FEEDING ECOLOGY OF THE SEAGRASS GRAZING NERITE *SMARAGDIA SOUVERBIANA* (MONTROUZER, 1863) IN SUB-TROPICAL SEAGRASS BEDS OF EASTERN AUSTRALIA

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FEEDING ECOLOGY OF *SMARAGDIA SOUVERBIANA*

Please note:
As Renee Rossini will be away in the field from the 23rd of June to the 26th of July José L. Rueda (jose.rueda@ma.ieo.es) *will act as corresponding author during this period.*

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Abstract

By amalgamating all seagrass-associated grazing invertebrates into an epiphyte-feeding guild the currently accepted model of seagrass trophic dynamics ignores the diverse range of invertebrates that feed directly on, and do considerable damage to seagrasses. Of the wide range of invertebrates documented to directly damage seagrass, the gastropod genus *Smaragdia* has adaptations and ecology that suggest it could be a completely seagrass feeding group, with two species documented to preferentially consume seagrass. This paper documents the dietary associations of *Smaragdia souverbiana,* one of the most widely distributed but least studied of the *Smaragdia* species, in the sub-tropical east-Australian part of its range. Using field-based assessments of grazing damage and targeted laboratory feeding trials we assess the dietary associations, digestibility and feeding preferences of *S. souverbiana* with local seagrasses (*Halophila ovalis, Zostera capricorni* and *Cymodocea serrulata*), demonstrating that this species consumes and damages all available species but shows a strong preference for the most abundant and moderately digestible *Zostera capricorni*. Although it avoids highly epiphytised seagrass tissues in a laboratory context, considerable amounts of epiphytic material were found in the faeces of field-caught individuals. Grazing and digestibility of seagrass cells was higher in *Z. capricorni* and *H. ovalis*, and the former seems to be prefered when both of them were available. This study adds to the growing body of literature that demonstrates *S. souverbiana* is one amongst potentially many other grazing invertebrates that, rather than targeting epiphytes cause considerable damage to seagrasses directly.

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Introduction

Grazing influences the health of seagrass ecosystems, however the classical model used to describe trophic links may be overlooking the functional complexity of this grazing guild (Heck and Valentine, 1999; Valentine and Heck, 1999; Duffy, 2006; Valentine and Duffy, 2006;). In this model grazers in seagrass systems are generally partitioned into two components: the large roving vertebrate grazers that remove seagrass, and the small herbivorous invertebrates that target the epiphytes that colonize seagrass blades (Heck and Valentine, 1999; Valentine and Duffy, 2006). By removing epiphytes, this invertebrate guild has the two-fold effect of protecting seagrass from overgrowth by epiphytes, especially when eutrophication increases, and transferring energy from this highly productive element of the system on to secondary consumers (Heck and Valentine, 1999; Hays, 2005; Tomas, Turon and Romero, 2005; Marco-Mendez, *et al.*, 2012; Verhoeven, *et al.*, 2012).

It is questionable whether all herbivorous invertebrates occupying this system contribute to this same positive functional role (Nakaoka, 2005; Vizzini, 2009; Jaschinski and Sommer, 2010). Several grazing invertebrate species have been documented to damage seagrasses inadvertently whilst grazing (Fredriksen, Christie and Bostrom, 2004; Marco-Mendez, *et al.*, 2012), when harvesting seagrass to build shelter (van Tussenbroek and Brearley, 1998; Brearley, Kendrick and Walker, 2008) or when feeding directly on seagrass tissues (Wassenberg, 1990; Guidetti, 2000; Jormalainen, Honkanen and Heikkila, 2001; Hickman, 2005; Rueda and Salas, 2007; Brearley, *et al.*, 2008; Rueda, *et al.*, 2009; Unabia, 2011; Reynolds, Carr and Boyer, 2012). Whilst the guild of epiphyte-grazing invertebrates within seagrass proposed by Valentine and Heck undoubtedly benefit seagrass, the distinct role of those that cause negative effects on seagrass need to be experimentally and theoretically acknowledged.

