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Burnell, Owen William; Connell, Sean Duncan; Irving, Andrew D.; Russell, Bayden D. Asymmetric patterns of recovery in two habitat forming seagrass species following simulated overgrazing by urchins, *Journal of Experimental Marine Biology and Ecology*, 2013; 448:114-120.

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16th August, 2013

<http://hdl.handle.net/2440/79177>

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

3

4 Owen W. Burnell^{1,*}, Sean D. Connell¹, Andrew D. Irving² & Bayden D. Russell¹

5

6 ¹Southern Seas Ecology Laboratories, Darling Building (DP418), School of Earth &

7 Environmental Sciences, University of Adelaide, South Australia 5005, Australia

8 ²School of Medical and Applied Sciences, Central Queensland University, Bruce Highway,

9 Rockhampton, Queensland 4702, Australia

10

11 * Corresponding author: owen.burnell@adelaide.edu.au

12 Tel: +61 (0) 8 8313 7035, Fax: +61 (0) 8 8313 4364

13 **Abstract**

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

30

31 **Key words:**

32 phase-shift, herbivory, habitat loss, meristem, *Amphibolis antarctica*, *Posidonia sinuosa*

33

34 **1. Introduction**

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

48

49 On southern Australian coasts, the seagrasses *Amphibolis spp.* and *Posidonia spp.* can form
50 extensive co-occurring habitats on moderately wave-exposed shores (Bryars and Rowling,
51 2009; Shepherd and Womersley, 1981). Widespread historical losses (Walker and McComb,
52 1992), limited re-colonisation (Bryars and Neverauskas, 2004; Kendrick, et al., 2002; Irving,
53 2013), and slow meadow expansion rates (Marba and Walker, 1999) have demonstrated the
54 susceptibility of these seagrasses to natural and anthropogenic impacts. Among natural
55 effects, overgrazing has been observed but appears to disproportionately impact *Amphibolis*
56 *antarctica* (author’s pers. obs.), possibly because it is a species that produces a canopy of
57 accessible meristematic leaf clusters while *Posidonia spp.* meristems are protected below
58 ground (Alcoverro and Mariani, 2002; Marba and Walker, 1999; Short and Duarte, 2001).

59

60 The purpose of this study was to determine how sea urchin grazing causes the loss and affects
61 the recovery of the seagrasses *A. antarctica* and *Posidonia sinuosa*. In southern Australia, the
62 short-spined sea urchin *Amblypneustes pallidus* can over-graze *A. antarctica* with little to no
63 recovery for at least 3 years (author's pers. obs.). However, grazing effects on *P. sinuosa*,
64 which often co-exist in mixed or adjacent meadows with *A. antarctica*, appear less
65 pronounced despite urchins often occurring in greater densities in *P. sinuosa*. We
66 experimentally tested the hypothesis that a fixed density of *A. pallidus* would
67 disproportionately impact the biomass of *A. antarctica* relative to *P. sinuosa*. In a second
68 experiment we tested the hypothesis that *P. sinuosa* would show greater recovery than
69 *A. antarctica* following simulated grazing damage.

70

71 **2. Materials & Methods**

72 *2.1 Study region*

73 All experiments were done at Lady Bay (35°28.036 S, 138°17.198 E), approximately 70 km
74 South-West of the city of Adelaide, South Australia. Seagrass meadows in this region are
75 largely intact and unaffected by anthropogenic impacts more common to the Adelaide
76 metropolitan coastline (e.g. eutrophication). Meadows comprise a mosaic of *P. sinuosa*,
77 *Posidonia angustifolia* and *A. antarctica*, interspersed by small rocky outcroppings and sand
78 patches. The short-spined sea urchin *A. pallidus* is a small (i.e. 2 - 4 cm) and often
79 inconspicuous seagrass grazer that is normally sparsely distributed but occasionally greatly
80 exceeds background densities (author's pers. obs.). All sampling was done between depths of
81 4 - 5.5 m.

82

83

84

85 2.2 Field sampling

86 Following a sea urchin over-grazing event in *A. antarctica* meadows during February 2010,
87 seagrasses were sampled within affected and unaffected meadows. Meadows were defined as
88 ‘affected’ where urchins had been observed actively grazing seagrass and there was clear
89 evidence of defoliation and dead shoots. Meadows were defined as ‘unaffected’ where
90 urchins had not been observed grazing seagrass and there was limited evidence of defoliation
91 or dead shoots. Five 0.0625 m² quadrats of *A. antarctica* were harvested at the substratum,
92 transported on ice under dark conditions and frozen for later analysis of above ground
93 biomass and shoot density.

