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Asymmetric patterns of recovery in two habitat forming seagrass species following simulated overgrazing by urchins

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Abstract

The persistence of seagrass meadows reflects variation in factors that influence their productivity and consumption. Sea urchins (*Amblypneustes pallidus*) can over-graze seagrass (*Amphibolis antarctica*) to create sparse meadows in South Australia, but this effect is not observed in adjacent *Posidonia sinuosa* meadows despite greater densities of inhabiting urchins. To test the effect of urchin grazing on seagrass biomass, we elevated the density of urchins in meadows of *A. antarctica* and *P. sinuosa* and quantified seagrass decline. Urchins removed similar amounts of biomass from both seagrass species, but the loss of leaf meristems was 11-times greater in *A. antarctica* than *P. sinuosa*. In a second experiment to assess the recovery of seagrass, we simulated urchin grazing by clipping seagrass to mimic impacts measured in the first experiment, as well as completely removing all above ground biomass in one treatment. Following simulated grazing, *P. sinuosa* showed a rapid trajectory toward recovery, while *A. antarctica* meadows continued to decline relative to control treatments. While both *A. antarctica* and *P. sinuosa* were susceptible to heavy grazing loss, consumption of the exposed meristems of *A. antarctica* appears to reduce its capacity to recover, which may increase its vulnerability to long-term habitat phase-shifts and associated cascading ecosystem changes.

Key words: phase-shift, herbivory, habitat loss, meristem, *Amphibolis antarctica, Posidonia sinuosa*
1. Introduction

Species that create widespread habitats provide the foundations for entire ecological systems (Barbier, et al., 2011), but tend to be susceptible to disturbances that increase their consumption relative to productivity (Connell, et al., 2011; Steneck, et al., 2002). This disruption to the balance between productivity and consumption is perhaps most renown along sub-tidal rocky coasts where sea urchins can overgraze kelp forests to create ‘barrens’ (Chapman and Johnson, 1990; Ling, et al., 2009; Pearse, 2006). Overgrazing can occur where the rate of herbivory exceeds the rate of plant productivity for sustained periods, resulting in diminished ecosystem functions (Eklof, et al., 2008). In seagrass meadows, overgrazing by urchins can cause phase-shifts to bare habitats (Eklof, et al., 2008; Rose, et al., 1999), which can impact coastal productivity, biodiversity, food-webs, sediment stabilisation, turbidity, nutrient cycling, and carbon sequestration (Duarte, 2002; Orth, et al., 2006). Such impacts may be persistent given that many species can take decades to centuries to recover from disturbance (Bryars and Neverauskas, 2004; Kirkman and Kuo, 1990; Irving, 2013).

On southern Australian coasts, the seagrasses *Amphibolis* spp. and *Posidonia* spp. can form extensive co-occurring habitats on moderately wave-exposed shores (Bryars and Rowling, 2009; Shepherd and Womersley, 1981). Widespread historical losses (Walker and McComb, 1992), limited re-colonisation (Bryars and Neverauskas, 2004; Kendrick, et al., 2002; Irving, 2013), and slow meadow expansion rates (Marba and Walker, 1999) have demonstrated the susceptibility of these seagrasses to natural and anthropogenic impacts. Among natural effects, overgrazing has been observed but appears to disproportionately impact *Amphibolis antarctica* (author’s pers. obs.), possibly because it is a species that produces a canopy of accessible meristematic leaf clusters while *Posdionia* spp. meristems are protected below ground (Alcoverro and Mariani, 2002; Marba and Walker, 1999; Short and Duarte, 2001).
The purpose of this study was to determine how sea urchin grazing causes the loss and affects the recovery of the seagrasses *A. antarctica* and *Posidonia sinuosa*. In southern Australia, the short-spined sea urchin *Amblypneustes pallidus* can over-graze *A. antarctica* with little to no recovery for at least 3 years (author’s pers. obs.). However, grazing effects on *P. sinuosa*, which often co-exist in mixed or adjacent meadows with *A. antarctica*, appear less pronounced despite urchins often occurring in greater densities in *P. sinuosa*. We experimentally tested the hypothesis that a fixed density of *A. pallidus* would disproportionately impact the biomass of *A. antarctica* relative to *P. sinuosa*. In a second experiment we tested the hypothesis that *P. sinuosa* would show greater recovery than *A. antarctica* following simulated grazing damage.

