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Variation in seagrass biomass estimates in low and high density settings: implications for the selection of sample size

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Abstract

Few seagrass biomass monitoring studies have considered the adequacy of monitoring intensity in their design. Power analysis is now widely used in ecological monitoring to determine sample size (replication) and the power (probability of not making a Type II error) of the monitoring design to detect change (effect size). We investigated seasonal variation of above-ground biomass of Zostera species at Woolooware Bay, Botany Bay, NSW and Ukerebagh Channel, Tweed River, NSW to show that seagrass biomass varies significantly between sites and seasonally. By conducting preliminary power analysis at each study site we found that our sampling design would only detect 70% change at Woolooware Bay, while <10% change would be detected at Ukerebagh Channel with the same intensity of sampling. We demonstrate the potential efficiency of harvesting as a means of estimating biomass in high biomass situations, where percentage cover may provide less discrimination between sampling sites.

Keywords

seagrass, sample, variation, selection, implications, settings, density, high, low, estimates, biomass, size

Disciplines

Medicine and Health Sciences | Social and Behavioral Sciences

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Variation in seagrass biomass estimates in low and high density settings: Implications for the selection of sample size

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KEYWORDS *Zostera*, seagrass biomass, seasonality, power analysis, statistical power

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Introduction

Seagrasses are an important and often dominant component of estuaries and shallow coastal waters of Australia (Larkum 1977; Long *et al*. 1994) and seagrass meadows are considered to be among the most valuable ecosystems in terms of the services they provide (Costanza *et al*. 1997; Orth et al. 2006). Seagrass in Australia and internationally may exhibit high productivity and support abundant communities, including many commercially important wildlife (Charpy-Roubaud and Sournia 1990; Smith, 1981; Thayer *et al*. 1975). Aside from acting as nursery grounds, adult habitat and food for commercially important prawns and fish (Bell and Pollard 1989; Blaber *et al*. 1992; Young 1978), wading birds and dugongs (Preen *et al*. 1992) seagrass also contributes large amounts of detritus and dissolved organic matter (Moriarty *et al*. 1984; Orth et al. 2006) to estuarine foodwebs. While they are present in only 0.15% of the ocean's surface (Charpy-Roubaud and Sournia 1990), they contribute 1% of the net primary production of the global ocean (Duarte and Cebrian 1996). Reliable and accurate estimates of seagrass biomass are essential for estimating productivity and establishing links among other estuarine components.

Zostera capricorni is among the common seagrass species dominating seagrass beds in southeastern Australia. Biomass of *Z. capricorni* has been reported for Lake Illawarra (Harris et al. 1979), Port Hacking (Kirkman et al. 1982), Botany Bay (Larkum et al. 1984) Tuggerah Lakes, NSW (Higginson 1965) and Moreton Bay, Queensland (Kirkman 1978). Sampling protocols, in particular quadrat size and replication, varied significantly among studies (Table 1), but the most commonly used method was sampling biomass within 0.25 m x 0.25 m quadrats (Kirkman and Cook 1982; Larkum et al. 1984; Larkum and West, 1990; Mellors 1991; Neverauskas 1987; Lanyon and Marsh 1995) with 10 replicate quadrats established within plots (Larkum et al. 1984).

Table 1: Size of quadrats used in seagrass biomass studies in Australia.

Source	Location	Dominant Species	Quadrat size (m^2)
Kirkman and Cook 1982	Port Hacking, NSW	*Zostera capricorni*	0.0625 (0.25x0.25)
Larkum *et al.* 1984	Botany Bay, NSW	*Z. capricorni*	0.0625 (0.25x0.25)
Larkum and West 1990	Quibray Bay and Silver Beach, NSW	*Posidonia* species	0.0625 (0.25x0.25)
Neverauskas 1987	Gulf St. Vincent, SA	*Posidonia* species *Amphibolis* species	0.0625 (0.25x0.25)
Lanyon and Marsh 1995	North Queensland	Seagrass species	0.0625 (0.25x0.25)
Mellors 1991	Green Island, Qld	*Halophilla uninervis*	0.25 (0.5x0.5)

Power analysis has been utilized in the design of monitoring programs to determine if a statistical test based on a given sample size has a high probability of detecting significant effects (Green 1989; Peterman 1990) or to determine sample size required to identify a change in effect size (Underwood 1997). The importance of undertaking preliminary power analysis to determine the efficacy of a monitoring program sampling design is becoming well accepted (Fairweather 1991; Thomas 1997). However, in spite of the importance of this task few studies internationally have considered the statistical power of monitoring sampling designs to determine change in seagrass cover or biomass (Kirkman 1996). Heidelburg and Nelson (1996) applied power analysis to contrast the statistical power of different techniques of biomass estimation (coring, shoot counting, percentage cover) to conclude that percent cover was the most cost efficient technique, while Mumby et al. (1997) assessed the statistical power of seagrass cover estimation from remote sensing platforms. One recent Australian seagrass study (McDonald et al. 2006), reviewed the statistical power of video assessment of seagrass cover finding differences in the minimum detectable change between uniform and variable seagrass cover.

