NUTRIENT PROCESSING ON TIDAL FLATS IN WESTERN PORT: INTERACTIONS WITH ECOLOGY AND IMPLICATIONS FOR BAY-WIDE NUTRIENT BUDGETS

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Chinaman Inlet seagrass meadow at low tide (Western Port)

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Photos: Victor Evrard

EXECUTIVE SUMMARY

Benthic habitats play an important role in aquatic ecosystem function. These habitats are fundamentally important in mediating nutrient cycling, which in turn influences the flora and fauna present and vice versa. Approximately one third of the area of Western Port is tidal flats and we have a very limited knowledge of nutrient cycling in such environments and of the relative importance of managing nutrient loads discharged from local waterways. The aims of this study were to: 1) quantify rates of nitrogen loss through denitrification in different habitat types on tidal flats in Western Port; 2) identify controlling factors such as faunal abundance that influence denitrification rates; 3) quantify the flux of nutrients between the sediment and water column in different habitat types; 4) quantify the fluxes of nutrients between the tidal flats and Western Port; and, 5) quantify macrofaunal abundances and their sources of nutrition using stable isotope analysis. This study was undertaken on two tidal flats: Watson Inlet, which is subject to high catchment-derived nitrogen loads from Watson Creek; and Chinaman inlet, which has no terrestrial inputs. The key findings of this study are summarized below and in a conceptual diagram (Fig. 1).

1. Bare tidal flat sediments had the highest rates of denitrification at both sites, and seagrass vegetated sediments typically had the lowest rates. The highest rates of denitrification were most likely associated with the high abundance of the burrowing shrimp *Biffarius arenosus*, which, by creating burrows, increased the surface area favourable to the activity of denitrifying bacteria. Scaling up the measured rates of denitrification to the whole of Western Port yields an estimated total annual removal rate of ~ 29 t per year compared to an estimated annual catchment load of ~600 t per year.
2. Rates of nitrogen fixation (a source of nitrogen to the system) by seagrass were ~1 mg N m^{-2} d^{-1} which scales up to ~12.5 t per year on a bay-wide basis.
3. Measurements of material exchange between the tidal flats and Western Port showed that the tidal flats were generally a source of dissolved nutrients including nitrogen and phosphorus (from the catchment and the flats). This was most marked at Watson Inlet during November when Watson Creek was flowing. A mass balance of NOx (the dominant form of bioavailable nitrogen) coming in from the catchment via Watson Creek compared to that leaving Watson Inlet showed that there was little retention within the inlet.
4. Small-scale flux measurements in core incubations showed there was generally a net uptake of nutrients from the water column by the sediments. This contrasts with the whole scale ecosystem-scale surveys, which showed a net release of nutrients, in particular phosphorus, highlighting the importance of drainage processes through the tidal flat (mostly through porewater flow enhanced by tidal pumping) in mediating nutrient fluxes.
5. In general, the macrofauna was more abundant at Chinaman Inlet, dominated by polychaetes (e.g. *Nephtys* sp.) and the ghost shrimp *B. arenosus,* with densities highest in the bare habitat. At Watson Inlet, the macrofauna was dominated by polychaetes (*Capitella* sp.) and *B. arenosus* with higher densities in the mangrove habitat. At both inlets, individuals of the mussel *Brachidontes erosus* were the most abundant in the seagrass habitat. Stable isotope food web analysis showed that benthic macrofauna relied on both seagrass and microphytobenthos (between 20-40%) as a source of organic matter in both vegetated and un-vegetated sediment.

6. The food web in Watson Inlet was consistently enriched in ^{15}N nitrogen (2.7‰) relative to Chinaman Inlet, reflecting a significant input NO_3^- with a heavier stable isotopic signature ($\delta^{15}N$ = 16.6‰) from the catchment, consistent with an anthropogenic source (fertilizers, waste water treatment plants, urban stormwater effluent). A two end-member mass balance suggests 18 % of the nitrogen assimilated into the foodweb is derived from Watson Creek while the remainder is derived from oceanic or coastal N.

We conclude that although internal nitrogen removal rates through denitrification are low on the tidal flats, relatively little nitrogen entering the system from land is assimilated into primary producers and the foodweb owing to high rates of tidal flushing within the bay. This means that localized hotspots of nutrient input show little impact of nutrient enrichment (as indicated by minimal change in the benthic infauna). It is, however, possible that less flushed areas (such as Corinella) may suffer from adverse water quality. This requires further investigation using a combination of hydrodynamic modeling and fieldwork.

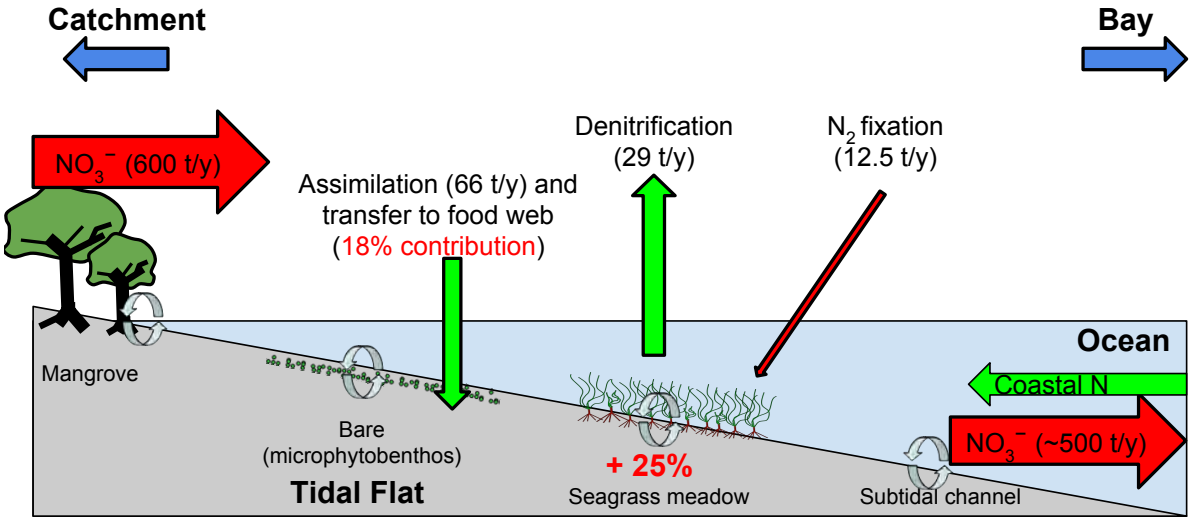


Fig. 1. Conceptual diagram of nutrient exchange between tidal flats and the bay in Western Port. Most anthropogenic N from the catchment entering the system (Watson Inlet) through fresh water discharge is exported to the bay (red arrows represent N inputs to the tidal flats and the bay). The flat offers little attenuation: where fresh water input occurs, anthropogenic N contributes ~ 18% of N assimilated by the intertidal food web; seagrass N content is 25% higher than at sites not influenced by anthropogenic N input (Chinaman Inlet) indicating that seagrass can store excess anthropogenic nitrogen, potentially buffering inputs to Western Port.

INTRODUCTION

Understanding if and where nutrients accumulate in marine systems is an important element of any environmental management strategy. Increased nutrient concentrations are often responsible for coastal eutrophication, a major issue leading to the loss of marine life and diversity, which ultimately can have a negative socio-economical impact. In Port Phillip, for example, extensive studies of nutrient flows have shown that seafloor sediments and organisms allow the Bay to cope with current inputs from Werribee Treatment Plant and other significant sources of anthropogenic nutrients (stormwater drains, streams and rivers).

Western Port, however, is very different to Port Phillip Bay and our understanding of nutrient flows there is inadequate. For example, one third of Western Port is intertidal seagrass and mudflats yet we know little of nutrient flows in those environments (elsewhere in the world, similar mudflats have been shown to be either sources or suppliers of nutrients to the marine ecosystems). Large changes in the extent of seagrass cover in recent decades and significant harvesting of ghost shrimps for fishing bait are two examples of subtantial environmental changes in Western Port (Contessa & Bird 2004; Keough 2011), yet we do not know how such changes will influence nutrient flows and what the broader impacts might be. Practically, our limited understanding of nutrient flows constrains our ability to prioritize nutrient reduction against other potential water quality improvement actions.

Here we contrasted the ecological and biogeochemical function of two tidal flats, one receiving high loads from the catchment (Watson Inlet, WI) and the other receiving no significant nutrient loading (Chinaman Inlet, CI).

This work aims to:

1. Quantify rates of nitrogen loss from tidal flats through denitrification in different habitat types on tidal flats in Western Port and identify controlling factors such as faunal abundance.
2. Quantify the flux of nutrients between the sediment and water column in different habitat types.
3. Quantify the fluxes of nutrients between the tidal flats and Western Port.
4. Quantify macrofaunal abundances and their sources of nutrition using stable isotope ratios.

METHODS

SAMPLING SITES

Two contrasting study sites were selected for the purpose of this research (Fig. 2). Watson Inlet (WI), situated off Yaringa, was selected as a high nutrient study site: Watson Creek (WC), which flows out into WI is a significant point of discharge for a catchment mainly comprised of agricultural lands. Chinaman Inlet (CI), situated off Warneet, was selected as a low nutrient study site as it doesn't receive any fresh water input from the land.

Fig. 2. Northern part of Western Port (Photo: www.nearmap.com). Site locations and 24 h offshore survey positions for Watson and Chinaman inlets (w and c, respectively). g corresponds to the location of the gauging station on Watson Creek.

PELAGIC FLUXES

Surface water flowing in and out of WI and CI was sampled offshore from RV Orca II during two tidal cycles in 24h time-series on 22nd – 24th November 2011 and on 21st – 23rd February 2012. Samples were analysed for chlorophyll a, total nitrogen (TN), total phosphorus (TP), nitrate/nitrite (NOx), filterable reactive phosphorus (FRP) and ammonium (NH_4^+) at 30 min intervals during the first field campaign and 60 min intervals during the second field campaign. Surface chlorophyll a, turbidity, salinity, dissolved O_2 and pH were recorded at 2 min intervals using a Hydrolab DX probe mounted on the side of the boat.

RV Orca II and onboard lab bench (Western Port)

Suspended particulate matter (SPM) was sampled at 1 h intervals by filtering seawater on 25 mm GF/F filters until clogged. Filters were subsequently analysed for organic C and N contents.

At each site, an Acoustic Doppler Current Profiler (ADCP; Argonaut-XR, Sontek YSI) was deployed at the bottom of the tidal channel and recorded water current velocities and water height at 1 Hz. A bathymetry survey of the cross section of the channel where the ADCP was deployed was conducted to allow the volume of water exchange between the inlet and the bay to be calculated (Figure 2).

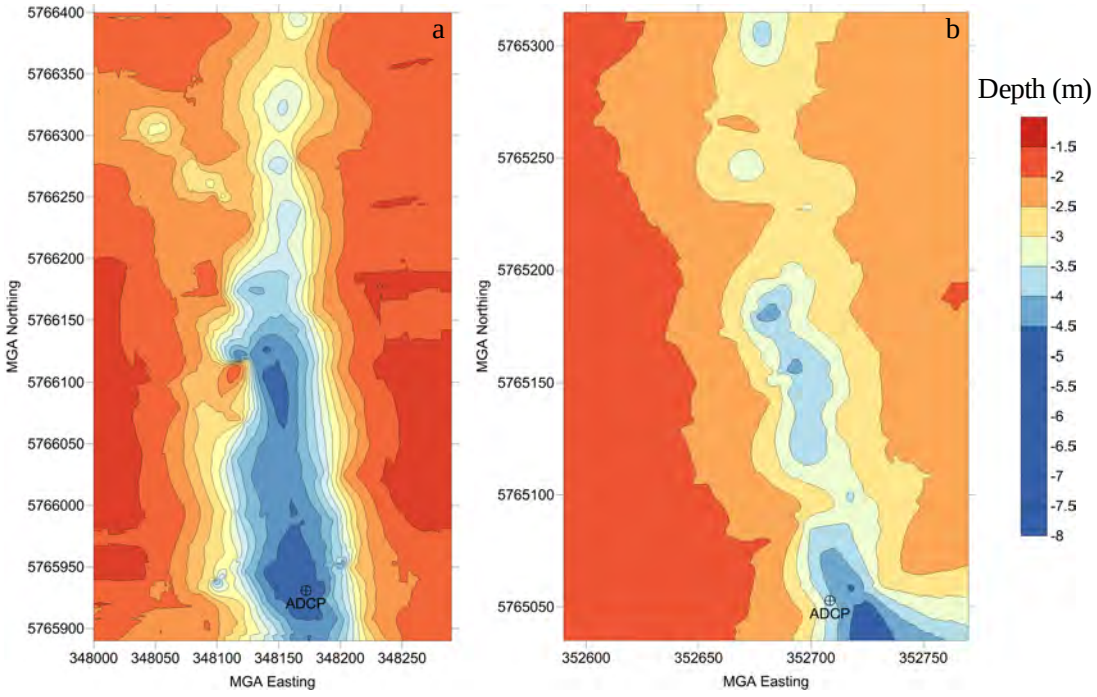


Fig. 3. Bathymetry survey and ADCP locations at a) Watson Inlet and b) Chinaman Inlet.

