Vol. 63: 53–63, 1990 | MARINE ECOLOGY PROGRESS SERIES
Mar. Ecol. Prog. Ser. | Published May 5

Abundances of benthic microfauna in relation to outwelling of mangrove detritus in a tropical coastal region

Daniel M. Alongi

Australian Institute of Marine Science, PMB No. 3, Townsville M. C., Queensland 4810, Australia

ABSTRACT: Mangrove litter outwells from tidal forests and deposits onto the adjacent coastal seafloor within the central Great Barrier Reef lagoon in tropical northeastern Australia. Previous studies in this region indicate that this detrital loading greatly influences edaphic characteristics (e.g. C/N ratios and POC-soluble tannin concentrations) and supports highly abundant and productive, sedimentary bacterial communities. Mean ciliate and flagellate densities (> 20 μm) within surface (0 to 1 cm) sediments in this region ranged from 23 to 511 cells cm^{-3} and from 40 to 806 cells cm^{-3}, respectively. Densities of nanoprotozoans (5 to 20 μm) ranged from below detection limits to 260.5×10^3 cells cm^{-3}. Seasonal fluctuations of all microfaunal groups were relatively minor. Total faunal densities generally declined with sediment depth, but low densities of sarcodinids, amoeboflagellates, and yeasts and yeast-like cells were discovered below the sediment surface at all of the stations sampled. Experimental additions of aged mangrove litter to mixed laboratory populations of *Euplotes* spp., hymenostomid and hypostomatid ciliates, and zooflagellates resulted in either no or poor growth, suggesting that outwelled litter is not a nutritious food. Densities of microfauna in these sediments are low compared to densities in temperate coastal habitats, and appear to be influenced more by preferences in sediment type than by detrital outwelling or by proximity to the mangrove forests. Poor food quality may partially explain the low densities and lack of a discernible effect of outwelling on the microfauna. Several factors such as low dissolved nutrient levels, high C/N ratios and low microalgal abundances are also cited to account for the low densities, but low water content and poor nutritional quality of litter are considered to be the major factors regulating the benthic microfauna in this region.

INTRODUCTION

Investigations on the ecological role of microfaunal assemblages in marine and estuarine sediments have greatly lagged behind analyses of larger benthic organisms. Much of our knowledge of the ecology of marine microfauna in sediments has been derived from the comprehensive works of Fenchel (1967, 1969; see additional references in Fenchel 1987) conducted in Scandinavian waters.

One reason for the lack of benthic microfaunal studies is that successful extraction and enumeration of protozoans and other small eucaryotes inhabiting soft bottoms has been a consistent problem for many years (Uhlig 1964, Bak & Nieuwland 1989). Recently, the use of silica gel (Percoll) as an extractant has resulted in improved extraction efficiencies, yielding significantly greater densities of microfauna than most other extraction methods (Alongi 1986). This technique has been used in a variety of habitats and sediment types within the central Great Barrier Reef province (Alongi 1986, 1987, 1988a, b, 1989) to enumerate large (> 20 μm) microfauna, mainly ciliates and flagellates.

One of the most extensively investigated study sites within the central Great Barrier Reef (GBR) province are the mangrove forests on Hinchinbrook Island, which is a large (>50 km long) continental island located in tropical north Queensland, Australia (Fig. 1). A recent study has indicated that as much as 25 000 tonnes of mangrove litter carbon are directly exported from the island (and from mangroves lining coastline adjacent to the island) into neighboring bays and channels of the central GBR lagoon (Robertson et al. 1988). In Missionary Bay adjacent to the northern end of the island, only 140 tonnes C yr^{-1} or 52 gC m^{-2} yr^{-1} deposits to the seabed with most of the remaining litter diluted over a wide area of the GBR lagoon. This amount may be small compared with the total volume

0171-8630/90/0063/0053/$ 03.00

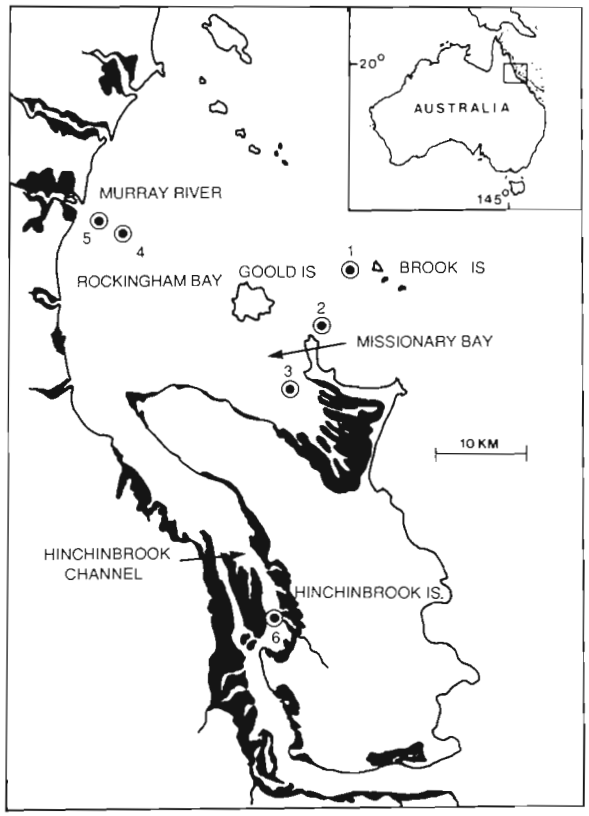

Fig. 1. Missionary Bay-Rockingham Bay-Hinchinbrook Channel region in north Queensland, Australia, with station locations (see Table 1 for latitude, longitude, water depths and sediment characteristics). Blackened areas are mangrove forests

exported to the entire lagoon, but it is likely to be the greatest contributor of detritus to the subtidal benthos in the bay and, over the long-term, appears to support highly abundant and productive, sedimentary bacterial communities (Alongi et al. 1989). In this companion paper, I document the responses of the benthic microfauna, including nanoprotozoans (5 to 20 µm), to this detrital outwelling by examining their temporal and spatial patterns in proximity to the mangrove forests, and the growth of mixed protozoan populations fed a diet of mangrove litter in laboratory culture.

