Nutrient status of seagrasses cannot be inferred from system-scale distribution of phosphorus in Shark Bay, Western Australia

Matthew W. Fraser[A,E], *Gary A. Kendrick*[A], *Pauline F. Grierson*[B],
James W. Fourqurean[C], *Mathew A. Vanderklift*[D] and *Diana I. Walker*[A]

[A]School of Plant Biology and The UWA Oceans Institute, The University of Western Australia,
  Crawley, WA 6009, Australia.
[B]School of Plant Biology and Ecosystem Research Group, The University of Western Australia,
  Crawley, WA 6009, Australia.
[C]Department of Biological Sciences and Southeast Environmental Research Center,
  Florida International University, North Miami, FL 33181, USA.
[D]CSIRO Wealth from Oceans Flagship, Private Bag 5, Wembley, WA 6913, Australia.
[E]Corresponding author. Email: matthew.fraser@uwa.edu.au

Abstract. Differences in phosphorus (P) availability can influence the ecology and physiology of seagrass communities; and are usually inferred from changes in the relative P content in seagrass leaves. Shark Bay is a subtropical marine embayment, with decreasing P concentrations in the water column and sediments from north to south across the entire embayment. We examined the P and nitrogen (N) content of seagrass leaves and P content of sediments across the Faure Sill and Wooramel delta region of Shark Bay, to determine whether the leaf content of seagrasses in Shark Bay also decreased from north to south over smaller spatial scales. Nutrient content of *Amphibolis antarctica* and *Halodule uninervis* were highly variable and were not strongly correlated with sediment P concentrations. Mean N : P ratios of seagrasses ($<$33.5) were not indicative of P limitation, as has been previously assumed for Shark Bay. We conclude that availability of P for uptake by seagrasses across Shark Bay may be highly localised and cannot be predicted from system-scale gradients ($>$100 km) of sedimentary P distributions. We suggest that P availability to seagrasses is more likely a complex function of differing nutrient inputs, rates of delivery to the plants and cycling rates.

Additional keywords: *Amphibolis antarctica*, calcareous, carbonate, C : N : P ratios, *Halodule uninervis*, river floods, salinity.

Introduction

Low P availability strongly limits primary productivity in many marine embayments (Atkinson 1987; Fourqurean and Zieman 1992). Seagrasses growing in marine embayments with calcareous sediments are particularly susceptible to P limitation to growth (Smith 1984; Howarth 1988; Fourqurean and Zieman 1992). Consequently, even small changes in the amount of P for uptake by benthic primary producers (bioavailability) across large embayments can significantly affect the structure and composition of the benthic community (Johnson *et al.* 2006). For example, P-addition experiments in Florida Bay have demonstrated that under increased concentrations of P, seagrass meadows shift from dominance by the slower-growing *Thalassia testudinum* Banks ex Konig to being dominated by the faster-growing *Halodule wrightii* Ascherson (Fourqurean *et al.* 1995). However, *T. testudinum* increased in biomass relative to *H. wrightii* when P concentrations returned to pre-fertilisation levels (Herbert and Fourqurean 2008). In extreme cases,

eutrophication of coastal embayments can occur after discharge of wastewater and agricultural runoff, leading to increased epiphytic and phytoplankton growth that in turn result in shading and loss of seagrasses (Ralph *et al.* 2005). However, the benthic ecology of any P-limited embayment, in particular the growth and dominance of individual seagrass species, is likely to be sensitive to changes in P bioavailability, even over small spatial scales.

Seagrass morphology and physiology can also change with increasing bioavailability of P (Herbert and Fourqurean 2009). Recent studies of *T. testudinum* have demonstrated that leaf P content and allocation of aboveground biomass relative to belowground biomass increase after P addition (Armitage *et al.* 2011). Thus, characteristics such as nutrient content of leaves and biomass allocation can be useful for estimating the extent of P limitation of seagrasses at any one site. Seagrasses, generally, have leaf tissues lower in P than in carbon (C) and N where light and other nutrients are not limiting seagrass growth. As such,

spatial differences in the nutrient content and elemental ratios of N and P of seagrass leaves across marine embayments can be useful for determining gradients in nutrient availability to primary producers (Atkinson and Smith 1983; Duarte 1990; Ferdie and Fourqurean 2004). In addition, N : P ratios in seagrass leaves can give an indication of nutrient limitation in seagrasses, with ratios >60 : 1 indicating P limitation (Armitage *et al.* 2005).

Phosphorus entering subtropical marine embayments from the adjacent ocean water is quickly removed from the water column and incorporated into sediments or benthic biomass (Fourqurean *et al.* 1993). For example, in the eastern embayment of Shark Bay, concentrations of P decrease southward into the Bay, in both the sediments and the water column (Atkinson 1987). Dissolved inorganic P (DIP) in the water column decreases from $0.2\,\mu M$ in the northern areas of the Bay with the lowest salinities of ~36, to undetectable ($<0.02\,\mu M$) limits in hypersaline reaches with salinities in excess of 65 (Atkinson 1987). In addition, C : P content of the benthic communities increased from north to south across the eastern embayment (Atkinson 1987). As such, it has been assumed that the main source of P to seagrass in Shark Bay is from oceanic water entering the Bay in the north (Atkinson 1987). However, the C : P content of benthic communities was determined only at four sites across the entire eastern embayment (>100 km; Atkinson 1987). Thus, significant patterns in P availability to seagrasses over smaller spatial scales may have been overlooked. In addition, the C : P ratios of benthic communities in Atkinson (1987) included an average across all benthic plants, including different seagrass and algal species. As such, species-specific differences in C : P ratios (Duarte 1990) may be preventing accurate interpretations regarding P availability. One area of Shark Bay where P concentrations are likely to be highly heterogeneous both spatially and temporally is across the Faure Sill, a shallow bank composed of calcareous sediments stretching across the eastern embayment of Shark Bay. The presence of the Faure Sill significantly alters the movement of water through the Bay, with substantial attenuation and dissipation of seawater occurring at the Sill (Burling *et al.* 2003), potentially altering nutrient-delivery rates. As such, the Faure Sill may be important in determining the availability of nutrients to primary producers.

Localised inputs of freshwater from river runoff or reduced light associated with sediment loads from flooding rivers may also contribute to spatial patterns in the nutrient availability in the near-shore systems. In semiarid regions, nutrient inputs from rivers to near-shore systems can be highly episodic and are generally associated with infrequent flood events (Mitchell *et al.* 1997). However, these terrestrial subsidies can potentially account for over 84% of annual P import (McKee *et al.* 2000). In addition, these flood events can import suspended sediments into embayments, decreasing light availability. This decreased light availability can then lead to a decrease in C : P ratios of seagrass leaves (Johnson *et al.* 2006). The only river flowing directly into Shark Bay is the Wooramel River; however, periods of river flow are restricted to episodic flood events related to summer cyclones (Smith and Atkinson 1983) and winter storms (Nott 2011). Episodic flooding of the Wooramel River could potentially subsidise the low P concentrations within the eastern embayment in Shark Bay, and increase the

availability of P at more local scales. We might thus expect seagrass adjacent to the mouth of the Wooramel River to have a lower C : P ratio than do seagrass communities at similar salinities elsewhere in Shark Bay.

The present study examined relationships between the spatial patterns of leaf nutrient content of two dominant seagrass species, *Amphibolis antarctica* (Labill.) Sonder et Aschers. and *Halodule uninervis* (Forssk.) Ascherson, with DIP in the water column and with total sediment P across the Faure Sill and Wooramel delta regions of Shark Bay. We hypothesised that (1) leaf P content of seagrasses would decrease from north to south across the study site, corresponding to an increasing salinity and decreasing oceanic P-supply gradient, (2) seagrass leaf P contents would reflect the sediment P content and (3) N : P ratios of seagrasses across the Faure Sill would be indicative of P limitation. We also investigated whether seagrass communities adjacent to the mouth of the recently flooded Wooramel River had an elevated P content relative to other seagrasses in the study site, as a result of terrestrial inputs contributing P to these adjacent seagrass communities.