94

95 As no conspicuous effects of grazing were observed in *P. sinuosa* meadows, we tested for a
96 relationship between urchin and *P. sinuosa* biomass, on three different dates, from December
97 2011 to March 2012. At four sites, each separated by a minimum of 1 km, urchin abundance
98 was counted in five 10 × 1 m transects. Twenty urchins were then haphazardly collected and
99 weighed while wet to estimate total biomass m⁻². Five 0.0625 m² quadrats of *P. sinuosa* were
100 then harvested at the substratum to quantify above ground biomass. We undertook the same
101 sampling procedure in nearby *A. antarctica* meadows, however, urchin numbers were so low
102 (zero at 66% of sites) that no relationship could be established, therefore only average urchin
103 densities are presented.

104

105 2.3 Urchin grazing (Experiment 1)

106 Manipulative experiments testing the effect of *A. pallidus* grazing on seagrass biomass were
107 done within nearby monospecific meadows of *A. antarctica* and *P. sinuosa*, where the
108 naturally occurring urchin populations observed during the experimental period (March to

109 August 2011) were zero. For each seagrass species, fifteen experimental plots were randomly
110 interspersed over 500 m² to separate plots by at least four meters. Five plots were designated
111 as treatments of elevated grazing by adding two urchins within a wire mesh cage anchored by
112 fence droppers (mesh size 0.02 × 0.02 m, cage size 0.4 × 0.4 × 0.4 m) to create urchin
113 densities within the natural range observed at Lady Bay (12.5 m⁻²; average wet urchin
114 biomass = 29.45 ± 0.40 g). Urchins were collected from nearby beds of *Posidonia spp.* and
115 *A. antarctica*, with weekly replacement of dead individuals if necessary. A procedural control
116 was established in another five plots of each species using a partial cage to test for caging
117 artefacts on seagrass. The remaining five plots were un-manipulated and served as controls.
118 Cages were cleaned of algal growth weekly. One grazing replicate from each species of
119 seagrass was lost due to storm damage during June. These treatments were not replaced.

120

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

129

130 Seagrass biomass was measured by removing any fleshy epiphytes with a razor blade, rinsing
131 in MilliQ water to remove salts and sediment, and then drying at 60°C for 48 h. For
132 *A. antarctica*, vertical rhizomes and leaves were separated prior to drying to determine above
133 ground leaf biomass in grams of dry weight per square metre (gdw m⁻²). Leaf meristems were

134 counted in *A. antarctica* if the apical meristem was intact on each leaf cluster, determined by
135 the survival of the youngest leaf in the cluster. Leaf meristems of *P. sinuosa* were counted if
136 sheaths contained at least one living leaf. In the final samples all *A. antarctica* shoots were
137 classified as either alive (i.e. containing living meristems) or dead. As urchins can graze *A.*
138 *antarctica* directly at the shoot, this was the best way to estimate rates of grazing damage. For
139 *P. sinuosa* 20 individual leaves were selected and the leaf tips identified as grazed if
140 possessing an apex consistent with urchin grazing scar.

141

142 *2.4 Grazing experiment statistical analyses*

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

151

152 Two-factor ANOVA was used to test for the effects of urchin grazing and seagrass species on
153 below ground biomass, meristem survival and grazing damage using PERMANOVA+ for
154 Primer v6. Urchin grazing and seagrass species were treated as fixed factors in the orthogonal
155 design. Cochran's *C*-test was used to test for homogeneity of variance prior to analysis.
156 Below ground biomass and meristem survival violated the assumption of homogeneity of
157 variance, therefore data was log-transformed prior to analysis. Grazing damage was

158 ArcSin (%) transformed prior to analysis. Pairwise tests were used where significant
159 interactions terms were detected.

160

161 *2.5 Recovery from simulated grazing (Experiment 2)*

162 To quantify seagrass recovery from overgrazing, urchin grazing was simulated in both
163 *A. antarctica* and *P. sinuosa* using scissors to clip above ground biomass. The recovery
164 experiment was completed in different monospecific beds of *A. antarctica* and *P. sinuosa*
165 adjacent to where the grazing experiment was undertaken. Three treatments were established:
166 control (no biomass removed), partial removal (removal equal to the urchin effects sampled
167 in the grazing experiment; see section 2.6) and complete removal (100 % removal of above
168 ground biomass). Five replicate plots $0.4 \times 0.4\text{m}$ (0.16 m^2) for each treatment were
169 established in both seagrass species, randomly interspersed over a 500 m^2 area for each
170 species and marked using star droppers. The experiment commenced seven weeks after the
171 grazing experiment and continued for 109 days from October 2011 to January 2012, after
172 which above ground biomass was harvested by destructively sampling within 0.0625 m^2
173 quadrats before processing seagrass, as described above.