The estimation of biomass from percentage cover has the advantages of relatively high statistical power, ease of capture and minimal interference. However, there are at least two reasons to retain harvesting as an additional technique in seagrass biomass estimation. First, percentage cover may not provide sufficient resolution among sites of relatively high biomass, all being of complete coverage and yet differing stand density and height. Secondly, the standing biomass may be a more direct measure of the ecosystem service being considered, where this service relates directly to primary productivity, as might be the case in determination of carbon flow in estuarine environments. This study is the first to apply power analysis to the determination of sample size by contrasting high and low biomass sites, one tropical and the other temperate. The study has two aims; first; to determine an optimal sampling design for monitoring changes in seagrass above-ground biomass in sites of high and low percentage cover. Power analysis was conducted to determine the optimal number of replicate quadrats (sample size) required to detect change (effect size) in seagrass above-ground biomass. Secondly, we investigated the seasonal variation of above ground biomass of *Zostera spp.* at two sites in southeastern Australia.

Methods

Study sites

This study was undertaken at two sites in southeastern Australia; Woolooware Bay, situated on the temperate east Australian coast with variable percentage cover, and Ukerebagh Channel situated on the subtropical east Australian coast with consistently high percentage cover (Figure 1). While Woolooware Bay (located on Botany Bay, 34°00'S, 151°11'E), is described as a marine dominated open embayment and Ukerebagh Channel (located on the Tweed River, 28°10'S, 153°33'E) is a barrier estuary (Roy et al. 2001), they are geomorphically similar, being situated on sandy marine deltas. Seagrass communities in Botany Bay are dominated by *Posidonia australis* and *Zostera capricorni* (Larkum and West 1990), while the Tweed River is dominated by monospecific stands of *Zostera* species (Saintilan and Rogers 2008).

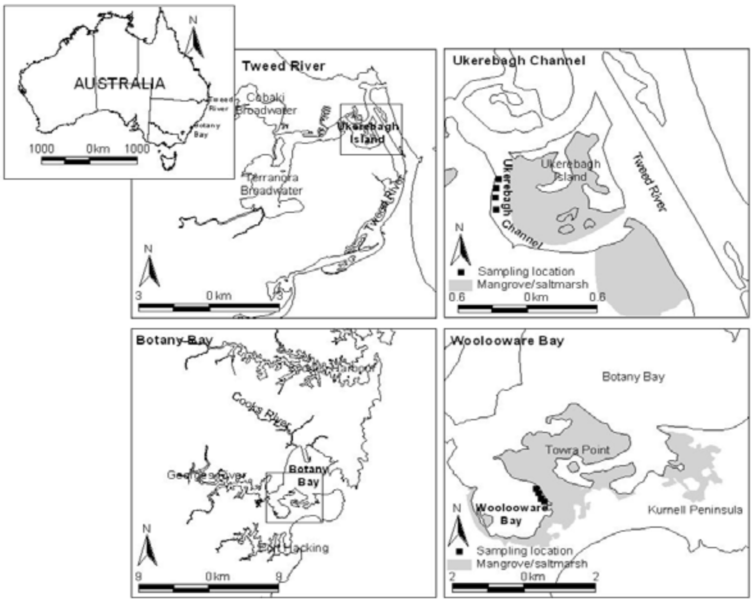

Figure 1: Location of Ukerebagh Channel, Tweed River and Woolooware Bay, Botany Bay.

Sampling Methods

As sampling was designed to maximise precision of biomass estimation over time, small quadrats with many replications were used in preference to a few large quadrats, as recommended by Downing and Anderson (1985). In both Woolooware Bay and Ukerebagh Channel, four plots were established in contiguous seagrass beds. Within each plot, eight quadrats of 0.25 m X 0.25 m were deployed for collecting samples. Re-randomised samples were collected on four occasions to represent each season between June 2002 and June 2003. Care was taken to ensure that re-randomised samples did not coincide with previously sampled sites.