Materials and methods

Field site description

The study was conducted at Shark Bay, Western Australia ($25°55'60''S, 113°32'32''E$), a 13 000-km^2 marine embayment located ~800 km north of Perth, which is divided by Peron Peninsula into an eastern and a western embayment (Fig. 1). Shark Bay is dominated by calcareous sediments and receives negligible terrestrial inputs of water in most years. The climate is hot and dry, where annual potential evaporation (2000 mm) exceeds precipitation (200 mm) by an order of magnitude (Logan and Cebulski 1970; Burling *et al.* 1999). Limited exchange with the ocean, coupled with high evaporation rates, has resulted in a strong increasing salinity gradient with increasing distance from the open ocean (Logan and Cebulski 1970). This salinity gradient (36 to >65) is particularly pronounced in the eastern embayment, resulting in the following three distinct zones across the eastern embayment of Shark Bay: an oceanic region, a metahaline region and a hypersaline region (Logan and Cebulski 1970; Atkinson 1987). The metahaline and hypersaline regions of the eastern embayment of Shark Bay are divided by the Faure Sill, a bank composed of calcareous sediments (Walker 1989) that runs from the mainland to the eastern coast of the Peron Peninsula. The Faure Sill restricts circulation in the eastern embayment, resulting in a distinct increase in salinity southward across the Sill. The Faure Sill is adjacent to the Wooramel delta, which extends northward from the Sill adjacent to the coastline, and the infrequently flowing Wooramel River (Fig. 1*c*).

The Wooramel River discharges directly into Shark Bay but freshwater flows are restricted to periods of cyclonic events and winter storms. On 15 December 2010, a monsoonal trough created over tropical waters off north-western Australia moved over the Gascoyne region, depositing heavy rainfall in the catchment until 18 December 2010 (Bureau of Meteorology 2011). The heavy rainfall resulted in the highest flood on record for the Gascoyne River, 120 km north of the Wooramel River. Locations within the Wooramel catchment received their mean annual rainfall of 300 mm in 1 or 2 days during the flooding
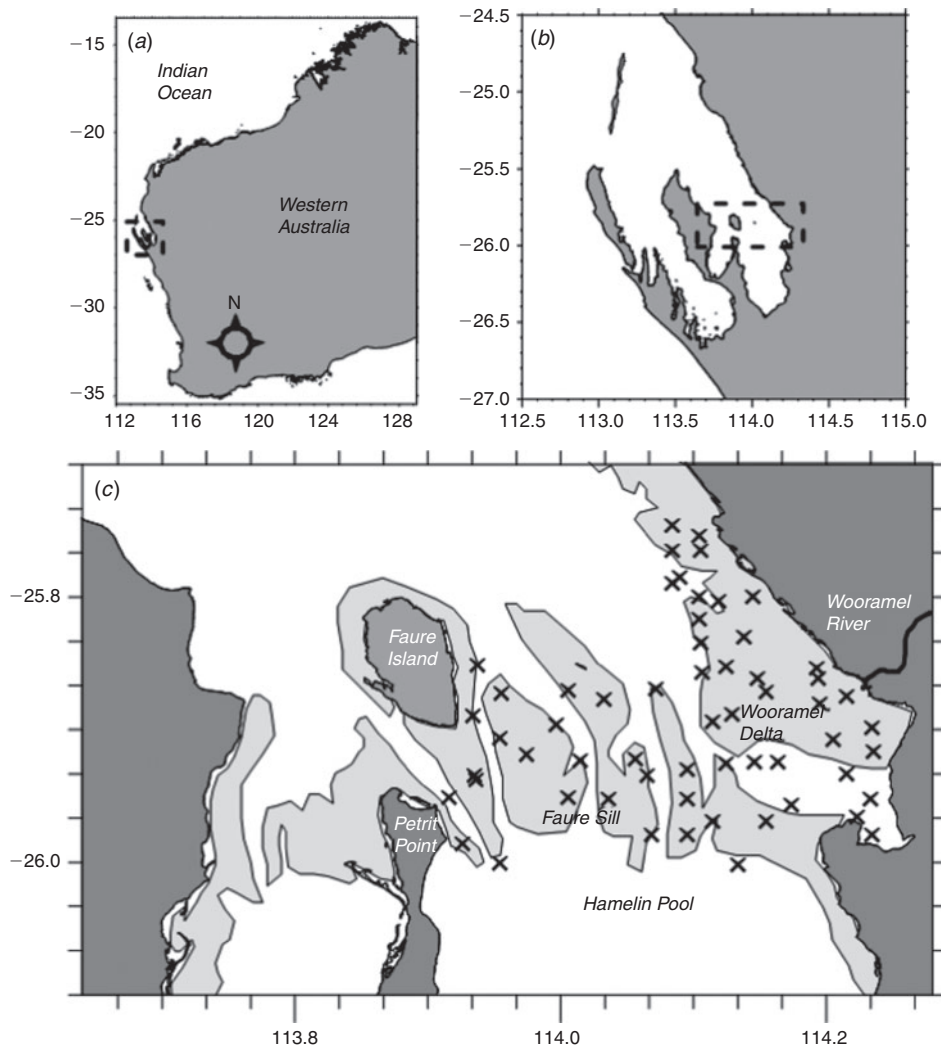
Fig. 1. Location of the study site. (*a*) Location of Shark Bay. (*b*) Location of the study site within the eastern embayment of Shark Bay. (*c*) Location of sites across the overall study site. Crosses show sites that were sampled in March–April 2011. Light shading indicates the Faure Sill, and darker shading shows the Wooramel delta.

event (Bureau of Meteorology 2011). Consequently, terrestrial inputs into Shark Bay are likely to have been higher than average in the months before the present study and, given the magnitude of the event, also greater than in many preceding years.

Shark Bay contains some of the most extensive and species-rich seagrass meadows in the world (Walker *et al.* 1988). Shark Bay is dominated by monospecific meadows of *A. antarctica*, which covers 3700 km^2 of the Bay. Both *Posidonia australis* Hook.f. and *H. uninervis* also occur in large monospecific meadows in the Bay (Walker 1989). *A. antarctica* is the dominant seagrass species across the Faure Sill, although *P. australis* is also present in channels across the Faure Sill (Walker *et al.* 1988). The Wooramel delta is dominated by monospecific meadows of *H. uninervis*, which also occurs as an understorey to both *A. antarctica* and *P. australis*, and in sand patches between meadows across the rest of the Bay (Walker 1989).

Experimental design and sampling

Field sites in the eastern embayment were sampled once in March and April 2011, during late summer. This sampling period is when seagrass nutrient limitation is generally highest, and seagrass nutrient content should deviate from the Redfield ratio most (Fourqurean *et al.* 2005). Four main sampling areas were identified across the Faure Sill, capturing gradients in salinity as well as different distances to the mouth of the Wooramel River (Fig. 1*c*). Overall, a total of 54 sites was sampled, with a minimum distance of 800 m between any two sites (Fig. 1*c*).

Both *A. antarctica* and *H. uninervis* were collected at each site, by taking one 25-cm-diameter core from the centre of each of five 0.25-m^2 quadrats dominated by each species. Plots were chosen at random by dropping a quadrat from the boat, and flipping the quadrat three times for each individual plot moving in the direction of the front of the boat. The entire contents of the

core (including belowground seagrass biomass and sediment) were extracted, washed free of sediment and sorted into species. Additional plants were collected by hand at sites where seagrass biomass in cores was insufficient for nutrient analysis.

A Seabird SBE 19 conductivity–temperature–depth profiler (CTD; Sea-Bird Electronics, Inc., Bellevue, WA, USA) was used to measure conductivity, temperature, and depth *in situ* at each site. In addition, nutrient concentrations of both sediments and the water column were measured at each site. Two sediment samples were also taken at each site for nutrient determination, using a 50-mL syringe with a cut top as a sediment corer. Nutrient concentrations were determined through analysis of two 50-cm^3 water samples, one filtered (through pre-rinsed 0.45-µm GF/C filters; Whatman Ltd., Maidstone, UK) to determine DIP, and one unfiltered to determine total P in the water column. All samples were stored on ice in the field, and then frozen immediately until processing was possible.

Detailed analysis of sediment composition (e.g. dry bulk density, carbonate content) is provided elsewhere in this issue (Fourqurean *et al.* 2012), and is not given here to avoid replication.