Organisms that directly feed or bore into seagrass are seldom subjects of enquiry or discussion in the literature. The few organisms whose effects have been the subject of empirical study remove considerable amounts of live seagrass tissue (Zimmerman, *et al.*, 1996; Brearley, *et al.*, 2008; Rueda, *et al.*, 2009; Holzer, *et al.*, 2011a; Holzer, *et al.,* 2011b; Reynolds, *et al.*, 2012), often

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from sensitive parts of the plant such as reproductive tissues (Wassenberg, 1990; Hickman, 2005; Nakaoka, 2005; Reynolds, *et al.*, 2012) or young leaves (Rueda, *et al.*, 2009), causing manifold damage to seagrass health by reducing fecundity and photosynthetic activity or increasing susceptibility to infection (Zimmerman, *et al.*, 1996; Holzer, *et al.*, 2011a; Holzer, *et al.,* 2011b). Seasonal pulses in the abundance of these grazers have been associated with peaks in damage to seagrass blades affecting over 80% of leaves, with up to 40% of the leaf surface removed (Zimmerman, *et al.*, 1996; Brearley, *et al.*, 2008; Rueda, *et al.*, 2008; Bendell, 2011; Unabia, 2011) . By consuming seagrass directly, these grazers are not only having a directly negative influence on seagrass population dynamics but are acting as an alternative trophic pathway passing energy assimilated from seagrass directly on to smaller secondary consumers. By lumping such seagrass specialists with epiphyte feeders into a general epiphyte-grazing guild we are overlooking their specialised role.

Gastropods are a diverse and abundant component of the invertebrate fauna within seagrass habitats, and at least four different families have been documented to feed directly on live seagrass tissues (Neritidae, Lottidae, Nacellidae, Plakobranchoidea)(see Holzer, *et al.*, 2011b for review). Studies pertaining to these confirmed seagrass feeders are limited, and rarely manipulative in nature (Holzer, *et al.*, 2011b). Of those that have been studied, the Nerite genus *Smaragdia* seems to be exclusively associated with seagrasses, and seagrass feeding has been documented in two of the ten currently accepted species (*Smaragdia viridis*, *S. bryanae*), however very little peer-reviewed ecological information is available on most *Smaragdia* species. These neritids are found globally in tropical and sub-tropical regions, with the majority of literature attention on their feeding ecology focussed in species occurring in the Caribbean (Holzer, *et al.*, 2011a; Holzer, *et al.,* 2011b), Hawaii (Unabia, 2011) and Mediterranean (Rueda and Salas, 2007; Rueda, *et al.*, 2009; Rueda, Salas and Gofas, 2011), where all have been shown to ingest a range of available seagrass species and actively avoid macro-algae and epiphytes. Their small size and radula morphology makes them well adapted to a seagrass specific diet (Rueda and Salas, 2007; Unabia, 2011), with few and strong cusps on lateral teeth that rasp open multiple columns of cell walls with each stroke, ensuring cell contents

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are directly ingested. Some species feed across a range of available seagrasses (Rueda and Salas, 2007; Holzer*, et al.*, 2011a), or preferentially upon those with greater digestibility (Rueda*, et al.*, 2011), whilst others associate specifically with just one seagrass species (Unabia, 2011). Those with trans-regional distributions (e.g. *Smaragdia viridis*) show location specific associations with completely different seagrass species and genera (Rueda and Salas, 2007; Holzer *et al.*, 2011a). Despite this, seagrass feeding and dietary preference for one seagrass species occuring in each region seems, so far, to be common.