MATERIALS AND METHODS

Study area and sediment characteristics. The study area was the Missionary Bay-Rockingham Bay-Hinchinbrook Channel region along the north Queensland coast of Australia (Fig. 1). Six subtidal stations (see Table 1 for station locations and depths) were established in the region: (A) Stns 1 to 3 on a transect from the shallow shore of Missionary Bay off the northern end of Hinchinbrook Island out to the area between Goold Island and the Brook Islands, (B) Stns 4 and 5 near the mouth of the nearby Murray River in northern Rockingham Bay and (C) Stn 6 near the mouth of the Herbert River within Hinchinbrook Channel. Stns 2, 3 and 6 are near the extensive, marine deltaic mangrove basins on the island and in the channel (see blackened areas in Fig. 1). In contrast, Stns 4 and 5 are near the riverine, fringing *Rhizophora*-dominated forests lining the Murray River, which are subjected to seasonal variations of freshwater input.

Sediments in the study area have been characterized as medium to very fine sands (Stns 1, 3 and 4; Table 1) or as coarse silts (Stns 2, 5 , 6; Table 1). All stations were shallow (< 15 m), moderately anaerobic with relatively low water content and moderate concentrations of calcium carbonate (Table 1), derived mainly from shell debris and localized patches of relict coral (e.g. near Stn 3). Differences in organic carbon and nitrogen concentrations, C/N ratios and soluble tannin content (polyphenolic acids derived from mangrove litter) among stations are mainly the result of spatial differences in the deposition of mangrove detritus (Alongi et al. 1989).

A significant relationship between soluble tannins and POC (particulate organic carbon) in the sediments provided a reasonable index of the relative loading of litter input to each station (Alongi et al. 1989). The degree of litter deposition decreases among the stations in the following order: Stn 6 > 3 > 5 > 4 > 2 > 1. The stations .closest to the mangroves have the highest POC and extractable tannin concentrations (Table 1) and receive more litter input than the more distant stations (Robertson et al. 1988). Bacterial densities and productivity in surface sediments at these sites are high (grand means of 6.6×10^{10} cells g^{-1} sediment dry wt and 632 mg C m^{-2} d^{-1}, respectively) but turnover times (range: 3 to 9 d) are low to moderate (Alongi et al. 1989). Dissolved organic carbon concentrations in the porewaters are low (Table 1), as are fresh microalgal and phaeopigment concentrations (as chlorophyll *a* and acid-treated degradation products; Table 1).

Sampling procedure and laboratory analysis. Sampling was conducted seasonally from August 1986 to May 1988 using a 0.027 m^2 boxcorer. At each station, 3 subcores were taken from 1 or 2 undisturbed boxcores to a depth of 1 cm using plastic Terumo^R syringes (1.1 cm inner diameter) with the needle end cut off. In February 1988, vertical distribution of microfauna was examined at each station by subsampling from stainless steel cores (7 cm inner diameter) inserted to a depth of 20 cm into boxcore samples. The cores were sectioned at 2 cm intervals and three 1 cc samples from each depth interval were processed for large (>20 µm) microfauna as described below; nanoprotozoa were not examined. Preliminary sampling during the first 2

Table 1. Mean and range (in parentheses) of surface sediment factors (summarized from Alongi et al. 1989) and plant pigment concentrations (μg g^{-1} sediment dry wt) at Stns 1 to 6 over the study period. POC: particulate organic carbon; TN: total nitrogen; DOC: dissolved organic carbon

Variable	Station					
	1	2	3	4	5	6
Latitude	18° 08′ S	18° 10′ S	18° 14′ S	18° 07′ S	18° 07′ S	18° 24′ S
Longitude	146° 15′ E	146° 14′ E	146° 12′ E	146° 04′ E	146° 03′ E	146° 12′ E
Water depth (m)	15	13	4	8	8	6
pH	7.3 (6.8–8.0)	7.3 (7.0–7.8)	7.4 (7.0–7.8)	7.5 (7.0–7.7)	7.5 (7.1–7.7)	7.1 (6.6–7.6)
Eh (mV)	+228 (−63+362)	+86 (−120+264)	+7 (−145+83)	+76 (−133+125)	+42 (−86+91)	+31 (−103+132)
Tannins[a]	0.002 (0.001–0.004)	0.008 (0.008–0.017)	0.014 (0.01–0.017)	0.009 (0.006–0.013)	0.013 (0.006–0.019)	0.033 (0.025–0.037)
POC[a]	0.22 (0.11–0.33)	0.70 (0.21–0.94)	1.32 (0.74–1.72)	0.82 (0.59–1.32)	1.41 (0.60–1.97)	2.60 (2.40–2.81)
TN[a]	0.04 (0.01–0.06)	0.08 (0.01–0.13)	0.08 (0.01–0.13)	0.09 (0.04–0.12)	0.12 (0.08–0.16)	0.12 (0.04–0.18)
C/N	5.5	8.8	16.5	9.1	11.7	21.7
Water content[a]	30.1 (28.5–31.7)	46.7 (43.6–48.6)	39.5 (35–46.3)	48.0 (45.9–49.1)	61.2 (59.2–63.6)	49.5 (45.6–52.2)
$CaCO_3$ content[a]	14.5 (11.6–16.2)	11.8 (7.9–12.9)	33.3 (15.2–44.9)	21.3 (13.3–29.2)	12.6 (4.2–22.1)	7.2 (1.2–10.3)
Grain size (mm)	0.30 (0.27–0.32)	0.06 (0.03–0.10)	0.16 (0.12–0.20)	0.11 (0.7–0.14)	0.06 (0.05–0.08)	0.04 (0.02–0.06)
% Gravel	5.1 (1.9–7.9)	0.5 (0–1.9)	8.2 (4.3–11.2)	2.1 (1.1–4.2)	1.0 (1.2–4.3)	0.5 (0–2.0)
% Sand	69.1 (12.1–86.6)	21.0 (7.0–31.1)	45.6 (35.9–55.3)	48.8 (21.2–56.9)	31.0 (12.3–51.2)	52.1 (39.6–61.2)
% Silt	12.3 (8.3–17.3)	60.1 (50.6–67.9)	33.7 (22.3–51.5)	37.4 (28.7–52.1)	39.5 (22.8–42.1)	22.9 (18.6–32.1)
% Clay	13.5 (2.0–32.0)	18.4 (6.3–19.9)	12.5 (5.4–21.7)	11.7 (6.2–15.8)	28.5 (6.9–34.9)	24.5 (1.7–31.2)
DOC (mg l^{-1})	4.6 (3.5–6.0)	4.1 (4.0–4.2)	3.6 (3.1–4.1)	4.5 (2.4–6.5)	3.8 (3.1–4.4)	3.8 (3.2–4.3)
Chlorophyll *a*	0.65 (0–2.5)	1.1 (0–3.0)	1.4 (0–6.2)	1.2 (0–5.1)	1.9 (0–9.2)	1.0 (0–3.5)
Phaeopigments	3.7 (1.4–5.3)	3.9 (0.2–8.5)	3.3 (0.3–8.9)	3.1 (1.2–5.8)	8.4 (0.2–12.6)	7.8 (5.2–14.3)

