR grade HCl). The samplers were mounted on either the ship's super-structure or an unobstructed corner of the AIMS building. The samplers were acid-soaked prior to cruises or periods of use and were kept covered with a tight fitting lid or plastic bag when no rain was falling. Regardless of the sampler used, replicate subsamples were taken whenever possible. The samples were stored frozen until analysed in acid-washed plastic test tubes or scintillation vials. No record was kept of the amount of rainfall associated with particular rain collections or the total volume collected. Rainfall over the GBR sections considered herein was estimated from historical records of the Bureau of Meteorology for Low Isles, Green Island and Fitzroy Island (J. Lough, pers. comm.).

5.7 Analytical Procedures

Inorganic nutrient (NH_4, NO_2, NO_3, PO_4, $Si(OH)_4$) concentrations were determined by standard wet chemical procedures (Treguer and Le Corre, 1975) implemented on a Skalar 20/40 segmented flow analyzer (adapted from Ryle et al., 1981). Frozen samples were thawed to room temperature immediately prior to analysis. Baselines were run against an artificial seawater (for marine samples) or deionized water (for river and rain water samples). Beginning in 1991, river water samples were run first for nitrogen and phosphorus species, then allowed to stand for several days at room temperature to allow the silicate polymers produced by freezing to re-equilibrate to monomeric or dimeric species measurable by normal colorimetric methods. In most recent samples separate subsamples were collected for silicate analyses and stored at room temperature. Dissolved organic nitrogen (DON) and phosphorus (DOP) concentrations were calculated by difference after oxidation of the samples with high intensity UV light (Armstrong et al., 1966; Walsh, 1989). Seawater samples were oxidized for ca. 7-8 hours while river water samples were oxidized overnight (ca. 14 hours). Irradiated water samples were re-frozen until the re-analysis of DIN and DOP was carried out. Nowicki (1986) has shown that

this results in negligible losses of DIN and DIP. Total nitrogen in the irradiated samples was calculated as the sum of NO_2+NO_3+NH_4 (Walsh, 1989).

Particulate nitrogen concentrations were determined by high temperature combustion of the organic matter collected on glass fibre filters using an ANTEK Model 707 Nitrogen Analyzer. The filters were freeze dried before analysis. The sample was inserted into the primary combustion oven of the analyzer and ramp heated (150°C min^{-1}) to 650°C in an oxygen-argon atmosphere. The combustion gases were swept into an oxygenated secondary oven (1050°C) with a ceramic catalytic surface. The nitrogen oxides produced (NO_x) were dried and quantified by chemiluminescence after mixing with ozone in the detector. Initially, sample filters were combusted in pre-ashed sample boats pressed from heavy gauge aluminium foil. From mid-1990 onward, pre-combusted brass punchings used to make .22 calibre rimfire cartridge cases (Winchester Australia) were used as sample boats. A semi-automated sample loader that could be pre-purged with argon and hold up to 30 samples in cartridges was fabricated by the AIMS workshops. The nitrogen analyzer was initially standardized with crystals of AR grade EDTA weighed out on a micro-balance. After the introduction of the brass cartridges as sample boats, standards were made up by blotting small volumes (ca. 10-100 µl) of an EDTA standard solution onto a glass fibre filter pre-combusted in a cartridge casing. The standard samples were oven dried and run in a manner similar to field samples. At the detector attenuation setting normally used, neither the brass cartridge cases or pre-fired filters produced measurable N blanks. A correction for adsorption of dissolved nitrogen (organic and inorganic) to the sample filters and from water retained by the filters was made by blotting small volumes (ca. 1 ml) of filtered surface seawater onto 25 ml GF/F filters at sea, sucking them dry and processing them in parallel with sample filters. Measurable nitrogen in these wet filter blanks (WFB) was on the order of 0.25-0.3 µg nitrogen per filter.