      Smaragdia souverbiana is one of the most frequent and widely distributed species of the genus but very few published accounts of its association with seagrasses exist to date along its circum-global distribution, including Japan (Higo and Goto, 1999), New Caledonia (Crosse, 1894; Heros, 2007), eastern Africa (Chelazzi, 1980; de Boer and Prins, 2002), the Middle-East (Dekker, 2000) and Australia (Barnes, 2010; Barnes 2013), among other areas. Within Australian waters, populations span tropical to sub-tropical regions of the east and west Indo-Pacific (Australian Museum Malacology Collection Records). Australian populations of *S. souverbiana* have been documented to associate with *Halophila ovalis, Halodule uninervis* and *Nanozostera muelleri capricorni* (synonymous with *Zostera capricorni*) throughout tropical and subtropical areas (Barnes, 2010; Bendell, 2011; Barnes, 2013), however existing studies only document their presence and provide no evidence regarding their feeding ecology. Like some northern hemisphere *Smaragdia* species, it is likely that *S. souverbiana* will consume seagrass directly and, therefore play a previously ignored direct role in the trophic dynamics of seagrass systems in this region. As *S. souverbiana* has a broad range covering regions with different seagrass assemblages, it is also possible that local associations with different seagrasses may be present. Dietary observations of *S. souverbiana* will aid in assessing the seagrass associations of *Smaragdia* as a genus as well as determine how the feeding ecology and ecological role of this broadly distributed member may, like *S. viridis,* vary across its expansive range.

      This study documents the abundance and feeding ecology of *Smaragdia souverbiana* inhabiting sub-tropical seagrass beds in eastern Australia,

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specifically in the Moreton Bay region, with the specific aim to discern its diet, ability to damage live seagrass tissues and any potential dietary preferences. We hypothesised that, like other *Smaragdia* species, *S. souverbiana* will readily feed upon and cause damage to common seagrasses present in this region. Of those seagrasses available we predict that their digestibility will differ, and *S. souverbiana* will show preference for a certain seagrass species.

Material and Methods

Study site

This study was completed in the eastern part of Moreton Bay, Queensland (Australia) at three sites spanning the western coast of North Stradbroke Island – Amity Point (27°24'42.2"S, 153°26'14.2"E), Dunwich (27°29'37.83"S, 153°23'52.78"E) and Myora (27°27'59.37"S, 153°25'18.49"E). Sampling and seagrass collection occurred in intertidal seagrass beds during the winter (August) of 2009 and autumn (May) and spring (October) of 2012. Seagrass beds at the sampling sites used by this study were predominantly composed of *Zostera capricorni* (synonymous with *Nanozostera muelleri capricorni*), with a coverage at Dunwich of 73.51±4.57% and at Myora of 75.99±3.17%, and of *Halophila ovalis*, with a coverage at Dunwich of 0.76±0.5% and at Myora of 4.74±1.4%. Small patches of *Cymodocea serrulata* also occurred in deeper areas (low intertidal and subtidal). Unless otherwise stated, individuals of *S. souverbiana* and seagrasses used in this study were randomly selected across all three sites.

Field density estimates of S. souverbiana *and seagrass damage*

Density estimates were completed using two methods. The standard coring methodology was not used in this study because preliminary observations found *S. souverbiana* individuals to be patchy and the amount of core extraction would be highly destructive for the seagrass bed with high by-catch. Instead, a visual census and epibenthic-harvesting methodology was employed. One meter squared quadrats (divided into one hundred 10cm²quadrants) were placed haphazardly throughout the seagrass bed (winter n=20 quadrats, spring n=20

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per site). Visual census spanned for 10 minutes extracting any *S. souverbiana* that could be seen in the different quadrants. Although small (some individuals with less than 2mm), *S. souverbiana* were usually found perched upon blades. In the spring sampling, in addition to visual census, abundance was estimated by collecting 5 randomly selected 10cm^2 epibenthic-harvests of seagrass blades from each quadrat. All blades with sheath within the five 10cm^2 quadrant sample areas were detached from the rhizome and placed immediately into a plastic sample bag. All blades in each bag were searched exhaustively in the laboratory for *S. souverbiana* by suspending the sample in filtered seawater and sorting through the blades*.*

