Effects of multiple disturbances in seagrass meadows: shading decreases resilience to grazing

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Abstract. Ecosystem shifts are often associated with multiple disturbances, but limited knowledge on the mechanisms involved hampers management. This study investigated how short-term shading affected the resilience of the seagrass *Halophila ovalis* to grazing by black swans (*Cygnus atratus*) – a historically dominant grazer currently recovering from hunting and habitat loss – in south-western Australian estuaries, using field surveys and a grazing-shading experiment. Black swans were heterogeneously distributed and, in a high-density site, consumed 23% of seagrass production. Seagrasses recovered rapidly from a single disturbance (i.e. short-term shading or grazing), even though shading alone halved carbohydrate content. When seagrasses were exposed to both disturbances, recovery depended on the type of grazing; where grazing was confined to leaves, leaf densities recovered within 3 weeks, but where grazing was on both leaves and rhizomes there was no recovery. Shading increased the frequency of apex initiation, as did rhizome grazing, but only if the plants had not been shaded. This indicates that shading alters the flow of energy needed to produce apices and leaves following disturbance on rhizomes. Based on the historical swan densities and continuing recurring phytoplankton blooms, management actions reintroducing swans without controlling algal blooms could have an on impact seagrass resilience and associated organisms.

Additional keywords: black swan, carbohydrates, *Cygnus atratus*, *Halophila ovalis*, recovery, Western Australia.

Introduction

Ecosystem degradation and shifts to undesirable and persistent regimes have been observed in many ecosystems (Scheffer *et al.* 2001). The major cause is often a gradual loss of resilience in habitat-forming species, typically caused by additive or synergistic effects of multiple disturbances – anthropogenic as well as anthropogenic superimposed on natural (Paine *et al.* 1998). Yet, there are few studies that have experimentally tested the mechanisms behind the interacting effects of multiple disturbances, which hampers management efforts.

Among the marine ecosystems that have suffered significant losses are seagrass ecosystems. Seagrasses form diverse and productive coastal ecosystems (Green and Short 2003) supporting a range of ecosystem services (Costanza *et al.* 1997). They are, however, in global decline due to anthropogenic disturbances (Orth *et al.* 2006) that cause shifts to undesirable regimes like recurring macro- and micro-algal blooms (Valentine and Duffy 2006). Even though multiple disturbances are considered a key factor behind habitat loss and regime shifts, especially in urbanised areas (e.g. Lotze *et al.* 2006), few studies experimentally address multiple disturbances on seagrasses (see e.g. Bintz *et al.* 2003; Ibarra-Obando *et al.* 2004; Macinnis-Ng and Ralph 2004). This limits the understanding of seagrass

disturbance dynamics and can lead to potentially interacting disturbances being managed separately.

Light limitation is a major driver behind seagrass decline, primarily caused by eutrophication-induced blooms of epiphytic and/or free-floating algae and sedimentation from land run-off (Orth *et al.* 2006). Seagrass survival during shading is dependent on their ability to access stored energy reserves in the form of carbohydrates, in large ‘climax’ species (e.g. Collier *et al.* 2008) as well as small ‘pioneer’ species (e.g. Longstaff *et al.* 1999). However, climax species have greater carbohydrate storages (due to thicker rhizomes) and can therefore withstand longer shading (Ralph *et al.* 2007). In contrast, herbivory is a natural and intrinsic disturbance on seagrasses (for a review, see Valentine and Duffy 2006). While grazing generally reduces biomass (Hughes *et al.* 2004), there is generally a parabolic response in seagrass growth to grazing intensity (Valentine *et al.* 1997; Cebrián *et al.* 1998; Ibarra-Obando *et al.* 2004); moderate to intermediate grazing can increase production through use of stored carbohydrates. The nature of the response curve differs extensively between populations, probably because carbohydrate content is affected by environmental conditions (Cebrián *et al.* 1998) including grazing history (Eklöf *et al.* 2008a). The response also varies between species as larger ‘climax’ species of seagrass have

greater carbohydrate reserves to utilise compared with smaller 'pioneer' species. The pivotal role of carbohydrates – in 'climax' as well as 'pioneer' species (Cebrián *et al.* 1998) – indicates that disturbances co-occurring with grazing (e.g. light reduction) could affect the response of seagrasses to grazing, and emphasises the need to experimentally evaluate the role of carbohydrates in grazing responses (van Tussenbroek *et al.* 2006).

Halophila ovalis R. Brown is a common seagrass species in tropical, subtropical and temperate regions. This fast-growing 'pioneer' species tolerates shading in the short term by accessing and using stored carbohydrates, but is affected in the long term, evident as leaf loss (Longstaff and Dennison 1999; Longstaff *et al.* 1999). Nevertheless, *H. ovalis* has been found to persist in relatively stressful environments with frequent light limitation (Hillman *et al.* 1995 and references therein), most likely because of its rapid recovery capability. Because of its relatively low C : N ratio, *H. ovalis* is commonly fed on by various grazers such as fish (Mariani and Alcoverro 1999), dugongs (Nakaoka and Aioi 1999) and turtles (Kuiper-Linley *et al.* 2007), and appears – at least under natural conditions – to be highly 'grazing resistant' (Kuiper-Linley *et al.* 2007).