174

175 *2.6 Partial removal treatment*

176 Due to the different morphologies of *Amphibolis spp.* and *Posidonia spp.* unique removal
177 techniques were utilised to accurately replicate grazing loss for each species. While
178 *Amphibolis spp.* have woody vertical rhizomes, with a number of short shoots and leaf heads
179 on each stem, the above ground biomass of *Posidonia spp.* consists entirely of strap like
180 leaves. For a detailed summary of the morphology of these species see Marba and Walker
181 (1999). Because urchins can remove either entire or partial sections of vertical rhizomes and
182 leaves, clipping of both entire and partial vertical rhizomes (*A. antarctica*) and leaves

183 (*P. sinuosa*) was required to accurately replicate grazing loss. Firstly, clipping of entire
184 vertical rhizomes (*A. antarctica*) and leaves (*P. sinuosa*) at the substrate was undertaken to
185 match loss recorded between control and urchin grazing treatments in Experiment 1.
186 Secondly, clipping of individual leaf heads (*A. antarctica*) and partial leaves (*P. sinuosa*) was
187 undertaken to account for the removal of partial vertical rhizomes and leaves from
188 Experiment 1. For each experimental plot ($0.4 \times 0.4\text{m}$) in *A. antarctica*, 90 entire vertical
189 rhizomes (560 m^{-2}) at an average $0.19 \text{ gdw vertical rhizome}^{-1}$ and 132 individual leaf heads
190 (825 m^{-2}) at an average $0.07 \text{ gdw leaf head}^{-1}$ were removed for a total removal of 26.28 gdw
191 plot^{-1} (164.2 g m^{-2}). For each experimental plot ($0.4 \times 0.4\text{m}$) in *P. sinuosa* 197 entire leaves
192 (1232 m^{-2}) at an average of $0.06 \text{ gdw leaf}^{-1}$ and 435 partial leaves (2720 m^{-2}) at an average of
193 $0.03 \text{ gdw partial leaf}^{-1}$ were removed for a total removal of $24.77 \text{ gdw plot}^{-1}$ (154.8 g m^{-2}).
194 Average weights of vertical rhizomes and leaf heads (*A. antarctica*) and leaves (*P. sinuosa*)
195 were calculated from Experiment 1.

196

197 *2.7 Recovery from simulated grazing statistical analyses*

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

207

208

209 **3. Results**

210 *3.1 Field sampling*

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

223

224 *3.2 Grazing experiment (Experiment 1)*

225 Urchin grazing reduced the above ground leaf biomass of *A. antarctica* and *P. sinuosa*. After
226 169 days there was no difference in the remaining leaf biomass between the two species (Fig.
227 1A,B Table 1), while, the rate of decline appeared faster in *P. sinuosa* than in *A. antarctica*;
228 this effect was not significant in the repeated measures ANOVA (Table 1, Time × Species ×
229 Grazing interaction). The procedural controls had no effect on the leaf biomass of seagrass at
230 either halfway or final sampling point (Fig. 1A, B, Table 1).

231

232 Grazing damage, quantified by the percentage of dead shoots (*A. antarctica*) and leaves with
233 grazing scars (*P. sinuosa*), increased where urchins were present, however, there was no

234 difference between the two species (Fig. 2A,B , Table 2). It is worth noting that data for both
235 *A. antarctica* and *P. sinuosa* may underestimate the frequency of grazing damage; given that
236 in *A. antarctica* all leaf heads had to be removed for a shoot to be classified as dead and in
237 *P. sinuosa* some consumed leaves may have been totally removed leaving no evidence of
238 grazing.

239

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

245

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

248

249 3.3 Seagrass recovery from simulated grazing (Experiment 2)

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

256

257

258

259

260 **4. Discussion**

261 Grazing by sea urchins has often been considered important for shaping the composition and
262 extent of habitats among sub-tidal rock and coral reefs. While seagrass habitats were long
263 believed to be predominantly bottom-up controlled, recent literature demonstrates that top-
264 down forces can also play an important role in regulating seagrass ecosystems (Eklof, et al.,
265 2008; Valentine and Duffy, 2006). Here, we identify that sea urchins living at elevated
266 though naturally occurring densities can switch habitat from dense to sparse seagrass
267 meadows through overgrazing, highlighting the susceptibility of seagrasses to top-down
268 grazing under certain conditions. While net grazing effects were consistent across both
269 species of seagrass, rates of recovery following simulated grazing were far greater in *P.*
270 *sinuosa* than in *A. antarctica*. Such asymmetric patterns in seagrass recovery from
271 overgrazing suggest that the duration of phase shifts from seagrass to bare habitats caused by
272 overgrazing, and any cascading ecosystem effects, can be dependent on the identity of
273 species which is lost.

274

275 The substantial loss of *A. antarctica* during a natural overgrazing event, compared to the
276 apparent stability of *Posidonia spp.* biomass in adjacent meadows, suggested that urchin
277 grazing effects may be species-specific. Therefore, the similar decline in seagrass biomass for
278 both *A. antarctica* and *P. sinuosa* during the grazing experiment was initially surprising. The
279 strong positive trajectory toward the recovery of *P. sinuosa* following simulated grazing,
280 however, was in stark contrast to *A. antarctica*, which showed minimal recovery. An elevated
281 capacity for regrowth is a successful strategy used by many terrestrial plants exposed to high
282 grazing pressure (Strauss and Agrawal, 1999; Vandermeijden, et al., 1988), and may better
283 prepare some species for herbivore driven disturbances. Given variability in urchin densities,

284 periods with reduced urchin presence are common (Pearse and Hines, 1987; Rose, et al.,
285 1999). Therefore, the combination of per capita grazing intensity, urchin return or recruitment
286 interval and rates of seagrass recovery from grazing may all be crucial to long-term seagrass
287 habitat persistence (Alcoverro and Mariani, 2002; Burnell et al., 2013).