Carbon, nitrogen and phosphorus analyses of seagrass and sediment samples

Seagrass samples were thawed and adherent epiphytes and sediments removed from seagrass leaves by gently scraping with a scalpel under flowing deionised water. *A. antarctica* samples were split into leaves, shoots, rhizomes and roots; *H. uninervis* samples were split only into aboveground and belowground components because of their smaller size. Seagrass and epiphyte fractions were then oven-dried separately at 60°C until constant weight. Then, a subsample of seagrass leaves was randomly chosen and ground to a fine powder with a ball mill. C and N contents, and δ^{13}C and δ^{15}N isotope signatures were determined for duplicate subsamples of seagrass and sediments by using an Automated C/N Analyser–Mass Spectrometer consisting of a 20/22 mass spectrometer connected to an ANCA-S1 preparation system (Sercon, Crewe, UK) at the Western Australian Biogeochemistry Centre at the University of Western Australia. All samples were standardised using multipoint normalisation against a secondary reference of Radish collegate (3.167% N, δ^{15}N 5.71‰, 41.51% C, δ^{13}C 28.61), which was in turn standardised against primary analytical standards (International Atomic Energy Agency (IAEA) Vienna) (Paul *et al.* 2007). The external error of analyses (one standard deviation) was no more than 0.1 for C : N ratio, 0.15‰ for δ^{13}C, and 0.3‰ for δ^{15}N.

Phosphorus contents of seagrass and sediment samples were measured using a modification of the method of Solórzano and Sharp (1980), which involves dry-oxidation and acid hydrolysis, followed by a colorimetric analysis of the phosphate concentration of the extract (Murphy and Riley 1962). Phosphate concentrations of the samples were determined colorimetrically using the ascorbic acid–molybdate technique (Murphy and Riley 1962), and were compared with Australasian Soil and Plant Analysis Council (ASPAC), Victoria, Australia Standards 102 (*Pinus radiata*) and 105 (*Eucalyptus* sp.), which were treated accordingly. We found this technique to yield

Table 1. Summary statistics for abiotic observations
BD = below detection, DIP = dissolved inorganic phosphorus (P), N = nitrogen, TP = total P

Variable	Mean	s.e.	Minimum	Maximum
Temperature (°C)	26.2	0.12	24.2	27.9
Salinity	48.6	0.57	42.1	54.9
Water column DIP (µM)	0.007	0.004	BD	0.19
Water column TP (µM)	0.059	0.024	BD	0.86
Sediment TP (µg P g^{-1})	11.4	0.8	3.1	25.3
Sediment N (µg N g^{-1})	129	10	34	542

96–105% of the reported P content of the ASPAC Standard 102 and 105 leaves. Elemental contents of seagrass and sediment samples were calculated as a percentage of dry weight, and elemental ratios were calculated on a mol : mol basis.

Water analysis

DIP was measured colorimetrically on filtered and unfiltered samples as above (Murphy and Riley 1962), with an error of ±0.02 µM. Total P concentrations were measured on unfiltered water samples, using a modification of the method presented by Solórzano and Sharp (1980) for dissolved total P determination. P analysis was then performed using automated colorimetric techniques (Technicon 1977).

Data analysis

Relationships among seagrass elemental ratios, salinity and sediment nutrient concentrations were examined using correlation analysis. Partial correlation coefficients were used to describe relationships between pairs of variables, while taking the effect of latitude into account. Data were log-transformed where necessary to improve homogeneity of variances and the significance level was set at $P = 0.05$ unless otherwise stated. R (version 2.13.1; R Development Core Team 2012) was used for all statistical analysis. The detection limit for DIP using the molybdate method is 0.02 µM, owing to background concentrations of arsenate in seawater, potentially creating a false positive reading (Broberg and Pettersson 1988). Subsequently, any DIP concentrations found below this limit were excluded from the statistical analysis.

Contour plots of seagrass N and P contents, sediment nutrient concentrations and salinity were generated using a kriging routine (Surfer, version 10, Golden Software, Inc., CO, USA) to determine spatial patterns of the elemental ratios in *A. antarctica* and *H. uninervis* leaves.

Results

Water-quality and sediment parameters

Salinity always exceeded 42, with maximum salinities >54 (Table 1). As expected, there was a strong north to south salinity gradient across the study site, ranging from 42.1 in the north to 54.9 in the south in March–April (Fig. 2a). Salinities were comparable to those measured previously across the Faure Sill (Burling *et al.* 1999), suggesting negligible influence of freshwater inputs from the Wooramel River 3 months post-flood.
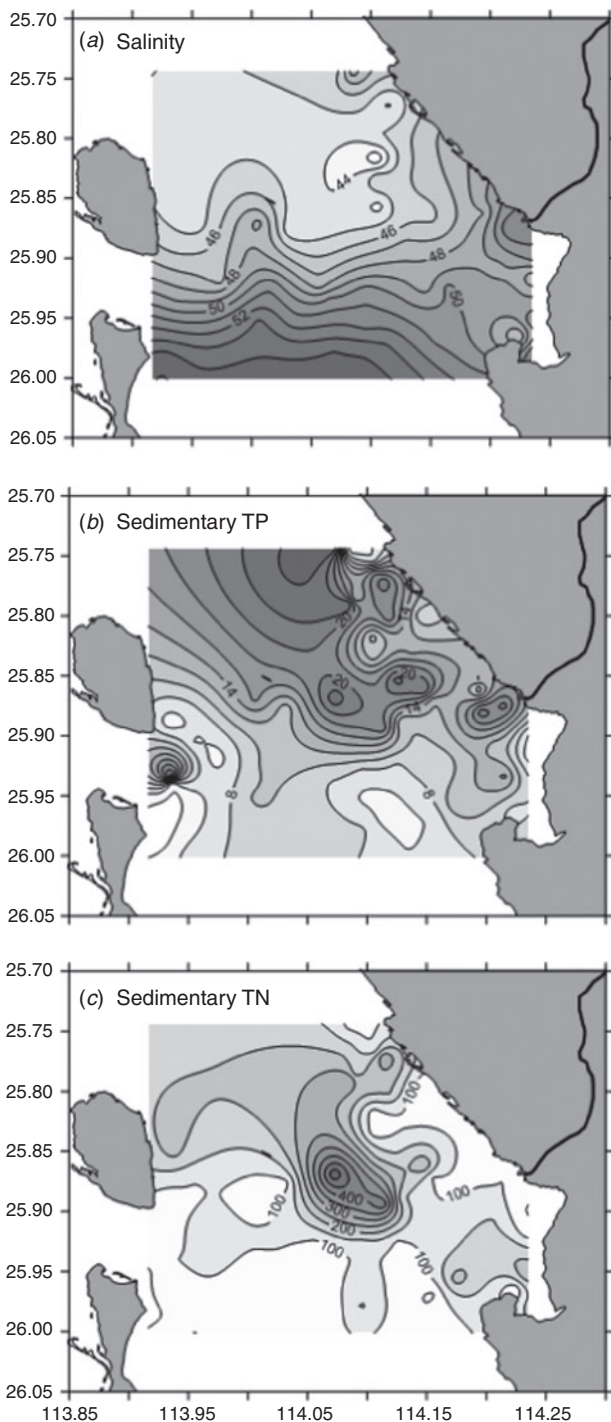
Fig. 2. Spatial patterns of abiotic variables across the Faure Sill and Wooramel delta region of Shark Bay, using a kriging routine. (*a*) Salinity distribution, with contour lines separated by increments of 1. Darker areas indicate higher salinities. (*b*) Sedimentary phosphorus (P) concentrations, with contour lines separated by increments of $2\,\mu g\,P\,g^{-1}$. Darker areas indicate higher sedimentary P concentrations. (*c*) Sedimentary nitrogen (N) concentrations, with contour lines separated by increments of $50\,\mu g\,N\,g^{-1}$. Darker areas indicate higher sedimentary N concentrations.

DIP in the water column was above the detectable concentration only at five sites, ranging from a maximum of $0.19\,\mu M$ adjacent to the Wooramel River to below detectable concentrations ($<0.02\,\mu M$) at 50 other sites (Table 1). Total P in the water column was slightly higher, ranging from a maximum of $0.86\,\mu M$ in the north of the study area to below detectable concentrations ($<0.02\,\mu M$) at 48 sites. There were insufficient data to quantify the relationship between seagrass nutrient concentrations and P in the water column.