The black swan (*Cygnus atratus*) is a herbivorous water fowl native to Australia (del Hoyo *et al.* 1992). The swans graze *H. ovalis* and other estuarine seagrasses in two distinct modes: (1) 'lawn mowing', where only leaves are ripped off, and (2) 'rhizome grazing', where primarily rhizomes but also leaves are dug up from circular pits (Brearley 2005). Because rhizomes are the main carbohydrate storage organs in most seagrasses, these two modes of grazing could affect seagrass responses in quite different ways.

In Western Australia, black swans were historically one of the most common estuarine waterfowls, but excessive hunting and habitat loss (partly caused by eutrophication) decimated populations from the 19th century onwards (Brearley 2005 and references therein). Because of its iconic status in Western Australia, directed management action (e.g. reduced nutrient inputs and habitat restoration) is now resulting in a gradual recovery of swan populations (Brearley 2005). At the same time, light limitation events caused by a combination of phytoplankton blooms, accumulation of floating plants and resuspension of sediments are still common throughout the Swan River estuarine system (Hillman *et al.* 1995; Brearley 2005). Consequently, there is a risk that management actions reintroduce a frequent natural disturbance (swan grazing) that, when superimposed on an anthropogenic disturbance (frequent light reduction events), causes synergistic and unforeseen effects on seagrasses and management outcomes.

The aim of the present study was to investigate how short-term shading affects the resilience of *H. ovalis* to grazing. We hypothesised that (1) black swan distribution is partly determined by distribution of seagrasses, (2) swans are significant grazers on *H. ovalis* and (3) short-term shading, by depleting carbohydrate reserves, decreases the resilience of *H. ovalis* to grazing.

Materials and methods

Study area

The study was conducted in the lower 'Swan-Canning' estuary (31°00'S, 115°49'E; Fig. 1), flowing through metropolitan

Perth in south-western Australia, hereafter referred to as the Lower Swan River. *Halophila ovalis* is the dominant seagrass in the lower estuary (Brearley 2005), covering ~500 ha (20% of the main basin) from 0- to 2.5-m depth (Hillman *et al.* 1995), and co-occurs with less common *Ruppia megacarpa* R. Mason and *Zostera muelleri* Irmisch ex Asch. (previously *Zostera mucronata*, see Jacobs *et al.* 2006). Phytoplankton and macroalgal blooms are common in the entire area due to cultural eutrophication and regularly shade seagrasses over periods from days to weeks (Brearley 2005).

Field survey: swan distribution and habitat characteristics

Swan abundance was investigated by repeated visual surveys at 45 sites (December 2006 to January 2007, $n = 12$), stratified by time of day (morning, midday, evening). On each occasion, the number of swans feeding was recorded, and the area (km²) and swan density (km⁻²) were calculated. A benthic habitat characterisation (seagrass cover, biomass, species composition and nutrient content (C : N ratio)) was conducted in the summer of 2007 from a subset of sites ($n = 26$). Seagrass cover and species composition (*Halophila ovalis*, *Ruppia megacarpa* or *Zostera muelleri*) was estimated by the transect point method using four, randomly located 20-m transects with measures every 1 m, within a depth of <1.0 m, the maximum swan grazing depth (Brearley 2005). Seagrass biomass (above and below ground) was estimated from six cores ($\varnothing = 9.5$ cm, depth = 10 cm) randomly located on the four transects. Dried, ground and homogenised tissue was analysed for carbon and nitrogen (%C or N DW) in a continuous flow isotope ratio mass spectrometer (20–20 IRMS, Europe, Crewe, UK).

To assess whether there were any relationships between habitat type and swan grazing pressure, standard Pearson Product Moment correlation coefficients were calculated between black swan feeding density and seagrass biomass, species cover and C : N ratio, with $\alpha = 0.05$, $n = 26$ ($n = 20$ for C : N). No transformations were performed.

Experiment 1: black swan grazing pressure

A 14-day experiment to investigate rates of swan grazing was conducted at Point Walter in April 2007 (Fig. 1). Fixed 2 × 2-m plots were marked in a shallow (<1 m) area where swans were actively grazing, 10 in monospecific *Halophila ovalis* areas ('*Halophila*') and seven in mixed *H. ovalis* and *Ruppia megacarpa* areas ('Mixed'). Initial grazing scars were mapped and measured (to the nearest 0.5% of the plot). Seagrass production was estimated by tagging five apices of *H. ovalis* and *R. megacarpa* (using plastic-coated garden wire) within 1 m of all '*Halophila*' and 'Mixed' plots (Short and Coles 2001). Attempts to assess *R. megacarpa* leaf growth using the leaf puncture method failed because of difficulty in relocating needle marks on the narrow (<0.5 mm) leaves, so we report only *H. ovalis* growth rates. After 14 days, all new grazing scars were measured, and the seagrass biomass sampled within and outside grazing scars in each plot, using a corer ($\varnothing = 10.5$ cm, depth = 10 cm). All marked apices and shoots were collected, inspected for signs of grazing, brought to the laboratory and cleaned in salt water. The tissue produced during the experiment was excised, dried (24 h at 60°C) and weighed.