NUTRIENT MASS BALANCE

A mass balance of the nutrients entering Watson Inlet from Watson Creek and leaving at the study site was calculated. The loads of TN, TP, NOx, NH4+ and FRP in Watson Creek were based on continuous flow measured at the Melbourne Water gauging station (G, Fig. 2) and water samples taken from the creek over the sampling period during the November field trip only. The flow in the days immediately prior to the field trip was steadily decreasing after a rain event (Fig. 4). The loads into Watson Inlet were estimated in the 24 h prior to the field trip. The loads leaving Watson Inlet were calculated from concentrations of the relevant constituents at the study site in the inlet multiplied by the flow at the site calculated from the measured velocities and the channel cross sectional area. The fluxes were corrected for change in storage of the inlet between the start and the end of the time series. The mass balance equation is:

$$M = \sum_{t=0.5}^{24} C_t \times Q_t \times t - \Delta V \times C_{inlet}$$

where M = load, C_t = constituent concentration at time interval t, Q_t = average discharge over the time interval t, ΔV = change in volume of the inlet between the start and the end of the time series, C_{inlet} = the concentration of the constituent at the same phase of the tidal cycle for which the storage correction was made

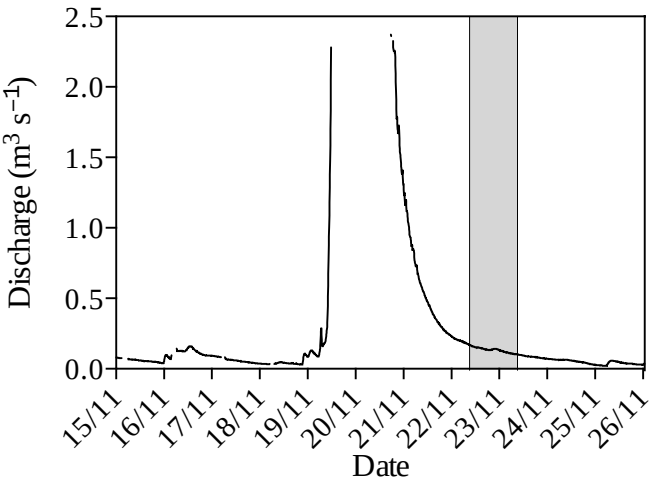
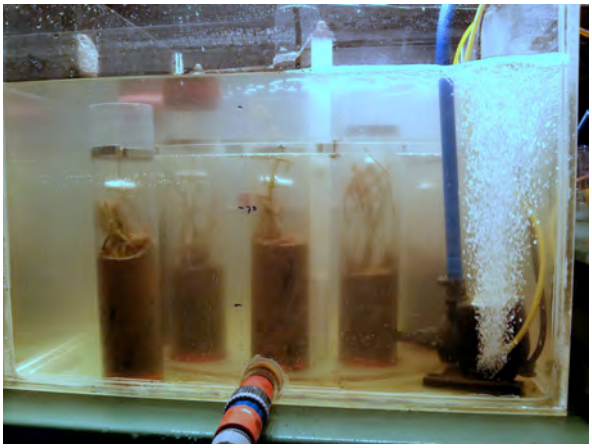


Fig. 4. Gauging station records of Watson Creek flow in November 2011. The shaded grey area indicates the 24h time series at Watson Inlet.

BENTHIC FLUXES



Seagrass sediment core incubation

Intact sediment cores (6.6 cm ID x ~15 cm h) from 3 different benthic intertidal habitats (mangrove, bare and seagrass) and channels were taken using acrylic core liners (7 cm OD x 30 cm h) and brought back to the laboratory for chamber incubation experiments. The sediment cores were incubated for 5 h in both light (188 µmol photons m^{-2} s^{-1}) and dark setup and dissolved inorganic carbon (DIC), dissolved oxygen (DO), total dissolved nitrogen (TDN), NH_4^+, total dissolved phosphorus (TDP), PO_4^{2-} and NOx fluxes were measured. Denitrification was quantified using the isotope pairing technique (Nielsen 1992). Core sampling and benthic flux experiments were carried out on the 13th of February and 12th of March 2012 for CI and WI respectively. Subsequent core sampling was carried out at both sites in March 2013 to assess N fixation in the seagrass habitat. N fixation was quantified following the acetylene reduction assay on whole intact cores using the methods in Capone (1982) and a 3:1 acetylene:N_2 ratio (Welsh 2000).

BENTHOS HABITATS SURVEY AND MACRO-INVERTEBRATE FOOD WEB STUDY

At both intertidal locations, 4 replicate 50 x 50 cm quadrats were randomly sampled in the seagrass dominated habitat. All above- and below-ground seagrass materials (leaves, roots and rhizomes) were collected in plastic bags and analysed for gross dry mass, organic C, N, and ^{13}C and ^{15}N stable isotopes.

The sediment surface (top 5 mm) was sampled from the mangrove, bare and seagrass habitats and analysed for Chl *a*, organic C, N, and ^{13}C and ^{15}N stable isotopes. The samples for isotopes were analysed on an ANCA GSL2 elemental analyser interfaced to a Hydra 20-22 continuous-flow isotope ratio mass-spectrometer (Sercon Ltd., UK). The precision is ±0.1‰ for ^{13}C and ±0.2‰ for ^{15}N (SD for n=5). Stable isotope data are expressed in the delta notation ($\delta^{13}C$ and $\delta^{15}N$), relative to the stable isotopic ratio of Vienna Pee Dee Belemnite standard (R_{VPDB}= 0.0111797) for C and atmospheric nitrogen (R_{Air} = 0.0036765) for nitrogen. Stable isotope data of the food web at both site and across habitats were analysed using the SIAR package in R (Parnell & Jackson 2011).

Chl *a* present in the top 5 mm of sediment at each habitat was measured using the method of Jeffrey and Humphrey (1975). Quadrats (50 × 50 cm) were used to estimate seagrass biomass in the seagrass habitat at CI and WI (whole plant in g DW m^{-2}).

The macrofauna (macro-invertebrates retained on 1 mm mesh sieve) was sampled by taking five replicate sediment cores (15cm diameter x 10 cm deep) in each habitat from which abundance and diversity were estimated. The macrofauna was also collected in each habitat for C and N stable isotope analysis. All individuals were identified to the lowest taxonomic level possible (genus or species for the numerically abundant taxa, and family or order for the less common taxa). Macrofauna community data were analysed using a factorial PERMANOVA (Anderson 2001) with two fixed factors (Site and Habitat). Analyses were based on Bray-Curtis dissimilarity measures and data were fourth root-transformed prior to analyses. Multivariate data were graphically represented by principal components ordination. Individual taxa that occurred in at least 5% of samples were analysed separately using two-factor analysis of variance tests that compared differences in abundances and biomass (wet weight per individual) between Habitats and Site. In all analyses, factors were fixed with three levels of Habitat (bare sediment, seagrass, mangrove) and two levels of Site (Chinaman Inlet and Watson Inlet). The homogeneity of variances assumption was tested using Cochran's test and boxplots and, when assumptions were not met, data was either square root or fourth root transformed (see Table 3 for details). Based on our experience undertaking macrofauna surveys on intertidal mudflats, we predicted that a 50% change from one habitat compared to the other two habitats would represent a significant biological difference. Since we were most interested in differences between Habitat rather than differences between Sites, power was calculated using an effect size of 50% increase in fauna from the sediment habitat compared to the other two habitats (seagrass and mangrove). The statistical package R version 2.13.1 (R Development Core Team, 2011) with the GAD package was used for all univariate data analyses and Microsoft Excel 2011 for graphs. Primer v6 was used for multivariate analyses and graphical presentation (Plymouth Marine Laboratory).

RESULTS

PELAGIC FLUXES

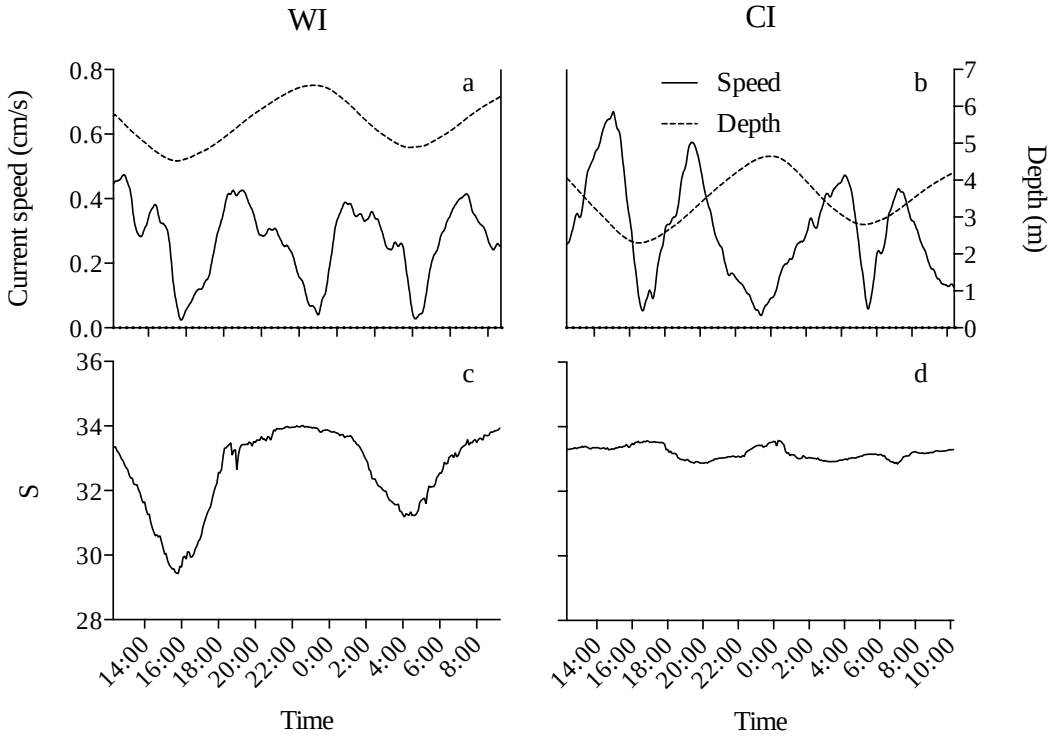


Fig. 5. November 2011 field campaign. Water level and current speed time series recorded by the Acoustic Doppler Current Profiler at the mouth of a) Watson Inlet (WI) and b) Chinaman Inlet (CI). Salinity time series recorded by the hydrolab at c) WI and d) CI sampling sites.

Despite different channel bathymetry, tidal amplitudes were similar at both sites during the November 2011 survey (Fig. 5a,b). Current speeds were lower at WI than at CI. Current speed at WI showed a double peak-pattern, consistent with a buffer effect from the creek's flow. Salinities were tightly coupled to the tides and varied between 30-34 (Fig. 5c,d). At WI, lower salinities were observed at low tide, consistent with the exchange of fresh water from the creek with seawater from the bay (Fig. 5d). Conversely, salinities were slightly higher at low tide at CI, consistent with evaporation during mudflat emersion (Fig. 5d). These salinity patterns were emphasized during the February 2012 survey, where increased salinities due to evaporation were clearly visible at low tide and significant at WI, consistent with the absence of flow in Watson Creek during this survey (Fig. 6).