[a] % by dry weight

cruises indicated a sample precision of ±50% (1 standard deviation) of total mean faunal densities using 3 cores. To increase precision to ±20% of the mean would have required more than 40 samples per site – a highly impractical number. Therefore, a sample precision of ±50% was considered adequate on subsequent cruises in order to determine station and seasonal differences, and to minimize extensive sorting and counting on board.

Each sediment sample (total volume of 0.95 cm^3) was immediately extruded into 30 ml centrifuge tubes (or into scintillation vials and transferred later) containing 2 ml of a Percoll-sorbitol mixture (Alongi 1986). The samples were then gently hand-mixed for 1 to 2 min, allowed to stand for 1 h and then centrifuged at 490 × *g* for 20 min. A 1 ml aliquot was placed into a scintillation vial containing 50 µl of a 5% sodium tetraborate-buffered formalin solution with either methyl green or nigrosin black for estimation of nanoprotozoan numbers (Kudo 1977, Lee et al. 1985). (These stains mix well in silica gel and formalin, unlike Lugol's solution which tends to clump in the gel mixture limiting identification to only the largest cells.) The sample was then gently mixed and kept cool (5°C) in the dark until counted in the laboratory (see below). The other 1 ml portion was poured into a small (4 cm diameter) Petri dish with the glass bottom lined into 0.5 cm^2 grids. The extraction procedure was repeated again and the 2 ml supernatant was added to the first 1 ml solution in the dish. A fixed number of grids (n = 10 of 24) were counted randomly for large (>20 µm) cells. To facilitate counting under a dissecting microscope, a 50% (w/v) $MgCl_2$ solution in seawater was added dropwise into the dish to slow mobility of the organisms. Counts were recovery-corrected using the extraction efficiencies determined earlier in the region by Alongi (1986).

For enumeration of nanoprotozoans (5 to 20 µm), two 5 to 10 µl aliquots from each 1 ml formalin-preserved aliquot per sample were counted on duplicate 0.1 mm^3 blocks on a hemocytometer at 400× (Collins & Lyne 1976). Including dilutions, the minimum number of cells which could be accurately counted was 4.2×10^4 cells ml^{-1}. Recovery of cultured cells from sterile sediments (see recovery methodology in Alongi 1986) was 54±13% Counts were recovery-corrected using this value.

Mangrove litter experiment. A laboratory experiment was conducted in tissue culture flasks to determine the effect of mangrove litter on population growth of benthic protozoans. Mixed populations of the hypotrich ciliate, *Euplotes* spp. (ca 50 cells per flask), small hymenostomid and hypostomatid ciliates (ca 100 cells per flask) and small (5 to 20 µm) zooflagellates (ca 1×10^5 per flask) were taken from stock cultures (Alongi 1986) and inoculated into the same 25 cm^2 tissue culture flasks containing 10 ml of aged 0.45 µm filtered seawater (35‰). The experiment was segregated into 2 sets of 4 replicate flasks for each of 2 food types: mixed oat cereal (pablum) as a control and mangrove litter. The flasks were provided with a ration level of 10 mg N m^{-2} d^{-1} (equivalent to 389 and 1587 mg dry wt m^{-2} d^{-1} of cereal and litter, respectively). This food level is considerably above the input rate (1 to 5 mg N m^{-2} d^{-1}) estimated for the area by Robertson et al. (1988). The mangrove litter was obtained from within Missionary Bay during one of the first cruises. The litter is an amalgamation of mainly well-aged, mangrove bark, twigs, leaf and root matter typically found at the stations. Both foods were oven-dried (60°C for 24 h) and milled (120 µm) prior to the experiment. The mixed cereal has an average C/N ratio of 17 and the mangrove litter has an average ratio of 52. The ration was provided every 10 d. The experiment was conducted for 30 d and maintained in the dark at 26°C.

The protozoans were counted every 5 d. *Euplotes* spp. were counted from duplicate 0.5 ml aliquots per flask in a counting well under a dissecting microscope. The smaller ciliates were narcotized with $MgCl_2$ and counted from duplicate 10 µl aliquots per flask in 0.1 mm^3 blocks on a hemocytometer at 200×. The flagellates were similarly counted from replicate (n = 2 to 3) 5 to 10 µl aliquots per flask on a hemocytometer at 400×.

Data analysis. Comparisons of microfaunal densities among seasons and stations, and between the 2 food treatments (excluding Day 0) in the laboratory experiment, were carried out using standard 2-way ANOVA. Comparisons of protozoan densities with sediment depth at each station were conducted using a 1-way ANOVA. Each ANOVA was followed by a Student-Newman-Keuls (SNK) multiple comparisons test if a significant ($p<0.05$) temporal or spatial effect was found. The data were log (x+1) transformed before analysis because prior F_{MAX} tests indicated heterscedasticity. Simple correlations (r) between faunal densities and sediment characteristics were determined using Pearson's product-moment correlation coefficient. All statistical analyses were conducted as described by Sokal & Rohlf (1981) Further details are provided in the legends to Figs. 2 to 4.