During sampling for *S. souverbiana* in October 2012, estimates of naturally occurring damage to seagrass were also made using quadrats. For each quadrat, percentage cover of each seagrass species was assessed using the point-intercept methodology. Proportion of damaged leaves was assessed by haphazardly sampling 10 blades of each species present from each quadrat. Leaves were transported to the laboratory and immediately inspected under dissection microscope. Number of leaves with signs of grazing damage was annotated and all damaged leaves were photographed to assess the proportion of the surface area damaged (see methodology for damage assessment under 'Assessing leaf damage'). As the damage to each species is not independent within quadrat, damage across species was analysed by subsampling from existing quadrats (giving n=10 per site) and compared using a two-factor ANOVA with site and species as fixed factors. Data for the proportion of leaves damaged failed the Cochran C test for heterogeneity of variance which was remedied by Arcsin transformation.

Feeding trials on different seagrass species

To asses whether epidermal tissues of seagrasses were ingested by this neritid, to characterize the morphology of the radular marks on each seagrass species and to determine whether *S. souverbiana* grazed preferentially on young un-epiphytised leaves. Thirty individuals with aperture widths ranging from 1 to 3 mm were collected from the field whilst sampling at Dunwich and Amity Point. The first set of experiments was completed using 12 of these individuals. After

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24h starvation period, individuals were placed in ca. 2.5 L plastic trays containing filtered seawater and shoots of different seagrass species with no grazing marks on their leaves (4 individuals with shoots of *Zostera capricorni*, 4 with *Halophila ovalis* and 4 with *Cymodocea serrulata*). At the end of each experiment, seagrass blades were examined for radular marks created by the neritids using a dissection microscope, and those areas on leaves containing feeding trails were photographed and fixed in Lugol. The amount of epiphytes in the different seagrass leaves that had radular marks was annotated using ranked categories (1: low epiphyte load when < 10 % of leaf surface area was covered by epiphytes; 2: intermediate epiphyte load when ca. 50% of leaf surface area was covered by epiphytes and 3: high epiphyte load when more than 90% of the seagrass leave was covered by epiphytes). The experiments were repeated three times on three sets of individuals. After the experiments were completed snails were released back to the field.

Rates of damage to different seagrass species in laboratory conditions

A second set of experiments similar to the 'Feeding trials' were carried out using 18 individuals in far more controlled conditions to ascertain if differing amounts of damage were done to the three common seagrass species. Individual of *S. souverbiana* were placed alone in a 50mL perforated sample jar suspended in an aerated and circulating 1000L tank in an outdoor aquarium (under Perspex rooved field station) and left to starve and acclimatise for 24hrs. Six replicate treatments of control (seagrass blade with no snail, in identical perforated jars) and treatment (seagrass and snail) were established by fastening ~4g of blot dried undamaged and un-epiphytised green seagrass tissues of the three most common seagrass species (*Z. capricorni, H. ovalis* and *C. serrulata*) into each jar using a small cable tie (giving a total of 48 jars). After 48hrs seagrass was removed and photographed for damage assessment (see methodology for damage assessment under 'Assessing leaf damage'). Control treatments showed no damage and were removed from the analysis. The amount of damage inflicted on each species was analysed using one-factorANOVA with seagrass species as a fixed factor. Data for the proportion of the leaf surface damaged

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failed the Cochran C test for heterogeneity of variance, which was remedied using an Arcsin transformation.