Total P concentration of the sediment decreased as salinity increased (partial $r^2 = 0.14$, $P = 0.0067$, $n = 49$, Fig. 2*b*). The concentration ranged from $25.3\,\mu g\,P\,g^{-1}$ in the north of the study area to $3.1\,\mu g\,P\,g^{-1}$ in the south of the study area. Total P concentrations of the sediment were relatively low compared with those in other embayments in Western Australia such as Oyster Harbour ($230\,\mu g\,P\,g^{-1}$; Cambridge and Kendrick 2009) and Princess Royal Harbour ($60\,\mu g\,P\,g^{-1}$; Cambridge and Kendrick 2009). The south-western section of the study site, situated between Faure Island and Petit Point, had relatively high P concentrations compared with other sampling points. The spatial patterning of N concentrations of the sediments was different from both the distributions of salinity and sedimentary P concentrations (Fig. 2*c*). Total N concentration of the sediments ranged from $542\,\mu g\,N\,g^{-1}$ in the centre of the Faure Sill to $34\,\mu g\,N\,g^{-1}$ in the south-east of the study site. However, the spatial pattern of higher N concentrations in the centre of the study site was driven by one point.

Seagrass distribution and nutrient ratios

Seagrass grew at 38 of the 54 sites sampled. *H. uninervis*, and *A. antarctica* were the dominant seagrass species across the study area. *H. uninervis* occurred at 32 sites, whereas *A. antarctica* was found only at 23 sites. Only two other seagrass species were found across the study area; *P. australis* was found at one site on the edge of a channel 2 km east of Faure Island, whereas *Cymodocea angustata* Ostenfeld was found at two sites on the Wooramel delta. Owing to their relatively low abundance, these species were not used for further analysis. Of the 16 sites sampled with no seagrasses, 11 were on the Wooramel delta, six of which were adjacent to the mouth of the Wooramel River.

Seagrass nutrient ratios ranged appreciably across the study site for both *A. antarctica* and *H. uninervis*. Both *A. antarctica* and *H. uninervis* across the Faure Sill had mean N:P ratios of about 20:1, with maximum N:P ratios being just over 30:1, suggesting that seagrasses are not nutrient-limited around the Faure Sill (Table 2). Leaf C:N of *A. antarctica* was correlated with salinity, whereas both C:P and N:P were not correlated with salinity (Fig. 3, Table 3). Variation was high for C:N, C:P and N:P ratios of *A. antarctica* seagrass leaves across the salinity gradient (Fig. 3). Nutrient contents and C:N, C:P and N:P ratios of *A. antarctica* leaves were not correlated with total P concentrations of the sediments (Fig. 3, Table 3). Similarly, nutrient ratios of *H. uninervis* leaves were not significantly correlated with either salinity or total P concentrations of the sediments (Table 3, Fig. 4). The only exception was a negative relationship between leaf C:N of *H. uninervis* and sediment total P. No significant relationships were detected

Table 2.   Summary statistics for seagrass nutrient concentrations and stable isotopes

All elemental ratios are presented as mol : mol

Species and variable	Mean	s.e.	Minimum	Maximum
Amphibolis antarctica				
C : N	22.2	0.79	16.44	30.82
C : P	516.26	27.28	279.20	832.98
N : P	23.16	0.67	17.68	28.81
δ^{13}C	−12.98	0.20	−15.47	−11.38
δ^{15}N	2.21	0.33	−1.55	4.16
Halodule uninervis				
C : N	21.55	0.58	17.02	31.58
C : P	459.8	19.59	222.89	722.77
N : P	21.25	0.84	11.35	33.52
δ^{13}C	−13.23	0.39	−19.31	−9.18
δ^{15}N	−4.24	0.41	−7.65	2.89

between δ^{13}C or δ^{15}N of *A. antarctica* and *H. uninervis* and salinity (Table 3).

Distinct spatial patterning in nutrient ratios of seagrass leaves was evident across the study site. The C : N of *A. antarctica* leaves tended to decrease further south in the Bay, from a maximum of >30 : 1 in the north to <17 : 1 in the south of the study site (Fig. 5, top left). Additionally, both C : N and C : P ratios of *A. antarctica* leaves tended to be lower nearer land (Fig. 5), with C : N of <17 : 1 and C : P of <300 : 1 found at sites between Faure Island and Petit Point. Leaf N : P of *A. antarctica* was highest in the centre of the study site, furthest from shore, with sites nearer land tending to be enriched in P (Fig. 5, bottom left). *H. uninervis* leaf C : P and N : P ratios were slightly lower nearer land than at sites further from shore, with seagrass leaves from near-shore sites generally being more enriched in P (Fig. 5, right).
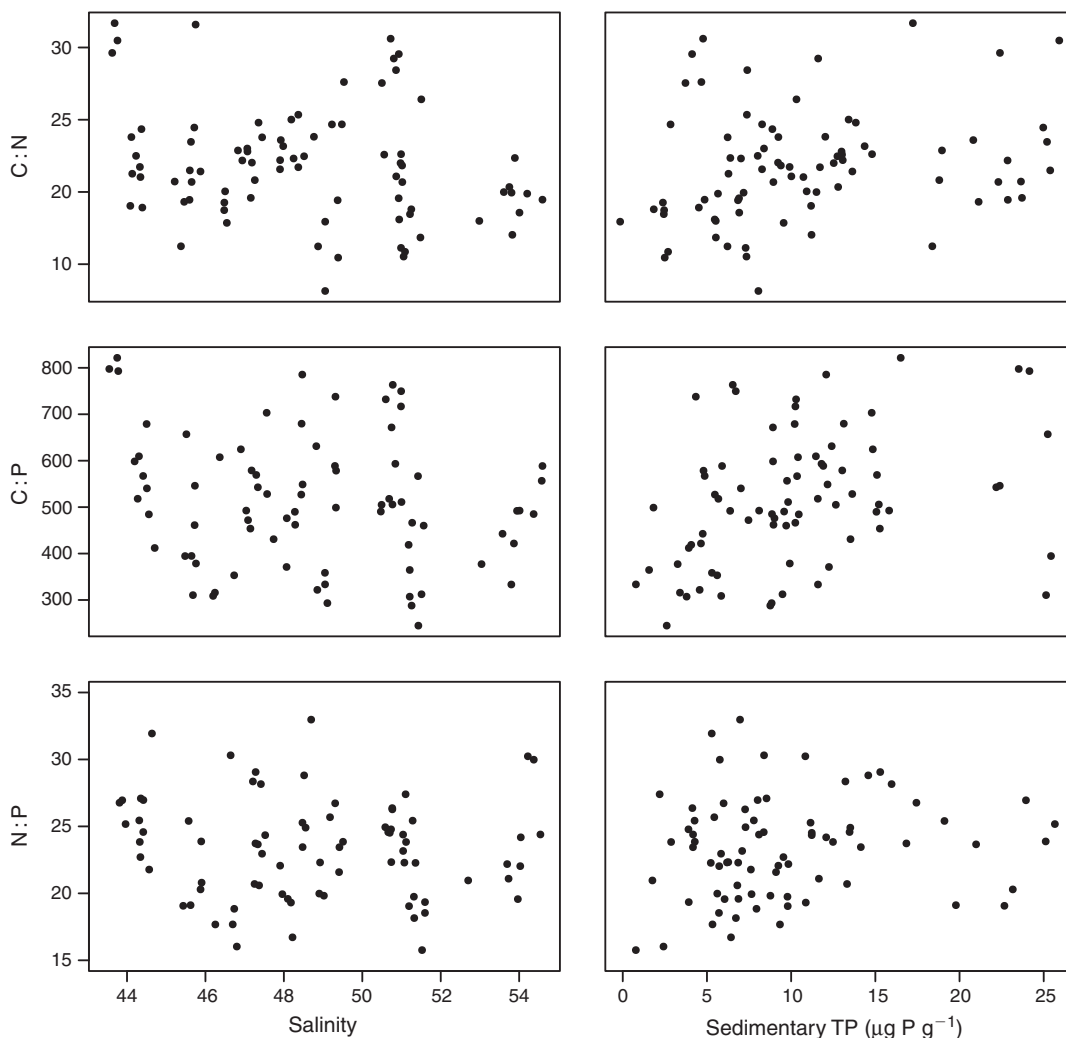
Fig. 3.   Scatterplots comparing *Amphibolis antarctica* nutrient ratios with salinity and total sedimentary phosphorus (P) concentrations. *A. antarctica* leaf carbon : nitrogen (C : N) ratio against salinity (top left) and total P concentration of the sediment (top right). Leaf C : P ratio against salinity (middle left) and total P concentration of the sediment (middle right). Leaf N : P against salinity (bottom left) and total P concentration of the sediment (bottom right). Black circles represent individual samples from March and April 2011.