Fig. 1. Map of the Lower Swan River Estuary (WA, Australia) showing the average black swan density at the 45 survey sites, and the location of the experimental sites Point Walter and Dalkeith.

Above- and below-ground production (g DW m^{-2}) was estimated from the weight of new tissue (per apex, including above- and below-ground components) and the apex density. The total biomass removed by grazing (g DW m^{-2}) was estimated by multiplying the total area (m^2) grazed by the difference in biomass (m^{-2}) between ungrazed areas and within grazing scars. Finally, the proportion of daily production (% of growth day^{-1}) removed by grazing was calculated by dividing the biomass removed (g DW m^{-2}) by the total production ($\text{g DW m}^{-2} \text{ day}^{-1}$). Since leaf production of *R. megacarpa* was not included, total production is underestimated and, therefore, the estimate of grazing rate in the 'Mixed' plots is an over-estimation to be treated with caution.

Soluble sugar and starch contents (% DW) of dried and ground *R. megacarpa* (from the 'Mixed' plots) and *H. ovalis* (from 'Halophila' plots; $n=5$ per treatment) were measured using the anthrone colourimetric method with an amylase pre-digest for starch measurements (Yemm and Willis 1954). Carbon and nitrogen contents (%) were determined as described above.

Differences in all variables between the two treatments ('Halophila' and 'Mixed') were investigated using Student's *t*-tests (with $\alpha=0.05$), after checking for normality and homoscedasticity (Quinn and Keough 2002).

Experiment 2: effects of shading and simulated swan grazing

The experiment was conducted in a shallow (0.8–1 m) *Halophila ovalis* meadow at Dalkeith in April 2007 (Fig. 1). We imposed two levels of shading (Unshaded control (U) and Shaded (S)), and three levels of simulated grazing: Controls (C) with no grazing; Leaf grazing (L) simulating the 'lawn mowing' grazing by removing all leaves; and Rhizome grazing (R) simulating grazing, which removes some leaves and rhizomes (Fig. 2). Simulated rather than actual swan grazing was used to: (1) be able to control where and when grazing occurred, and (2) impose the two different grazing treatments (because they have differing impact on rhizomes, which are the major carbohydrate storage organ). Simulated grazing is commonly used to test grazing effects on seagrasses (see e.g. Cebrián *et al.* 1998; Eklöf *et al.* 2008a), but it cannot be ruled out that effects of actual grazing differ from those reported here (see Ibarra-Obando *et al.* 2004 and references therein). Treatments were established in an orthogonal and replicated design, resulting in six treatments (UC, UL, UR, SC, SL and SR).

Experimental plots (1 × 1-m, placed 2 m apart) were established in five rows (perpendicular to the beach) with six plots per row ($n=30$), and each treatment was replicated once per row. Shading was imposed by erecting 1 × 1-m shade screens

Fig. 2. Schematic of experiment testing interactive effects of shading and simulated swan grazing on *Halophila ovalis*. Following 4 days of shading (U, unshaded v. S, shaded) of 1 x 1-m plots, three levels of simulated grazing (C, control; L, leaf grazing; R, rhizome removal) were imposed onto 20 x 20-cm plots in the centre. This resulted in six treatment combinations (UC, UL, UR, SC, SL, SR), testing effects of shading, grazing and their interaction. Symbol courtesy of IAN Library, Maryland University, USA.

(cloth with 80% light reduction) 0.3 m above the bottom in three randomly chosen plots per row (15 plots in total). The remaining 15 plots acted as controls for shading. After 3 days (corresponding to a typical shading event in the area, Hillman *et al.* 1995), three mature leaf pairs (produced before the shading was imposed) were collected from within three plots of each treatment (Shaded and Unshaded). In the laboratory, the soluble sugar content (per leaf) was analysed using the anthrone method (Yemm and Willis 1954), showing that sugar content was 50% lower in shaded than unshaded leaves (*t*-test; $P < 0.001$, $n = 3$). The following day (4 days of shading), the shade screens were removed and the grazing treatments imposed in the centre 20 x 20-cm area within each plot (marked with metal rods). This design ensured that >0.4-m shaded *Halophila ovalis* beds surrounded the plots in every direction, which should exclude carbohydrate translocation from unshaded ramets outside (see Marbà *et al.* 2006). The number of leaves within each area was counted (time = 0), revealing that shading had not affected leaf density (*t*-test: $P = 0.55$). Swan grazing was then simulated using the three treatments in 15 of the plots ($n = 5$ per treatment). In the 'lawn-mowing' treatments (UL and SL), all leaves within each 20 x 20-cm area were cut at the sediment level. In the 'rhizome grazing' treatment (UR and SR), four sediment cores ($\varnothing = 4$ cm, totalling 12.5% of the surface area; depth 10 cm) were taken randomly within each plot and the rhizomes in the 20 x 20-cm area were severed in the shape of a cross (5 x 5 cm) using a knife, to simulate mechanical disturbance from the grazing. Leaves

removed from the UL and SL treatment plots were collected, frozen and brought to the laboratory for analyses. Finally, the control plots (UC and SC) were left undisturbed throughout the experiment.