RESULTS

Temporal and spatial variations

Mean ciliate and flagellate (>20 µm) densities within surface (0 to 1 cm) sediments ranged from 23 to 511 cells cm^{-3} (grand mean = 157) and from 40 to 806 cells

Table 2. (a) Variation in ciliate densities (cells cm^{-3}) with season and station. Each value is a mean (± 1 SD). (b) Summary of 2-way ANOVA for log (x+1) transformed ciliate density data. (c) Summary of SNK test for ciliate densities by stations

(a) Ciliate densities

Season	Station	Density	Season	Station	Density
Winter	1	232 (18)	Winter	1	322 (111)
(August 1986)	2	47 (16)	(August 1987)	2	38 (39)
	3	150 (68)		3	51 (55)
	4	31 (14)		4	73 (31)
	5	64 (26)		5	112 (62)
	6	416 (190)		6	218 (94)
Spring	1	275 (86)	Spring	1	214 (26)
(October 1986)	2	23 (14)	(October 1987)	2	56 (22)
	3	322 (48)		3	253 (96)
	4	56 (25)		4	128 (104)
	5	108 (68)		5	181 (84)
	6	116 (46)		6	150 (42)
Summer	1	382 (92)	Summer	1	219 (125)
(February 1987)	2	57 (22)	(February 1988)	2	103 (49)
	3	460 (207)		3	197 (76)
	4	64 (21)		4	111 (46)
	5	95 (40)		5	103 (38)
	6	511 (280)		6	167 (76)
Autumn	1	283 (28)	Autumn	1	236 (43)
(May 1987)	2	73 (75)	(May 1988)	2	86 (95)
	3	176 (105)		3	155 (69)
	4	34 (26)		4	60 (26)
	5	125 (80)		5	26 (19)
	6	146 (80)		6	43 (23)

(b) Summary of 2-way ANOVA

Source	df	MS	F
Seasons	7	2.62	1.96^{ns}
Stations	5	16.18	12.07^{***}
Seasons × Stations	35	1.55	1.16^{ns}
Error	96	1.34	

*** $p < 0.001$; ^{ns} not significant at $\alpha = 0.05$ level

(c) SNK results (2-tailed test; $p < 0.05$)

Stations:

2 4 5 < 3 6 1

cm^{-3} (grand mean = 185), respectively (Tables 2a and 3a). Only significant station differences were found for ciliates (Table 2b); both station and seasonal differences were found for flagellates (Table 3b). Significant interaction effects were found for the flagellates indicating that density differences among stations varied with different seasons. For the ciliates, differences among seasons and stations accounted for 6.5 and 28.7 % of the variance, respectively, with interactions and within-group error accounting for 19.2 and 45.6 % of the variance. Ciliate densities were highest at Stns 1, 6, and 3 followed by equivalent abundances at Stns 5, 4 and 2 (Table 2c). Ciliates did not correlate with any other variables measured.

Seasonal and site differences for the flagellates accounted for 9.9 and 12.5 % of the variance, whereas interaction effects and within-group error accounted for 33.6 and 44.0 % of the variance, respectively (Table 3b). Highest flagellate densities occurred at the muddiest stations (5, 2, 6 and 4) with significantly lower abundances at the sandier sites (Stns 1 and 3), reflecting a high negative correlation ($r = -0.80$; $p<0.01$) with grain size. There were significant seasonal differences for flagellates, but there were no consistent trends such as both summers being greater than both winters (Table 3c).

Densities of nanoprotozoans ranged from less than the detection limit (4.2×10^4 cells cm^{-3}) to 260.5×10^3 cm^{-3} (Table 4a). The statistical results indicate only station (6.8 % of variance) differences; variations with

Table 3. (a) Variation in flagellate densities (cells cm^{-3}) with season and station. Each value is a mean (± 1 SD). (b) Summary of 2-way ANOVA for log (x + 1) transformed flagellate density data. (c) Summary of SNK tests for flagellate densities by stations and seasons

(a) Flagellate densities

Season	Station	Density	Season	Station	Density
Winter	1	114 (47)	Winter	1	44 (48)
(August 1986)	2	449 (170)	(August 1987)	2	51 (57)
	3	102 (50)		3	141 (70)
	4	459 (100)		4	120 (85)
	5	326 (145)		5	172 (119)
	6	53 (23)		6	459 (182)
Spring	1	146 (65)	Spring	1	80 (42)
(October 1986)	2	431 (53)	(October 1987)	2	319 (134)
	3	137 (69)		3	114 (49)
	4	286 (168)		4	101 (76)
	5	357 (76)		5	128 (66)
	6	145 (82)		6	89 (55)
Summer	1	52 (44)	Summer	1	123 (42)
(February 1987)	2	123 (69)	(February 1988)	2	300 (100)
	3	124 (52)		3	137 (41)
	4	242 (103)		4	57 (47)
	5	224 (113)		5	806 (245)
	6	459 (76)		6	119 (19)
Autumn	1	40 (37)	Autumn	1	62 (22)
(May 1987)	2	132 (29)	(May 1988)	2	106 (41)
	3	119 (70)		3	48 (36)
	4	93 (81)		4	79 (23)
	5	168 (31)		5	128 (65)
	6	265 (60)		6	66 (21)

(b) Summary of 2-way ANOVA

Source	df	MS	F
Seasons	7	3.05	3.08••
Stations	5	5.41	5.46•••
Seasons × Stations	35	2.07	2.09•
Error	96	0.99	

••• $p < 0.001$; •• $p < 0.01$; • $p < 0.05$

(c) SNK results (2-tailed test; $p < 0.05$)

Stations:

1 3 < 4 6 2 5

Seasons:

5/88 5/87 10/87 8/87 2/87 8/86 10/86 2/88

season (4.4 % of variance) and interaction effects (27.1 % of variance) were not significant (Table 4b).

Vertical distribution

Vertical profiles of ciliates (Fig. 2) and flagellates (Fig. 3) at all of the stations indicated either low and variable densities throughout the sediment column or rapid attenuation of densities with depth. Excluding flagellates at Stns 4 and 6, most density decreases with depth were significant (see Figs. 2 & 3 and legends). Low densities of the other microfauna, namely sarcodinids (47 % of subsurface densities), amoeboflagellates (22 %) and yeasts and yeast-like cells (31 %), were found almost exclusively in subsurface sediments (Fig. 4).