Sample collection was conducted at low tide (<0.4 m above mean sea level) to ensure ease of access, minimize water turbidity and maximise visibility of seagrass beds. Above-ground seagrass biomass for all ramets located within the quadrats was removed. The above-ground biomass was cleaned using freshwater to remove all salts, sediments and epiphytes. Biomass was oven dried to constant weight at 80^{o}C and immediately weighed after removal from ovens to avoid rehydration of samples.

As a guide to optimal sampling design, power analysis was conducted on samples collected in June 2002 at both study sites. While there is debate about appropriate confidence and power for monitoring (Di Stefano 2003; Field et al. 2007), here we used a confidence of 0.95 and a minimum power of 0.8 as a guide for selecting sample size as these values have become a convention in ecological monitoring. Furthermore, since there is little guidance on the effect size which enables a prompt and effective management response to detrimental change in seagrass biomass, above-ground biomass data from initial sampling at each study site were manipulated to have an effect size change in increments of 10%. Repeated measures analysis of variance was conducted to determine the sample size for detecting an effect size change in above-ground seagrass biomass.

Univariate analysis (ANOVA) and post-hoc Student-Newmans-Kules (SNK) tests were performed on all samples to assess differences in biomass among seasons within sites, while two-way analysis of variance was used to determine differences between sites and among seasons.

Results

The statistical power of the analysis on seagrass biomass collected at Woolooware Bay was significantly less than that at Ukerebagh Channel (Figure 2). This is likely to be due to greater variance at Woolooware Bay (coefficient of variance = 44%) compared to Ukerebagh Channel (coefficient of variance = 40%). As many as ten quadrats per plot would be required to detect a 50% change in seagrass above-ground biomass at Woolooware Bay, while only seven quadrats are required to detect 10% change in seagrass biomass at Ukerebagh Channel. The current sampling design of eight quadrats per plot would detect 70% change in seagrass above-ground biomass at Woolooware Bay, while <10% change would be detected at Ukerebagh Channel.

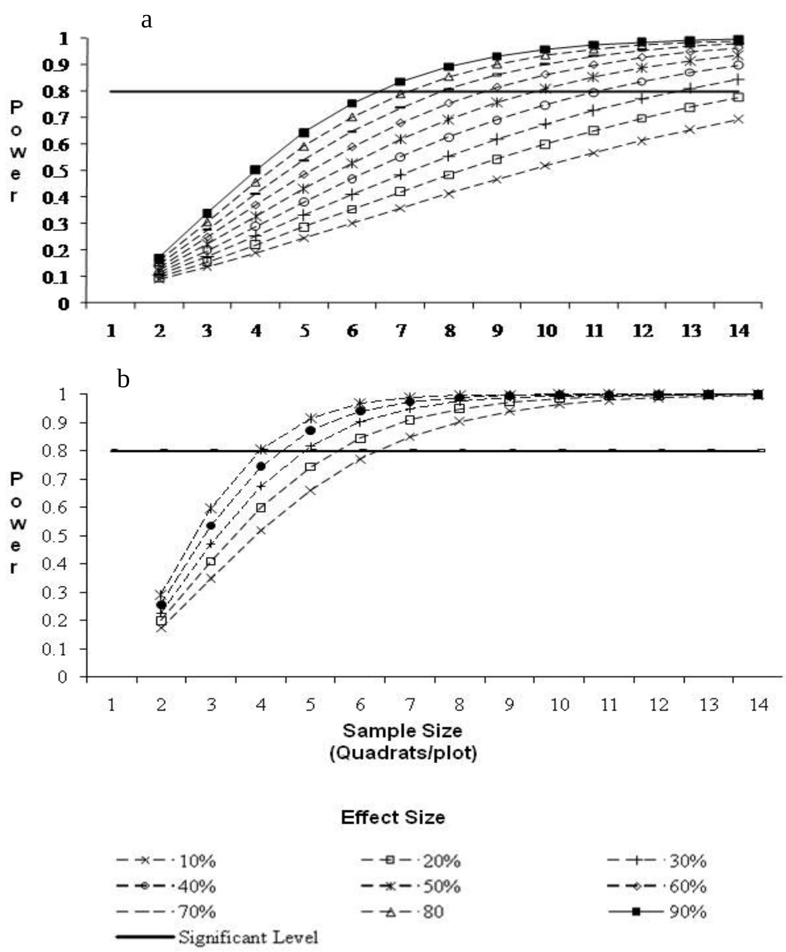

Figure 2: Sample size (quadrats per plot) versus power for seagrass biomass at a) Woolooware Bay and b) Ukerebagh Channel