Leaf density was counted (within each 20 x 20-cm plot), after 7, 14 and 21 days. At termination (21 days), all seagrass material was retrieved, sieved, frozen and taken to the laboratory. One UL treatment plot was excluded because of physical disturbance occurring between 14 and 21 days ($n = 4$). All seagrass material was initially cleaned (using salt water). Leaf width and length (nearest 0.5 mm) were measured on 10 leaves collected from (1) the UL and SL treatments after the 4 days of shading and (2) from each treatment 21 days after grazing (only using leaves produced during the experiment). Leaf surface area was calculated in the shape of an ellipse using the formula $A \times B \times \pi$ (where $A = \text{leaf width} \times 0.5$ and $B = \text{leaf length} \times 0.5$). This showed that 4 days of shading alone had no effect on leaf size (*t*-test: $P = 0.14$).

The number of nodes, initiated apices (no internodes produced) and newly produced branches (containing at least one and a maximum of three internodes) were counted from the rhizome, and the internode length was measured (average from 10 newly produced branches, 0.5-mm accuracy). Branching frequency and frequency of apex initiation were calculated as the number of new branches and new apices produced per node, respectively. After this, the biomass was estimated (nearest 0.001 g) following drying (60°C to constant biomass). Carbohydrate (sugar and

Table 1. Meadow characteristics and grazing pressure of black swans

Measured in fixed plots ('*Halophila*' and 'Mixed' (*Halophila* and *Ruppia*)) at Point Walter. AG, above-ground (leaves); BG, below-ground (roots and rhizomes); Total, both in combination ($n = 10$ for '*Halophila*', 7 for 'Mixed', means ± 1 s.e.)

Treatment	' <i>Halophila</i> '			'Mixed' (<i>Halophila</i> + <i>Ruppia</i>)		
	Total	AG	BG	Total	AG	BG
Meadow characteristics						
Seagrass bottom cover (%)	92.3 $\pm$ 1.5	—	—	95.4 $\pm$ 1.6	—	—
Biomass <i>Halophila ovalis</i> (g DW m ⁻²)	61.4 $\pm$ 4.5	12.7 $\pm$ 0.6	48.7 $\pm$ 4.3	41.1 $\pm$ 2.6	9.0 $\pm$ 1.1	32.1 $\pm$ 2.5
Biomass <i>Ruppia megacarpa</i> (g DW m ⁻²)	—	—	—	9.8 $\pm$ 2.3	3.4 $\pm$ 0.95	6.4 $\pm$ 1.6
Total seagrass production (g DW m ⁻² day ⁻¹)	3.1 $\pm$ 0.3	1.5 $\pm$ 0.2	1.6 $\pm$ 0.2	0.11 $\pm$ 0.03	0.08 $\pm$ 0.03	0.03 $\pm$ 0.01
Total carbohydrate content (%)	—	6.8 $\pm$ 3.4 ^A	12.6 $\pm$ 6.3 ^A	—	2.6 $\pm$ 1.3 ^B	2.5 $\pm$ 1.3 ^B
Total carbohydrates (g DW m ⁻²)	2.6 $\pm$ 0.2	1.5 $\pm$ 0.1	1.1 $\pm$ 0.1	2.23 $\pm$ 0.15	1.29 $\pm$ 0.15	0.93 $\pm$ 0.01
Nitrogen content (%)	—	1.46 $\pm$ 0.06 ^A	0.44 $\pm$ 0.02 ^A	—	1.25 $\pm$ 0.05 ^B	0.79 $\pm$ 0.06 ^B
Total nitrogen (g DW m ⁻²)	0.37 $\pm$ 0.02	0.17 $\pm$ 0.01	0.20 $\pm$ 0.02	0.35 $\pm$ 0.02	0.16 $\pm$ 0.02	0.183 $\pm$ 0.005
C : N ratio	—	18.1 $\pm$ 0.6 ^A	46.3 $\pm$ 4.7 ^A	—	20.5 $\pm$ 0.7 ^B	23.3 $\pm$ 1.0 ^B
Grazing pressure						
Surface area grazed (%)	6.4 $\pm$ 1.7	—	—	4.6 $\pm$ 0.9	—	—
<i>Halophila ovalis</i> biomass grazed (%)	4.4 $\pm$ 1.1	5.9 $\pm$ 1.6	4.0 $\pm$ 1.02	2.0 $\pm$ 0.5	3.5 $\pm$ 0.8	1.6 $\pm$ 0.6
<i>Ruppia megacarpa</i> biomass removed (%)	—	—	—	3.5 $\pm$ 0.6	3.6 $\pm$ 0.5	3.3 $\pm$ 0.8
Production removed (% of daily production)	23.2 $\pm$ 6.1	12.9 $\pm$ 3.0	34.6 $\pm$ 9.8	—	—	—
Biomass <i>Halophila ovalis</i> removed (g DW m ⁻² day ⁻¹)	0.17 $\pm$ 0.04	0.05 $\pm$ 0.01	0.12 $\pm$ 0.03	0.05 $\pm$ 0.01	0.019 $\pm$ 0.003	0.03 $\pm$ 0.01
Biomass <i>Ruppia megacarpa</i> removed (g DW m ⁻² day ⁻¹)	—	—	—	0.025 $\pm$ 0.007	0.008 $\pm$ 0.001	0.017 $\pm$ 0.005

^A*Halophila ovalis* only.