288

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

298

299 Morphology and growth strategy can be important for seagrass regeneration and proliferation
300 (Tomlinson, 1974). While both *A. antarctica* and *P. sinuosa* grow by continually replacing
301 old leaves (Short and Duarte, 2001), the leaf meristems of *Amphibolis spp.* are elevated in the
302 canopy, whereas those of *Posidonia spp.* are below-ground (Marba and Walker, 1999). The
303 exposure of leaf meristems on vertical shoots above the sediment can result in strong grazing
304 impacts (Alcoverro and Mariani, 2002). Since seagrass regeneration is reliant on active
305 meristems (Tomlinson, 1974), seagrasses that have lost raised apical meristems can only
306 regenerate by creating new shoots from existing rhizomes (Alcoverro and Mariani, 2002;
307 Rose, et al., 1999). Consequently, when *A. antarctica* is overgrazed until the majority of
308 shoots are completely defoliated, its recovery becomes reliant on new shoot growth from

309 below ground rhizome extension, whereas basal meristems of *Posidonia spp.* may continue to
310 produce new leaves in a largely uninterrupted way. This species-dependent capacity for re-
311 growth based on meristem location appears a likely explanation for the patterns of recovery
312 we observed and is analogous to findings for the recovery of other seagrasses exposed to
313 significant meristem damage (i.e. *Syringodium filiforme*, *Zostera marina* & *Thalassodendron*
314 *ciliatum*) (Alcoverro and Mariani, 2002; 2005; Rivers and Short, 2007; Rose, et al., 1999). In
315 fact, grazing pressure may even play a significant role in determining seagrass species
316 distribution (Vonk, et al., 2008). The tropical seagrass *T. ciliatum*, which has a similar
317 morphology to *A. antarctica* and a high vulnerability to urchin grazing (Alcoverro and
318 Mariani, 2002; 2005), is dominant in zones protected from fishing where urchin populations
319 remain low (McClanahan, et al., 1994), but lower in unprotected zones where urchin
320 abundance is greater (Alcoverro and Mariani, 2004). Such patterns suggest that morphology
321 may interact with grazing pressure to shape the distribution of some seagrass species.

322

323 Recovery of *P. sinuosa* biomass during the simulated grazing experiment was greater where
324 more seagrass was removed, whereas in *A. antarctica* biomass recovery declined as removal
325 increased. Seagrass growth in response to changing grazing intensity is rarely linear (Cebrian,
326 et al., 1998; Eklof, et al., 2008) and can be highly species-specific (Alcoverro and Mariani,
327 2005; Cebrian, et al., 1998). The greater loss of leaf meristems in *A. antarctica* where more
328 seagrass was removed is likely to have limited the availability of active meristems that could
329 produce new growth. In comparison, simulated grazing in *P. sinuosa* potentially induced an
330 overcompensatory growth response, where recovery rate increased as a function of removal.
331 Alternatively, natural senescence and shedding of older leaves by *P. sinuosa* in the control
332 and partial removal treatment could explain the lower recovery, in comparison to the
333 complete removal treatment where no older leaves remained.

334

335 While it appears that meristem damage from grazing may modify the vulnerability and post-
336 grazing recovery of *A. antarctica*, inherent biological differences between the two species
337 should not be discounted and could also have contributed to the species-specific differences
338 we found (Strauss and Agrawal, 1999). The greater below ground resources we recorded for
339 *P. sinuosa*, which is consistently evident when compared with *A. antarctica* (Cambridge,
340 1999), could have aided rapid recovery from defoliation as resources can be translocated
341 from roots and rhizomes to help leaf growth and recovery (Cebrian, et al., 1998; Strauss and
342 Agrawal, 1999). Regardless of the mechanism to recovery, however, it appears evident that
343 *P. sinuosa* has a greater tolerance to urchin grazing than *A. antarctica*.

344

345 It is important to note that simulated grazing is rarely an exact substitute for actual grazing
346 (Eklof, et al., 2009; Ibarra-Obando, et al., 2004; Strauss and Agrawal, 1999), suggesting the
347 recovery we recorded should be interpreted cautiously. It was necessary to use new
348 experimental plots for the recovery experiment as we destructively sampled seagrass to
349 quantify urchin effects during the grazing experiment. Therefore, any deterioration of below
350 ground resources that occurred during the grazing experiment was not replicated in the
351 simulated grazing experiment. While *A. antarctica* showed a greater loss of below ground
352 biomass than *P. sinuosa* during the grazing experiment, albeit non-significantly, replicating
353 this effect in the simulated grazing experiment would only have exacerbated the species
354 differences we observed, as fewer resources would have been available to *A. antarctica* for
355 recovery. Simulated grazing can also underestimate potential benefits of herbivory. For
356 example, urchin faeces can increase seagrass productivity by enhancing the rate of nutrient
357 cycling, effectively fertilising any remaining plants (Koike, et al., 1987; Vonk, et al., 2008).
358 The absence of this effect could have potentially underestimated seagrass recovery during the

359 simulated grazing experiment, although this effect should be consistent across both species
360 and is therefore unlikely to alter the inter-specific differences seen here. In addition, the rapid
361 re-growth of *P. sinuosa* suggests that plants at the study site were unlikely to be nutrient-
362 limited.