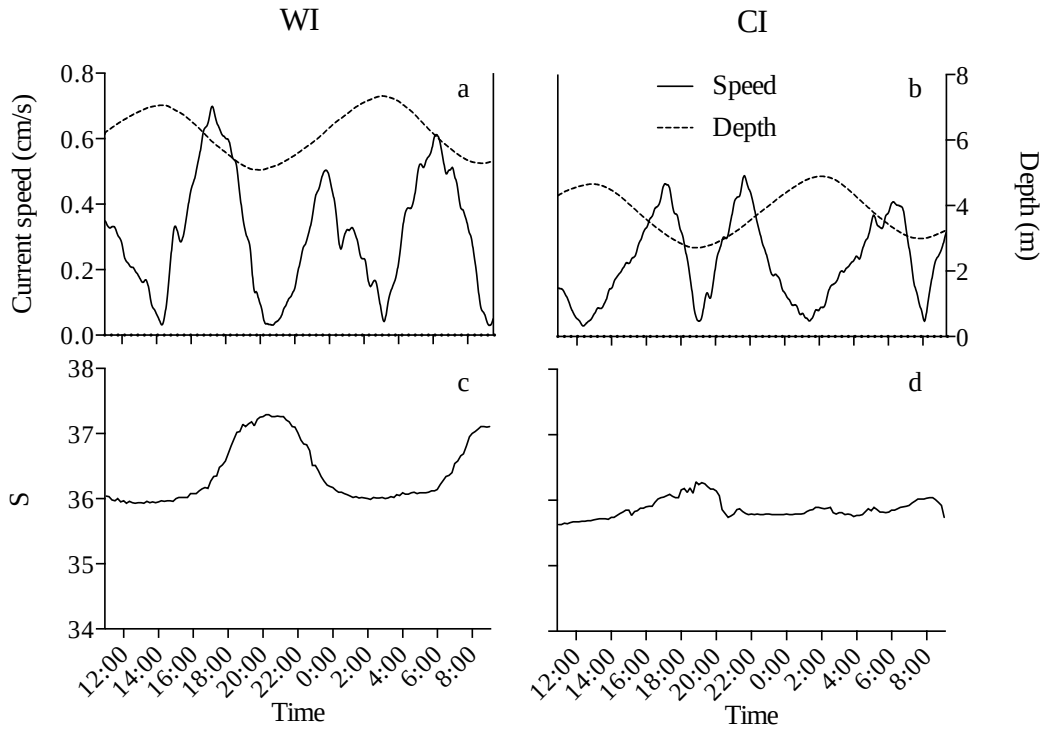


Fig. 6. February 2012 field campaign. Water level and current speed time series recorded by the Acoustic Doppler Current Profiler at the mouth of a) Watson Inlet (WI) and b) Chinaman Inlet (CI). Salinity time series recorded by the hydrolab at c) WI and d) CI sampling sites.

During the November 2011 survey, nutrient concentrations were tightly coupled to the tides at both sites, with higher concentrations at low tide and lower concentrations at high tide (Fig. 7). This pattern was more pronounced at WI than at CI, consistent with the recent flow event at Watson Creek. TN varied between ~0.2 and 0.9 mg L^{-1} at WI and between ~0.2 – 0.4 mg L-1 at CI (Fig. 7a-b). Total phosphorus (TP) and dissolved organic phosphorus (DOP) followed the same pattern, respective to each site. Nitrate (NOx) concentrations at WI were one order of magnitude higher than those reported for CI, consistent with Watson Creek's discharge (Fig. 7 c-d). During the February survey, nutrient concentrations were similar at both sites and slightly lower than in November (Fig. 8).

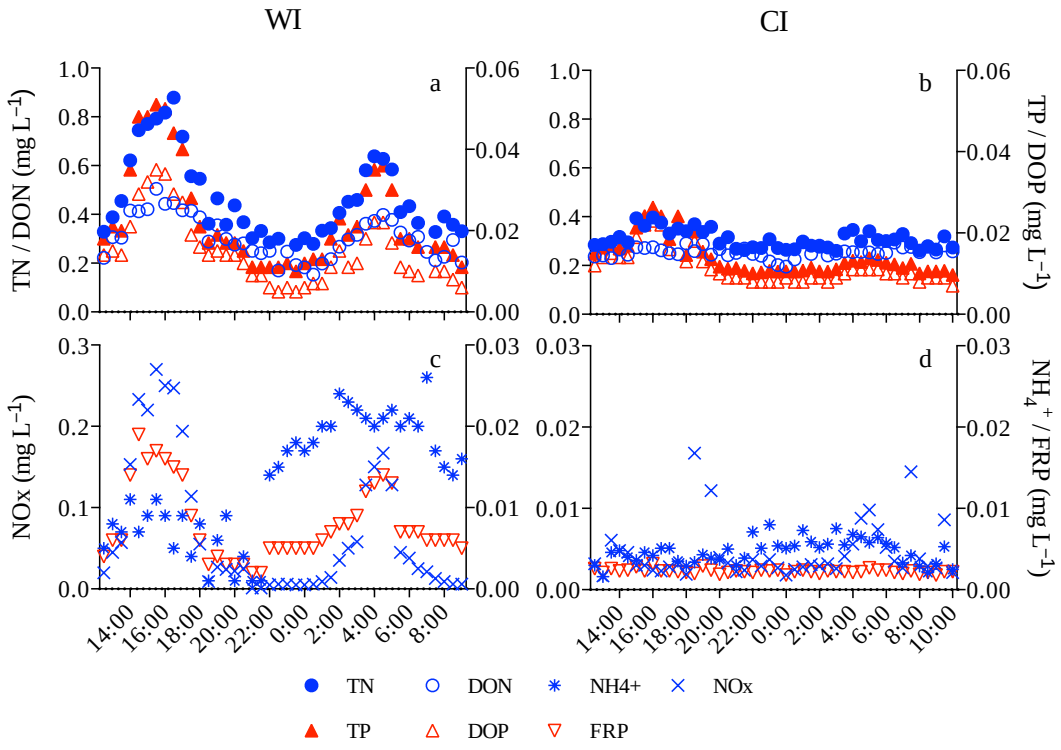


Fig. 7. Total nitrogen (TN), dissolved organic nitrogen (DON), NH_4^+, NOx, total phosphorus (TP), dissolved organic phosphorus (DOP) and filterable reactive phosphorus (FRP) concentration time series in surface water recorded at a,c) Watson inlet (WI) and b,d) Chinaman inlet (WI) during the November 2011 field campaign.

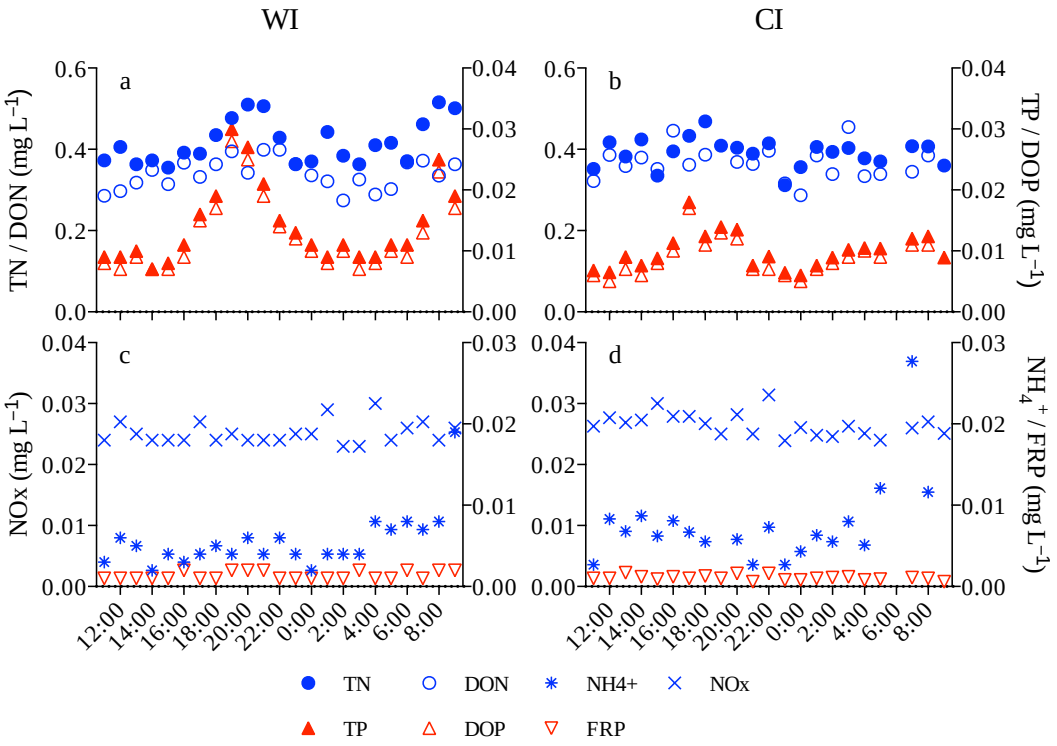


Fig. 8. Total nitrogen (TN), dissolved organic nitrogen (DON), NH_4^+, NOx, total phosphorus (TP), dissolved organic phosphorus (DOP) and filterable reactive phosphorus (FRP) concentration time series in surface water recorded at a,c) Watson inlet (WI) and b,d) Chinaman inlet (WI) during the February 2012 field campaign.

Chlorophyll *a* (Chl *a*) and dissolved oxygen (DO) recorded continuously in the water column showed patterns consistent with light availability (for photosynthesis) and tides (Fig. 9). Dissolved oxygen concentrations were typically lower at the beginning of the day, increased throughout the day and decreased throughout the night. Chl *a* concentration generally showed a delayed response to light availability and were higher at high tide, consistent with input from the bay. Although dissolved oxygen concentrations were similar at both locations and during both surveys, Chl *a* concentrations were slightly higher at WI in November (Fig. 9a).

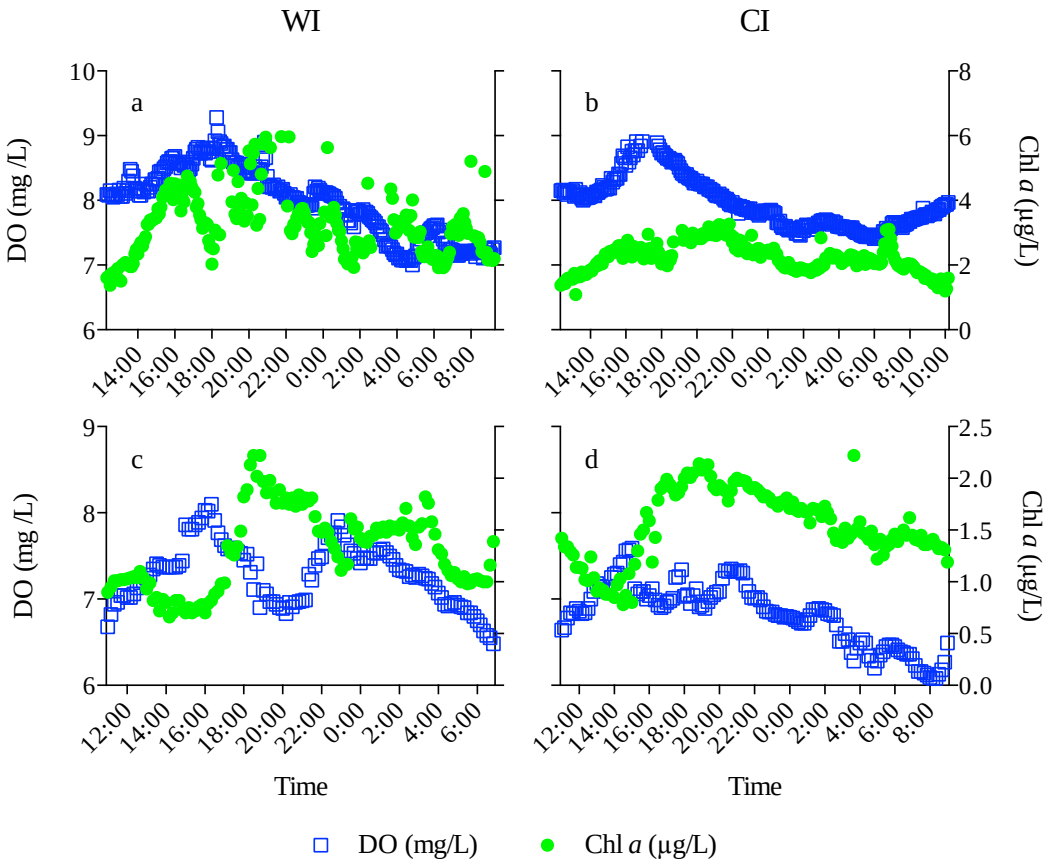


Fig. 9. Dissolved oxygen (DO) and chlorophyll *a* (Chl *a*) concentrations time series recorded at at Watson (WI) and Chinaman (CI) inlets in November 2011 (a and b, respectively) and in February 2012 (c and d, respectively).