^B*Ruppia megacarpa* only.

starch), nitrogen and carbon content of dried, ground leaves and rhizomes was estimated from samples collected in all plots after 3 weeks of recovery, using the methods described above.

Effects of shading on leaf density recovery from the two different forms of simulated grazing were investigated using 'repeated-measures ANOVA' with two factors: Treatment (six levels) and Time (three levels: 7, 14 and 21 days). Because of large within-treatment variation in shoot density, we used '% of shoot density at day 0' (i.e. just before applying the grazing treatments) as the response variable. To test whether the rate of recovery from Day 7 to 21 differed between grazing treatments and whether shading affected the recovery, linear regression analysis was first conducted on each separate treatment ($n = 5$ plots $\times$ 3 time periods = 15), and pairwise tests of slopes and intercepts were then conducted using Student's *t*-tests (Wuensch *et al.* 2002).

Effects of simulated grazing and shading on variables sampled at the end of the experiment were tested with two-way orthogonal ANOVAs with Grazing (fixed, three levels) and Shading (fixed, two levels) as factors. ANOVA assumptions were first tested with Cochran's C test and Levene's test, respectively. When assumptions were not met, the data was transformed or the violation was reported (because there is no non-parametric analogue to a two-way ANOVA with replication). Percentage change in above-ground biomass and leaf N content were heteroscedastic and could not be appropriately transformed. Following significant ANOVA effects, post hoc comparisons were conducted using the Fisher's post hoc test. The significance level was set at $\alpha = 0.05$, and all analyses were run using Statistica (Statsoft, Inc., Tulsa, OK, USA) ver. 7.0 for Windows. Reported measures of spread are standard errors.

Results

Field survey: swan distribution and habitat characteristics

On average, 78 black swans were present in the Lower Swan River Estuary on each sampling occasion, of which 69% were feeding. Swan distribution was not uniform; densities > 90 km⁻² were commonly observed at three sites, while no swans were ever observed at other sites (Fig. 1). Black swans were observed feeding in both single-species (*Halophila ovalis*) and mixed-species meadows (with *H. ovalis*, *Ruppia megacarpa* and *Zostera muelleri*). Across sites, seagrass cover ranged from 35 to 100% (average, $80 \pm 4\%$), and biomass ranged from 40 to 440 g DW m⁻² (average, 180 ± 24 g DW m⁻²). *H. ovalis* was the most common species (average cover, $80 \pm 5\%$), followed by *R. megacarpa* ($12 \pm 5\%$) and *Z. muelleri* ($5 \pm 2\%$). C : N ratios of the seagrass ranged from 17.0 to 34.6 (average, 24.4 ± 0.8). There was a weak although significant positive correlation ($r^2 = 0.23$, $P < 0.05$) between the density of feeding black swans and *Ruppia* cover, but no significant correlations with cover of the other two species, all three species pooled, or seagrass C : N ratio ($r^2 = 0.02$ – 0.11 , $P > 0.05$, respectively).

Experiment 1: black swan grazing pressure

Over the 14-day period, 10–15 adult black swans – corresponding to 1 individual ha⁻¹ – were observed at Point Walter. Swans grazed $6.5 \pm 1.7\%$ ('*Halophila*') and $4.5 \pm 0.9\%$ ('Mixed') of the total surface area of meadow over the 14 days (Table 1), equating to $4.5 \pm 1.1\%$ ('*Halophila*') and $2.0 \pm 0.5\%$ ('Mixed') of the total standing crop (Table 2). There was no difference in cover of seagrass removed in the two meadow types (*t*-test; $P = 0.40$). Assuming a constant biomass in the

Table 2. Results of (1) a repeated measures ANOVA on leaf density after 3 weeks, and two-way ANOVAs on (2) frequency of apex initiation, (3) branching frequency, (4) internode length, (5) leaf blade area, (6–11) carbohydrate content (sugar, starch and total carbohydrates (CH) in leaves and rhizomes) and (12–15) leaf and rhizome %N and C:N ratio, after 3 weeks of recovery from shading and simulated swan grazing
P-values in **bold** indicate significant effects ($\alpha = 0.05$)