Discussion

Are seagrasses across the Faure Sill really P-limited?

The hypothesis that the N : P ratios of seagrasses across the Faure Sill would be indicative of P limitation was rejected, adding to evidence that P limitation is not universal in subtropical embayments dominated by calcareous sediments (Erftemeijer *et al.* 1994; Udy *et al.* 1999; Fourqurean and Zieman 2002; Ferdie and Fourqurean 2004). Seagrass leaf N : P ratios have been used to infer the nutrient that is possibly limiting growth (Armitage *et al.* 2005; Armitage and Fourqurean 2006). For example, N : P > 96 : 1 indicated severe P limitation in Florida Bay, whereas P and N availability was considered balanced at sites with N : P of ~31 : 1 (Armitage *et al.* 2005). The maximum N : P ratio of seagrass leaves sampled in the present study was <34 : 1 (Table 2), which, on the basis of studies in other embayments, suggests that seagrass growth in Shark Bay is not entirely P-limited, as originally proposed by Smith (1984). Traditionally, seagrasses growing in subtropical marine embayments dominated by calcareous sediments have been assumed to be limited by low P availability, because of the high affinity of calcareous sediments for inorganic P, removing dissolved phosphate ions from the water column (Short 1987). However, the relatively low N : P ratios in the present study suggest that another resource may be limiting seagrass production, preventing nutrient limitation. For example, the relatively low N : P ratio may suggest that light is limiting seagrass production (Johnson *et al.* 2006). In addition, stressors such as salinity may be preventing high seagrass production in Shark Bay (Walker 1985), and this may also lead to a relatively low N : P ratio. Nutrient limitations must be confirmed by nutrient-addition experiments (Armitage *et al.* 2005; Cambridge and Kendrick 2009; Hovey *et al.* 2012), which should focus on addition of a range of nutrients, including at least P, N and Fe, to match the growing realisation that managers need to concentrate

on the effects of changes in a range of nutrients, and not just the one that is predicted to be limiting growth (Elser *et al.* 2007). Nevertheless, simplified assumptions about nutrient limitations of seagrass communities based on embayment locality and sediment composition are inadequate for a detailed biogeochemical understanding of seagrass ecology.

Leaf nutrient content is spatially variable across the Faure Sill

The availability of P for uptake by seagrasses did not decrease southward along the Faure Sill and Wooramel delta, contrary to the first hypothesis. This hypothesis was based on the only previous published patterns of P distribution in biota, sediments and water column through the eastern embayment of Shark Bay (Atkinson 1987). However, the lack of a similar southward decrease in seagrass P content in the present study may be due to the smaller spatial extent of the study site. Sampling over large spatial extents allows system-scale changes in nutrient bio-availability to be captured well, but underestimates small-scale variability. For example, across the entire salinity gradient of the eastern embayment (~35–65, ~100 km), there was a significant decrease in the concentration of DIP in the water column (Atkinson 1987). However, over salinities similar to those in the present experiment (~44–55), DIP concentrations were more variable (Atkinson 1987), with the variation being similar to that in the nutrient content of seagrass leaves found in the present study. Consideration of local-scale variation in nutrient bioavailability is crucial for effective understanding and management of benthic communities in subtropical marine embayments, because the responses of benthic communities to addition of nutrients may be underestimated in some areas if only broad, system-scale patterns of nutrient are considered (Fourqurean and Zieman 2002). Therefore, simplified nutrient budgets based on system-scale patterns of nutrient availabilities and

Table 3. Summary statistics for partial correlation analysis, correcting for the effect of latitude (Pearson's correlation statistic)
$*P \leq 0.05, **P \leq 0.01, ***P \leq 0.001$

Variable 1	Variable 2	Partial correlation coefficient	P	No. of observations (n)
Nutrient content				
A. antarctica leaf C : N	Salinity	0.33	0.003**	78
A. antarctica leaf C : P	Salinity	0.22	0.052	78
A. antarctica leaf N : P	Salinity	−0.045	0.69	78
A. antarctica leaf C : N	Sediment TP	0.14	0.22	78
A. antarctica leaf C : P	Sediment TP	0.19	0.10	78
A. antarctica leaf N : P	Sediment TP	0.20	0.08	78
H. uninervis leaf C : N	Salinity	0.06	0.71	42
H. uninervis leaf C : P	Salinity	0.12	0.46	42
H. uninervis leaf N : P	Salinity	0.08	0.63	42
H. uninervis leaf C : N	Sediment TP	−0.33	0.03*	42
H. uninervis leaf C : P	Sediment TP	−0.20	0.18	42
H. uninervis leaf N : P	Sediment TP	0.015	0.92	42
Stable isotope				
δ^{15}N of *Amphibolis antarctica*	Salinity	−0.045	0.69	78
δ^{13}C of *Amphibolis antarctica*	Salinity	0.03	0.79	78
δ^{15}N of *Halodule uninervis*	Salinity	−0.11	0.52	42
δ^{13}C of *Halodule uninervis*	Salinity	0.052	0.76	42
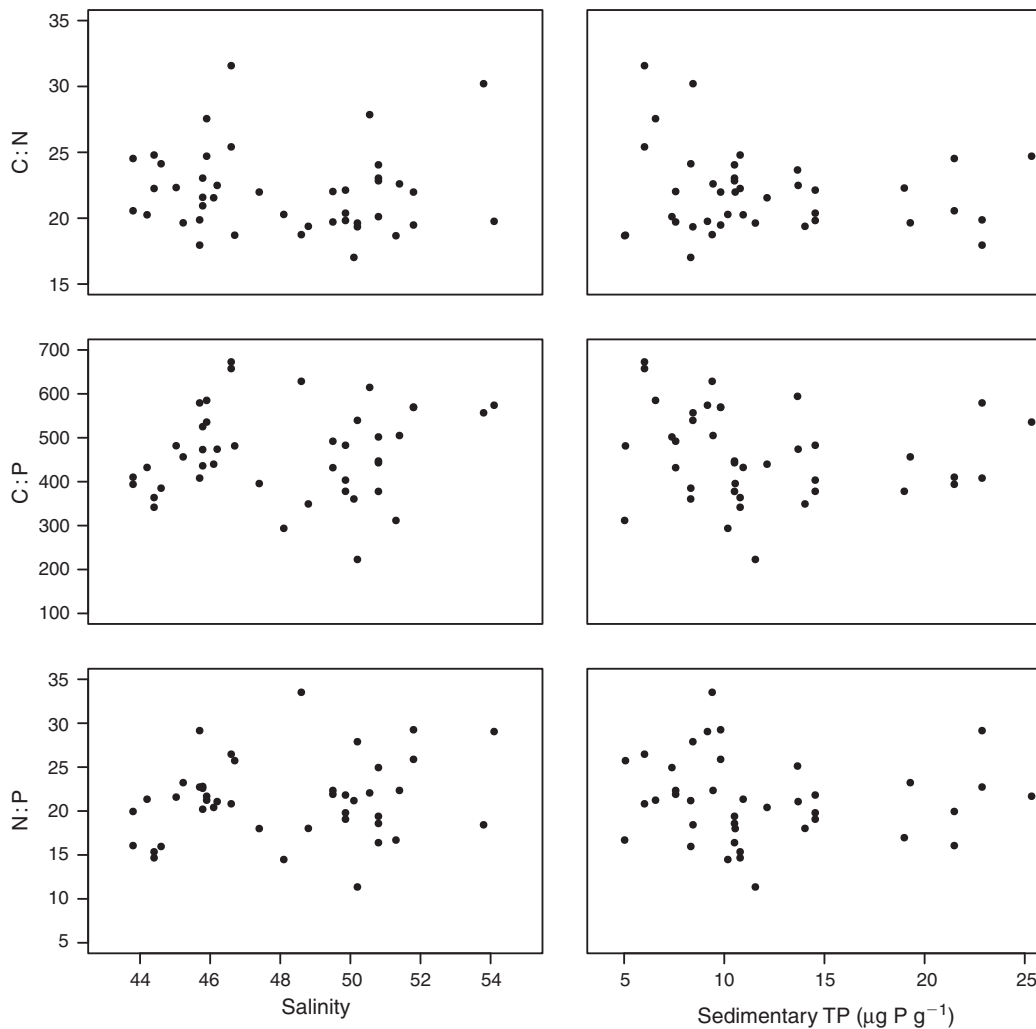
Fig. 4. Scatterplots comparing *Halodule uninervis* nutrient ratios with salinity and total sedimentary phosphorus (P) concentrations. *H. uninervis* leaf carbon : nitrogen (C : N) ratio against salinity (top left) and total P concentration of the sediment (top right). Leaf C : P ratio against salinity (middle left) and total P content of the sediment (middle right). Leaf N : P against salinity (bottom left) and total P concentration of the sediment (bottom right).