WATSON INLET NUTRIENT MASS BALANCE

NOx, FRP and NH_4^+ were generally extremely low over the entire tidal cycle indicating an insignificant exchange between Western Port and the tidal flats. When Watson Creek was flowing in November, Watson Inlet was clearly a source of nutrients to Western Port (Table 1), although the export was slightly lower than the input from Watsons Creek for NOx and FRP indicating a small net retention for these constituents.

Table 1. Watson (WI) and Chinaman Inlet (CI) net nutrient export to the bay of Western Port, and Watson's Creek (WC) load contributions (mol) over two tidal cycles. Negative values represent a net retention of the nutrient.

	NH4+	NOx	FRP
Nov-11			
WC	389	25611	1649
WI	2757	-1096	-654
CI	365	-1286	46
Feb-12			
WI	1830	175	165
CI	945	-31	2

BENTHIC FLUXES

BENTHIC METABOLISM

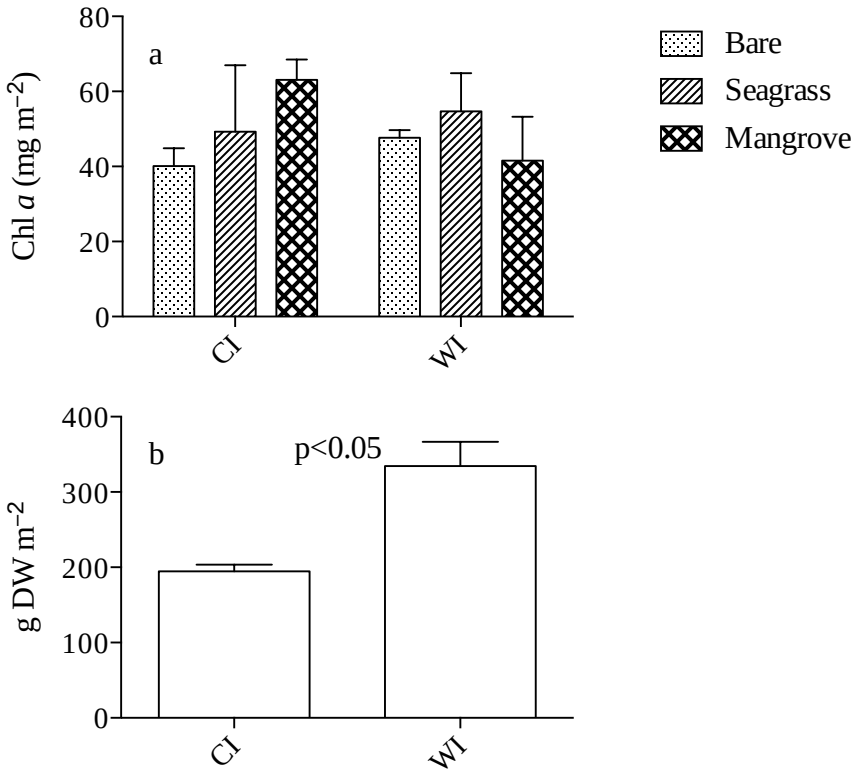


Fig. 10. a) Benthic Chl *a* (mg m^{-2}; mean ± SE, n = 3) in the top 5 mm of sediment in 3 different types of habitats in the intertidal area at Chinaman Inlet (CI) and Watson Inlet (WI). b) Seagrass biomass at CI and WI (g DW m^{-2}; mean ± SE, n = 4).

Benthic metabolism is an important parameter in aquatic ecosystems because it tells us whether the sediment is likely to be a net source of nutrients (net heterotrophic) or a sink for nutrients (net autotrophic). It has also been shown that the most autotrophic sediments have the lowest rates of denitrification (Risgaard-Petersen 2003). The upper 5 mm of sediment contained ~40-60 mg m^{-2} of Chl *a* and there were no significant differences between habitats and sites (Fig. 10). Seagrass-vegetated sediments were the most productive at both sites with net DIC fluxes of ~1300 and 2300 µmol m^{-2} h^{-1} at CI and WI respectively (Fig. 11a-b). WI was consistently the most productive site with DIC uptake occurring in all habitats (Fig. 11a). Unexpectedly, CI consistently had the highest rate of DIC production and O_2 uptake, indicating that the input of detrital material to this system may be important (Fig. 11b). This was consistent with high O_2 consumption and higher DIC production found in all habitats in dark incubations (Fig. 11d). Production to respiration ratios (P:R) were all positive at WI, with the exception of the channel habitat, suggesting that WI was net autotrophic in general (Fig. 11d). On the other hand the P:R ratios were all negative at CI, suggesting that CI was net heterotrophic (Fig. 11d).

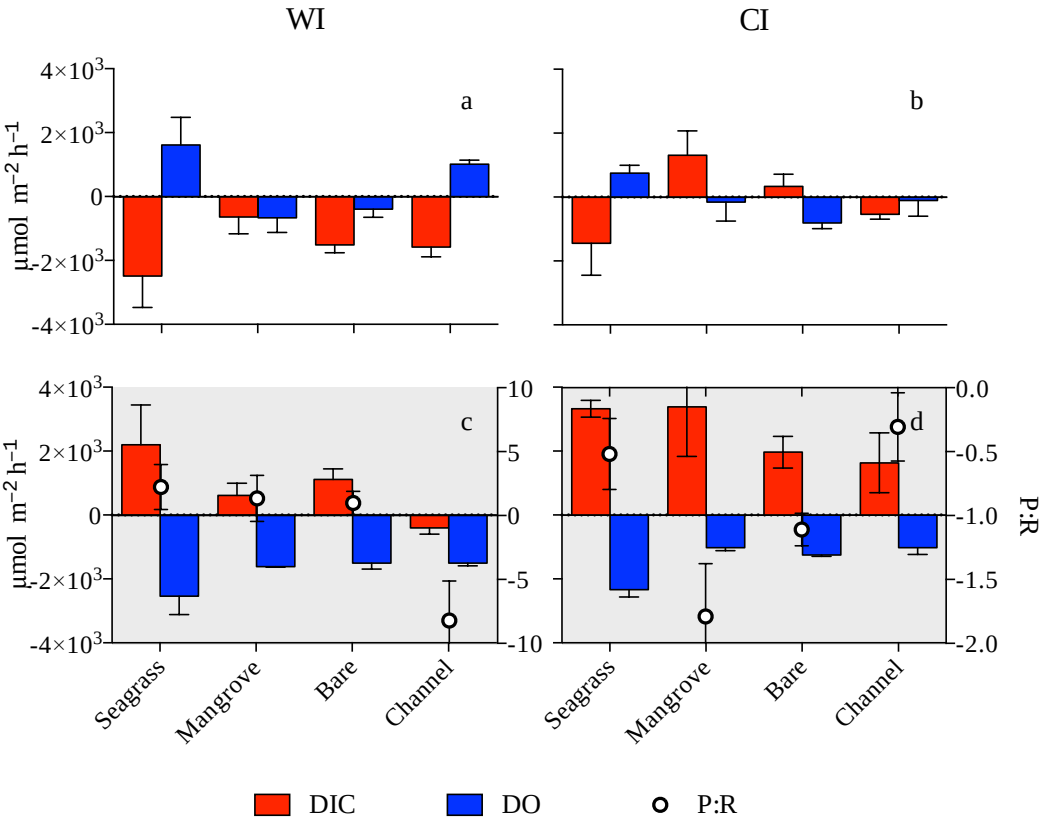


Fig. 11. a,b) Benthic metabolism (µmol m^{-2} h^{-1}; mean ± SE, n = 3) measured in core incubations in the light and c,d) in the dark (greyed background) with production to respiration ratio (P:R, no unit; mean ± SE, n = 3), at Watson (WI) and Chinaman (CI) inlets respectively.

NUTRIENTS

The sediments at WI were generally a sink for DIN in the light, and a slight source in the dark (Fig. 12a,c). Sediments at CI acted as both a nets source and sink in the light and a net source in the dark (Fig. 12b,d). TDN fluxes were highly variable showing a slight tendency to be a sink at WI in the light, and a source at CI in the light. There was no clear flux direction for TDN in the dark incubations. The sediments were consistently a sink for TDP and FRP in both the light and the dark (Fig. 12e-h), with the exception of the channel sediments that tended to be a source in the dark channel site at CI (Fig. 12h).

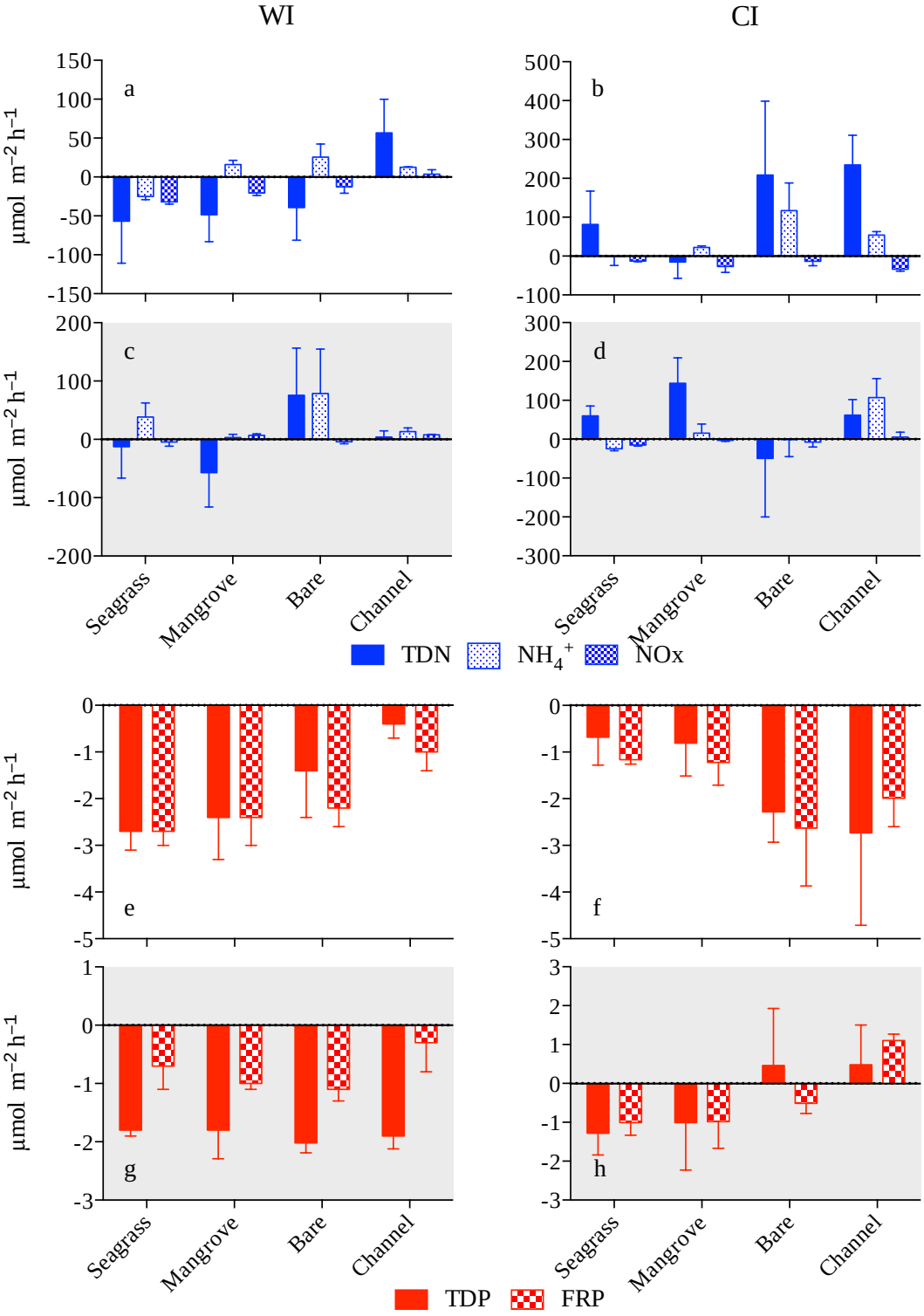


Fig. 12. Benthic nutrient fluxes (µmol m^{-2} h^{-1}; mean ± SE, n = 3) measured in core incubations in the light and in the dark (greyed background) at Watson and Chinaman inlets (WI left and CI right plots, respectively).