### 2. Materials & Methods

#### 2.1 Study region

All experiments were done at Lady Bay (35°28.036 S, 138°17.198 E), approximately 70 km South-West of the city of Adelaide, South Australia. Seagrass meadows in this region are largely intact and unaffected by anthropogenic impacts more common to the Adelaide metropolitan coastline (e.g. eutrophication). Meadows comprise a mosaic of *P. sinuosa*, *Posidonia angustifolia* and *A. antarctica*, interspersed by small rocky outcroppings and sand patches. The short-spined sea urchin *A. pallidus* is a small (i.e. 2 - 4 cm) and often inconspicuous seagrass grazer that is normally sparsely distributed but occasionally greatly exceeds background densities (author’s pers. obs.). All sampling was done between depths of 4 - 5.5 m.
2.2 Field sampling

Following a sea urchin over-grazing event in *A. antarctica* meadows during February 2010, seagrasses were sampled within affected and unaffected meadows. Meadows were defined as ‘affected’ where urchins had been observed actively grazing seagrass and there was clear evidence of defoliation and dead shoots. Meadows were defined as ‘unaffected’ where urchins had not been observed grazing seagrass and there was limited evidence of defoliation or dead shoots. Five 0.0625 m$^2$ quadrats of *A. antarctica* were harvested at the substratum, transported on ice under dark conditions and frozen for later analysis of above ground biomass and shoot density.

As no conspicuous effects of grazing were observed in *P. sinuosa* meadows, we tested for a relationship between urchin and *P. sinuosa* biomass, on three different dates, from December 2011 to March 2012. At four sites, each separated by a minimum of 1 km, urchin abundance was counted in five 10 × 1 m transects. Twenty urchins were then haphazardly collected and weighed while wet to estimate total biomass m$^{-2}$. Five 0.0625 m$^2$ quadrats of *P. sinuosa* were then harvested at the substratum to quantify above ground biomass. We undertook the same sampling procedure in nearby *A. antarctica* meadows, however, urchin numbers were so low (zero at 66% of sites) that no relationship could be established, therefore only average urchin densities are presented.

2.3 Urchin grazing (Experiment 1)

Manipulative experiments testing the effect of *A. pallidus* grazing on seagrass biomass were done within nearby monospecific meadows of *A. antarctica* and *P. sinuosa*, where the naturally occurring urchin populations observed during the experimental period (March to
August 2011) were zero. For each seagrass species, fifteen experimental plots were randomly interspersed over 500 m² to separate plots by at least four meters. Five plots were designated as treatments of elevated grazing by adding two urchins within a wire mesh cage anchored by fence droppers (mesh size 0.02 × 0.02 m, cage size 0.4 × 0.4 × 0.4 m) to create urchin densities within the natural range observed at Lady Bay (12.5 m², average wet urchin biomass = 29.45 ± 0.40 g). Urchins were collected from nearby beds of *Posidonia spp.* and *A. antarctica*, with weekly replacement of dead individuals if necessary. A procedural control was established in another five plots of each species using a partial cage to test for caging artefacts on seagrass. The remaining five plots were un-manipulated and served as controls. Cages were cleaned of algal growth weekly. One grazing replicate from each species of seagrass was lost due to storm damage during June. These treatments were not replaced.

Destructive sampling of seagrass was done at 82 and 169 days. Small 0.01 m² samples of seagrass (i.e. 6 % of experimental plots) were harvested after 82 days and their location marked to prohibit re-sampling. Larger 0.0625 m² samples were taken after 169 days. Above ground biomass was harvested at the substratum, transported on ice under dark conditions and frozen for later measurement of above ground leaf biomass, meristem survival and grazing damage. Below ground biomass was only sampled at the conclusion of the experiment (i.e. 169 days) by excavating roots and rhizomes in 0.0625 m² quadrats to a depth of 20 cm.

Seagrass biomass was measured by removing any fleshy epiphytes with a razor blade, rinsing in MilliQ water to remove salts and sediment, and then drying at 60°C for 48 h. For *A. antarctica*, vertical rhizomes and leaves were separated prior to drying to determine above ground leaf biomass in grams of dry weight per square metre (gdw m⁻²). Leaf meristems were
counted in *A. antarctica* if the apical meristem was intact on each leaf cluster, determined by
the survival of the youngest leaf in the cluster. Leaf meristems of *P. sinuosa* were counted if
sheaths contained at least one living leaf. In the final samples all *A. antarctica* shoots were
classified as either alive (i.e. containing living meristems) or dead. As urchins can graze *A.
antarctica* directly at the shoot, this was the best way to estimate rates of grazing damage. For
*P. sinuosa* 20 individual leaves were selected and the leaf tips identified as grazed if
possessing an apex consistent with urchin grazing scar.