363

364 We considered it important to quantify the rate of biomass decline in both species despite the
365 possibility that sub-sampling halfway through the grazing experiment may have impacted
366 adjacent seagrasses due to the clonal nature of ramets. Importantly, *Amphibolis* and
367 *Posidonia* rhizomes form a complex matrix where many individual plants overlap, rather than
368 being a single clonal individual (Bryars, et al., 2011), making the removal of small amounts
369 of above ground biomass unlikely to destroy entire plants. While sub-sampling itself does not
370 appear to send seagrass meadows into significant decline (Bryars, et al., 2011; Valentine and
371 Heck, 1991; Vonk, et al., 2008), it is plausible that biomass removal could have had a larger
372 effect where seagrasses were already under stress from urchin grazing, possibly exacerbating
373 grazing impacts. Yet we anticipate that our results are not an overestimation of grazing
374 impacts for two reasons. First, only a small amount of above-ground biomass was removed
375 (6% of plots) and belowground biomass was undamaged so there is no reason to assume that
376 translocation of belowground resources was unduly interrupted. Second, while we removed
377 6% of the areal cover in all treatments, seagrass cover and biomass were already reduced in
378 grazing treatments and by sampling in a standard area more biomass was actually removed in
379 the control treatments. Nonetheless, non-destructive estimates of grazing impact, such as
380 shoot density and canopy height could be used in future studies, though this can be inherently
381 difficult due to species-specific morphology and seasonal changes in biomass composition.

382

383

384 **5. Conclusion**

385 The current rate of decline in seagrass meadows places them among the most threatened
386 ecosystems worldwide (Waycott, et al., 2009). While some species of seagrass have evolved
387 traits to tolerate high grazing intensity, such as rapid regeneration and basal leaf meristems
388 (Valentine and Duffy, 2006), others such as *A. antarctica* appear vulnerable to long lasting
389 impacts from sudden increases in grazing pressure due to a reduced capacity for recovery
390 post-disturbance. Habitats created by such species appear particularly vulnerable to long-
391 term, top-down induced phase-shifts. The cascading ecosystem effects of wholesale seagrass
392 habitat loss threaten coastal productivity and biodiversity, through a reduction in sediment
393 stabilisation, nutrient cycling and support of trophic food webs (Duarte, 2002; Orth, et al.,
394 2006). Understanding the causes of sudden urchin population increases, and under what
395 conditions seagrasses may be more or less susceptible to overgrazing, may help us predict
396 and prepare for the effects of seagrass loss.

397

398 **Acknowledgements**

399 Thanks to Eric Drew, Samuel Owen and Kingsley Griffin for the many hours of diving
400 assistance. The Field Naturalists Society of South Australia provided two Lirabenda
401 Endowment Fund Grants to O.W.B. that helped fund the research. S.D.C. and B.D.R. were
402 funded by an ARC grant and S.D.C. an ARC Future Fellowship.

403

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505

506 **Table 1.** Repeated measures analysis of variance comparing the effect of urchin grazing
 507 (control, procedural control v. grazing), seagrass species (*Amphibolis antarctica* v. *Posidonia*
 508 *sinuosa*) and time (halfway v. final) on the above ground leaf biomass of seagrasses.
 509 Significant effects indicated in bold where $p < 0.05$.

Source	df	MS	F	p
Leaf Biomass				
Between Subjects				
Species	1	0.064	0.528	0.475
Grazing	2	4.779	39.536	0.000
Species × Grazing	2	0.087	0.716	0.499
Residual	24	0.121		
Within subjects				
Time	1	0.423	4.626	0.042
Time × Species	1	0.253	2.772	0.109
Time × Grazing	2	0.157	1.714	0.202
Time × Species × Grazing	2	0.181	1.986	0.159
Residual	24	0.091		

510 **Pairwise comparison: Leaf Biomass**

511 **Urchin grazing:** control = procedural control > grazing

512

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

Source	df	MS	F	p
Grazing Damage				
Grazing	1	1.557	194.610	0.001
Species	1	0.003	0.388	0.526
Grazing × Species	1	0.027	3.380	0.092
Residual	16	0.008		
Leaf Meristem Survival				
Grazing	1	0.543	61.834	0.001
Species	1	0.016	1.794	0.213
Grazing × Species	1	0.463	52.778	0.001
Residual	16	0.009		
Below Ground Biomass				
Grazing	1	0.072	0.715	0.407
Species	1	2.623	26.086	0.001
Grazing × Species	1	0.135	1.344	0.296
Residual	16	0.101		

517 **Pairwise comparisons: Leaf Meristem Survival**

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

519 **Seagrass species:** control, *A. antarctica* > *P. sinuosa*; grazing, *A. antarctica* < *P. sinuosa*.