Average seagrass biomass was significantly higher in Ukerebagh Channel (mean (SE) 69.59 (6.15) g dry wt. m^{-2}) than at Woolooware Bay (mean (SE) 26.01 (2.30) g dry wt. m^{-2}) ($p<0.05$, Table 2). The significant interaction between season and site ($p<0.05$) indicates a unique pattern of seasonal variability between sites. This seasonal variability was evident at both Woolooware Bay ($p<0.05$), where biomass was higher in spring than all other seasons and lower in winter than all other seasons ($p<0.05$, Table 3), and Ukerebagh Channel ($p<0.05$, Table 3) where biomass was higher in summer than autumn and winter (Figure 3). Contrary to the preliminary power analysis, the monitoring sampling strategy detected in excess of 50% change in seagrass biomass at Wooloware Bay.

Table 2: Two-way analysis of variance results for seagrass biomass with factors of site (Woolooware Bay, Ukerebagh Channel) and season (spring, summer, autumn and winter).

Source of variation	Type III Sum of Squares	Degrees of Freedom	Mean Squares	F-statistic	P-value
Corrected Model	621.73(a)	7	88.82	28.03	0.000
Intercept	2284.96	1	2284.96	721.130	0.000
Season	103.56	3	34.520	10.89	0.000
River	474.80	1	474.80	149.85	0.000
Season * River	43.37	3	14.46	4.56	0.004
Error	785.81	248	3.170		
Total	3692.50	256			
Corrected Total	1407.54	255			

(a) R Squared = 0.442 (Adjusted R Squared = 0.426)

Table 3: Univariate analysis of variance results for seagrass biomass at a) Woolooware Bay (Cochranes Test C = 0.3556) and b) Ukerebagh Channel (Cochranes Test C = 0.4955).

Site	Source of Variation	Degrees of Freedom	Mean Squares	F-statistic	P-value
a)	Season	3	9.86	23.13	0.0001
	Residual	124	0.43		
	Total	127			
b)	Season	3	39.16	6.62	0.0003
	Residual	124	5.911		
	Total	127			

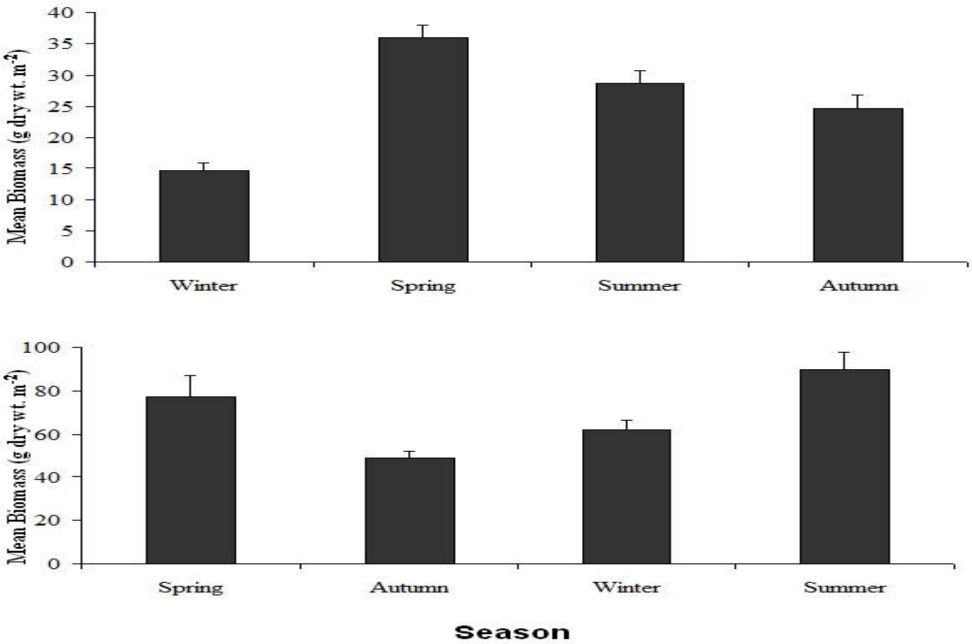

Figure 3: Mean biomass (+ standard error) of seagrass (dry weight per square metre) at a) Woolooware Bay and b) Ukerebagh Channel. n=128

Discussion

Power Analysis

The power analysis presented here demonstrates the need to conduct preliminary analyses on results at each study site to determine the power and sample size required for detecting change. The greater variance at Woolooware Bay meant that sampling effort should be significantly greater than that at Ukerebagh Channel, a site of relatively high and consistent biomass. More specifically, 10 quadrats per plot would be required to detect a 50% change in biomass at Woolooware Bay, while only 4 quadrats per plot would be required at Ukerebagh Channel to significantly detect the same degree of change.