2.4 Grazing experiment statistical analyses

Repeated measures ANOVA was used to compare the effects of grazing and seagrass species
on leaf biomass for both the halfway (82 day) and final (169 day) sampling dates of the
grazing experiment using SPSS. Urchin grazing, seagrass species (between subject factors)
and time (within subject factor) were treated as fixed. Halfway and final above ground leaf
biomass were fourth-root transformed to satisfy the assumption of homogeneity of variances
and normality, which were analysed using Levene’s test and Shapiro-Wilk’s test,
respectively. Sphericity could not be evaluated as there were only two levels of the repeated
measure time.

Two-factor ANOVA was used to test for the effects of urchin grazing and seagrass species on
below ground biomass, meristem survival and grazing damage using PERMANOVA+ for
Primer v6. Urchin grazing and seagrass species were treated as fixed factors in the orthogonal
design. Cochran’s C-test was used to test for homogeneity of variance prior to analysis.
Below ground biomass and meristem survival violated the assumption of homogeneity of
variance, therefore data was log-transformed prior to analysis. Grazing damage was
ArcSin (%) transformed prior to analysis. Pairwise tests were used where significant interactions terms were detected.

2.5 Recovery from simulated grazing (Experiment 2)

To quantify seagrass recovery from overgrazing, urchin grazing was simulated in both A. antarctica and P. sinuosa using scissors to clip above ground biomass. The recovery experiment was completed in different monospecific beds of A. antarctica and P. sinuosa adjacent to where the grazing experiment was undertaken. Three treatments were established: control (no biomass removed), partial removal (removal equal to the urchin effects sampled in the grazing experiment; see section 2.6) and complete removal (100% removal of above ground biomass). Five replicate plots 0.4 × 0.4m (0.16 m²) for each treatment were established in both seagrass species, randomly interspersed over a 500 m² area for each species and marked using star droppers. The experiment commenced seven weeks after the grazing experiment and continued for 109 days from October 2011 to January 2012, after which above ground biomass was harvested by destructively sampling within 0.0625 m² quadrats before processing seagrass, as described above.

2.6 Partial removal treatment

Due to the different morphologies of Amphibolis spp. and Posidonia spp. unique removal techniques were utilised to accurately replicate grazing loss for each species. While Amphibolis spp. have woody vertical rhizomes, with a number of short shoots and leaf heads on each stem, the above ground biomass of Posidonia spp. consists entirely of strap like leaves. For a detailed summary of the morphology of these species see Marba and Walker (1999). Because urchins can remove either entire or partial sections of vertical rhizomes and leaves, clipping of both entire and partial vertical rhizomes (A. antarctica) and leaves
(P. sinuosa) was required to accurately replicate grazing loss. Firstly, clipping of entire vertical rhizomes (A. antarctica) and leaves (P. sinuosa) at the substrate was undertaken to match loss recorded between control and urchin grazing treatments in Experiment 1. Secondly, clipping of individual leaf heads (A. antarctica) and partial leaves (P. sinuosa) was undertaken to account for the removal of partial vertical rhizomes and leaves from Experiment 1. For each experimental plot (0.4 × 0.4m) in A. antarctica, 90 entire vertical rhizomes (560 m²) at an average 0.19 gdw vertical rhizome⁻¹ and 132 individual leaf heads (825 m²) at an average 0.07 gdw leaf head⁻¹ were removed for a total removal of 26.28 gdw plot⁻¹ (164.2 g m⁻²). For each experimental plot (0.4 × 0.4m) in P. sinuosa 197 entire leaves (1232 m²) at an average of 0.06 gdw leaf⁻¹ and 435 partial leaves (2720 m²) at an average of 0.03 gdw partial leaf⁻¹ were removed for a total removal of 24.77 gdw plot⁻¹ (154.8 g m⁻²).

Average weights of vertical rhizomes and leaf heads (A. antarctica) and leaves (P. sinuosa) were calculated from Experiment 1.