Denitrification

Rates of denitrification were generally highest in the bare and channel habitats at both sites and lowest in the mangrove and seagrass sediments (Fig. 13). For each habitat type, rates were generally

highest in the dark compared to the light and were consistently higher at CI compared to WI, for each habitat type (Table 2).

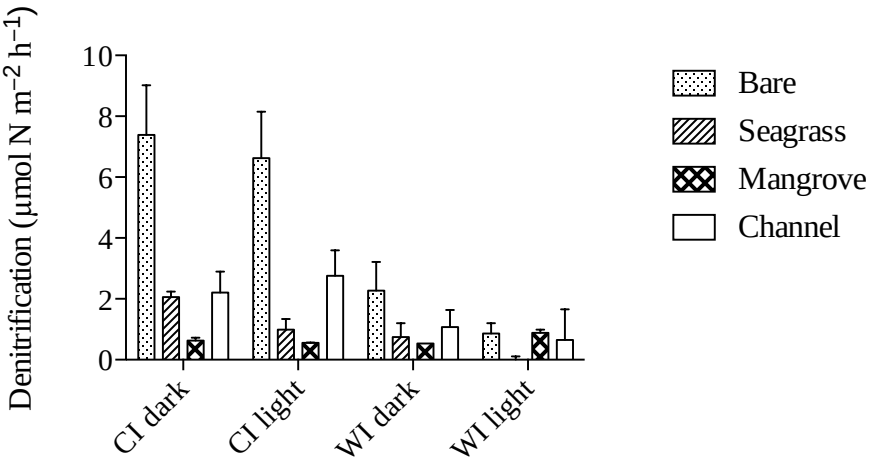


Fig. 13. Denitrification (µmol N m^{-2} h^{-1}; mean ± SE, n = 3) measured from light and dark incubations at Watson (WI) and Chinaman (CI) in all habitats.

NITROGEN FIXATION IN SEAGRASS MEADOWS

N fixation was low and there were no significant differences between sites (Fig. 14). However, N fixation in the dark was significantly higher than in the light ($F(1, 8) = 15.49$; $P = 0.0043$). N fixation represented a small contribution to the daily budget of N fluxes (Table 2)

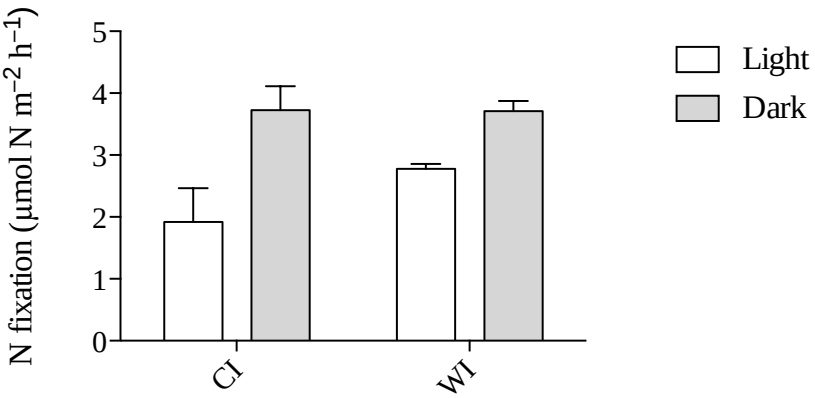


Fig. 14. Nitrogen fixation rates (µmol N m^{-2} h^{-1}; mean ± SE, n = 3) at Chinaman Inlet (CI) and Watson Inlet (WI) measured from both light and dark incubations.

Table 2. Nutrient fluxes, denitrification (D) and N fixation rates (µmol N m^{-2} d^{-1}) calculated using a 12:12 (h:h) light:dark cycle. Denitrification efficiency = 100 × D / (NH_4^+ + NO_x + D). Note that N fixation is negative to reflect the flow of N towards the sediment.

		NH_4^+	NO_x	D	N fixation	Denitrification efficiency
WI	Seagrass	161	-436	9	-6	-3
	Mangrove	230	-160	17		19
	Bare	1253	-202	38		3
	Channel	311	138	21		4
CI	Seagrass	-296	-331	37	-7	-6
	Mangrove	455	-351	14		12
	Bare	1389	-249	168		13
	Channel	1941	-334	60		4

MACROFAUNA

BIOMASS

Twenty-nine taxa were found in the macrofauna sediment cores. These included mostly polychaetes (10 taxa) and crustaceans (8 taxa), with some bivalves (3 taxa), gastropods (5 taxa) and fish (3 taxa). Multivariate analyses showed that differences in abundances and biomass between habitats varied between the two sites (Site x Habitat, $p < 0.05$, Table 3). Ordination of the abundance data revealed a clear separation between the assemblage of the mangrove habitat on one hand and those of the bare sediment and seagrass habitats on the other hand (Axis 1, Fig. 15a). The ordination on Axis 2 revealed a separation between CI and WI sites. The ordination of the biomass data showed clear separation between habitats on both axes 1 and 2, irrespective of sites (Fig. 15b). Total numbers of individuals did not vary between Habitat or Site (Table 3, Fig. 16). Total biomass was greater in seagrass habitats at both sites and overall slightly great at WI compared to CI (Table 3, Fig. 17).

Table 3. Results of PERMANOVA comparing multivariate data for macrofauna abundances and biomass between sites and habitats. Significant P-values < 0.05 are bold.

		Abundance		Biomass	
	df	MS	P (perm)	MS	P (perm)
Site	1	5430	**0.002**	3626	**0.0094**
Habitat	2	9008	**0.0001**	10345	**0.0001**
Site × Habitat	2	2552	**0.0073**	2712	**0.0147**
Residual	24	1049		1221	

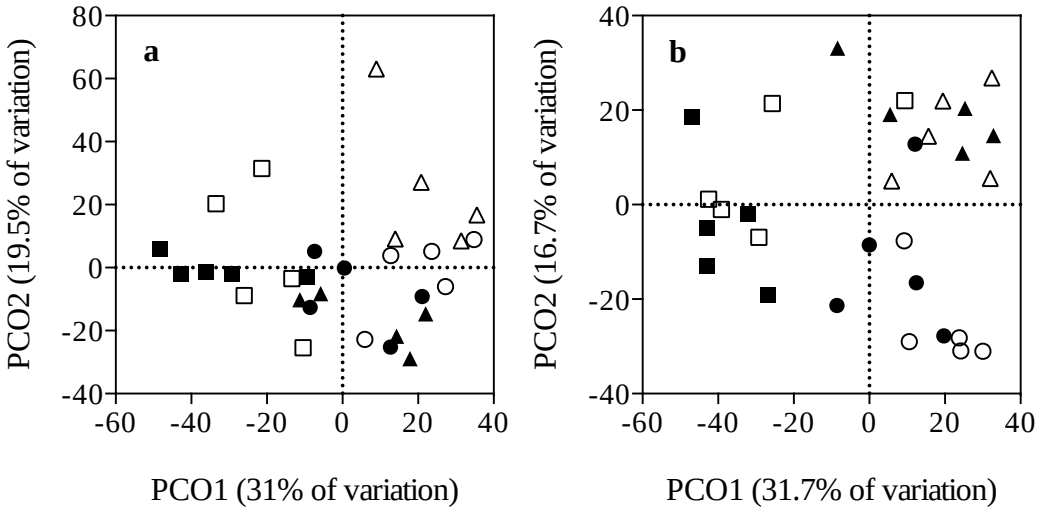


Fig. 15. Principal coordinate analysis (axes 1 and 2) of fauna assemblages for a) abundances and b) biomass. Symbols represent bare sediment (circle), seagrass (square) and mangrove (triangle) habitats, at Chinaman Inlet (white) or Watson Inlet (black).

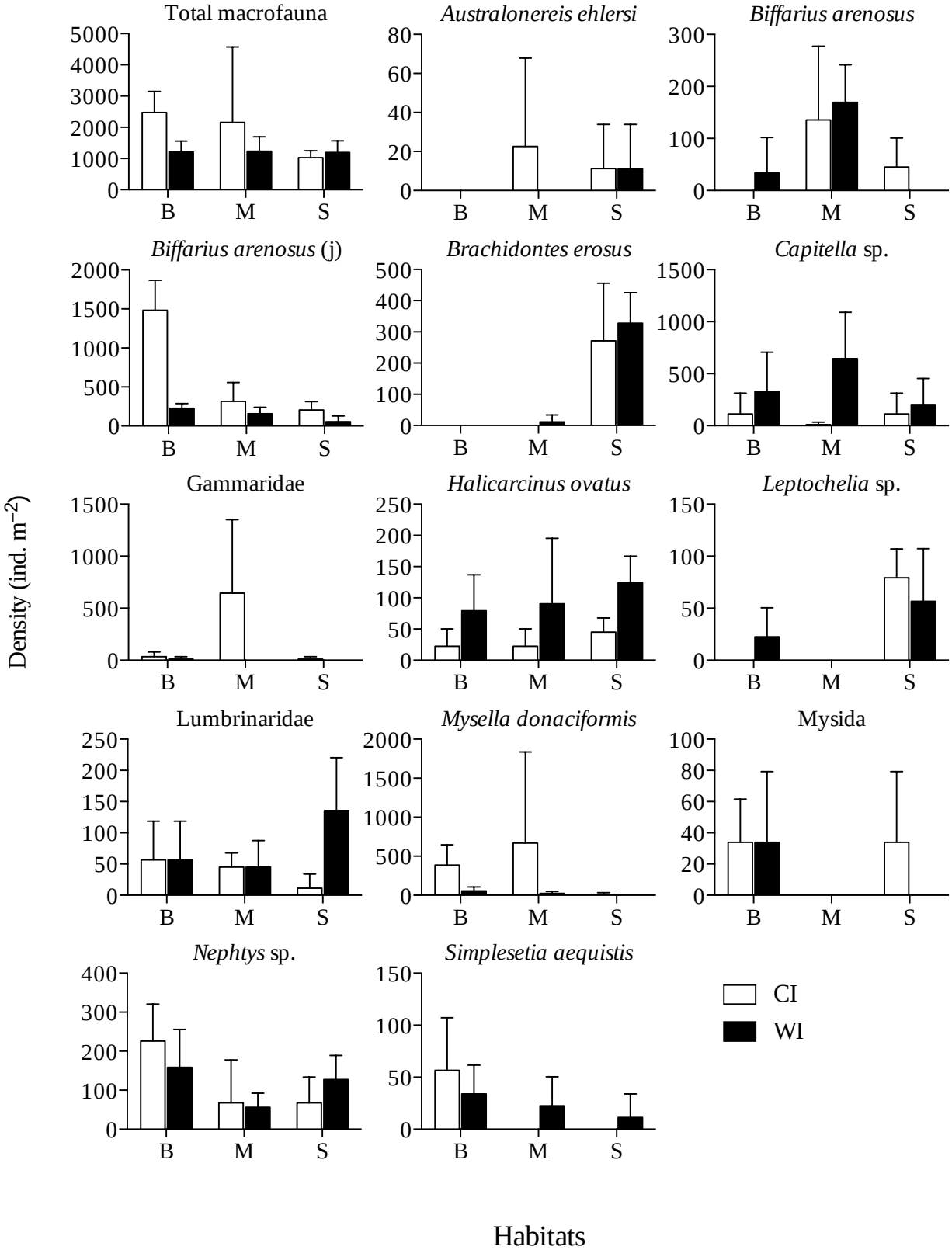


Fig. 15. Macrofauna density (ind. m^{-2}; mean + SD, n=5) in bare sediment (B), mangrove (M) and seagrass (S) habitats at Chinaman Inlet (CI, white bars) and Watson Inlet (WI, black bars).