Faecal content analyses

In order to contrast the digestibility of the different seagrass species, *S. souverbiana* were collected and starved as in the previous experiments, but on this occasion each snail was fed a monospecific diet of either *Z. capricorni, H. ovalis* or *C. serrulata* (n=4 snails per seagrass species) for 24hr. Faeces egested by all *S. souverbiana* individuals in a seagrass species tray were collected using a Pasteur pipette and fixed in Lugol for further microscopic analyses. Empty seagrass cells (without cytoplasm and chloroplasts) and intact cells (with cytoplasm and chloroplasts) were counted in 10 faecal samples from different individuals that fed on each seagrass species. The potential assimilation of the three seagrass species was then estimated as the percentage of empty seagrass cells compared to the total number of seagrass cells (empty and intact). Data was analysed as the average proportion of empty or intact cells across the ten replicates, using a two-factor ANOVA with seagrass species and cell status as fixed factors.

For studying the faecal content of *S. souverbiana* in the field, ten individuals were collected randomly from the seagrass beds at the Dunwich and Myora sites during sampling (on two occasions: May and June 2012) with the majority of individuals being found on *Z. capricorni*. Each individual was placed immediately into its own 5mL sample jar filled with filtered seawater and left to defecate for 24 hours before being released back to the field. Faeces were immediately collected using Pasteur pipette then wet-mounted on slides to be observed under compound microscope. Faecal contents were compared to pre-prepared slides of available food sources collected from the field at the same time (*H. ovalis, Z. capricorni, C. serrulata,* and smears of epiphyte scrapes that contained macro and micro algae, cyanobacteria, diatoms and a matrix of indefinable detritus). Presence/absence of each food type was noted for each individual and collated as the proportion of all individuals sampled that had egested each food type.

Preference for a seagrass species

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Experiments regarding preference focussed only on *Z. capricorni* and *H. ovalis*, due to very low grazing rates on *C. serrulata* observed in the afore mentioned experiments. Using the same circulating seawater system as the feeding trials for assessing seagrass damage, ten replicate jars of each treatment were set up by placing a single *S. souverbiana* individual into each jar and leaving it to starve for 24h. Seagrass was fixed into containers by fastening a total of ~4g blot-dried pieces of the designated seagrass species (*Z. capricorni, H. ovalis* or both seagrasses) in either control (seagrass no snail) or treatment (seagrass and snail) jars (giving a total of 60 jars) using small cable ties and snails were left to feed for 24hrs. Seagrass was removed from the jar, all leaves photographed and damage inflicted assessed using the methods described in 'Assessing leaf damage'. Control treatments experienced some senescence but no epidermal damage and therefore were removed from analysis. As the relative amounts of each seagrass species within choice treatments is not independent, direct comparison within the choice treatment between seagrasses cannot be made. These choice-no-choice experiments were loosely designed after the simplest iteration of the recommended methodology for assessing preference of Underwood and Clarke (2005) (used by Jackson and Underwood, 2007). Statistical comparisons were made regarding whether the amount of damage caused to each species was the same when offered in isolation, or when a choice was available, using the Student t-test.

Assessing leaf damage

All leaves to be assessed for damage were wet mounted between two microscope slides and photographed under dissecting microscope with the light source emanating from below and using a Cannon Powershot digital camera. Proportion of the leaf surface suffering damage was then assessed using image analysis in ImageJ software by tracing the total leaf area, and the area of the leaf where the blade was thinned by grazing. Senescent and browning tissues were included in the total leaf area, but not the grazed component.

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Results

Smaragdia souverbiana (Figure 1.) was found at all study sites in all sampling intervals. Visual estimation within quadrats *in situ* underestimated abundance by 95% (mean±SE; 0.85±0.34 individuals m^{-2} for visual vs. 16.92±6.71 individuals m^{-2} for epi-benthic harvest methodology) so any abundance measures using the former method have been excluded due to its low accuracy. Abundance in the spring sampling interval was patchy at both within and between-site scale, with large variance in abundance per m^2 within a site. This within site variance contributed to there being no significant differences between abundance at each site, regardless of a markedly higher mean at Dunwich (mean±SE; 26.15± 8.59 individuals m^{-2}) than Myora (7.69 ± 4.82 individuals m^{-2})(One-factor ANOVA: n=13, F=3.51, P=0.73) and individuals being encountered more frequently in samples from Dunwich (54% of samples) than Myora (23%).