Particulate phosphorus retained on glass fibre filters was determined colorimetrically (Parsons et al., 1984) after acid-persulfate digestion (e.g. Menzel and Corwin, 1965) of the organic matter on the filters (taking suggestions from Smith et al., 1981). Filters were placed in acid-washed glass scintillation vials with 5 ml of 5% w/v potassium persulfate. The persulfate was refluxed to dryness using an aluminium block heater holding 100 vials. Acid-washed cat's eye glass marbles were used as stoppers for the vials. Following the digestion, 5 ml of deionized water were added to each vial and the filter and salt residue resuspended and pulverized to dissolve all soluble material. The residue in the vials was cleared by centrifugation and the inorganic phosphorus determined colorimetrically in aliquots of supernatant. Organic and inorganic phosphorus standards were run with each batch of samples. In the case of sediments, weighed subsamples of agate ground sediment were placed in acid-washed scintillation vials, acidified with 25% v/v AR grade HCl and refluxed to dryness to remove all carbonates and extraneous acid. The residue was then redissolved in 5% persulfate and treated as above.

Chlorophyll on filters was determined fluorometrically after grinding in 90% (v/v) acetone (Parsons et al., 1984). The fluorometer (Turner Designs-005R) was standardized spectrophotometrically (Jeffrey and Humphrey, 1975) against extracts of pigments from exponentially growing cultures of the diatom *Chaetoceros simplex* (chlorophylls *a* and *c*).

Suspended solids concentrations were determined gravimetrically from the difference between loaded and unloaded filter weights after the filters were dried overnight at 80°C. Wet filter salt blanks were subtracted from the resulting weight.

5.8 Primary Production Measurements

Water column primary production and coupled nutrient demand in the Cairns and Tully boxes were estimated from the uptake of ^{14}C bicarbonate by phytoplankton (Furnas and Mitchell,

1987, 1989, unpubl. data). Subsurface water samples were collected with General Oceanics Niskin or Go Flo bottles. Surface samples were occasionally collected with acid-cleaned plastic buckets or with an acid and seawater pre-soaked Niskin bottle. Depending upon depth, up to six water samples were collected at depths with nominal mid-day *in situ* irradiance levels on the order of 100, 50, 30, 20, 8 and 4 percent of surface irradiance. Three to nine 250 ml polycarbonate bottles (pre-soaked with 0.2% Baker Instra-analysed HCl and deionized water) were filled with water from each sampling depth and spiked with 5 µCi (185 kBq) of radioactive bicarbonate. A third of the bottles from each depth were wrapped in aluminium foil as dark bottles. The bottles were incubated at simulated *in situ* irradiance levels (as above) corresponding to the depth of collection in seawater cooled deck incubators screened with neutral density shadecloth. At the end of the incubation, the samples were filtered onto either Whatman GF/F (0.4 µm pore diameter) or Nuclepore filters (2 and 10 µm pore size). After counting, the filters were placed in scintillation vials and the remaining inorganic carbon removed by addition of 0.1 ml of 1N HCl (Hitchcock, 1986). Radioactivity remaining on the filters was determined by liquid scintillation counting. Hourly primary production rates were calculated according to Parsons et al. (1984). Daily production was estimated by dividing the production measured during the experiment by the proportion of daily irradiance impinging during the ca. 4 hour incubation period. Nutrient demand was estimated by dividing the daily production estimated by the appropriate C:N (6.6:1) and C:P (106:1) ratios (Redfield et al., 1963) after converting the primary production estimate to a molar rate.

5.9 Frequency and Magnitude of Intrusive Activity off Cairns

Sea Data Temperature-Depth Recorders (TDR) were moored approximately 1 m off the bottom at shelfbreak sites adjacent to the entrances to Grafton Passage (seaward of Euston Reef: 56-58 m depth) and Trinity Opening (north of Norman Reef: 52 m depth). The instruments were deployed for periods of 5-7 months and were set to sample at intervals of 15-20 minutes. Two years of data were collected at each site. The instruments were calibrated in the laboratory to derive relationships between instrument digital counts and *in situ* temperature. The calibrations were checked in the field against CTD casts or discrete *in situ* temperature measurements using reversing thermometers.