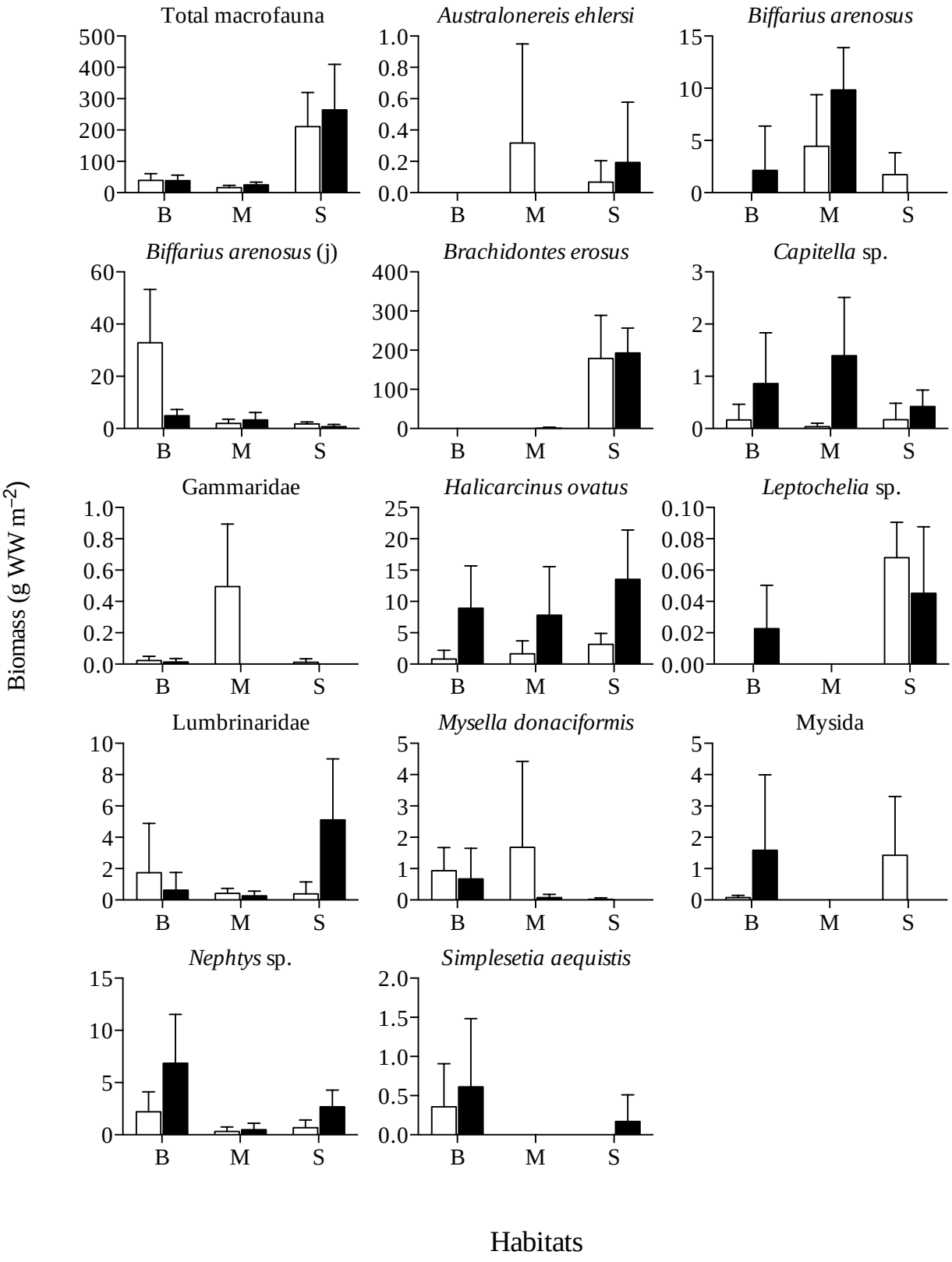


Fig. 16. Macrofauna biomass (g WW m^{-2}; mean + SD, n=5) in bare sediment (B), mangrove (M) and seagrass (S) habitats at Chinaman Inlet (CI, white bars) and Watson Inlet (WI, black bars).

Juvenile ghost shrimp, *Biffarius arenosus*, were most abundant in bare sediments at CI and comprised 50-70% of individuals (Fig. 16), while in the other habitats at CI and all the habitats at WI, it accounted for 16 ± 12% of individuals (average ± std error). Biomass of juvenile *B. arenosus* was variable among Habitats and between Sites (Table 4) with higher biomass in bare sediment at CI (Fig. 17). There were few adult *B. arenosus* (total of 34 individuals) and these occurred mostly in the mangrove habitats (Fig. 16). There was a significant effect of Habitat on the abundance and biomass of adult *B. arenosus* (Table 4).

Abundance and biomass of the crab *Halicarcinus ovatus* were significantly higher at WI compared to CI, irrespective of habitat (Table 4, Figs. 15 & 16). Abundances and biomass of Gammaridae amphipods were significantly different among habitats depending on site (Table 4). Fifty-seven individuals were found in the CI mangrove habitat, while only one or two individuals were found at the other sites (Fig. 16). Isopods, *Leptochelia* sp., and Mysida made up <3% of total numbers of individuals and 1% of total biomass and occurred only in the seagrass and bare sediment habitats (Figs. 15 &16).

There were significantly higher abundances and biomass of the mussel, *Brachidontes erosus*, in the seagrass habitats at both sites (Table 4, Figs. 15 &16). The polychaete *Capitella* sp. was more abundant overall in the WI habitats compared to CI (Table 4, Fig. 16). This pattern was also reflected in the biomass data for *Capitella* sp. (Fig. 17). There was an overall main effect of habitat on abundances and biomass of *Nephtys* sp. (Table 4), which tended to increase from mangrove to seagrass to bare sediment habitats (Fig. 16 & 16). In addition, biomass of *Nephtys* sp. was significantly higher at WI compared to CI (Fig. 17). *Simplesetia aequistis* was more abundant in the bare sediment habitat compared to the other habitats at both sites (Table 4, Fig. 16), while there were no significant differences in biomass (Table 4, Fig. 17). There was no effect of Site or Habitat on the abundance or biomass of *Australonereis ehlersi* (Table 4). The bivalve *Mysella donaciformis* occurred at CI in the mangrove and bare sediment habitats (Table 4, Fig. 16), with a similar pattern reflected in the biomass measurements of higher biomass in the mangrove and bare sediment habitats, irrespective of site (Table 4, Fig. 17).

Table 4. Results of analysis of variance comparing macrofauna abundances and biomass among Habitats (Sediment, Seagrass, Mangroves) at each Sites (Chinaman Inlet, Watson Inlet). Significant p-values (<0.05) are bold. Response variables were fourth root transformed prior to analysis unless indicated (* = square root transformed, ^ = no transformation). Power values are based on 50% change from one habitat compared to the other two.

	Abundance					Biomass				
	Site (df=1)	Habitat (df=3)	Site × Habitat (df=2)	$MS_{residual}$ (df=24)	Power	Site (df=1)	Habitat (df=3)	Site × Habitat (df=2)	$MS_{residual}$ (df=24)	Power
Total Macrofauna	0.146	0.193	0.211	0.091	0.98	**0.02**	**<0.001**	0.327	0.021	-
Australonereis ehlersi	0.526	0.424	0.666	0.114	0.1	0.6	0.445	0.582	0.013	0.33
Biffarius arenosus	0.858	**<0.001**	0.108	0.196	-	0.15	**<0.001**	**0.027**	<0.001 ^	-
Biffarius arenosus (j)	**0.002**	**<0.001**	0.068	0.194	-	0.29	**0.001**	**0.047**	<0.001 ^	-
Brachidontes erosus	0.193	**<0.001**	0.608	0.119	-	0.24	**<0.001**	0.683	0.039	-
Capitella sp.	**0.013**	0.492	0.156	31.500 ^	0.98	**0.01**	0.812	0.944	0.001 *	0.23
Gammaridae	**<0.001**	**0.001**	**<0.001**	0.165	-	**0.01**	0.088	**0.032**	<0.001 ^	0.07
Halicarcinus ovatus	**0.037**	0.155	0.937	0.262	0.42	**0.01**	0.28	0.582	0.002 ^	0.11
Leptochelia sp.	0.999	**<0.001**	**0.048**	0.116	-	1	**<0.001**	0.283	0.003	-
Lumbrineridae	0.073	0.584	**0.046**	1.150 ^	0.14	0.29	0.21	**0.012**	0.005 *	0.16
Mysella donaciformis	**0.018**	**0.005**	0.492	0.381	-	0.31	**0.006**	0.995	0.013	-
Mysida	0.34	0.078	0.402	0.317 ^	0.32	0.43	0.09	0.222	0.023	0.28
Nephtys sp.	0.662	**0.01**	0.484	2.717 ^	-	**0.02**	**0.041**	0.581	0.005 *	-
Simplisetia aequistis	0.292	**0.029**	0.496	0.198	-	0.14	0.203	0.707	0.004 *	0.21

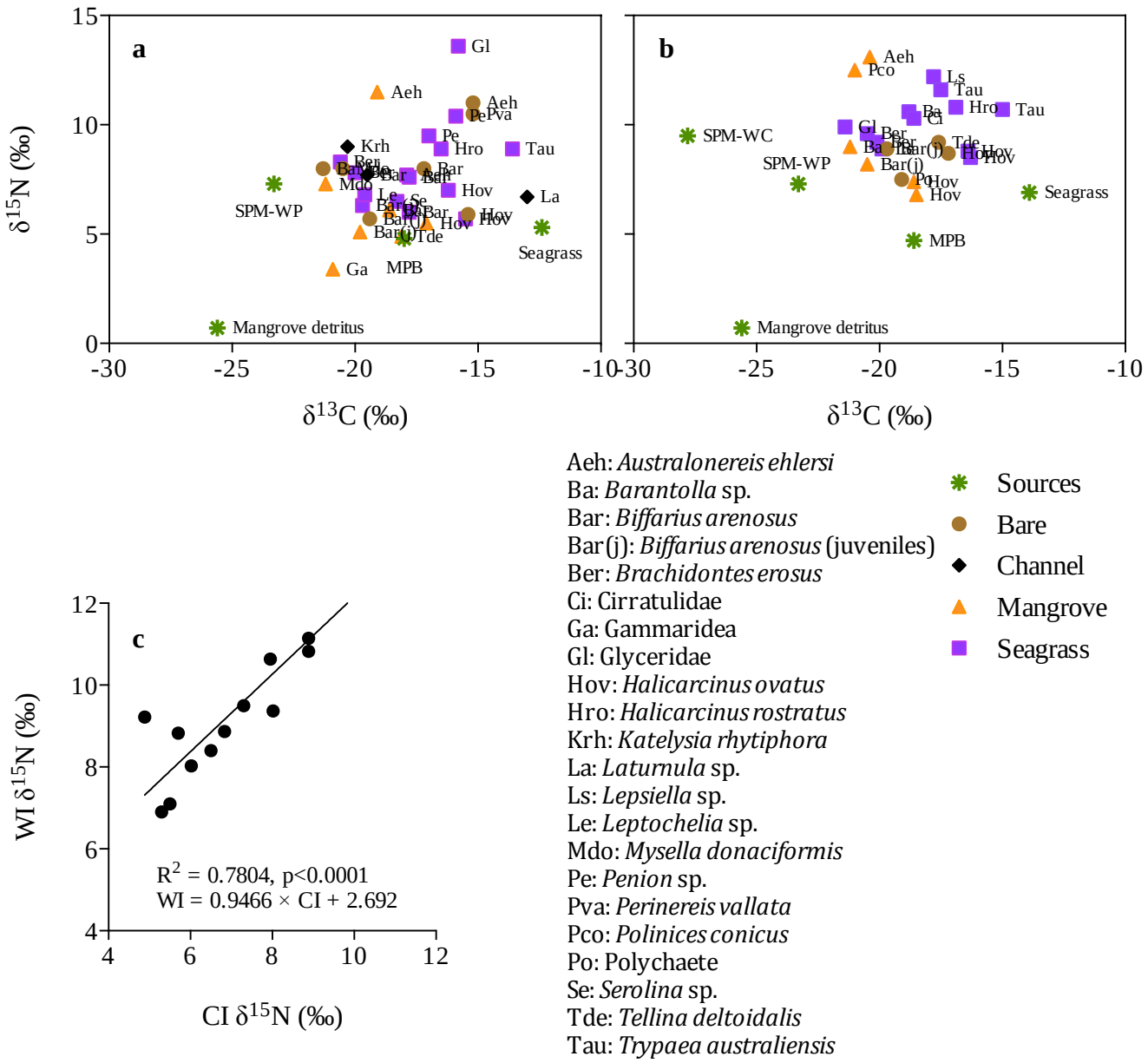


Fig. 18. ^{13}C and ^{15}N stable isotope values of resources (seagrass; MPB: microphytobenthos; SPM-WP: suspended particulate matter from Western Port; SPM-WC: suspended particulate matter from Watson Creek) and consumers at a) Chinaman (CI) and b) Watson (WI) inlets. c) Relationship between $\delta^{15}N$ values of resources and consumers at CI and WI.