Sampling effort may be reduced by increasing the effect size that could be detected (e.g. from 50% to 70%). However, this undermines the ability of the monitoring to promptly detect change (Di Stefano 2003; Field et al. 2007; Mapstone 1995). Alternatively, sampling effort may be reduced by decreasing the confidence interval (eg from 0.95 to 0.90; Underwood 1994). Trade-offs in the degree of change detectable and confidence is not necessary at Ukerebagh Channel which has relatively high statistical power, but may be appropriate at Woolooware Bay. Some discussion has been given to appropriate trade-offs which increase the power of detecting change, without greatly increasing the cost or effort (Field et al. 2005, 2007; Mapstone 1995). In the case of Woolooware Bay, since the cost of making Type I and Type II error is unknown it may be appropriate to use an iterative process of reducing the confidence level and recalculating the power until they are equal (Field et al. 2007; Mapstone 1995).

While we demonstrate the need for preliminary statistical power analysis of seagrass biomass monitoring, a monitoring program based on this design will only trigger a management response at Woolooware Bay when biomass either decreases or increases by 70% or more. An effect size of 70% or more may fail to allow managers to respond quickly and effectively to negative or detrimental changes. In addition, sampling design at Woolooware Bay cannot be altered according to the iterative process (Field et al. 2007; Mapstone 1995) described above as it assumes that the effect size to be detected has been clearly defined in the objectives of the monitoring (Field et al. 2007). To encourage adequate design of seagrass biomass monitoring and to aid prompt and effective management action, we urge for further consideration to be given to the effect size that monitoring of seagrass biomass should detect.

Few studies have quantified the success of seagrass recovery, possibly due to a lack of long-term data from monitoring programs or because recovery has been unsuccessful or extremely slow (Short and Wyllie-Echeverria 1996); however, rapid recovery of *Zostera marina* has been documented (Plus *et al.* 2003). *Zostera marina* may reproduce both sexually and asexually, with higher rates of sexual reproduction reportedly compensating for slower rates of horizontal rhizome extension (Phillips et al. 1983; Rasheed 2004). Recovery success is therefore dependent on seed availability, either from parent plants or seed banks, and conditions suitable for seedling establishment and rhizome extension, which may require disturbance minimization. As seed availability and suitable conditions for seedling establishment and vegetative expansion will vary among sites, an appropriate site-specific effect size should be selected on the basis of these variable factors. Monitoring should be designed to detect the suitable effect size and then consideration should be given to changes in power and/or confidence.

However, the relatively high statistical power of the high biomass Ukerebagh site would suggest that harvesting as a form of biomass estimation is a useful alternative to applying percentage cover over sites where this technique provides little discrimination. In lower biomass situations, the relative benefit of percentage cover over harvesting increases as suggested by Heidelburg and Nelson (1996).

Seagrass Biomass

The seasonal variation in biomass at Woolooware Bay is similar to results reported for *Zostera capricorni* measured over the same seasons in Botany Bay (Larkum et al. 1984) and nearby Port Hacking (Kirkman et al. 1982). Seasonal variation in results was somewhat different at Ukerebagh Channel than other sites in southeastern Australia (Kerr and Strother 1990; Kirkman et al. 1982; Larkum et al. 1984), however, they still indicate that productivity increases in response to favourable growing conditions, particularly warmer temperatures (Hillman et al. 1989).

Conclusion

Since seagrass above-ground biomass varied between sites and seasonally it is appropriate for preliminary analyses of statistical power to be completed at each study site before undertaking any monitoring of seagrass biomass. Rather than replicating sampling designs used in previous studies, preliminary analyses of the degree of replication should be completed for each study site so as to adequately design monitoring programs that have the statistical power to detect change. The relatively higher statistical power at high biomass sites may present harvesting as a useful alternative to percentage cover in biomass estimation in these situations.

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