In the laboratory *S. souverbiana* left signs of feeding upon *Z. capricorni, H. ovalis* and *C. serrulata* (Figure 2)*.* Grazing damage made by *S. souverbiana* results in a thinning of the seagrass blade usually in small, disjointed patches across the blade. Most radular marks occurred on leaves with low amounts of epiphytes (generally young leaves) compared to those with high loads (generally very old

leaves) in *Z. capricorni* (proportion of 0.77 grazing marks where epiphytes were low; values ranging from 0 to 1), *H. ovalis* (proportion of 0.6 grazing marks where epiphytes were low) and *C. serrulata* (proportion of 0.81 grazing marks where epiphytes were low). The faeces of half of the field-collected individuals contained cells of these three seagrass species, but all *S. souverbiana* individuals also egested food types associated with epiphytes (e.g. cyanobacteria, diatoms, filamentous algae). Of the seagrass portion, cells of *Z. capricorni* were found most frequently, followed by those of *H. ovalis* (proportion of 0.15 individuals overall, but only in spring) and *C. serrulata* (proportion of 0.30 individuals overall, but only in winter). However, these three seagrass species do not seem to be equally digestible (multi-factor ANOVA species x cell status interaction, n=4, F=86.1, p<0.001) and faeces of individuals fed monospecific diets of *H. ovalis* displayed the highest average percentage of empty seagrass cells, followed

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Fig. 3

by those of individuals that fed on *Z. capricorni* and finally those that fed on *C. serrulata* (Figure 3).

When individuals of *S. souverbiana* were offered single seagrass species treatments in the laboratory, they caused equal amounts of damage to the leaf surface area in *Z. capricorni* and *H. ovalis,* but caused significantly less damage to *C. serrulata* (One-factor ANOVA: $n=6$, F=12.48, $P=0.001$)(Figure 4). The same pattern occurred again when assessing preference in single species treatments, with a strong preference for *Z. capricorni* when offered a choice, but with no change in the amount of *Z. capricorni* consumed (Student t-test: $n=10$, F=0.08, $P=0.77$) and a significant reduction in the amount of *H. ovalis* consumed when *Z. capricorni* was also present (Student t-test: $n=10$, F=21.7, $P=<0.001$)(Figure 5).

Fig. 4

Fig. 5

Site-specific patterns of damage on seagrasses were found in the field (Two-factor ANOVA: proportion of leaves damaged, $n=10$, F=9.8, $P=0.003$; damage per leaf, $n=5$, F=6.01, $P=0.26$). At Dunwich, there was no difference in proportion of leaves of each species that showed signs of grazing (Tukey HSD post-hoc: $n=13$, $P=0.99$). In contrast at Myora, *H. ovalis* had significantly greater proportions of damaged leaves (Tukey HSD post-hoc: $n=13$, $P=0.008$,) and damage per leaf although these differences were not statistically significant (Figure 6). No *C. serrulata* was encountered during sampling, so the rate of damage occurring naturally to this species was not ascertained.

Fig. 6

Discussion

Smaragdia souverbiana were readily found at all sites and in all study periods, suggesting they are likely to be a common element of Moreton Bay seagrass assemblages. This study supports a growing body of literature concerning the genus *Smaragdia* demonstrating that, like other congeners, *S. souverbiana* readily feed on commonly available seagrasses showing a preference for the most locally abundant and more digestible species (*Z. capricorni*)(Rueda*, et al.*, 2011; Unabia, 2012). Unlike its congeners, there is evidence that this *Smaragdia* species also ingests a considerable amount of epiphytic material. We argue that the current amalgamation of seagrass feeders such as *Smaragdia* with epiphyte feeding invertebrates overlooks their functional individuality.