Four potential primary food sources were identified from both inlets. Mangrove detritus had the lowest stable isotope signatures ($\delta^{13}C$ = −25.6‰, $\delta^{15}N$ = 0.7‰; Fig. 18). Mangrove detritus didn't appear to be a major contributor to the benthic macrofauna community and was therefore ignored in the following analysis. On the other hand, suspended particulate matter (SPM from Western Port; −23.3‰, $\delta^{15}N$ = 7.3‰), surface sediment ($\delta^{13}C$ ~−20‰, $\delta^{15}N$ ~ 3.5‰) and seagrass ($\delta^{13}C$ = −13.9/−12.4‰, $\delta^{15}N$ = 6.9/4.3‰; for WI and CI respectively) seemed to support most consumers. In

addition, SPM coming from Watson Creek was considered as an additional potential food source at WI.

At both sites, all consumers were well constrained within these 3 or 4 food sources suggesting contributions from phytoplankton, microphytobenthos (MPB) and seagrass. A clear shift of + 2.7 ‰ was observed between $\delta^{15}N$ values in CI and WI (Fig. 18a,b). This shift was consistent for seagrass leaves, SPM and consumers, suggesting that anthropogenic N inputs from the catchment delivered by the creek contributed significantly to the N requirements of primary producers,and subsequently the consumers in WI (Fig. 18c). With a $\delta^{15}N\text{-}NO_3^-$ measured at Watson Creek of 16.6‰, a consistent + 2.7 ‰ shift in primary producers and consumers suggests that Watson Creek's nitrogen loads contributed 16% of the assimilated N in the inlet's food web.

Food source contributions were assessed in each habitat for WI and CI (Fig. 19). The analysis revealed that sources generally contributed equally to the diet of the benthic fauna. However, suspended particulate matter (SPM) contributed more in the mangrove habitat. Seagrass and SPM seemed to be the main food source in the seagrass habitat at both CI and WI. When all habitats were pooled for the analysis, MPB contributed the least to the macrofauna diet at both sites.

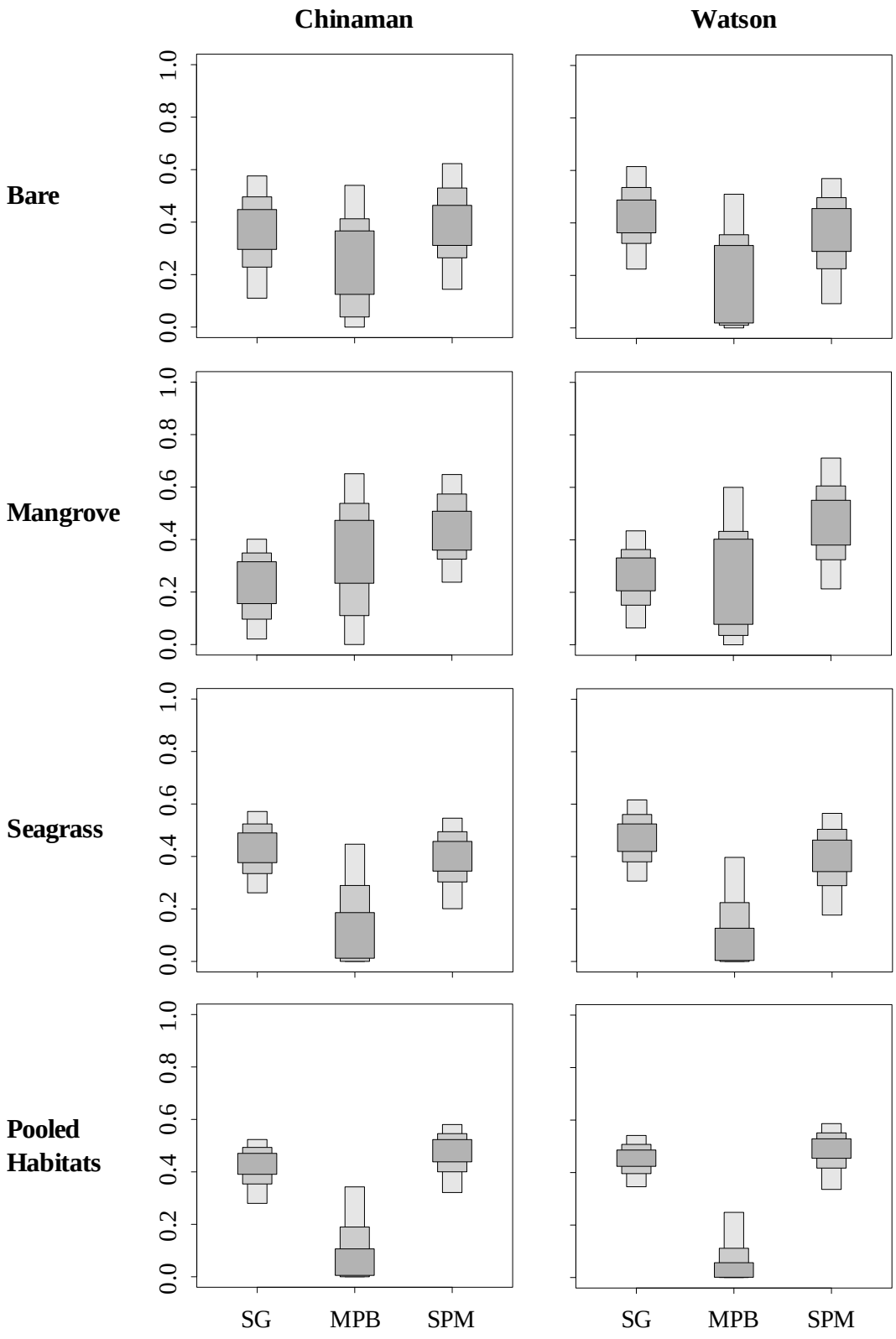
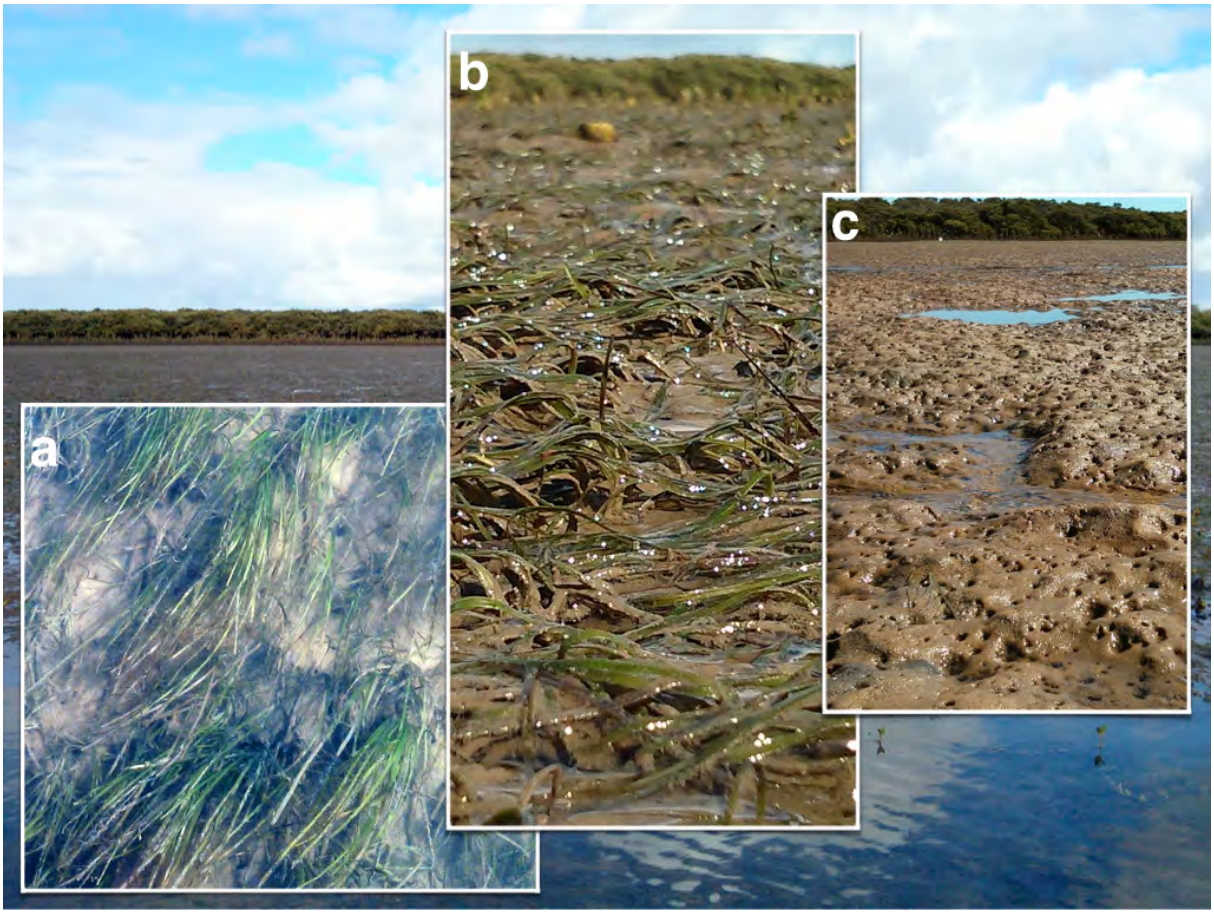


Fig. 19. Seagrass (SG), microphytobenthos (MPB) and suspended particulate matter (SPM) contributions (95, 75 and 25% credibility intervals) to the diet of the macrofauna in habitat-specific and pooled habitats at Chinaman (CI) and Watson (WI) inlets.

DISCUSSION

COMPARISON OF LARGE-SCALE EXCHANGE WITH BENTHIC FLUXES.

Unsurprisingly, the estimates of material exchange for the two inlets differed greatly in both size and magnitude, with the whole system estimates (Total Pelagic) generally showing a much greater export than the core incubations (Table 5). The most likely explanation for this discrepancy is that core incubations neglect the effects of sediment resuspension, porewater drainage through the tidal flats and water column-based biogeochemical processes (e.g. nutrient transformation through photosynthesis, bacterial and zooplankton assimilation). In addition, this exchange budget was estimated from two discrete 24 h time series and extrapolated to the whole system of the inlets.



a. Subtidal and b. intertidal seagrass (Zostera muelleri) at low tide at Watson Inlet. c. Bare sediment at Chinaman Inlet

Table 5. February daily exchange (mol) of total dissolved inorganic C (TCO_2), dissolved oxygen (DO), oxidised nitrogen (NOx), filterable reactive phosphorus (FRP) and denitrification (Dn) at Watson (WI) and Chinaman (CI) inlets over the course of 24 h assuming a 12:12 (h:h) light/dark cycle. Total pelagic nutrient fluxes over the course of 2 tidal cycles (~25 h) are included here for comparison. Percent habitat coverage is given between brackets: *D. Ball, pers comm; [†]areas estimated from Nearmap (www.nearmap.com). [#]CI Bare:Seagrass cover ratio of the non-mangrove intertidal area assumed similar to WI (43.7:47.7).