520

521 **Table 3.** Final seagrass dry weight from Experiment 1 (Grazing Experiment) and Experiment
 522 2 (Simulated Grazing Experiment) used to calculate biomass recovery during the Simulated
 523 Grazing Experiment. Recovery was calculated by subtracting leaf biomass at the conclusion
 524 of the Grazing Experiment from leaf biomass at the conclusion of the Simulated Grazing
 525 Experiment, given the the biomass removed in the partial removal treatment of the Simulated
 526 Grazing Experiment mimicked that removed by urchins.

Treatment	Urchin Grazing (Exp 1) (gdw m⁻²)	Simulated Grazing (Exp 2) (gdw m⁻²)	Calculated Recovery (Exp 2) (gdw m⁻²)
<i>Amphibolis antarctica</i>			
Control	219.84 ± 28.11	364.32 ± 23.99	144.48
Urchin Grazing/Partial Removal	55.64 ± 10.12	90.75 ± 6.64	35.11
Complete Removal	n/a	26.24 ± 3.17	26.24
<i>Posidonia sinuosa</i>			
Control	220.58 ± 21.47	363.78 ± 40.79	143.20
Urchin Grazing/Partial Removal	65.76 ± 5.72	226.62 ± 25.92	160.86
Complete Removal	n/a	204.96 ± 16.28	204.96

527

528

529 **Table 4.** Analysis of variance comparing the effect of simulated grazing (control v. partial
 530 removal and control v. complete removal) and species (*Amphibolis antarctica* v. *Posidonia*
 531 *sinuosa*) on above ground leaf biomass following 109 days of recovery. Significant effects
 532 indicated in bold where $p < 0.05$.

Source	df	MS	F	p
Leaf Biomass (Partial Removal)				
Grazing	1	210860	57.08	0.001
Species	1	22892	6.20	0.032
Grazing × Species	1	23262	6.30	0.024
Residual	16	3694		
Leaf Biomass (Complete Removal)				
Grazing	1	308630	98.20	0.001
Species	1	39683	12.63	0.006
Grazing × Species	1	40169	12.78	0.003
Residual	16	3143		