2.7 Recovery from simulated grazing statistical analyses

Two-factor ANOVA was used to test for the effects of simulated grazing and seagrass species on seagrass recovery using PERMANOVA+ for Primer v6. Species and simulated grazing were treated as fixed factors. Partial removal and complete removal treatments were compared independently of one another against controls in two separate analyses. This approach was taken as the partial removal treatment tested urchin effects from Experiment 1 (i.e. different pre-recovery biomass for each species) whereas the complete removal tested simulated grazing of all above ground biomass (i.e. identical pre-recovery biomass for each species). Cochran’s C was used to test for homogeneity of variance prior to analysis of variance. Pairwise tests were used where significant interaction terms were detected.
3. Results

3.1 Field sampling

Sampling of *A. antarctica* meadows following observations of elevated *A. pallidus* densities suggested that urchins may have been responsible for the loss of > 83% of above ground biomass (133.2 v. 21.7 gdw m$^{-2}$) and > 96% of living shoot density (360.6 v. 12.8 shoots m$^{-2}$) in un-affected v. affected meadows. While *P. sinuosa* meadows appeared un-impacted by urchin grazing during our initial observations, we later sampled a highly variable negative relationship between urchin and *P. sinuosa* biomass, with regression coefficient values ranging between $0.11 \leq r^2 \leq 0.90$ over three sampling dates. The maximum density of urchins recorded in *P. sinuosa* across the three sampling dates and sites was $8.76 \pm 0.50$ m$^{-2}$, with mean densities of $1.03 \pm 0.78$ m$^{-2}$, although densities as high as $34.6 \pm 3.88$ m$^{-2}$ were recorded two months prior to the first sampling date. During the same period in nearby *A. antarctica* meadows, urchin densities did not exceed $0.70 \pm 0.11$ m$^{-2}$, with mean densities of $0.12 \pm 0.11$ m$^{-2}$.

3.2 Grazing experiment (Experiment 1)

Urchin grazing reduced the above ground leaf biomass of *A. antarctica* and *P. sinuosa*. After 169 days there was no difference in the remaining leaf biomass between the two species (Fig. 1A, B Table 1), while, the rate of decline appeared faster in *P. sinuosa* than in *A. antarctica*; this effect was not significant in the repeated measures ANOVA (Table 1, Time × Species × Grazing interaction). The procedural controls had no effect on the leaf biomass of seagrass at either halfway or final sampling point (Fig. 1A, B, Table 1).

Grazing damage, quantified by the percentage of dead shoots (*A. antarctica*) and leaves with grazing scars (*P. sinuosa*), increased where urchins were present, however, there was no
difference between the two species (Fig. 2A,B, Table 2). It is worth noting that data for both
*A. antarctica* and *P. sinuosa* may underestimate the frequency of grazing damage; given that
in *A. antarctica* all leaf heads had to be removed for a shoot to be classified as dead and in
*P. sinuosa* some consumed leaves may have been totally removed leaving no evidence of
grazing.

Urchin presence significantly reduced meristem survival in *A. antarctica*, but had no impact
in *P. sinuosa*. While *A. antarctica* had a greater abundance of leaf meristems than *P. sinuosa*
where urchins were absent, this effect was reversed where urchin densities were elevated,
highlighting the significant loss of *A. antarctica* leaf heads and associated meristems due to
grazing (Fig. 2C,D, Table 2: Grazing × Species interaction).

Below ground biomass was greater in *P. sinuosa* than in *A. antarctica*, but the grazing
treatment had no impact on below ground biomass of either species (Fig. 2E,F, Table 2).

3.3 *Seagrass recovery from simulated grazing (Experiment 2)*

Recovery of biomass in *P. sinuosa* following simulated grazing exceeded that for
*A. antarctica* (56 and 62% v. 7 and 25% of controls in the complete and partial removal
treatments, respectively) (Fig. 3, Tables 3 & 4). Recovery of *P. sinuosa* was greater where
more seagrass was removed (a direct relationship between removal and recovery), whereas in
*A. antarctica* recovery declined as removal increased (an inverse relationship between
removal and recovery) (Table 3).
4. Discussion

Grazing by sea urchins has often been considered important for shaping the composition and extent of habitats among sub-tidal rock and coral reefs. While seagrass habitats were long believed to be predominantly bottom-up controlled, recent literature demonstrates that top-down forces can also play an important role in regulating seagrass ecosystems (Eklof, et al., 2008; Valentine and Duffy, 2006). Here, we identify that sea urchins living at elevated though naturally occurring densities can switch habitat from dense to sparse seagrass meadows through overgrazing, highlighting the susceptibility of seagrasses to top-down grazing under certain conditions. While net grazing effects were consistent across both species of seagrass, rates of recovery following simulated grazing were far greater in *P. sinuosa* than in *A. antarctica*. Such asymmetric patterns in seagrass recovery from overgrazing suggest that the duration of phase shifts from seagrass to bare habitats caused by overgrazing, and any cascading ecosystem effects, can be dependent on the identity of species which is lost.