	Habitat	TCO_2	DO	NH_4^+	NOx	FRP	D
Watson Inlet	Bare (43.7*)	-5985.3	-29006.9	1594.4	-256.6	-50.4	48.7
	Channel (8.4*)	-5820.3	-1448.9	76.1	33.8	-3.8	3.9
	Mangrove (0.2*)	-1.6	-159.6	1.3	-0.9	-0.2	0.1
	Seagrass (47.7*)	-4793.4	-15464.9	223.6	-605.8	-56.7	14.2
Total benthic (2913892 m^{2*})		-16600.6	-46080.2	1895.4	-829.5	-111.2	66.9
Total Pelagic				1829.7	175.0	165.2	
Chinaman Inlet	Bare (21.4[†#])	6620.4	-5905.9	332.4	-59.6	-9.0	44.2
	Channel (2.8[†])	415.4	-428.4	61.5	-10.6	-0.3	2.0
	Mangrove (52.6[†])	33155.0	-8318.9	267.7	-206.8	-15.5	9.6
	Seagrass (23.2[†#])	5860.8	-4915.8	-76.9	-85.8	-6.7	9.5
Total benthic (1118964 $m^{2†}$)		46051.6	-19569.0	584.7	-362.8	-31.6	65.3
Total Pelagic				945.5	-30.6	1.8	

ENVIRONMENTAL CONTROLS ON DENITRIFICATION

Denitrification is a key process of interest in this study because it represents a sink for nitrogen entering Western Port from the catchment. The patterns of denitrification and apparent controls observed here are consistent with previous studies in other systems:

1. Primary producers. Benthic microphytes are known to exert a critical control over denitrification because they compete with nitrifying and denitrifying bacteria for available nitrogen (Risgaard-Petersen 2003; Risgaard-Petersen et al. 2004). This effect was apparent in our study as evidenced by the highest rates of denitrification being observed at the most heterotrophic site (CI bare sediment) and the lower rates of denitrification that were generally observed in incubations in the light.
2. Consumers. Macrofauna is known to have a strong stimulatory effect on denitrification (Rysgaard et al. 1995). A similar effect was observed in our study where the highest rates of denitrification were correlated with highest abundances of the ghost shrimp *Biffarius arenosus* (Fig. 30). Burrowing thalassinidean shrimps have been known to enhance denitrification (Webb & Eyre 2004) and our results confirm the important role of fauna in maintaining ecosystem health.
3. Elevation has previously been shown to affect denitrification, with highest rates typically being observed in the lower intertidal compared to the higher intertidal zones (Cook et al. 2004). We observed this pattern here, with the lowest rates of denitrification occurring in the upper intertidal and relatively higher rates being found in the sub-tidal zone.

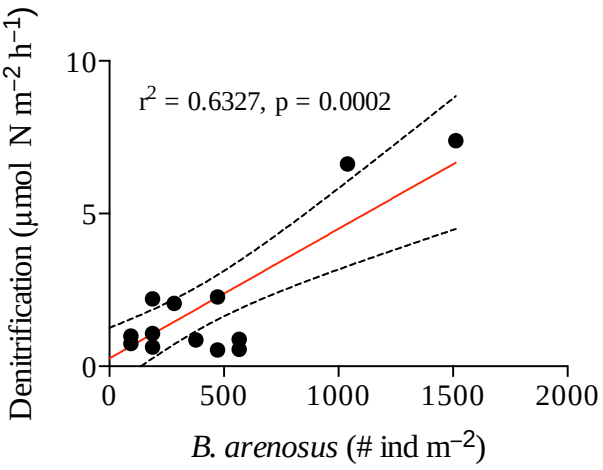


Fig. 30. Relationship between ghost shrimp *Biffarius arenosus* abundances and denitrification (with 95% confidence interval).

IMPORTANCE OF TIDAL FLATS IN CATCHMENT NITROGEN ATTENUATION.

One of the primary goals of this study is to evaluate the importance of the sediments in the nitrogen budget of Western Port. Scaling up the denitrification rates measured in this study to other tidal flats in the bay is highly uncertain, however it can give us a first order indication of the likely magnitude of denitrification compared to external inputs. Here we make this comparison on the scale of Watson Inlet, and the whole of Western Port.

The importance of tidal flats in attenuating nutrient input from the catchment can be best gauged on a whole system scale by comparing nutrient loads into WI from Watson Creek, with that exported from WI. In this study we quantified TN, NH_4^+, NOx, DON, TP and FRP. Here we focus our discussion on NOx, because it is the most bioavailable major form of nitrogen exported from Watson Creek. Furthermore, concentrations of NOx were extremely low in Western Port, greatly reducing the uncertainty in the net export. The total import of NOx from Watson Creek (incoming load) to Watson Inlet over two tidal cycles was about 25600 mol and the total export from WI to the bay was ~ 24500 mol, indicating that there was very little to no attenuation within Watson Inlet. This was consistent with the pattern observed in February for NOx, which showed a small export.

If we scale up the denitrification rates measured in WI and CI using habitat covers of 48% seagrass, 44% bare (the rest being composed of channel and mangrove habitats; D. Ball pers comm) over the study period, we calculate at total denitrification rate of 67 and 65 mol d^{-1} for WI and CI respectively (Table 5). At WI, this is negligible compared to the incoming load from WC and this is consistent with the whole system measurements, which shows that most N is exported to the bay. The small amount of nitrogen assimilated at Watson inlet is consistent with the isotope mass balance undertaken above that suggests only 18% of the nitrogen in the food web in Watson inlet is derived from Watson Creek (82% originates either from ocean sources, elsewhere in the bay or possibly N_2 fixation).

The estimated total area of intertidal flats in the northern half of Western Port is 70 km^2 (visual analysis with Nearmap). Scaling the mean rate of denitrification measured in this study (0.42 g m^{-2} y^{-1}) over this surface area and assuming the same habitat distribution correspond to an annual denitrification rate of 29 t which is about 5% of the estimated annual load to the bay (600 t). The low

attenuation rate of nitrogen by denitrification is consistent with previous studies which show low nitrogen removal rates in systems with low residence times (Nixon et al. 1996). The low residence times in Western Port, however, make this system less susceptible to eutrophication because nutrients are exported before they can be assimilated. In the case of Watson Inlet, this is exemplified by the low concentrations of chlorophyll a in the water column (Fig. 9) and the relatively small amount of terrestrial nitrogen assimilated by seagrass. There is, however, a risk that the exported nitrogen can lead to high chlorophyll a concentrations in 'downstream' water masses with high residence times. An example of such a region could be the Corinella section of Western Port in the south east of Western Port where high chlorophyll concentrations have been observed (Keough 2011). The possibility of this should be further investigated using both hydrodynamic models to understand water sources and residence times as well as investigate local nutrient sources.

SEAGRASS NUTRIENT DYNAMICS

NITROGEN FIXATION AND NUTRIENT UPTAKE

Nitrogen fixation rates, measured in whole core incubations, were 68 and 78 µmol N m^{-2} d^{-1} (average of ~1 mg m^{-2} d^{-1}) at WI and CI respectively. These rates are similar to those generally found in temperate areas (Welsh 2000). Assuming that seagrass meadows cover about 48% of the intertidal area of Western Port, N fixation would represent approximately 12.5 t y^{-1}. This suggests that seagrass N fixation does not appear to be a significant process in Western Port in comparison to total nitrogen loads of ~600 t.

Nitrogen uptake rates from core incubations were 4.6 and 6.1 mg N m^{-2} d^{-1} (Table 2) for CI and WI, respectively. This is equivalent to 57 and 75 t N y^{-1} (using the same scaling approach) for CI and WI, respectively. The N fixation rates were 1.1 and 0.9 mg m^{-2} d^{-1} (Fig. 14), which gives an estimated total N uptake of 5.72 and 7.05 mg m^{-2} d^{-1} at CI and WI, respectively. This shows that total N uptake at WI is 23% more than that of CI. This is consistent with the significant difference in %N of seagrass at CI and WI (1.61% vs 2.05%, respectively; $p = 0.0048$, data not shown) and suggests that WI seagrass takes up 27% more N than at CI. Despite CI and WI seagrass biomass being significantly different (188 vs. 323 mg dwt m^{-2}; $p = 0.0143$), whole plant growth rates were 356 and 345 mg dwt m^{-2} d^{-1}. This compared well with previously estimated values of 150 -1000 mg dwt m^{-2} d^{-1} for aboveground biomass growth rates of *Heterozostera* (Edmunds et al. 2006).

CONTRIBUTION OF NUTRIENTS FROM WATSON CREEK TO SEAGRASS AND THE FOODWEB

Nitrogen fixation is difficult to quantify directly because of its temporal and spatial variability. We believe stable isotopes can provide a first order estimate of the importance of N fixation and other nutrient uptake. While core incubations will give us relative contributions obtained from a discrete temporal point of view, stable isotopes will provide time-integrated contributions.

Chinaman Inlet (Western Port

In this study, assuming that seagrass $\delta^{15}N$ values are constrained solely by the relative uptake of atmospheric N (through N_2 fixation) and direct nutrient uptake, we can infer the relative contributions using the source end-members. $\delta^{15}N$ of newly fixed atmospheric N_2 is ~0 ‰ and nitrogen derived from other sources including oceanic, Watson Creek, the Bass River and Bunyip River have isotope values of 5-8‰ (Altabet 2007; Lourey et al. 2003), 17‰, 11‰ and 7‰ (Fiona Warry pers comm) respectively. In the present study, NO_3^- - $\delta^{15}N$ at Watson Creek was 16.6‰. Considering that seagrass at CI had a N stable isotope signature of 5.3 ‰ and that N fixation contributed 19% of its N demand (81% from nutrient uptake), we can infer that the average oceanic $\delta^{15}N$ was 6.5‰. This is in good agreement with the average seawater value (Altabet 2007). Using this value and considering that N fixation contributed 13% of WI N-demand (87% from nutrient uptake), we can derive that the average $\delta^{15}N$ of the nutrient source end-member at WI was 8‰. Assuming that this average end-member is constrained by both oceanic nutrients (6.5‰) and nutrient loads from Watson Creek (16.6‰), and that isotope fractionation is consistent with both sources, Watson Creek contributed 14% of the seagrass nutrient uptake (i.e. 86% coming from oceanic sources). This is in good agreement with the estimated 16% contribution of Watson Creek's nutrient load to the entire food web at WI.

Because the core incubation approach was only done once to estimate of NOx fluxes, it might be inconsistent with the time-integrated stable isotope mass-balance calculations. Therefore, NOx uptake rates and total N uptake rates at WI may be biased. However, there is another independent approach to estimate NOx uptake rates and the relative contributions of oceanic and WC nutrients. If we consider that the $\delta^{15}N$ of the oceanic nutrient source end-member is correct (6.5‰), the N budget for CI remains unchanged. If we assume that total N uptake rate is proportional to %N in the plant (also reflecting time-integrated processes), we can use this relationship at CI to estimate the total N uptake rate at WI. Total N uptake rate at WI = 5.72 / 1.61 × 2.05 = 12.52 mg N m^{-2} d^{-1}. This corresponds to a whole plant growth rate of 612 mg dwt m^{-2} d^{-1}. If we subtract N_2 fixation from the total N uptake, the NOx uptake rate is 11.57 mg N m^{-2} d^{-1}. This is equivalent to 142 t N y^{-1} and suggests that core incubations significantly underestimated these rates. Using the same $\delta^{15}N$ mass-balance approach shown above (between the $\delta^{15}N$ of the plant and that of the atmospheric N_2 end-member), we can also infer that the average $\delta^{15}N$ of the nutrient source end-member at WI was 7.5 ‰. This suggests that WC contributed 9% of the seagrass nutrient uptake and is slightly lower than the estimated 16% contribution of Watson Creek's nutrient load to the entire food web at WI.

COMMUNITY EFFECTS

The structure and composition of macrofauna assemblages are typically variable depending on a range of biotic and abiotic factors, such as nutrient levels (Posey et al. 2006), sediment properties and competitive interactions (Volkenborn & Reise 2007). This variability was reflected in these results with differences in abundance and biomass of the macrofauna assemblages between sites and habitats sampled within each site. The key difference between the two sites and the reason why they were chosen was higher nutrient levels at WI compared to CI. If nutrients were the main factor

driving the differences between the assemblages we expected WI to have low species diversity and dominance of the opportunistic polychaete worm, *Capitella* sp., an indicator species of nutrient enrichment (Morris & Keough 2003). We found *Capitella* sp. was more abundant at WI, but species diversity was higher at WI compared to CI. Therefore, whilst there does appear to be a slight impact at WI owing to nutrient loading it is only very small and consistent with the nutrient and isotope budgets that show most of the nutrients are exported owing to the low residence time of WI.

The ghost shrimp, *B.arenosus* was most abundant in the bare sediment habitat at CI. *B.arenosus* and other thalassinidean shrimp species (e.g. *Trypaea australiensis*) are numerically dominant taxa in many intertidal and shallow subtidal soft sediment habitats extending from Western Port, Victoria (Butler et al. 2009) to north Queensland (Webb & Eyre 2004). Thalassinidean shrimp are bioturbators and, as demonstrated by this study and others, their presence in the sediment is closely associated with high denitrification efficiencies (Webb & Eyre 2004). Any decline in the densities of ghost shrimp through activities such as bait pumping could have significant broader impacts on sediment properties and nutrient fluxes in Western Port (Keough 2011).

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