533 **Pairwise comparisons: Partial Removal**

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

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

536 **Pairwise comparisons: Complete Removal**

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

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

539 **Figure Captions**

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

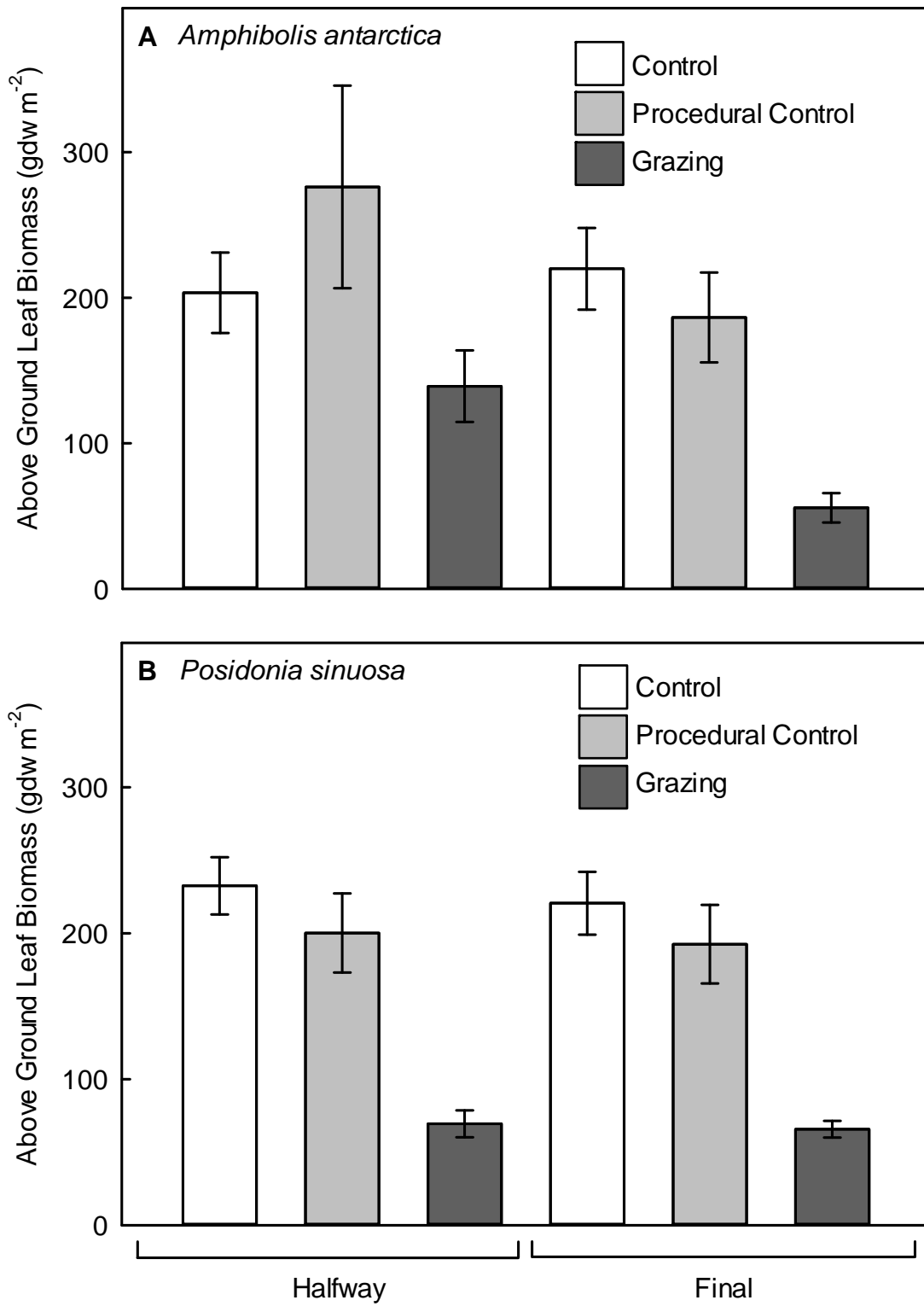
544

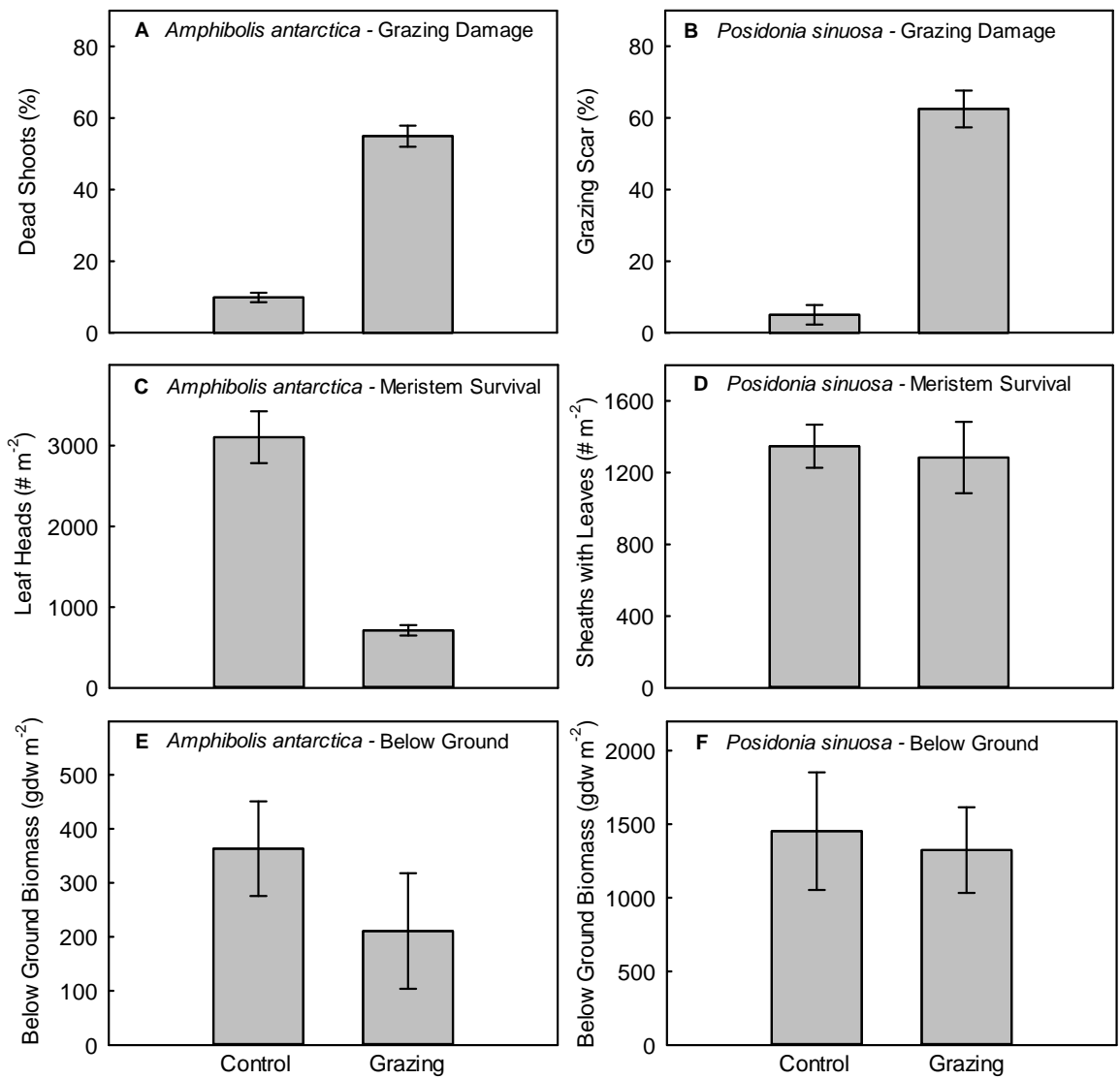
545 Figure 2. The effect of sea urchins *Amblypneustes pallidus* (control v. grazing), on grazing
546 damage (A. *Amphibolis antarctica*, percentage dead shoots; B *Posidonia sinuosa*, percentage
547 of leaves with grazing scar), leaf meristem survival (C. *Amphibolis antarctica*, # leaf heads;
548 D.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