concentrations are not adequate for a detailed understanding of the nutrient status of the system and for adequate management of benthic communities at smaller spatial scales.

Does the P content of sediments reflect the P content of seagrass leaves in Shark Bay?

The hypothesis that the P content of seagrass leaves would reflect total sediment P was rejected, again suggesting that light or salinity may be limiting seagrass production in Shark Bay, which would prevent significant relationships between sedimentary P and seagrass leaf P. However, we measured only total sedimentary P, and this may not represent the P available for uptake by seagrasses. For example, P is usually more available for seagrass uptake in terrigenous sediments than in calcareous sediments (Short 1987), and mineralisation of organic matter in sediments can also make P available to seagrasses (Holmer *et al.* 2001). As such, areas with higher total sedimentary P

concentrations may have lower levels of available P than areas with lower total sedimentary P, depending on P fractionation. Further studies using methodologies to fractionate P would help in determining fractions of P available for seagrass uptake, and may correlate to seagrass P concentrations even if total sedimentary P concentrations do not.

In addition, nutrient uptake through leaves may be as important as uptake through roots for seagrasses growing in low-nutrient, subtropical embayments such as Shark Bay (Erftemeijer and Middel 1995; Hemminga *et al.* 1999). Indeed, *A. antarctica* collected from Cockburn Sound, Western Australia, took up more nutrients from the water column through leaves than from the sediment through roots (Pedersen *et al.* 1997). Seagrass leaves can take up inorganic P in the water column at nano-molar concentrations (Nielsen *et al.* 2007), suggesting that, although low in P, the water column can still provide adequate concentrations of P to meet seagrass demands where there is a constant, albeit low, supply. Quantities of
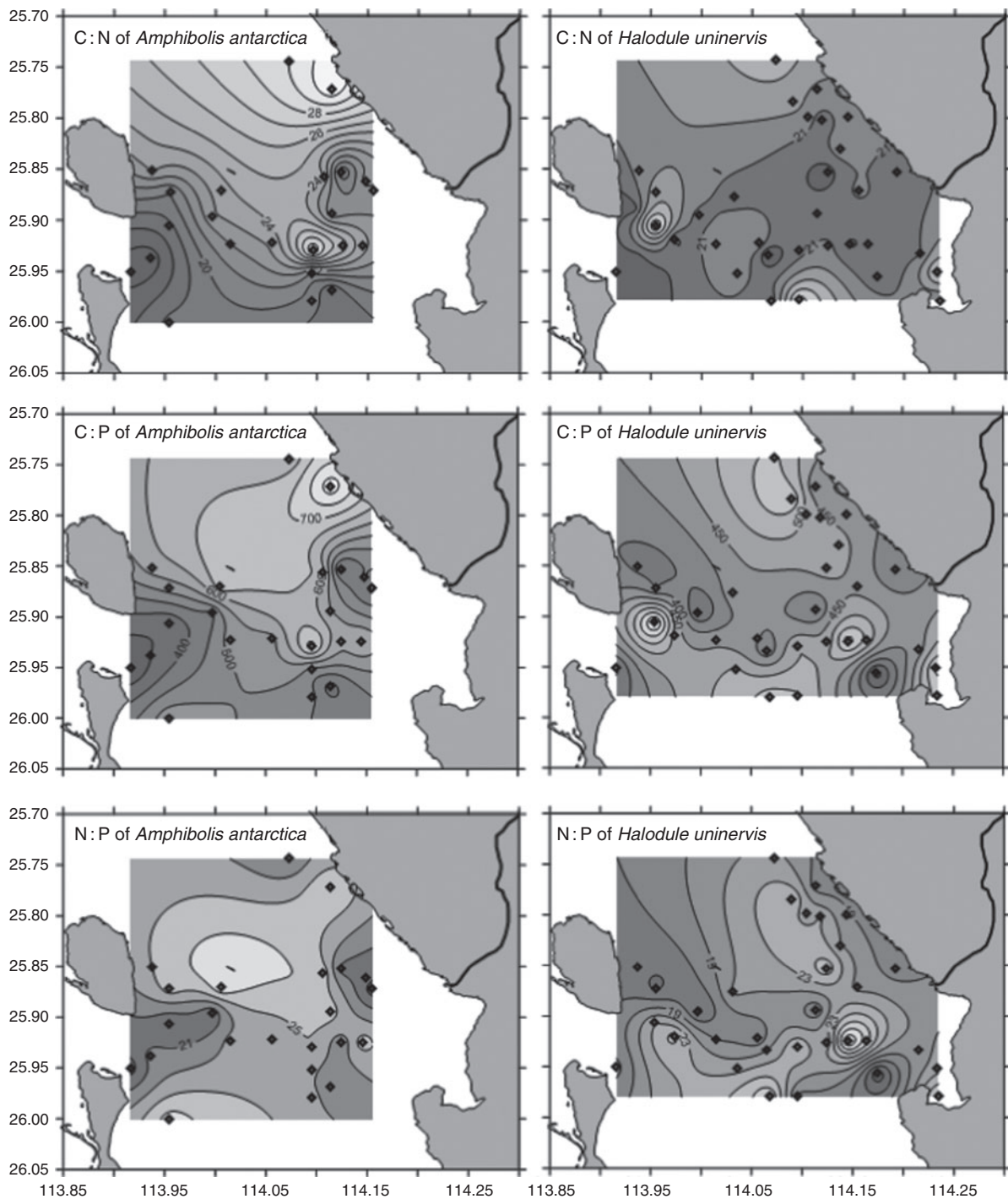
Fig. 5. Spatial pattern in nutrient ratios of *Amphibolis antarctica* (left) and *Halodule uninervis* leaves (right) using a kriging routine. Contour lines are separated by increments of 1 (carbon : nitrogen (C : N) ratio, top-left), 50 (C : phosphorus (P) ratio, middle-left) and 2 (N : P ratio, bottom left) for *Amphibolis antarctica*, and by increments of 1 (C : N ratio, top-right), 50 (C : P ratio, middle-right) and 2 (N : P ratio, bottom right) for *Halodule uninervis*. Shading lightens with increasing ratio. Symbols represent sampling locations.

P available for uptake would presumably be increased due to the fast (up to 8 kn) tidal currents across the Faure Sill (Walker *et al.* 1988). Thus, these currents could consistently replenish P around seagrass leaves, and alleviate potential P limitation of seagrasses. Furthermore, seagrasses can take up inorganic P at a

faster rate than it can be adsorbed to calcareous sediments (Nielsen *et al.* 2007). As such, sediment nutrient concentrations may not accurately reflect the bioavailability of nutrients to seagrasses. However, there is a distinct lack of fine-scale bathymetric and hydrological data across the Faure Sill region

of Shark Bay. Because nutrients can be advected in the water column (Koch *et al.* 2005), fundamental studies detailing bathymetry, currents, and tidal movements across the Faure Sill and Wooramel delta are essential so as to estimate the importance of water movement in delivering nutrients to benthic communities. Such information may help explain patterns of connectivity between potential nutrient sources and adjacent seagrass communities enriched in nutrients (Eyre *et al.* 2011).