As no extensive hydrographic sampling has been carried out during an intrusion event in the Cairns region, data from twenty-three (23) hydrographic sections sampled in Palm Passage between January, 1983 and October, 1987 (e.g. Figure 12) were used to derive empirical relation-ships between near-bottom temperature at the shelfbreak in Palm Passage and the calculated volume of intruded Subtropical Lower Water (SLW) on the shelf in Palm Passage. The volume of intruded SLW was estimated by a two-stage process which is summarized as follows. Water masses within the outer shelf of the GBR can be considered a mixture between three water types (Pickard et al., 1977): Lagoonal Water (LW), Coral Sea Surface Water (CSSW) and SLW (Figure 13). In the case of LW and CSSW water types, the temperature-salinity (T/S) characteristics on individual transects were taken as the means of temperatures and salinities between the depths of 10 and 25 m to minimize distortion due to near-surface diurnal heating effects or dilution by rainwater. Within the 10-25 metre depth stratum, temperatures and salinities are usually fairly constant. Over the course of the sampling program, a variety of T/S relationships were observed.

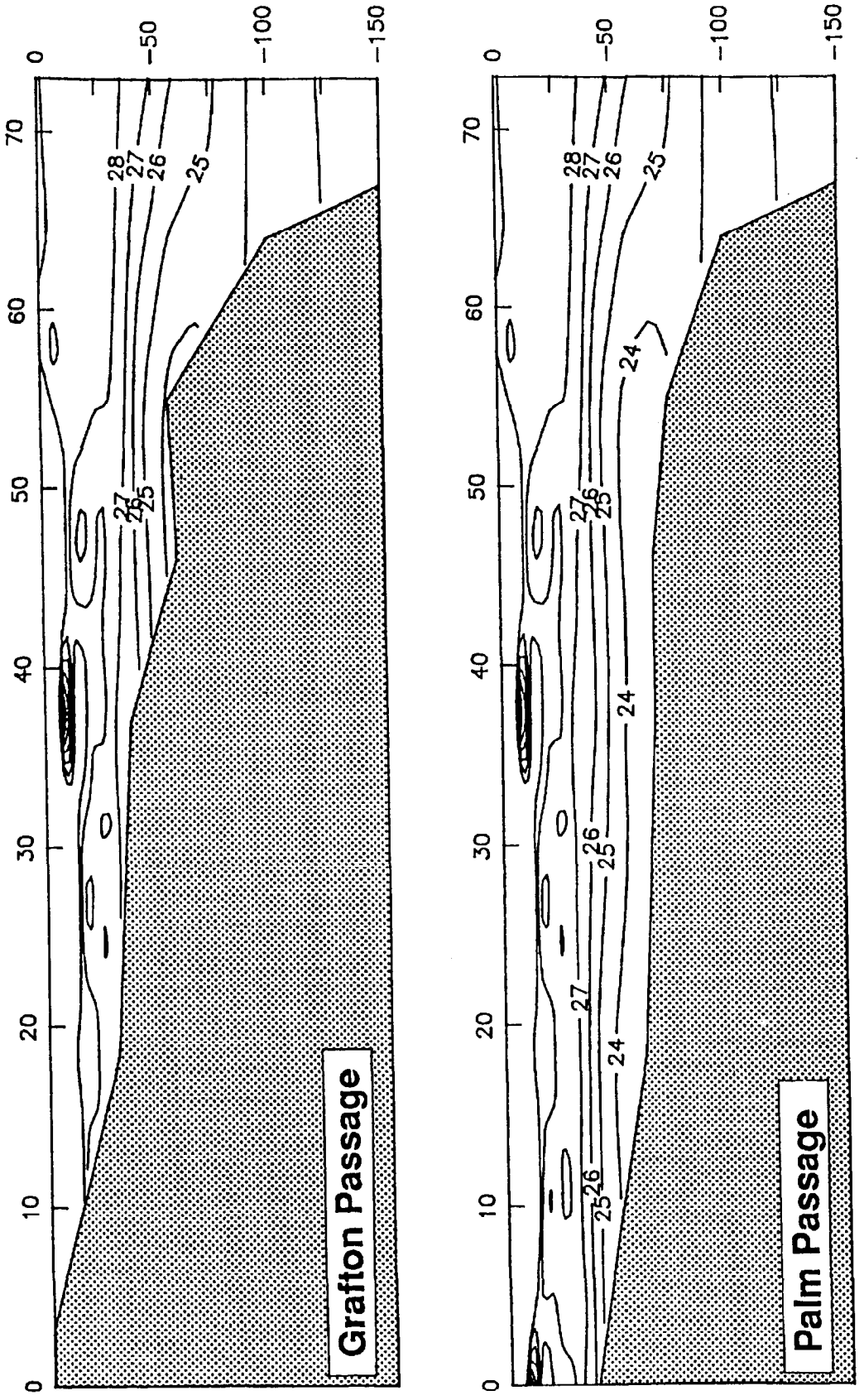

Figure 12. Bottom: Cross-shelf section through a cold-water intrusion into Palm Passage (19°S) during January 1983. Top: The same intrusion event masked with the bathymetry down the central axis of Grafton Passage.

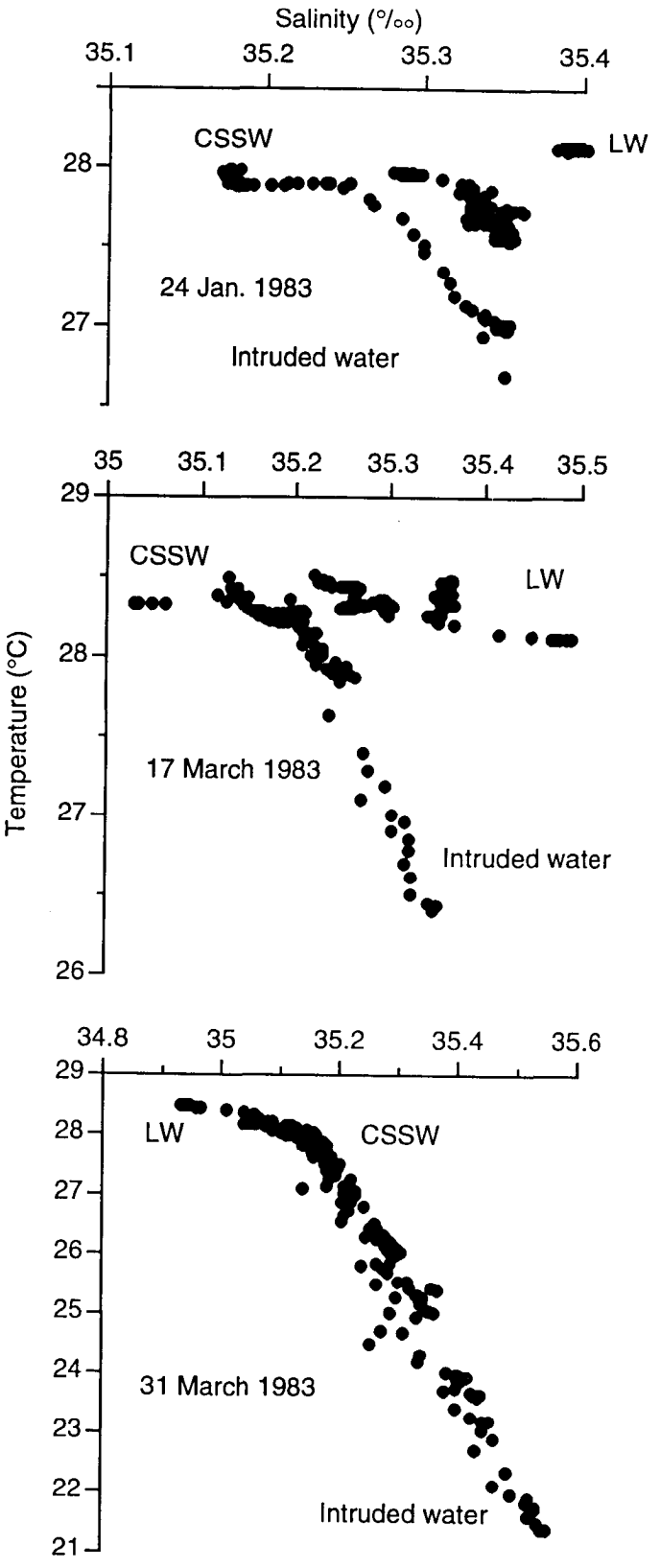

Figure 13. Temperature-salinity plots for water masses in Palm Passage on three occasions during the summer of 1983. Each T/S plot combines data from four stations spaced between the GBR Lagoon and the shelfbreak.