Source	d.f.	<i>F</i>	<i>P</i>	Source	d.f.	<i>F</i>	<i>P</i>
1. Leaf density				9. Rhizome sugar (%)			
Treatment	5	15.3	<0.001	Grazing (G)	2	0.383	0.68
Time	2	63.7	<0.001	Shading (S)	1	0.925	0.34
Treatment $\times$ Time	10	7.95	<0.001	G $\times$ S	2	1.399	0.26
2. Apex initiation				Error	23		
Grazing (G)	2	1.4175	0.26	10. Rhizome starch (%)			
Shading (S)	1	0.0233	0.88	Grazing (G)	2	0.365	0.69
G $\times$ S	2	4.6473	0.02	Shading (S)	1	0.719	0.40
Error	23			G $\times$ S	2	0.141	0.86
3. Branching frequency				Error	23		
Grazing (G)	2	4.3826	0.02	11. Rhizome total CH (%)			
Shading (S)	1	0.2839	0.59	Grazing (G)	2	0.257	0.77
G $\times$ S	2	0.1315	0.88	Shading (S)	1	0.639	0.43
Error	23			G $\times$ S	2	1.302	0.29
4. Internode length				Error	23		
Grazing (G)	2	0.1627	0.85	12. Leaf N (%)			
Shading (S)	1	3.2397	0.09	Grazing (G)	2	0.76	0.48
G $\times$ S	2	0.8421	0.44	Shading (S)	1	1.08	0.31
Error	23			G $\times$ S	2	0.91	0.42
5. Leaf area				Error	23	0.76	
Grazing (G)	2	1.160	0.33	13. Rhizome N (%)			
Shading (S)	1	4.955	0.04	Grazing (G)	2	2.7	0.09
G $\times$ S	2	0.818	0.45	Shading (S)	1	0.07	0.79
Error	23			G $\times$ S	2	1.19	0.32
6. Leaf sugar (%)				Error	23	2.7	
Grazing (G)	2	1.675	0.21	14. Leaf C:N			
Shading (S)	1	6.597	0.02	Grazing (G)	2	1.0	0.38
G $\times$ S	2	0.425	0.66	Shading (S)	1	1.78	0.19
Error	23			G $\times$ S	2	1.26	0.30
7. Leaf starch (%)				Error	23	1.0	
Grazing (G)	2	1.208	0.32	15. Rhizome C:N			
Shading (S)	1	1.078	0.31	Grazing (G)	2	3.82	0.04
G $\times$ S	2	0.99	0.38	Shading (S)	1	0.04	0.84
Error	23			G $\times$ S	2	0.28	0.76
8. Leaf total CH (%)				Error	23	3.82	
Grazing (G)	2	1.56	0.23				
Shading (S)	1	5.88	0.02				
G $\times$ S	2	0.70	0.50				
Error	23						

meadow throughout the experiment, 0.2 ± 0.04 g DW m⁻² was consumed in the ‘*Halophila*’ plots, and in the mixed plots 0.05 ± 0.01 g DW m⁻² of *Halophila* and 0.02 ± 0.01 g DW m⁻² of *Ruppia*. In the ‘*Halophila*’ plots, where total production was 3.1 ± 0.9 g DW m⁻² day⁻¹, this grazing rate corresponded to 23% of daily seagrass production (Table 1).

Total leaf carbohydrate content was 2.6 times higher in *Halophila* than *Ruppia* (6.8 v. 2.6% DW, $P < 0.001$), due to both higher sugar and starch. There was a similar difference between species in below-ground carbohydrates, with five times more total carbohydrates (12.6 v. 2.5% DW, $P < 0.001$) in *Halophila* due to more sugars. The two meadow types had similar amounts of carbohydrates per unit area, 2.6 ± 0.2 g DW m⁻² (‘*Halophila*’) and 2.2 ± 0.1 g DW m⁻² (‘Mixed’). Nitrogen content of *Halophila* was $1.5 \pm 0.06\%$ DW (leaves) and

$0.4 \pm 0.02\%$ DW (below ground), and $1.2 \pm 0.05\%$ DW (leaves) and $0.8 \pm 0.06\%$ DW (below ground) for *Ruppia*.

Experiment 2: effects of shading and simulated swan grazing

Rates of leaf density recovery

The effect of Treatment on leaf density depended on Time (significant Treatment $\times$ Time interaction; Table 2, Fig. 3). In treatments with no simulated grazing (UC v. SC), shading had no effect on leaf density at any of the three times (Fisher’s test, $P = 0.45$ – 0.69); in both treatments there was a $\sim 17\%$ increase in leaf density between 14 and 21 days ($P < 0.01$ and 0.02 , respectively). In leaf grazing (L) treatments, where all leaves were removed, there were no differences between the shaded

Fig. 3. Leaf density (% of density at $t = 0$) of *Halophila ovalis* in six treatments (unshaded: UC (control), UL (leaf grazing), UR (rhizome removal); shaded: SC (control), SL (leaf grazing), SR (rhizome removal)) after 7, 15 and 21 days (means + s.e., $n = 5$).

and unshaded plots at any time; in unshaded plots, leaf density increased to 21% after 7 days, 43% after 14 days, and full recovery occurred after 21 days (UL v. UC, $P = 0.06$), with a nearly identical trend in the shaded, leaf grazing treatment (SL v. UC, $P = 0.66$ – 0.98). However, in the rhizome grazing treatment (R), shaded and unshaded plots showed contrasting trends. In the unshaded plots (UR), leaf density increased over time and was not detectably different from the controls at any of the three times (UR v. UC, $P = 0.40$ – 0.93), whereas in shaded plots (SR), leaf density after 21 days was 32% lower than in unshaded plots (SR v. UR, $P = 0.03$), and 27% lower than the unshaded controls (SR v. UC, $P < 0.01$).

In unshaded plots, there was a linear increase in leaf density from 7 to 21 days after grazing was imposed (leaf (UL) or rhizome (UR)), but not in the controls (UC) (Table 3). Similar responses were observed in the shaded plots with leaf grazing treatments (SL) and no grazing controls (SC), but not for those subjected to rhizome grazing (SR) (Table 3). In treatments where there was a significant linear increase in leaf density, the rate of recovery was greater in leaf grazing (slope k : UL = 2.95, SL = 3.34) than in rhizome grazing treatments (k : UR = 0.99), the 3-fold difference between UL and UR being statistically significant ($P < 0.01$).