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Unlike other representatives of the *Smaragdia* genus that feed mostly on seagrass, the diet of *S. souverbiana* includes considerable amounts of epiphytic material. General observations made during faecal analysis of field-caught individuals found that the seagrass component of the faeces generally equal to the seagrass component (Rossini and Rueda, pers. obs.). Unlike *S. viridis*, in which a seagrass-rich seam was directly visible in faeces (Rueda and Salas, 2007), *S. souverbiana* faeces were homogenous brown. *Smaragdia souverbiana* will undoubtedly ingest epiphyte inadvertently when grazing seagrass, however the higher representation of this food type in *S. souverbiana* suggests it may play a more important role in the diet of this particular species. Distinct from other *Smaragdia* species, *S. souverbiana* may have the ability to have a two-fold influence on its habitat, acting as both an epiphyte and seagrass feeder.

Differences in methodology and study system between the present and previous studies mean this study may overestimate the contribution of epiphytes to the diet of *S. souverbiana* (Rueda*, et al.*, 2009; Holzer*, et al.*, 2011a; Holzer*, et al.*, 2011b; Rueda*, et al.*, 2011; Unabia, 2012). We present the probability of an individual egesting each food type whilst others present the proportion of the food type in the faeces of all individuals. Epiphytic material was also found in the faeces of most *S. viridis* in Mediterranean studies though it represented a small proportion of the faeces (Rueda, pers. obs.). Due to the methodology employed in this study, a small amount of epiphytes in the faeces of all individuals gives a high ranking to this food type, leading to a potential overestimate of the importance of epiphytes. Studies of *S. viridis* also harvested seagrass and *Smaragdia* from deeper sites (from 2 to 7m depth)(Rueda*, et al.*, 2009; Holzer*, et al.*, 2011a; Holzer*, et al.*, 2011b; Rueda*, et al.*, 2011), which are likely to have less developed epiphytic assemblages due to light attenuation compared to the intertidal seagrass beds of the present study. Studies of *S. souverbiana* in equitable habitats will aid in establishing if this higher representation of epiphytes in the diet of *S. souverbiana* is a reflection of its ecology or this difference in methodology.

Smaragdia souverbiana readily damages and consumes two of the most common seagrasses it associates with in this region, displaying a preference for *Z. capricorni*. Associations with the *Zostera* and *Halophila* genus have been found

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in other *Smaragdia* species such as that of *Halophila* with *S. bryannae* (Unabia, 2011) and *S. rangiana* (Zuschin and Hohenegger, 1998; Zuschin and Baal, 2009) and that of *Zostera* with *S. viridis* (Rueda and Salas 2007, 2008; Rueda *et al.*, 2009), but this study is the first to find a simultaneous association with both, and adequately demonstrate a preference for one of them. In the present study, preference was only observable when seagrasses were offered as a choice. Although *Smaragdia* has a preference, its diet is flexible and it will consume less preferred food types, a pattern of flexibility similar to that observed in *S. viridis* of the Caribbean (Holzer, *et al.*, 2011a) and the Alboran Sea (Rueda*, et al.*, 2011). This represents an advantage for colonizing new areas or against local extinction in those areas with changes in the presence/absence of certain seagrasses. Preference has been suggested in Mediterranean populations of *S. viridis* (Rueda*, et al.*, 2011) and the Hawaiian *S. bryannae* (Unabia, 2011) but unfortunately, these studies have not adequately tested for it in a choice-no choice style (for the ramifications of this see Underwood and Clarke, 2005). The results of experiments conducted here are testament to the importance of such designs, as consumption patterns of *S. souverbiana* only differed when a choice was offered. Without such information for other *Smaragdia* species it is difficult to determine if their dietary associations are generalist or preferential. Such information would contribute greatly to the contemporary urge to challenge the assumption that seagrass herbivore assemblages are predominantly generalist (Nakaoka, 2005).