To estimate the volume of SLW in individual Palm Passage sections, the local salinity and temperature characteristics of surface water at a particular station were assumed to be derived from a conservative (linear) mixing relationship between CSSW and LW. The volume percent of intruded water at each 1 metre depth step at each station was then calculated from the T/S data assuming a conservative mixing relationship between the calculated "local" surface water T/S characteristics and the defined T/S character of SLW. Figure 14 shows two representative T/S plots from stations in the EAC seaward of the transect through Palm Passage. The seasonal changes in the temperature characteristics of CSSW are clearly evident, but the linear mixing relationships between CSSW and SLW in the profiles still apply. The calculated profiles of volume percent SLW were averaged between adjacent 1-metre depth increments and then horizontally between stations and finally multiplied by the distance between stations to calculate the volume of undiluted SLW at metre depth intervals between adjacent stations. Where the bottom intervened between stations, appropriate trapezoids were calculated for the depth increment. The total volume percent SLW for each transect was then numerically integrated.

To convert shelfbreak water temperature time series to estimates of SLW intrusion volumes in the Cairns and Tully boxes, a calculated "mean" bathymetry of the outer shelf for each box (Figure 15) was masked over each of the Palm Passage intrusions. The mean bathymetric shape of the outer shelf in the two boxes was derived from the cross-shelf transects used to calculate water volumes within each box. Figure 12 Top illustrates masking of a Palm Passage intrusion sampled in January, 1983 with a bathymetric section from the central axis of Grafton Passage. Because of the narrow width of the shelf in the Cairns box, the Palm Passage transect extended across the full width of the shelf. In the Tully box, the transect extended shoreward to the 10 m isobath. After the mean bathymetric transects were masked over each of the Palm Passage sections, the volumes of SLW remaining were integrated and plotted against the nominal shelfbreak water temperatures.

Temperature - nutrient relationships (Figure 16) determined at hydrographic stations located seaward of the shelfbreak in the EAC were used to calculate the mean nitrate (NO_3 - 4.77 μM) and phosphate (PO_4 - 0.64 μM) concentrations expected at the core of the SLW water layer. A similar inverse relationship also applied for silicate. Ammonia concentrations were near the limits of detection at all depths and showed no functional relationship to *in situ* temperature. A more limited data set (not shown) indicates that PP, PN, DOP and DON concentrations at the SLW core (and in Coral Sea waters in general) were similar to, or less than, concentrations in shelf waters. As a result, inputs of nitrogen and phosphorus from intrusions are almost exclusively due to NO_3 and PO_4 fluxes.

5.10 Sediment Trap Sampling

Sedimentation fluxes of particulate carbon, nitrogen and phosphorus were measured over an annual cycle (September 1988 - August 1989) using moored traps at four sites along a cross-shelf transect extending seaward from the Family Islands, just south of the Tully box (Figure 7 Bottom). Nutrient and particulate sedimentation rates in the Cairns box (Figure 7 Top) were measured with both moored traps (as above) and Lagrangian drifter traps (Knauer et al., 1979) between May, 1990 and July, 1991. For the Cairns box, only results from the drifting traps will be considered in detail. Inter-calibration runs between the two trap types were carried out in six series of paired deployments off Cairns in August, 1990.

In the case of carbon and phosphorus, the drifter traps collected approximately twice as much material per unit area as the moored traps (Figure 19). The situation for nitrogen was less clear, but on average, the area-normalized fluxes of PN into the drifting traps were 1.6 times the concurrent fluxes calculated for the moored traps. For consistency between series of

experiments, the fluxes measured with the moored traps have been multiplied by a factor of 2 to be equivalent to the rates measured with the drifting traps.