Effect of mangrove litter on protozoan laboratory populations

Populations of mixed species of *Euplotes* (Fig. 5A), smaller ciliates (Fig. 5B) and zooflagellates (Fig 5C) grew significantly ($p<0.001$) better on mixed cereal than on the mangrove litter obtained from Missionary Bay. In all cases, the F values in the 2-way ANOVAs were significant for time (F range = 4.3 to 17.6; 5 df)

Table 4. (a) Variation in nanoprotozoan densities (cells × 10^3 cm^{-3}) with season and station. Each value is a mean (± 1 SD). (b) Summary of 2-way ANOVA for log (x + 1) transformed nanoprotozoan densities. (c) Summary of SNK test for nanoprotozoan densities by stations

(a) Nanoprotozoan densities

Season	Station	Density	Season	Station	Density
Winter	1	77.1 (62.9)	Winter	1	46.8 (32.0)
(August 1986)	2	29.8 (33.8)*	(August 1987)	2	64.0 (45.6)
	3	37.5 (44.7)*		3	72.7 (26.3)
	4	131.1 (10.9)		4	55.5 (47.4)
	5	119.8 (11.8)		5	159.4 (112.0)
	6	145.5 (42.7)		6	187.7 (151.2)
Spring	1	80.2 (41.5)	Spring	1	94.1 (16.1)
(October 1986)	2	59.7 (28.4)	(October 1987)	2	171.3 (102.1)
	3	83.8 (31.6)		3	90.8 (38.7)
	4	42.7 (21.9)*		4	94.1 (16.1)
	5	106.9 (39.8)		5	78.7 (46.2)
	6	125.0 (88.7)		6	90.8 (38.7)
Summer	1	167.1 (65.6)	Summer	1	183.3 (25.3)
(February 1987)	2	191.8 (81.2)	(February 1988)	2	86.4 (43.6)
	3	25.7 (36.4)*		3	119.8 (26.6)
	4	55.5 (26.1)		4	77.1 (63.0)
	5	141.2 (42.2)		5	154.3 (62.9)
	6	260.5 (160.2)		6	193.6 (81.4)
Autumn	1	106.9 (38.6)	Autumn	1	34.2 (26.3)*
(May 1987)	2	98.5 (59.6)	(May 1988)	2	59.7 (61.8)
	3	102.6 (73.3)		3	84.6 (15.2)
	4	184.1 (71.2)		4	8.5 (12.0)*
	5	189.3 (66.5)		5	81.3 (26.6)
	6	137.1 (121.7)		6	90.8 (46.6)

* Below or at detection limit of individual sample

(b) Summary of 2-way ANOVA

Source	df	MS	F
Seasons	7	11.7	1.0^{ns}
Stations	5	28.1	2.4^{*}
Seasons × Stations	35	14.2	1.2^{ns}
Error	96	11.7	

* $p < 0.05$; ^{ns} not significant at $\alpha = 0.05$ level

(c) SNK results (2-tailed test; $p < 0.05$)

Stations:

3 4 2 1 5 6

indicating population fluctuations, food type (F range = 59.4 to 737.9; 1 df) and interaction effects (F range = 8.0 to 14.3; 5df). Significant interactions, at least for the ciliates, reflect the increasing numerical divergence of both populations over time. On the diet of mangrove litter, growth was barely observable for the ciliate populations; no growth was observed for the zooflagellates, except after Day 15.

DISCUSSION

Both the field data and laboratory experiments provide little evidence to indicate enhancement of microfaunal densities or growth in response to mangrove litter export, generally supporting the patterns of bacterial abundances and productivity, and dissolved organic carbon fluxes across the sediment/water interface as reported previously for these stations (Alongi et al. 1989). Most variations in microfaunal densities were accounted for by interactive effects and within-group error, rather than by station and seasonal differences. Station differences appear to reflect preferences for sediment type, as there were no strong or consistent trends among or within protozoan groups of significantly higher (or lower) densities in proximity to the mangrove forests (Tables 2, 3 and 4). For example, nanoprotozoan densities were highest in the Hinchin-

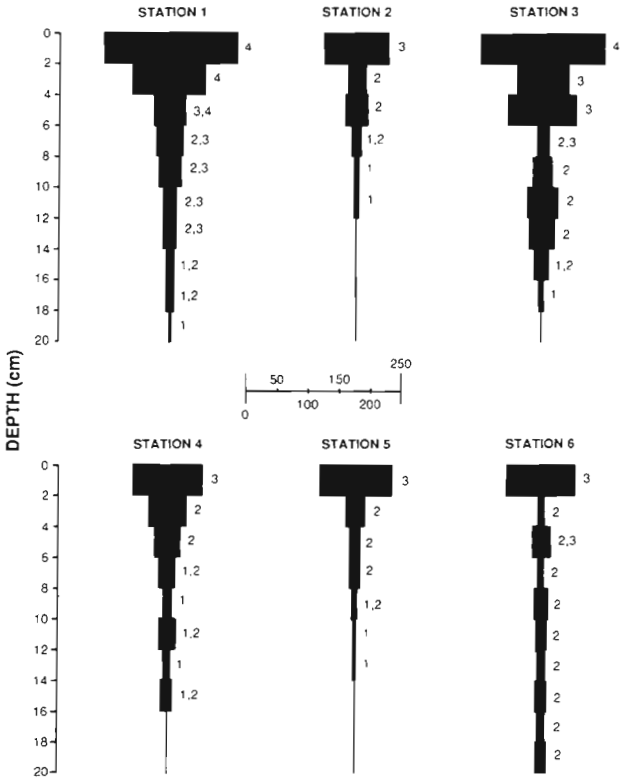

Fig. 2. Vertical distribution of ciliates (cells cm^{-3}) at Stns 1 to 6 over 2 cm intervals from 0 to 20 cm sediment depth, February 1988. Blocks with identical numbers denote densities which are not significantly ($p > 0.05$) different (SNK test). Higher numbers indicate significantly higher densities