The substantial loss of *A. antarctica* during a natural overgrazing event, compared to the apparent stability of *Posidonia spp.* biomass in adjacent meadows, suggested that urchin grazing effects may be species-specific. Therefore, the similar decline in seagrass biomass for both *A. antarctica* and *P. sinuosa* during the grazing experiment was initially surprising. The strong positive trajectory toward the recovery of *P. sinuosa* following simulated grazing, however, was in stark contrast to *A. antarctica*, which showed minimal recovery. An elevated capacity for regrowth is a successful strategy used by many terrestrial plants exposed to high grazing pressure (Strauss and Agrawal, 1999; Vandermeijden, et al., 1988), and may better prepare some species for herbivore driven disturbances. Given variability in urchin densities,
periods with reduced urchin presence are common (Pearse and Hines, 1987; Rose, et al., 1999). Therefore, the combination of per capita grazing intensity, urchin return or recruitment interval and rates of seagrass recovery from grazing may all be crucial to long-term seagrass habitat persistence (Alcoverro and Mariani, 2002; Burnell et al., 2013).

While only one period of natural overgrazing was observed in *A. antarctica* during intermittent visits to the study area over three years, recovery of seagrasses at the grazed sites appeared negligible during this time. Following natural overgrazing of *A. antarctica*, urchins were only present within adjacent *Posidonia spp.* suggesting the inability of *A. antarctica* to recover from grazing and hence provide a stable habitat may have resulted in urchin migration, starvation or predation. While grazing impacts appear persistent within *A. antarctica* meadows over the short-term, longer term studies would be required to accurately gauge if the frequency of these events exceed seagrass recovery from meadow expansion or recruitment.

Morphology and growth strategy can be important for seagrass regeneration and proliferation (Tomlinson, 1974). While both *A. antarctica* and *P. sinuosa* grow by continually replacing old leaves (Short and Duarte, 2001), the leaf meristems of *Amphibolis spp.* are elevated in the canopy, whereas those of *Posidonia spp.* are below-ground (Marba and Walker, 1999). The exposure of leaf meristems on vertical shoots above the sediment can result in strong grazing impacts (Alcoverro and Mariani, 2002). Since seagrass regeneration is reliant on active meristems (Tomlinson, 1974), seagrasses that have lost raised apical meristems can only regenerate by creating new shoots from existing rhizomes (Alcoverro and Mariani, 2002; Rose, et al., 1999). Consequently, when *A. antarctica* is overgrazed until the majority of shoots are completely defoliated, its recovery becomes reliant on new shoot growth from
below ground rhizome extension, whereas basal meristems of *Posidonia* spp. may continue to produce new leaves in a largely uninterrupted way. This species-dependent capacity for regrowth based on meristem location appears a likely explanation for the patterns of recovery we observed and is analogous to findings for the recovery of other seagrasses exposed to significant meristem damage (i.e. *Syringodium filiforme*, *Zostera marina* & *Thalassodendron ciliatum*) (Alcoverro and Mariani, 2002; 2005; Rivers and Short, 2007; Rose, et al., 1999). In fact, grazing pressure may even play a significant role in determining seagrass species distribution (Vonk, et al., 2008). The tropical seagrass *T. ciliatum*, which has a similar morphology to *A. antarctica* and a high vulnerability to urchin grazing (Alcoverro and Mariani, 2002; 2005), is dominant in zones protected from fishing where urchin populations remain low (McClanahan, et al., 1994), but lower in unprotected zones where urchin abundance is greater (Alcoverro and Mariani, 2004). Such patterns suggest that morphology may interact with grazing pressure to shape the distribution of some seagrass species.