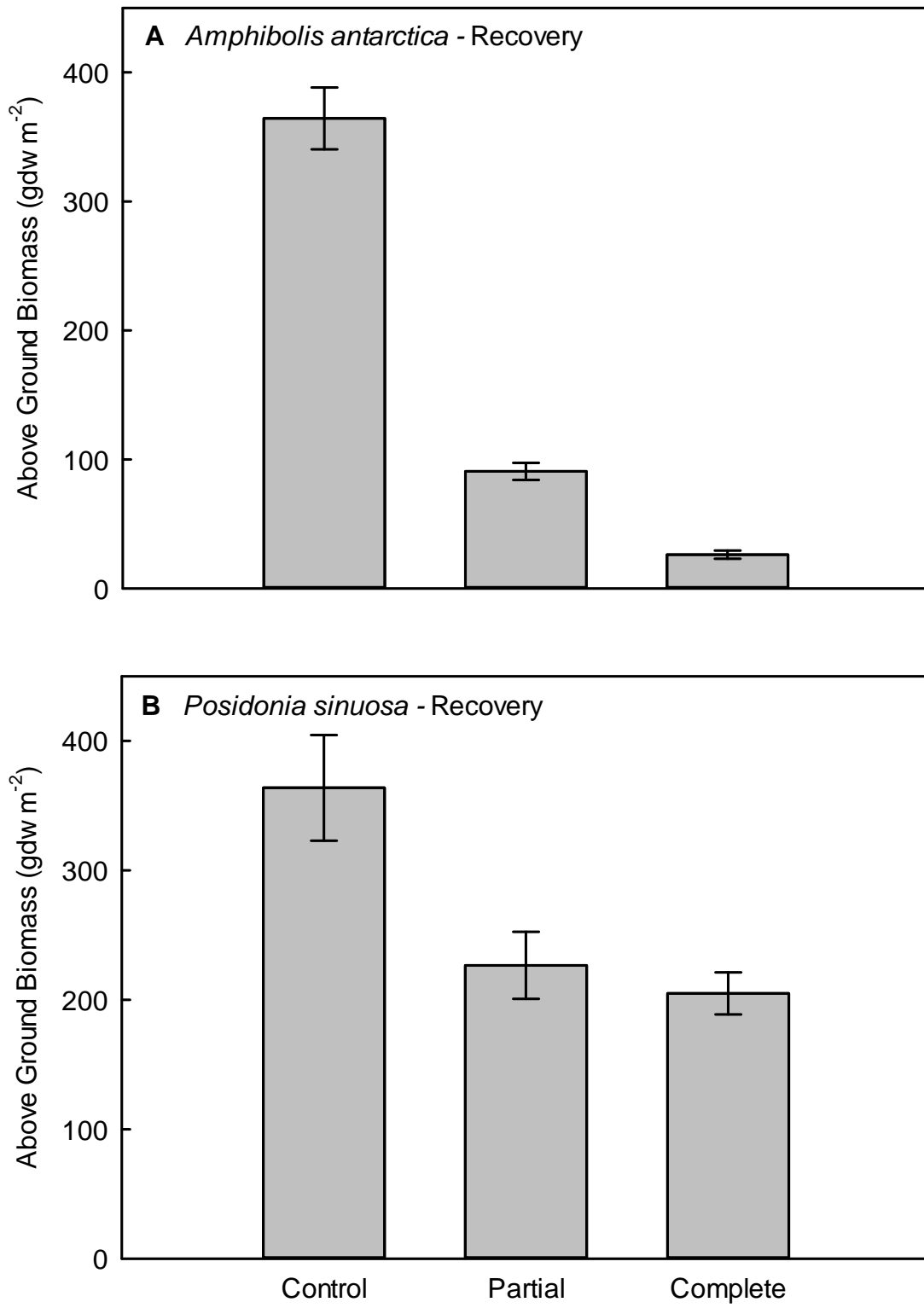
551

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

555







561

562