Do terrestrial inputs of nutrients alleviate nutrient limitations in Shark Bay?

As hypothesised, seagrass communities adjacent to the mouth of the recently flooded Wooramel River had relatively high P contents, potentially as a result of terrestrial inputs or low light availabilities. Terrestrial inputs of nutrients can supply bioavailable nutrients to near-shore seagrass communities (Mitchell *et al.* 1997), and thus may contribute to the variability in nutrient content of seagrasses. Inputs of nutrients associated with episodic flooding of the Wooramel River may increase in the near future, with weather projections suggesting that the frequency and intensity of severe storm events will increase around Australia as a consequence of climate change (Wernberg *et al.* 2011). Thus, predicting the response of near-shore seagrass communities to increased episodic flooding must be a research priority. In addition, reductions in light availability associated with inputs of suspended sediments after the flood event may have led to a decrease in the C : P ratio of seagrass leaves (Fourqurean and Rutten 2003; Johnson *et al.* 2006). However, the quantities of P and N, as well as freshwater, sediments and organic matter, that the Wooramel River delivers into Shark Bay, is not well known (but see Price *et al.* 2012). As such, determining the nutrient fluxes associated with the flooding of the Wooramel River should be considered a priority for further research.

Implications for the management of Shark Bay and other subtropical embayments

The combination of the regional-scale changes in sedimentary nutrients and high variability in nutrient bioavailability over local spatial scales underscores the need for management to monitor seagrass, and indeed ecosystem, responses to increased nutrients over a broad range of spatial scales. Appropriate spatial monitoring is particularly important in nutrient-deplete, subtropical marine embayments such as Shark Bay, which often contain a complex mosaic of benthic habitats (Eyre *et al.* 2011) that would respond differently to changes in factors such as nutrient delivery and availability. The disparity in sources of nutrients over these different spatial scales underscores the difficulty faced in properly managing benthic communities in vast, subtropical marine embayments; ensuring spatial resolution to capture locally significant trends while keeping monitoring comprehensive is difficult across large embayments (Fourqurean and Rutten 2003). As a result, we must attempt to focus sampling programs around areas potentially acting as nutrient sources to benthic communities. Spatial resolution should be increased at near-shore areas and near other suspected sources of nutrients in Shark Bay, similar to management plans in Florida Bay, because benthic communities in these areas

appear to be strongly affected by nutrient inputs (Fourqurean and Rutten 2003).

References

Armitage, A. R., and Fourqurean, J. W. (2006). The short-term influence of herbivory near patch reefs varies between seagrass species. *Journal of Experimental Marine Biology and Ecology* **339**, 65–74. doi:10.1016/J.JEMBE.2006.07.013

Armitage, A. R., Frankovich, T. A., Heck, K. L., and Fourqurean, J. W. (2005). Experimental nutrient enrichment causes complex changes in seagrass, microalgae, and macroalgae community structure in Florida Bay. *Estuaries* **28**, 422–434. doi:10.1007/BF02693924

Armitage, A. R., Frankovich, T. A., and Fourqurean, J. W. (2011). Long-term effects of adding nutrients to an oligotrophic coastal environment. *Ecosystems (New York, N.Y.)* **14**, 430–444. doi:10.1007/S10021-011-9421-2

Atkinson, M. J. (1987). Low phosphorus sediments in a hypersaline marine bay. *Estuarine, Coastal and Shelf Science* **24**, 335–347. doi:10.1016/0272-7714(87)90054-0

Atkinson, M. J., and Smith, S. V. (1983). C:N:P ratios of benthic marine plants. *Limnology and Oceanography* **28**, 568–574. doi:10.4319/LO.1983.28.3.0568

Broberg, O., and Pettersson, K. (1988). Analytical determination of orthophosphate in water. *Hydrobiologia* **170**, 45–59. doi:10.1007/BF00024898

Bureau of Meteorology (2011). 'Gascoyne river flood.' Availalble at http://www.bom.gov.au/wa/sevwx/gascoyne_river/gascoyne_river.shtml [accessed 25 October 2011].

Burling, M. C., Ivey, G. N., and Pattiaratchi, C. B. (1999). Connectively driven exchange in a shallow coastal embayment. *Continental Shelf Research* **19**, 1599–1616. doi:10.1016/S0278-4343(99)00034-5

Burling, M. C., Pattiaratchi, C. B., and Ivey, G. N. (2003). The tidal regime of Shark Bay, Western Australia. *Estuarine, Coastal and Shelf Science* **57**, 725–735. doi:10.1016/S0272-7714(02)00343-8

Cambridge, M. L., and Kendrick, G. A. (2009). Contrasting responses of seagrass transplants (*Posidonia australis*) to nitrogen, phosphorus and iron addition in an estuary and a coastal embayment. *Journal of Experimental Marine Biology and Ecology* **371**, 34–41. doi:10.1016/J.JEMBE.2009.01.001

Duarte, C. M. (1990). Seagrass nutrient content. *Marine Ecology Progress Series* **67**, 201–207. doi:10.3354/MEPS067201

Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., Ngai, J. T., Seabloom, E. W., Shurin, J. B., and Smith, J. E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters* **10**, 1135–1142. doi:10.1111/J.1461-0248.2007.01113.X

Erftemeijer, P. L. A., and Middel, J. J. (1995). Mass balance constraints on nutrient cycling in tropical seagrass beds. *Aquatic Botany* **50**, 21–36. doi:10.1016/0304-3770(94)00440-W

Erftemeijer, P. L. A., Stapel, J., Smekens, M. J. E., and Drossaert, W. M. E. (1994). The limited effect of *in situ* phosphorus and nitrogen additions to seagrass beds on carbonate and terrigenous sediment in South Sulawesi, Indonesia. *Journal of Experimental Marine Biology and Ecology* **182**, 123–140. doi:10.1016/0022-0981(94)90215-1

Eyre, B., Ferguson, A. J. P., Webb, A., Maher, D., and Oakes, J. M. (2011). Denitrification, N-fixation and nitrogen and phosphorus fluxes in different benthic habitats and their contribution to the nitrogen and phosphorus budgets of a shallow oligotrophic sub-tropical coastal system (southern Moreton Bay, Australia). *Biogeochemistry* **102**, 111–133. doi:10.1007/S10533-010-9425-6

Ferdie, M., and Fourqurean, J. W. (2004). Responses of seagrass communities to fertilization along a gradient of relative availability of nitrogen and phosphorus in a carbonate environment. *Limnology and Oceanography* **49**, 2082–2094. doi:10.4319/LO.2004.49.6.2082

Fourqurean, J. W., and Rutten, L. M. (2003). Competing goals of spatial and temporal resolution: monitoring seagrass communities on the regional scale. In 'Monitoring Ecosystems: Interdisciplinary Approaches for Evaluating Ecoregional Initiatives'. (Eds D. E. Busch and J. C. Trexler.) pp. 257–289. (Island Press: Washington, DC.)

Fourqurean, J. W., and Zieman, J. C. (1992). Phosphorus limitation of primary production in Florida Bay: evidence from C : N : P ratios of the dominant seagrass *Thalassia testudinum. Limnology and Oceanography* **37**, 162–171. doi:10.4319/LO.1992.37.1.0162

Fourqurean, J. W., and Zieman, J. C. (2002). Nutrient content of the seagrass *Thalassia testudinum* reveals regional patterns of relative availability of nitrogen and phosphorus in the Florida Keys USA. *Biogeochemistry* **61**, 229–245. doi:10.1023/A:1020293503405

Fourqurean, J. W., Jones, R. D., and Zieman, J. C. (1993). Processes influencing water column nutrient characteristics and phosphorus limitation of phytoplankton biomass in Florida Bay, FL, USA: inferences from spatial distributions. *Estuarine, Coastal and Shelf Science* **36**, 295–314. doi:10.1006/ECSS.1993.1018

Fourqurean, J. W., Powell, G. V. N., Kenworthy, W. J., and Zieman, J. C. (1995). The effects of long-term manipulation of nutrient supply on competition between the seagrasses *Thalassia testudinum* and *Halodule wrightii* in Florida Bay. *Oikos* **72**, 349–358. doi:10.2307/3546120