Recovery of *P. sinuosa* biomass during the simulated grazing experiment was greater where more seagrass was removed, whereas in *A. antarctica* biomass recovery declined as removal increased. Seagrass growth in response to changing grazing intensity is rarely linear (Cebrian, et al., 1998; Eklof, et al., 2008) and can be highly species-specific (Alcoverro and Mariani, 2005; Cebrian, et al., 1998). The greater loss of leaf meristems in *A. antarctica* where more seagrass was removed is likely to have limited the availability of active meristems that could produce new growth. In comparison, simulated grazing in *P. sinuosa* potentially induced an overcompensatory growth response, where recovery rate increased as a function of removal. Alternatively, natural senescence and shedding of older leaves by *P. sinuosa* in the control and partial removal treatment could explain the lower recovery, in comparison to the complete removal treatment where no older leaves remained.
While it appears that meristem damage from grazing may modify the vulnerability and post-grazing recovery of *A. antarctica*, inherent biological differences between the two species should not be discounted and could also have contributed to the species-specific differences we found (Strauss and Agrawal, 1999). The greater below ground resources we recorded for *P. sinuosa*, which is consistently evident when compared with *A. antarctica* (Cambridge, 1999), could have aided rapid recovery from defoliation as resources can be translocated from roots and rhizomes to help leaf growth and recovery (Cebrian, et al., 1998; Strauss and Agrawal, 1999). Regardless of the mechanism to recovery, however, it appears evident that *P. sinuosa* has a greater tolerance to urchin grazing than *A. antarctica*.

It is important to note that simulated grazing is rarely an exact substitute for actual grazing (Eklof, et al., 2009; Ibarra-Obando, et al., 2004; Strauss and Agrawal, 1999), suggesting the recovery we recorded should be interpreted cautiously. It was necessary to use new experimental plots for the recovery experiment as we destructively sampled seagrass to quantify urchin effects during the grazing experiment. Therefore, any deterioration of below ground resources that occurred during the grazing experiment was not replicated in the simulated grazing experiment. While *A. antarctica* showed a greater loss of below ground biomass than *P. sinuosa* during the grazing experiment, albeit non-significantly, replicating this effect in the simulated grazing experiment would only have exacerbated the species differences we observed, as fewer resources would have been available to *A. antarctica* for recovery. Simulated grazing can also underestimate potential benefits of herbivory. For example, urchin faeces can increase seagrass productivity by enhancing the rate of nutrient cycling, effectively fertilising any remaining plants (Koike, et al., 1987; Vonk, et al., 2008). The absence of this effect could have potentially underestimated seagrass recovery during the
simulated grazing experiment, although this effect should be consistent across both species and is therefore unlikely to alter the inter-specific differences seen here. In addition, the rapid re-growth of *P. sinuosa* suggests that plants at the study site were unlikely to be nutrient-limited.

We considered it important to quantify the rate of biomass decline in both species despite the possibility that sub-sampling halfway through the grazing experiment may have impacted adjacent seagrasses due to the clonal nature of ramets. Importantly, *Amphibolis* and *Posidonia* rhizomes form a complex matrix where many individual plants overlap, rather than being a single clonal individual (Bryars, et al., 2011), making the removal of small amounts of above ground biomass unlikely to destroy entire plants. While sub-sampling itself does not appear to send seagrass meadows into significant decline (Bryars, et al., 2011; Valentine and Heck, 1991; Vonk, et al., 2008), it is plausible that biomass removal could have had a larger effect where seagrasses were already under stress from urchin grazing, possibly exacerbating grazing impacts. Yet we anticipate that our results are not an overestimation of grazing impacts for two reasons. First, only a small amount of above-ground biomass was removed (6% of plots) and belowground biomass was undamaged so there is no reason to assume that translocation of belowground resources was unduly interrupted. Second, while we removed 6% of the areal cover in all treatments, seagrass cover and biomass were already reduced in grazing treatments and by sampling in a standard area more biomass was actually removed in the control treatments. Nonetheless, non-destructive estimates of grazing impact, such as shoot density and canopy height could be used in future studies, though this can be inherently difficult due to species-specific morphology and seasonal changes in biomass composition.
5. Conclusion

The current rate of decline in seagrass meadows places them among the most threatened ecosystems worldwide (Waycott, et al., 2009). While some species of seagrass have evolved traits to tolerate high grazing intensity, such as rapid regeneration and basal leaf meristems (Valentine and Duffy, 2006), others such as *A. antarctica* appear vulnerable to long lasting impacts from sudden increases in grazing pressure due to a reduced capacity for recovery post-disturbance. Habitats created by such species appear particularly vulnerable to long-term, top-down induced phase-shifts. The cascading ecosystem effects of wholesale seagrass habitat loss threaten coastal productivity and biodiversity, through a reduction in sediment stabilisation, nutrient cycling and support of trophic food webs (Duarte, 2002; Orth, et al., 2006). Understanding the causes of sudden urchin population increases, and under what conditions seagrasses may be more or less susceptible to overgrazing, may help us predict and prepare for the effects of seagrass loss.
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12381.
Table 1. Repeated measures analysis of variance comparing the effect of urchin grazing (control, procedural control v. grazing), seagrass species (*Amphibolis antarctica* v. *Posidonia sinuosa*) and time (halfway v. final) on the above ground leaf biomass of seagrasses. Significant effects indicated in bold where p<0.05.