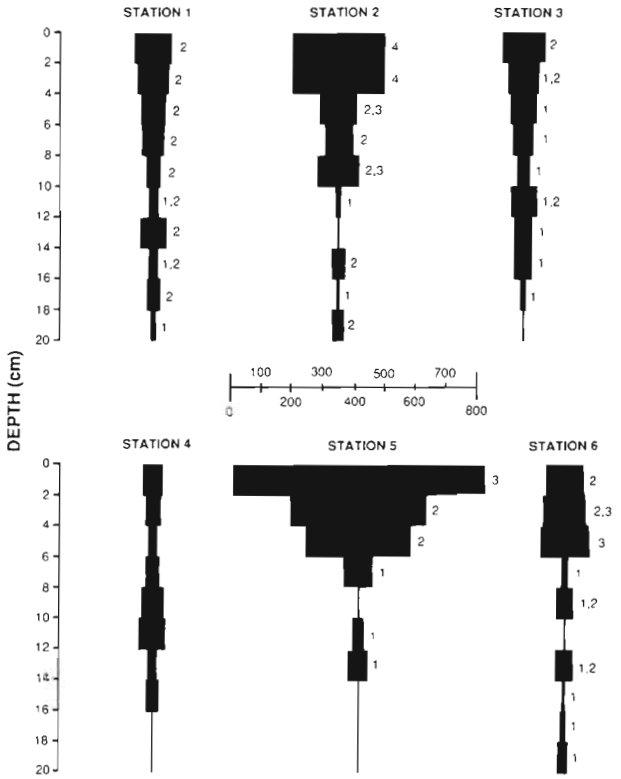

Fig. 3. Vertical distribution of flagellates (cells cm^{-3}) at Stns 1 to 6 over 2 cm intervals from 0 to 20 cm sediment depth, February 1988. Blocks with identical numbers denote densities which are not significantly ($p > 0.05$) different (SNK test). Higher numbers indicate significantly higher densities

brook Channel where the highest concentrations of exported litter are deposited, but the lowest densities occurred at Stn 3 which is closest to the Missionary Bay mangroves. One would have expected equally high (or nearly so) densities at Stns 3 and 5 if there was a clear effect of outwelling, but such was not the case.

There may well be differences in species composition of all groups among stations, but the fauna were not identified below the subphyletic level. The microfaunal assemblages generally did not correlate with sedimentary factors, bacterial parameters or with plant pigments, probably because faunal densities varied little (or not at all) seasonally and very little among stations, at least in comparison with temporal and sedimentary patterns exhibited by microfauna in most temperate habitats (e.g. Fenchel 1969, Bak & Nieuwland 1989). In fact, there were no significant differences with season for the sedimentary factors measured. There were sea-

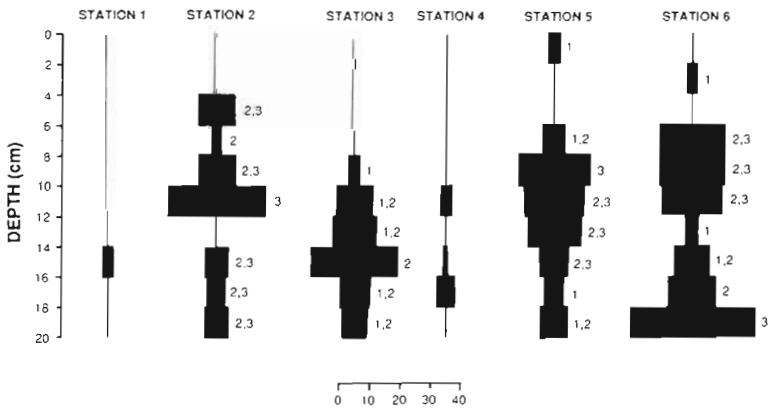

Fig. 4. Vertical distribution of other microfauna (cells cm^{-3}) at Stns 1 to 6 over 2 cm intervals from 0 to 20 cm sediment depth, February 1988. Blocks with identical numbers denote densities which are not significantly ($p > 0.05$) different (SNK test). Higher numbers indicate significantly higher densities

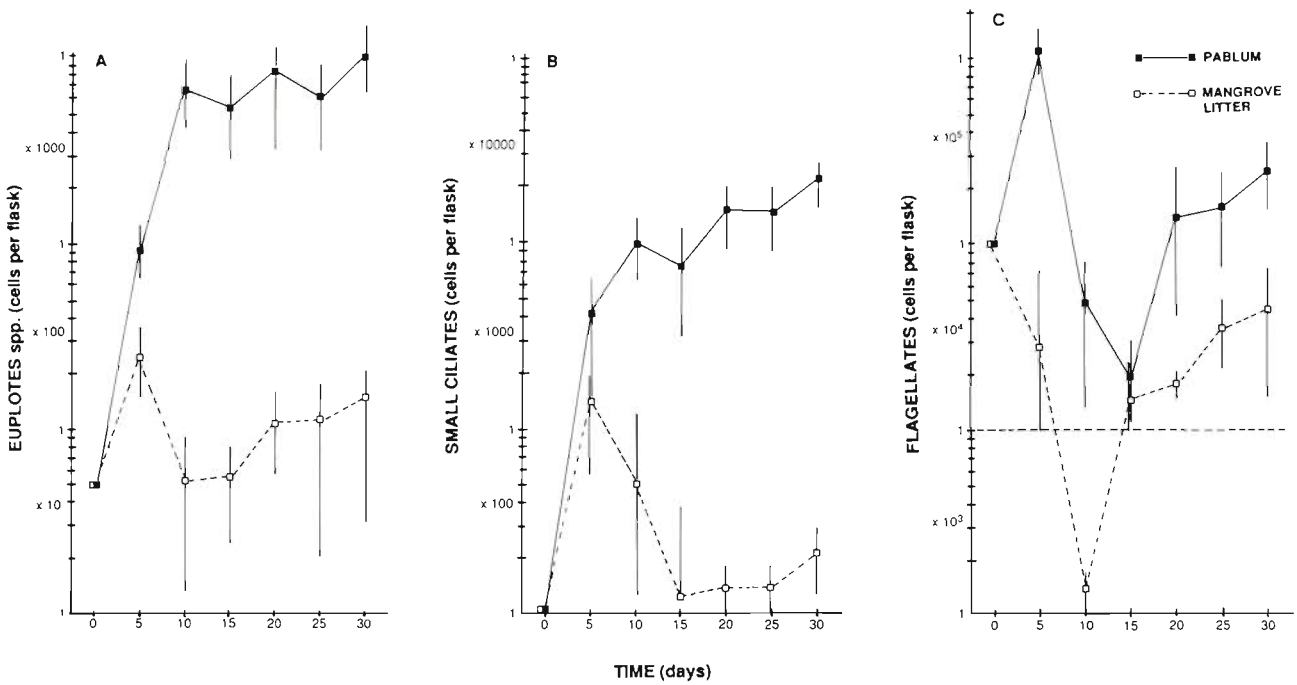

Fig. 5. Growth of mixed populations of (A) the ciliate *Euplotes* spp., (B) small hymenostomid and hypostomatid ciliates and (C) zooflagellates in tissue cultures with mixed cereal and with mangrove litter. The dashed horizontal line in (C) denotes the detection limit of an individual flask