Fourqurean, J. W., Escorcia, S. P., Anderson, W. T., and Zieman, J. C. (2005). Spatial and seasonal variability in elemental content, δ^{13}C, and δ^{15}N of T*halassia testudinum* from south Florida and its implications for ecosystem studies. *Estuaries* **28**, 447–461. doi:10.1007/BF02693926

Fourqurean, J. W., Kendrick, G. A., Collins, L. S., Chambers, R. M., and Vanderklift, M. A. (2012). Carbon, nitrogen and phosphorus storage in subtropical seagrass meadows: examples from Florida Bay and Shark Bay. *Marine and Freshwater Research* **63**, 967–983. doi:10.1071/MF12101

Hemminga, M. A., Marbà, N., and Stapel, J. (1999). Leaf nutrient resorption, leaf lifespan and the retention of nutrients in seagrass systems. *Aquatic Botany* **65**, 141–158. doi:10.1016/S0304-3770(99)00037-6

Herbert, D. A., and Fourqurean, J. W. (2008). Ecosystem structure and function still altered two decades after short-term fertilization of a seagrass meadow. *Ecosystems* **11**, 688–700. doi:10.1007/S10021-008-9151-2

Herbert, D. A., and Fourqurean, J. W. (2009). Phosphorus availability and salinity control productivity and demography of the seagrass *Thalassia testudinum* in Florida Bay. *Estuaries and Coasts* **32**, 188–201. doi:10.1007/S12237-008-9116-X

Holmer, M., Andersen, F. O., Nielsen, S. L., and Boschker, H. T. S. (2001). The importance of mineralisation based on sulfate reduction for nutrient

regeneration in tropical seagrass sediments. *Aquatic Botany* **71**, 1–17. doi:10.1016/S0304-3770(01)00170-X

Hovey, R. K., Cambridge, M. L., and Kendrick, G. A. (2012). Season and sediment nutrient additions affect root architecture in the temperate seagrasses *Posidonia australis* and *P. sinuosa. Marine Ecology Progress Series* **446**, 23–30. doi:10.3354/MEPS09483

Howarth, R. W. (1988). Nutrient limitation of net primary production in marine ecosystems. *Annual Review of Ecology and Systematics* **19**, 89–110. doi:10.1146/ANNUREV.ES.19.110188.000513

Johnson, M. W., Heck, K. L., Jr, and Fourqurean, J. W. (2006). Nutrient content of seagrasses and epiphytes in the northern Gulf of Mexico: evidence of phosphorus and nitrogen limitation. *Aquatic Botany* **85**, 103–111. doi:10.1016/J.AQUABOT.2006.02.003

Koch, E. W., Ackerman, J. D., Verduin, J. J., and van Keulen, M. (2005). Fluid dynamics in seagrass ecology – from molecules to ecosystem. In 'Seagrasses: Biology, Ecology and Conservation'. pp. 193–225. (Springer: Dordrecht, The Netherlands.)

Logan, B. W., and Cebulski, D. E. (1970). Sedimentary environments of Shark Bay, Western Australia. In 'Carbonate Sedimentation and Environments, Shark Bay, Western Australia'. (Ed. B. W. Logan.) pp. 1–36. (The American Association of Petroleum Geologists: Tulsa, OK.)

McKee, L. J., Eyre, B. D., and Hossain, S. (2000). Transport and retention of nitrogen and phosphorus in the sub-tropical Richmond River estuary, Australia – a budget approach. *Biogeochemistry* **50**, 241–278. doi:10.1023/A:1006339910533

Mitchell, A. W., Bramley, R. G. V., and Johnson, A. K. L. (1997). Export of nutrients and suspended sediments during a cyclone-mediated flood event in the Herbert River catchment, Australia. *Marine and Freshwater Research* **48**, 79–88. doi:10.1071/MF96021

Murphy, J., and Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* **27**, 31–36. doi:10.1016/S0003-2670(00)88444-5

Nielsen, O. I., Koch, M. S., and Madden, C. J. (2007). Inorganic phosphorus uptake in a carbonate-dominated seagrass ecosystem. *Estuaries and Coasts* **30**, 827–839.

Nott, J. (2011). A 6000 year tropical cyclone record from Western Australia. *Quaternary Science Reviews* **30**, 713–722. doi:10.1016/J.QUASCIREV.2010.12.004

Paul, D., Skrzypek, G., and Forizs, I. (2007). Normalization of measured stable isotopic compositions to isotope reference scales – a review. *Rapid Communications in Mass Spectrometry* **21**, 3006–3014. doi:10.1002/RCM.3185

Pedersen, M. F., Paling, E. I., and Walker, D. I. (1997). Nitrogen uptake and allocation in the seagrass *Amphibolis antarctica. Aquatic Botany* **56**, 105–117. doi:10.1016/S0304-3770(96)01100-X

Price, R. M., Skrzypek, G., Grierson, P. F., Swart, P. K., and Fourqurean, J. W. (2012). The use of stable isotopes of oxygen and hydrogen to identify water sources in two hypersaline estuaries with different hydrologic regimes. *Marine and Freshwater Research* **63**, 952–966. doi:1071/MF12042

R Development Core Team (2012). R: A Language and Environment for Statistical Computing (Version 2.13.1). R Foundation for Statistical Computing, Vienna, Austria. Available at http://www.R-project.org [accessed 28 September 12]

Ralph, P. J., Tomasko, D. A., Moore, K. A., Seddon, S., and Macinnis-Ng, C. M. O. (2005). Human impacts on seagrasses: eutrophication, sedimentation, and contamination. In 'Seagrasses: Biology, Ecology and Conservation'. (Eds A. W. D. Larkum, R. J. Orth and C. M. Duarte.) pp. 567–593. (Springer: Dordrecht, The Netherlands.)

Short, F. T. (1987). Effects of sediment nutrients on seagrasses: literature review and mesocosm experiment. *Aquatic Botany* **27**, 41–57. doi:10.1016/0304-3770(87)90085-4

Smith, S. V. (1984). Phosphorus limitation of net production in a confined aquatic ecosystem. *Nature* **307**, 626–627. doi:10.1038/307626A0

Smith, S., and Atkinson, M. (1983). Mass balance of carbon and phosphorus in Shark Bay, Western Australia. *Limnology and Oceanography* **28**, 625–639. doi:10.4319/LO.1983.28.4.0625

Solórzano, L., and Sharp, J. H. (1980). Determination of total dissolved phosphorus and particulate phosphorus in natural waters. *Limnology and Oceanography* **25**, 754–758. doi:10.4319/LO.1980.25.4.0754

Technicon (1977). Individual/Simultaneous determination of Nitrogen and/or Phosphorus in BD acid digests. Industrial Method No. 329-374 W/B. Technicon Industrial Systems, New York.

Udy, J. W., Dennison, W. C., Lee Long, W. J., and McKenzie, L. J. (1999). Responses of seagrass to nutrients in the Great Barrier Reef, Australia. *Marine Ecology Progress Series* **185**, 257–271. doi:10.3354/MEPS185257

Walker, D. I. (1985). Correlations between salinity and growth of the seagrass *Amphibolis antarctica* (labill.) Sonder & Aschers., in Shark Bay, Western Australia, using a new method for measuring production rate. *Aquatic Botany* **23**, 13–26. doi:10.1016/0304-3770(85)90017-8

Walker, D. I. (1989). Regional studies – seagrasses in Shark Bay, the foundations of an ecosystem. In 'Biology of Seagrasses: a Treatise on the Biology of Seagrasses with Special Reference to the Australian Region'. (Eds A. W. D. Larkum, A. J. McComb and S. A. Shepherd.) pp. 182–210. (Elsevier Science: Amsterdam.)

Walker, D. I., Kendrick, G. A., and McComb, A. J. (1988). The distribution of seagrass species in Shark Bay, Western Australia, with notes on their ecology. *Aquatic Botany* **30**, 305–317. doi:10.1016/0304-3770(88)90063-0

Wernberg, T., Russell, B. D., Moore, P., Ling, S. D., Smale, D. A., Campbell, A., Coleman, M. A., Steinberg, P. D., Kendrick, G. A., and Connell, S. D. (2011). Impacts of climate change in a global hotspot for temperate marine biodiversity and ocean warming. *Journal of Experimental Marine Biology and Ecology* **400**, 7–16.