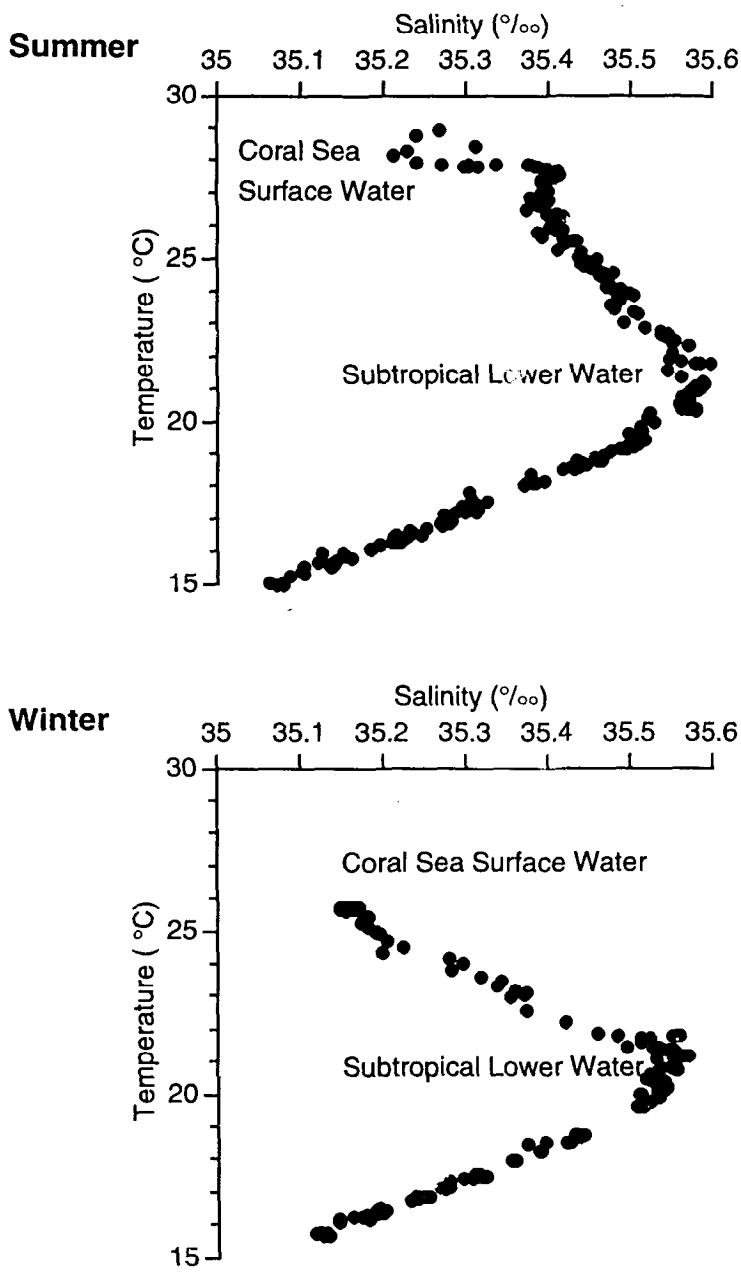

Figure 14. Representative seasonal temperature-salinity relationships from a station in the East Australian Current, immediately seaward of Palm Passage.