sonal temperature changes, but the range of variation was small as were most variations in bacterial numbers and rates of growth (Alongi et al. 1989).

Vertically, the few subsurface maxima observed may reflect episodic resuspension of sediments at these sites, as indicated in sediment slabs taken for X-radiographic analysis (Alongi unpubl.). Most of these vertical depth profiles are similar to those found in other studies (i.e. a decline with sediment depth), probably for the same reasons (lack of O_2, etc.), although some workers have observed abundant protozoan populations to depths as great as 1 m (e.g. Dye 1979). Thus, vertical distribution depends on sediment type, but it is improbable that densities in sediments deeper than 20 cm depth at these sites would be greater than or equal to densities in surface sediments because the boxcorer rarely penetrated deeper than 20 cm due to high compaction and low water content. The subsurface occurrences of amoeba, amoeboflagellates, yeasts and yeast-like cells have been observed in other habitats, particularly in the deep sea (Burnett 1981, Alongi 1987). The reasons for this phenomenon are not understood. Their presence at depth many reflect avoidance of predators or competitors, or attraction to moderately anaerobic conditions not tolerated by surface-dwelling microbes.

Despite the high bacterial densities at these sites, microfaunal densities are low compared with studies conducted in temperate and arctic latitudes, where protozoan densities frequently exceed 500 to 1000 cells cm^{-3} (Fenchel 1969, 1975, Dye 1979, Wyatt & Pearson 1982, Kemp 1988, Bak & Nieuwland 1989). Low densities of ciliates and flagellates within the >20 µm size range have been found in other GBR habitats, including on coral reefs (Hansen et al. 1987), mangroves (Alongi 1988a), sandflats (Alongi 1988b) and on the continental shelf proper (Alongi 1989). As in the other GBR habitats, small foraminifera were rarely observed, nearly all being meiofaunal in size and therefore not included in this study.

Several factors probably account for the low microfaunal densities: low dissolved nutrient levels (in the µmol range), high C/N ratios, low microalgal concentrations and low water content (see Table 1). These factors appear to be characteristic of tropical coastal sediments receiving some carbonate input (Alongi 1990). Microalgal concentrations are low probably because of high levels of turbidity and physical disturbances (storms, etc.) which naturally disrupt the seabed in this region. The low nutrient levels reflect high rates of bacterial and phytoplankton turnover (Furnas & Mitchell 1987), and generally low to moderate levels of sedimentation from the water column, excluding storm events (Furnas 1989). The high C/N ratios reflect the amounts of mangrove litter deposited as well as its generally poor nutritional quality. Although the tissue culture experiments are not directly comparable to the field situation and were conducted for a short time period, an examination of the flasks containing litter after Day 60 (rations were provided up to this time) detected high densities of bacteria (10^9 to 10^{10} ml^{-1}) but

no living protozoans in any of these flasks. In litter bag experiments, Robertson (1988) observed high (10^{10} g^{-1} dry wt) bacterial densities on decomposing mangrove leaves even after 348 d, but they were estimated to contribute < 2% of the measured leaf nitrogen. In addition, C/N ratios of detritus were similar after the first 156 d. Thus, it is probable that aged mangrove litter is not a nutritious food for most eucaryotes even with bacterial enrichment over time.

The low microfaunal densities in these sediments may also reflect predation by larger benthos, but abundances of meiofauna and macroinfauna are relatively low in this region (Alongi unpubl.). In the opposite vein, it is unlikely that the microfauna are bacterial food-limited, considering the high densities (mean = 6.6×10^{10} cells g^{-1} dry wt) of bacteria in these sediments.

Perhaps the most critical factor controlling benthic microfaunal densities is the amount of interstitial water. Generally, the more compact a sediment is, the fewer protozoans the interstices can support. Water content of tropical sediments containing various amounts of carbonate (as in this region) is generally low (< 50%) because carbonates tend to compact more readily than continental rock and lithify into limestone (Chilingar et al. 1979).

Comparisons of the nanoprotozoan data with other data are difficult owing to the very few studies available, the different methods used and cell sizes measured. The early studies of Mare (1942), Perkins (1958) and Lighthart (1969) used methods which were either not quantitative or measured only the largest cells. Previous studies by the author within the GBR province (Alongi 1986, 1987, 1988a, b, 1989) similarly did not measure cells smaller than about 20 µm because acid Lugol's solution was used as a preservative/stain mixture and tends to mix poorly, coagulating in the Percoll-sorbitol solution, and obscuring all but the largest cells on a hemocytometer. The use of a low concentration of formalin (concentrations > 5% cause the Percoll mixture to gel within minutes) and other stains, such as nigrosin black or methyl green, resulted in a clear and improved image quality. Cells as small as about 5 to 10 µm can be counted, but the high detection level (in this study, 4.2×10^4 cells ml^{-1}) limits its accuracy. The nanoprotozoan counts reported here are therefore probably underestimates as the hemocytometer technique does not permit detection of cells less than about 5 to 10 µm in size which may be the dominant size class of microfauna as in some temperate sediments (Bak & Nieuwland 1989). Epifluorescence microscopy would have been a more accurate technique (e.g. see Bak & Nieuwland 1989), but initial identification attempts failed because viable cells were obscured by fluorescence of the large number of mangrove detrital particles present in these sediments. Nevertheless, the low densities of larger microfauna suggest that the low nanoprotozoan density estimates are genuine, probably for the same reasons as for the larger fauna.