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<td>Time × Species</td>
<td>1</td>
<td>0.253</td>
<td>2.772</td>
<td>0.109</td>
</tr>
<tr>
<td>Time × Grazing</td>
<td>2</td>
<td>0.157</td>
<td>1.714</td>
<td>0.202</td>
</tr>
<tr>
<td>Time × Species × Grazing</td>
<td>2</td>
<td>0.181</td>
<td>1.986</td>
<td>0.159</td>
</tr>
<tr>
<td>Residual</td>
<td>24</td>
<td>0.091</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Pairwise comparison: Leaf Biomass**

**Urchin grazing:** control = procedural control > grazing
Table 2. Two factor analysis or variance comparing the effect of urchin grazing (control v. grazing) and seagrass species (*Amphibolis antarctica* v. *Posidonia sinuosa*) on grazing damage, leaf meristem survival and below ground biomass of seagrasses. Significant effects indicated in bold where p<0.05.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Grazing Damage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grazing</td>
<td>1</td>
<td>1.557</td>
<td>194.610</td>
<td>0.001</td>
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<tr>
<td>Species</td>
<td>1</td>
<td>0.003</td>
<td>0.388</td>
<td>0.526</td>
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<tr>
<td>Grazing × Species</td>
<td>1</td>
<td>0.027</td>
<td>3.380</td>
<td>0.092</td>
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<tr>
<td>Residual</td>
<td>16</td>
<td>0.008</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Leaf Meristem Survival</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grazing</td>
<td>1</td>
<td>0.543</td>
<td>61.834</td>
<td>0.001</td>
</tr>
<tr>
<td>Species</td>
<td>1</td>
<td>0.016</td>
<td>1.794</td>
<td>0.213</td>
</tr>
<tr>
<td>Grazing × Species</td>
<td>1</td>
<td>0.463</td>
<td>52.778</td>
<td>0.001</td>
</tr>
<tr>
<td>Residual</td>
<td>16</td>
<td>0.009</td>
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<td></td>
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<tr>
<td><strong>Below Ground Biomass</strong></td>
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<tr>
<td>Grazing</td>
<td>1</td>
<td>0.072</td>
<td>0.715</td>
<td>0.407</td>
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<td>Species</td>
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<td>2.623</td>
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<tr>
<td>Grazing × Species</td>
<td>1</td>
<td>0.135</td>
<td>1.344</td>
<td>0.296</td>
</tr>
<tr>
<td>Residual</td>
<td>16</td>
<td>0.101</td>
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</tr>
</tbody>
</table>

Pairwise comparisons: Leaf Meristem Survival

Urchin grazing: *A. antarctica*, control > grazing; *P. sinuosa*, control = grazing;