Growth: frequency of initiated apices and branching

Shading increased the frequency of initiated apices from 0.15 (UC) to 0.24 (SC; Fisher's test, $P = 0.03$). Rhizome grazing also increased the frequency of initiated apices from 0.15 (UC) to 0.27 (UR; Fisher's test, $P < 0.01$) in unshaded plots, but not in shaded plots (SC v. SR, $P = 0.28$). Leaf grazing had no effect

Table 3. Regression analysis for change in *Halophila ovalis* leaf density over time measured over 3 weeks in six treatments using experimental plots

P -values in **bold** indicate significant effects ($\alpha = 0.05$)

Treatment	k (slope)	R^2	P
Unshaded control (UC)	0.76	0.18	0.11
Unshaded leaf grazing (UL)	2.95	0.67	0.001
Unshaded rhizome grazing (UR)	0.99	0.3	0.03
Shaded control (SC)	1.04	0.13	0.18
Shaded leaf grazing (SL)	3.34	0.77	0.001
Shaded rhizome grazing (SR)	0.12	0.01	0.67

on apex initiation. These differences are reflected in a significant grazing $\times$ shading interaction for the frequency of initiated apices (Table 2, Fig. 4a). There was a significant effect of grazing on branching frequency (Fig. 4b, Table 2), with frequencies in the rhizome grazing treatments (UR and SR) 1.7 times higher than in the no grazing (UC and SC) and 2.3 times higher than in the leaf grazing (UL and SL) treatments.

Morphology: internode length and leaf area

There was no significant effect of any treatment on rhizome internode length (Table 2, Fig. 4c). There was, however, a significant effect of shading on leaf area (Table 2), shaded plots having 13% larger leaves than control plots (UC v. SC, $P = 0.04$; Fig. 4d), but no effect of grazing (Table 2).

Fig. 4. (a) Frequency of initiated apices, (b) branching frequency, (c) internode length and (d) leaf area of *Halophila ovalis* exposed to two levels of shading (unshaded v. shaded) crossed with three levels of simulated grazing (C, control; L, leaf grazing; R, rhizome removal) (means + s.e., $n = 5$).

Carbohydrate, carbon and nitrogen content

Leaf total carbohydrate content was 12% lower in shaded than unshaded plots (Table 2, Fig. 5e; Fisher's $P = 0.02$), caused by 17% lower sugar content (Table 2, Fig. 5a; Fisher's $P = 0.02$). There were no effects of shading or grazing on rhizome carbohydrate content (Table 2, Fig. 5f).

Leaf N content, C:N ratio and rhizome N content were all unaffected by grazing and shading (Table 2, Fig. 5g). Rhizome C:N ratio was, however, affected by grazing (Table 2, Fig. 5g) such that the leaf grazing treatment was 13% higher than the

ungrazed controls (42.1 v. 37.3, $P = 0.011$), but not significantly higher than in the rhizome grazing treatment ($P = 0.07$).

Discussion

The results show that black swans can, at least periodically, be significant seagrass grazers in the Lower Swan River estuary, removing 23% of total seagrass production. We cannot comment on the annual consumption of productivity as our study was confined to a single period of the year, in April. Maximum rates of productivity typically occur in early to mid-summer, when they can be up to three times of that observed in April, but are negligible from late autumn to mid-spring (Hillman *et al.* 1995). The rate of consumption we observed would therefore represent a much lower fraction of summer productivity, but a much greater proportion of the production typical of about half of the year.

Responses to shading

The results clearly indicate an interactive effect of multiple disturbances. Plants subjected to either shading or grazing alone showed responses different from those subjected to both disturbances. However, the nature of the grazing disturbance was an important determinant of the response. The responses of *H. ovalis* to shading are consistent with the responses of other small, fast-growing and ephemeral seagrasses. Shading caused a 50% drop in leaf sugars after 3 days, without affecting leaf density or size. This confirms previous reports that *H. ovalis* can cope with short-term shading through physiological rather than morphological adaptation (Longstaff and Dennison 1999; Longstaff *et al.* 1999). Following the 21-day recovery, the persistence of larger leaves with lower sugar content in shaded than in unshaded plants is consistent with shading triggering a delayed 'light-harvesting' response through increased surface area, as observed in other seagrasses (Ralph *et al.* 2007). The 60% higher initiation of apices with shading was probably a similar light stress response, which increased the potential for leaf production and harvesting of light, although this potential was not realised as there was no difference in the actual branching frequency (conversion of apices into new modules with leaves) between unshaded and shaded plants, and hence no difference in leaf density.