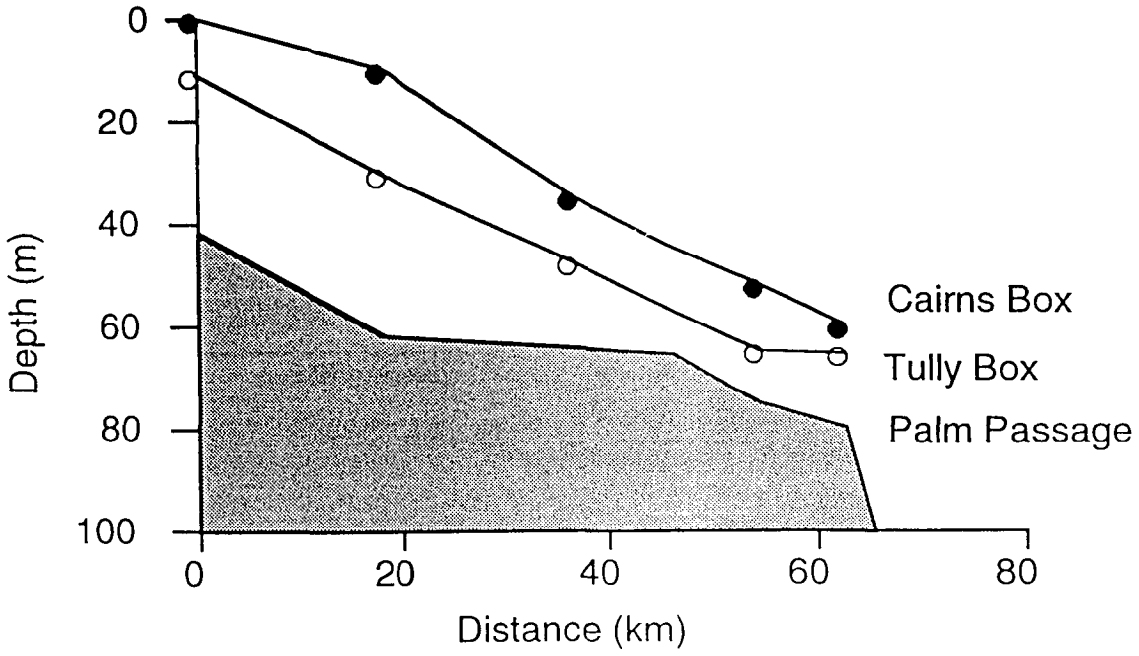

Figure 15. Mean cross-shelf bathymetric profiles for the Cairns and Tully boxes in comparison to the cross-shelf bathymetry down the central axis of Palm Passage.