Acknowledgements. I thank O. Dalhaus, P. Christoffersen, B. Burgher and F. Tirendi for field and laboratory assistance, and the masters and crew of the RV 'The Harry Messel'. Kim Truscott typed the manuscript, A. Robertson offered statistical help and C. Wilkinson reviewed an earlier draft. This project was supported by MST/ARC Grant 86/0708 to the author and by the Australian Institute of Marine Science. Contribution No. 495 from the Australian Institute of Marine Science.

LITERATURE CITED

Alongi, D. M. (1986). Quantitative estimates of benthic protozoa in tropical marine systems using silica gel: a comparison of methods. Estuar. coast. Shelf Sci. 23: 443–450

Alongi, D. M. (1987). The distribution and composition of deep-sea microbenthos in a bathyal region of the western Coral Sea. Deep Sea Res. 34: 1245–1254

Alongi, D. M. (1988a). Bacterial productivity and microbial biomass in tropical mangrove sediments. Microb. Ecol. 15: 59–79

Alongi, D. M. (1988b). Microbial-meiofaunal interrelationships in some tropical intertidal sediments. J. mar. Res. 46: 349–365

Alongi, D. M. (1989). Benthic processes across mixed terrigenous-carbonate sedimentary facies on the central Great Barrier Reef continental shelf. Cont. Shelf. Res. 9: 629–663

Alongi, D. M. (1990). The ecology of tropical soft-bottom benthic ecosystems. Oceanogr mar. Biol. A. Rev. 28: 381–496

Alongi, D. M., Boto, K. G., Tirendi, F. (1989). Effect of exported mangrove litter on bacterial productivity and dissolved organic carbon fluxes in adjacent tropical nearshore sediments. Mar. Ecol. Prog. Ser. 54: 133–144

Bak, R. P. M., Nieuwland, G. (1989). Seasonal fluctuations in benthic protozoan populations at different depths in marine sediments. Neth. J. Sea Res. 24: 37–44

Burnett, B. R. (1981). Quantitative sampling of nanobiota (microbiota) of the deep-sea benthos. III. The bathyal San Diego Trough. Deep Sea Res. 28: 649–663

Chilingar, G. V., Bissell, H. J., Wolf, K. H. (1979). Diagenesis of carbonate sediments and epigenesis (or catagenesis) of limestone, In: Larsen, G., Chilingar, G. V (eds.) Diagenesis in sediments and sedimentary rocks. Elsevier, Amsterdam, p. 249–422

Collins, C. H., Lyne, P. M. (1976). Microbiological methods. Butterworth's, London

Dye, A. H. (1979). Quantitative estimation of protozoa from sandy substrates. Estuar. coast. mar Sci. 8: 199–204

Fenchel, T. (1967). The ecology of marine microbenthos. I. The quantitative importance of ciliates as compared with metazoans in various types of sediments. Ophelia 4: 121–137

Fenchel, T (1969). The ecology of marine microbenthos. IV Structure and function of the benthic ecosystem, its chemical and physical factors and the microfauna communities with special reference to the ciliate protozoa. Ophelia 6: 1–182

Fenchel, T (1975). The quantitative importance of the benthic microfauna of an Arctic tundra pond. Hydrobiol. 46: 445–464

Fenchel, T (1987). Ecology of protozoa. The biology of free-living phagotrophic protists. Science Tech., Madison

Furnas, M. J. (1989). Cyclonic disturbance and a phytoplankton bloom in a tropical shelf ecosystem. In: Okaichi, T., Anderson, D. M., Nemoto, T (eds.) Red tides: biology, environmental science, and toxicology. Elsevier, Amsterdam, p. 273–276

Furnas, M. J., Mitchell, A. W. (1987). Phytoplankton dynamics in the central Great Barrier Reef. II. Primary production. Cont. Shelf Res. 7: 1049–1062

Hansen, J. A., Alongi, D. M., Moriarty, D. J. W., Pollard, P. C. (1987). The dynamics of benthic microbial communities at Davies Reef, central Great Barrier Reef. Coral Reefs 6: 63–70

Kemp, P. F. (1988). Bacterivory by benthic ciliates: significance as a carbon source and impact on sediment bacteria. Mar. Ecol. Prog. Ser., 49: 163–169

Kudo, R. R. (1977). Protozoology, 5th edn. Thomas, Springfield

Lee, J. J., Hunter, S. H., Bovee, E. C. (1985). An illustrated guide to the protozoa. Society of Protozoologists, Allen Press, Kansas

Lighthart, B. (1969). Planktonic and benthic bacteriovorous protozoa at eleven stations in Puget Sound and adjacent Pacific Ocean. J. Fish. Res. Bd Can. 26: 299–304

Mare, M. F. (1942). A study of marine benthic community with special reference to the microorganisms. J. mar biol. Ass. U. K. 25: 517–574

Perkins, E. J. (1958). The food relationships of the microbenthos with particular reference to that found at Whitstable, Kent. Ann. Mag. nat. Hist. 1 64–77

Robertson, A. I. (1988). Decomposition of mangrove leaf litter in tropical Australia J. exp. mar Biol. Ecol. 116: 235–247

Robertson, A. I., Alongi, D. M., Daniel, P. A., Boto, K. G. (1988). How much mangrove detritus reaches the Great Barrier Reef lagoon? Proc. 6th Int. Coral Reef Symp. 2: 601–606

Sokal, R. R., Rohlf, F. J. (1981). Biometry. The principles and practice of statistics in biological research. W. H. Freeman & Co., San Francisco

Uhlig, G. (1964). Eine einfache Methode zur Extraktion der vagilen, mesopsammalen Mikrofauna. Helgoländer wiss. Meeresunters. 11: 78–185

Wyatt, C. E., Pearson, T. H. (1982). The Loch Eil project: population characteristics of ciliate protozoans from organically enriched sea-loch sediments. J. exp. mar. Biol. Ecol. 56: 279–303

This article was presented by Dr J. S. Bunt, Cairns, Australia

Manuscript first received: October 24, 1989
Revised version accepted: February 15, 1990