Responses to grazing

Grazing treatments that simulated the two different modes of swan grazing resulted in what we label different 'response pathways'. In the 'lawn mowing' treatment, leaf density recovered within 21 days, strikingly similar to the 20 days needed for *H. ovalis* to recover from dugong grazing (Nakaoka and Aioi 1999). The 13% increase in rhizome C:N ratio due to leaf grazing likely indicates that N was translocated from below- to above-ground tissue to aid the production of new leaves. When rhizomes were removed as well as leaves, leaf density was comparable to that in controls after 7 days, again confirming the rapid recovery rate. The 80% higher frequency of initiated apices and 70% higher branching frequency, in combination with no discernable effect on leaf or rhizome carbohydrates after 21 days, suggest that grazed plants quickly invested carbohydrates in regaining below-ground tissue by producing new branches, although these reserves were back to normal levels before the

Fig. 5. Content (% DW) of (a) leaf sugars, (b) rhizome sugars, (c) leaf starch, (d) rhizome starch, (e) leaf total carbohydrates, (f) rhizome total carbohydrates, nitrogen of (g) leaves and (h) rhizomes, and C:N ratios of (i) leaves and (j) rhizomes of *Halophila ovalis* after 3 weeks of recovery from two levels of shading (unshaded v. shaded) crossed with three levels of simulated grazing (C, control; L, leaf grazing; R, rhizome removal) (means $\pm$ s.e., $n = 5$).

final sampling. It appears then, that under normal light conditions *H. ovalis* can maintain above- and below-ground biomass under levels of grazing comparable to the highest intensities we observed.

Responses to multiple disturbances

The resilience of plants subjected to multiple disturbances (shading followed by grazing) was highly dependent on the type of

grazing. Plants subjected to simulated leaf grazing showed similar responses whether or not they had been shaded, suggesting that the plant can cope with these disturbances and reinforces the results of previous studies (Nakaoka and Aioi 1999; Kuiper-Linley *et al.* 2007), indicating that *H. ovalis* is highly resilient to short-term disturbance. The stimulation of apex production is the mechanism used to re-establish following disturbance, either light reduction or physical disturbance to the rhizome from

grazing, and this appears to be possible despite the decline in carbohydrate reserves of the shaded plants, except where grazing is on the rhizomes.

When grazing affected the rhizomes, there was a very strong interactive effect of shading; leaf density was 30% lower than in controls and unshaded treatments after 21 days. There was an absence of the increased apex initiation observed in unshaded plants subjected to rhizome grazing, so the potential to produce new leaves was reduced. These observations are consistent with a pathway of response whereby depletion of carbohydrate reserves through short-term shading interferes with the flow of energy (probably through physical disruption to the vascular system) required for apex initiation and new leaf production. As a consequence, the rate of recovery was much lower under these conditions compared with plants that had not been shaded or had been exposed to the lawn mowing form of grazing. The reduction in leaf sugar levels of shaded plants was still apparent after 21 days, suggesting that this reduced capacity to cope with grazing could remain on the time scale of at least weeks. Furthermore, the study was conducted during a period with high light conditions (end of summer), and it is likely that during winter, when seagrass production and potentially carbohydrate content is much lower (Hillman *et al.* 1995), the relative swan grazing pressure (% of production consumed over time) and presumably the impact on seagrasses will be higher than reported here.

Swan–seagrass interactions in the historical and present setting

Historically, black swans were much more abundant in south-western Australia than now. Early European settlers reported flocks of several thousand swans grazing in the estuaries in the early 19th century (Hay 1906; Brearley 2005). In combination with the 23% removal of seagrass production by swans in a current high-density area (but most likely, a low-density area), the historically higher swan density must have been a more significant disturbance to seagrasses. We therefore suggest that the settlement of the region by Europeans, who hunted out the swans and initiated eutrophication of the estuaries (Brearley 2005), has likely resulted in a major shift in disturbance regime, from frequent swan grazing to shading (by phytoplankton blooms, floating plants and resuspended sediments). Based on the observed effects of shading together with the often occurring rhizome grazing, we therefore suggest that in the present day estuarine system, with regular light reduction events combined with increased swan grazing mediated through management aimed at increasing swan abundance, the resilience of *H. ovalis* populations may be detrimentally affected. Before taken as a fact, this must be corroborated by studies comparing impacts of actual swan grazing with those of simulated grazing on more variables (e.g. seagrass reproduction) over larger spatial-temporal scales. However, based on the results of the present study and other studies on swan grazing impacts, we suggest that re-establishing swan densities without simultaneously reducing the extent of light reduction events could have major and potentially unforeseen consequences. Even though ‘pioneer’ species like *H. ovalis* generally recover more quickly from grazing than larger ‘climax’ species (e.g. Eklöf *et al.* 2008b), and loss of benthic estuarine vegetation could decrease swan abundance

and indirectly reduce grazing pressure (McKinnon and Mitchell 1994), shading-induced community shifts to domination by persistent and recurring phytoplankton blooms can be maintained by even moderate black swan grazing pressure (Mitchell *et al.* 1994; Perrow *et al.* 1997).

Conclusions

Complex cause–effect pathways mediate the responses of *H. ovalis* to the investigated disturbances, where key adaptive mechanisms of plants (e.g. allocation of carbohydrate reserves) may be affected simultaneously by more than one pressure. In applying ecologically relevant levels of two common disturbances – shading and grazing – we have shown strikingly different responses to multiple co-occurring disturbances than to either of the disturbances alone, although this is highly dependent on the nature of the grazing disturbance. This emphasises that in environments where anthropogenic disturbances are superimposed onto natural disturbances, managers must understand the mechanistic pathways by which pressures affect organisms, then assess and manage the full suite of disturbances and their potential interactions to avoid undesirable ecosystem shifts and achieve long-term success.

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