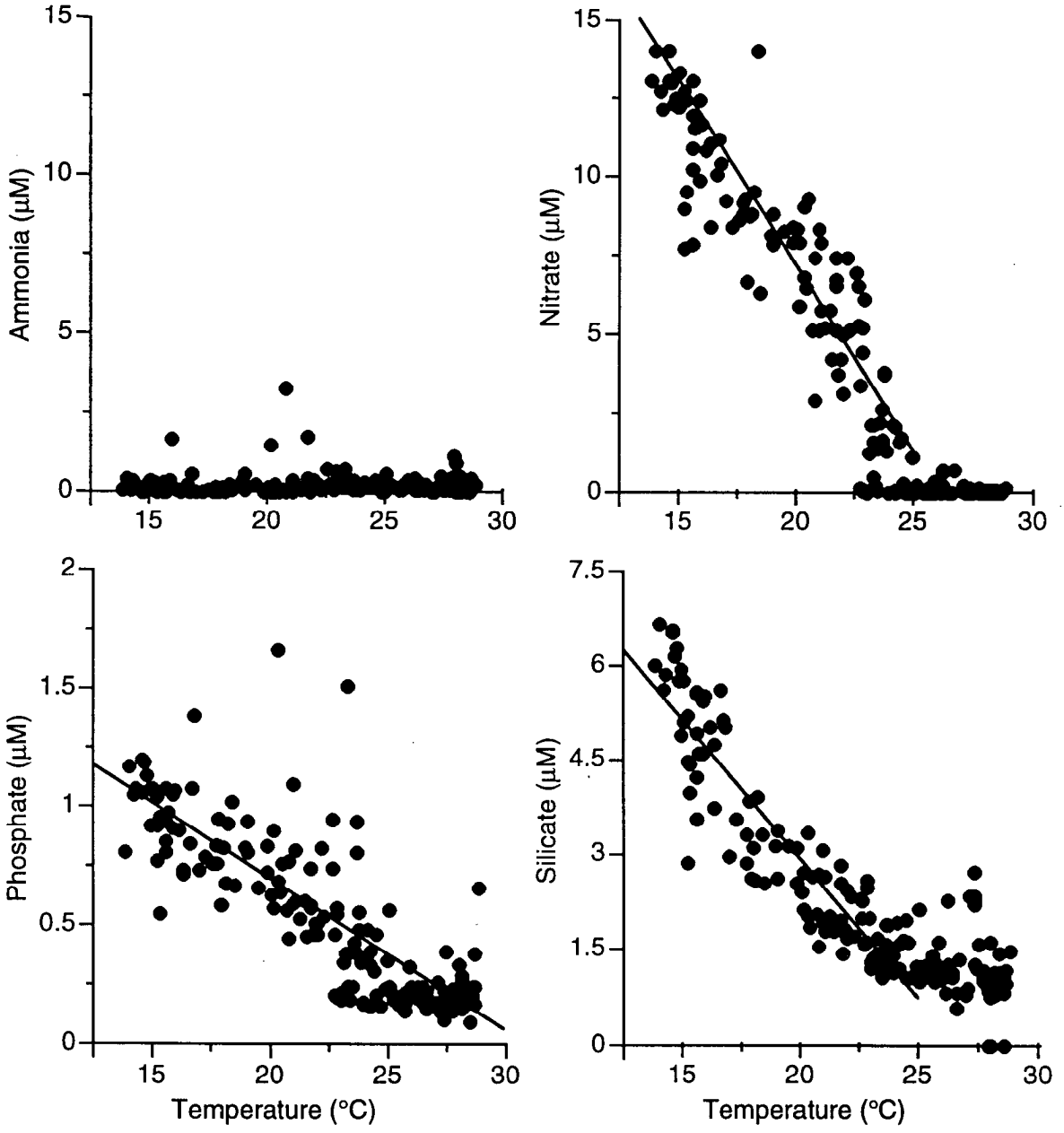

Figure 16. Temperature-inorganic nutrient relationships from stations in the East Australian Current during 1983. The linear regressions shown (for temperatures < 25°C) are: $NO_3 = 28.97 - 1.11$ (T), $r^2 = 0.840$; $PO_4 = 1.95 - 0.06$ (T), $r^2 = 0.484$; $Si(OH)_4 = 11.58 - 0.43$ (T), $r^2 = 0.842$.

5.11 Moored Traps

The moored traps (Figure 17) were right cylinders 38 cm in diameter with a long tapering cone at the bottom to concentrate sedimented material into a small volume sample cup. The traps and cones were constructed of rolled sheet steel which was hot dip galvanized and covered with an electrostatically applied polyester paint (Powdercote). To reduce resuspension within individual traps, the cylindrical portion of the traps had a length:diameter ratio of 3 (Butman, 1986; Butman et al., 1986). Each individual mooring was comprised of paired traps in a rigid wood and aluminium frame which included built-in buoyancy to keep the traps floating upright when deployed. Trap moorings were anchored to the bottom with a 75 kg steel weight and plow anchor. A 14 mm nylon or polyester line extending to a surface buoy with a radar reflector was attached to the top of the trap frame.

The traps were recovered by positioning the research vessel above the deployed trap, grappling the surface line aboard and winching the trap smoothly to the surface with the ship's trawl winch capstan. Flapper doors in the side of the cylindrical portion of the trap allowed water above the cone to escape when the traps were hauled through the surface. The traps were placed on the deck in a vertical position for processing of the contents. Visual examination of the water remaining within the conical section of the trap indicated that appreciable resuspension of particulate material did **not** occur during recovery.

Moored trap deployments were nominally 24 hours in duration. Because of the short duration of the deployments, no preservatives were added to the collection cups. When the recovered trap was returned to the ship's deck, water remaining within the cone portion of each trap (ca. 44-50 l) was drained into a polyethylene drum and retained. The sample cup at the bottom of each trap and the small volume of water remaining at the bottom of the cones were then drained through a tap into a plastic beaker. The interior of each trap was washed with a fine high pressure spray of surface seawater into the beaker containing the concentrated sediment. The volume of water drained from each cone and the combined volume of sediment and trap washings for each trap were noted.

Duplicate aliquots of well mixed water from each of the drained cones and each of the combined sediment + washing samples were filtered onto pre-weighed, ashed (400°C) glass fibre filters (Whatman GF/A, 47 mm). Visually obvious live zooplankton ("swimmers") were removed during and after filtration. In most cases, two litres of drum water and 250-500 ml of the sediment + washings were filtered. The filtered material was usually a mixture of clay-like inorganic and gelatinous organic matter. The filters were stored frozen in ashed petri dishes, dried at 60°C and reweighed before analysis. The total amount of mass, carbon, nitrogen or phosphorus sedimented in each individual trap was ultimately calculated taking account of both the concentrated material in the cups and the incremental amount of dispersed suspended material within the water drained from the cones.