Seagrass species: control, *A. antarctica* > *P. sinuosa*; grazing, *A. antarctica* < *P. sinuosa*.
Table 3. Final seagrass dry weight from Experiment 1 (Grazing Experiment) and Experiment 2 (Simulated Grazing Experiment) used to calculate biomass recovery during the Simulated Grazing Experiment. Recovery was calculated by subtracting leaf biomass at the conclusion of the Grazing Experiment from leaf biomass at the conclusion of the Simulated Grazing Experiment, given the the biomass removed in the partial removal treatment of the Simulated Grazing Experiment mimicked that removed by urchins.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Urchin Grazing (Exp 1)</th>
<th>Simulated Grazing (Exp 2)</th>
<th>Calculated Recovery (Exp 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphibolis antarctica</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>219.84 ± 28.11</td>
<td>364.32 ± 23.99</td>
<td>144.48</td>
</tr>
<tr>
<td>Urchin Grazing/Partial Removal</td>
<td>55.64 ± 10.12</td>
<td>90.75 ± 6.64</td>
<td>35.11</td>
</tr>
<tr>
<td>Complete Removal</td>
<td>n/a</td>
<td>26.24 ± 3.17</td>
<td>26.24</td>
</tr>
<tr>
<td>Posidonia sinuosa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>220.58 ± 21.47</td>
<td>363.78 ± 40.79</td>
<td>143.20</td>
</tr>
<tr>
<td>Urchin Grazing/Partial Removal</td>
<td>65.76 ± 5.72</td>
<td>226.62 ± 25.92</td>
<td>160.86</td>
</tr>
<tr>
<td>Complete Removal</td>
<td>n/a</td>
<td>204.96 ± 16.28</td>
<td>204.96</td>
</tr>
</tbody>
</table>
Table 4. Analysis of variance comparing the effect of simulated grazing (control v. partial removal and control v. complete removal) and species (*Amphibolis antarctica* v. *Posidonia sinuosa*) on above ground leaf biomass following 109 days of recovery. Significant effects indicated in bold where $p<0.05$.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>$F$</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Leaf Biomass (Partial Removal)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Grazing</td>
<td>1</td>
<td>210860</td>
<td>57.08</td>
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<tr>
<td>Species</td>
<td>1</td>
<td>22892</td>
<td>6.20</td>
<td>0.032</td>
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<tr>
<td>Grazing × Species</td>
<td>1</td>
<td>23262</td>
<td>6.30</td>
<td>0.024</td>
</tr>
<tr>
<td>Residual</td>
<td>16</td>
<td>3694</td>
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</tr>
<tr>
<td><strong>Leaf Biomass (Complete Removal)</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Grazing</td>
<td>1</td>
<td>308630</td>
<td>98.20</td>
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</tr>
<tr>
<td>Species</td>
<td>1</td>
<td>39683</td>
<td>12.63</td>
<td>0.006</td>
</tr>
<tr>
<td>Grazing × Species</td>
<td>1</td>
<td>40169</td>
<td>12.78</td>
<td>0.003</td>
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<tr>
<td>Residual</td>
<td>16</td>
<td>3143</td>
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</tr>
</tbody>
</table>

Pairwise comparisons: Partial Removal

**Grazing:** *A. Antarctica*, control > partial removal, *P. sinuosa*, control > partial removal;

**Species:** control, *A. antarctica* = *P. sinuosa*, partial removal, *A. Antarctica* < *P. sinuosa*;

Pairwise comparisons: Complete Removal

**Grazing:** *A. Antarctica*, control > complete removal, *P. sinuosa*, control > complete removal;

**Species:** control, *A. antarctica* = *P. sinuosa*, complete removal, *A. antarctica* < *P. sinuosa*. 
Figure Captions

Figure 1. The effect of sea urchins *Amblypneustes pallidus* (control, procedural control v. grazing) on the halfway (82 days) and final (169 days) above ground leaf biomass (A. *Amphibolis antarctica*; B. *Posidonia sinuosa*) of seagrasses (gdw m\(^{-2}\)). Bars indicate the mean and standard error.

Figure 2. The effect of sea urchins *Amblypneustes pallidus* (control v. grazing), on grazing damage (A. *Amphibolis antarctica*, percentage dead shoots; B *Posidonia sinuosa*, percentage of leaves with grazing scar), leaf meristem survival (C. *Amphibolis antarctica*, # leaf heads; D. *Posidonia sinuosa*, # sheaths with leaves) and final (169 days) below ground biomass (E. *Amphibolis antarctica*, gdw m\(^{-2}\); F. *Posidonia sinuosa*, gdw m\(^{-2}\)). Bars indicate the mean and standard error.

Figure 3. The effect of simulated urchin grazing (control, partial removal v. complete removal) on the above ground leaf biomass (A. *Amphibolis antarctica*; B. *Posidonia sinuosa*) of seagrass (gdw m\(^{-2}\)) after 109 days. Bars indicate the mean and standard error.
Figure 1

**A** *Amphibolis antarctica*

- Control
- Procedural Control
- Grazing

**B** *Posidonia sinuosa*

- Control
- Procedural Control
- Grazing

Above Ground Leaf Biomass (gdw m\(^{-2}\))

<table>
<thead>
<tr>
<th></th>
<th>Halfway</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Grazing</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 2

A. Amphibolis antarctica - Grazing Damage

B. Posidonia sinuosa - Grazing Damage

C. Amphibolis antarctica - Meristem Survival

D. Posidonia sinuosa - Meristem Survival

E. Amphibolis antarctica - Below Ground

F. Posidonia sinuosa - Below Ground
Figure 3

A  *Amphibolis antarctica* - Recovery

B  *Posidonia sinuosa* - Recovery