Dietary observations across the three seagrass species suggest that particular seagrasses could be preferred due to their potential nutritional value, as well as non-trophic reasons. The digestibility of seagrass tissues for *Smaragdia* is linked to their capability to break open cell walls whilst rasping, and large-celled species seem easier to break and provide greater cytoplasm per cell (Holzer, *et al.*, 2011a; Rueda*, et al.*, 2011). In the present study *H. ovalis* had the greatest cell size and digestibility but was not the most preferred under laboratory conditions. Other trophic reasons for selecting seagrass species may relate to the differential presence of phenolic compounds in *Zostera*, *Halophila* and *Cymodocea* (McMillan et al., 1980). For small organisms, such as *Smaragdia,* food sources also serve as habitat (Reynolds*, et al.*, 2012), provisioning shelter

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from fish predators that have been shown to control populations of small grazing invertebrates (Lewis and Anderson, 2012). Egg masses of *S. souverbiana* have only been found deposited at the base of sheaths of *Z. capricorni* (egg masses were found on four occasions, only on *Z. capricorni,* pers. obs.), indicating that associations with this seagrass species are multi-faceted. Analogous to terrestrial insect grazing systems (and demonstrated for *S. bryannae* in Unabia, 2011), sub-optimal hosts can and will be used but this can have major impacts on the reproductive success of adults, and subsequent fitness or survival of the offspring reared (Thompson, 1988). Although it seems *Smaragdia* can thrive on a suite of seagrass hosts, without adequate assessments of preference and studies of performance across the life cycle we cannot be sure whether populations can be sustained on non-preferred foods.

   The ability of *S. souverbiana* to damage seagrass was manifested in the field in a highly variable fashion, reflecting the patchiness in populations and potential role of other seagrass grazers. High variability in the abundance of *Smaragdia* and functionally similar micro-grazers occurs at both spatial (Rueda and Salas, 2008; Zimmerman*, et al.*, 1996), and temporal scales (Brearley*, et al.*, 2008; Rueda and Salas, 2008; Bendell, 2011; Rueda*, et al.*, 2011). This may manifest as highly variable patterns of damage to seagrass, often peaking during summer when abundance of dominant micro-grazers reach maxima (Rueda*, et al.*, 2009; Bendell, 2011; Unabia, 2011). In the present study, locations with high *S. souverbiana* abundances also displayed high rates of damage to the preferred *Z. capricorni*. In contrast, high damage rates on the less-preferred *H. ovalis* were found where *S. souverbiana* densities were low. An accurate assessment of the role of *S. souverbiana* would need to monitor the damage they cause through time, as both seasons addressed by the present study are likely to be outside of the potential grazing peak in other studied populations (i.e summer).

   Smaragdia souverbiana is not the only invertebrate feeding directly on seagrasses of eastern Australia, with each member of this suite of small seagrass grazers likely to have its own feeding ecology and habitat usage. Just as functional diversity in invertebrate grazers is overlooked when we amalgamate them into an epiphyte-only feeding guild, we must also be cautious of overlooking diverse functions within seagrass feeders as it is the

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complementarity of a diverse range of grazers, not the homogenous contribution of an identical set, that results in ecosystem level effects (Burkepile, 2013; Burkepile and Hay, 2008; Burkepile and Hay, 2011; Duffy, Richardson and Canuel, 2003; Schmitz, 2008). The present and other published studies inadvertently collected small isopods and sacoglossans with seagrass consistently (Barnes, 2010; Barnes, 2013; Bendell, 2011), both of which have also been recorded to cause direct damage to seagrasses. Enquiry into the feeding ecology and populations dynamics of the diverse representatives within this distinct seagrass-feeding guild must be documented before we can confidently amalgamate their functional roles. These seagrass specific invertebrate grazers have the potential to influence seagrass dynamics directly, differentiating them from epiphyte-controlling invertebrates who are in the same size and movement range, but also from the trophically similar but functionally distinct larger seagrass grazers (Bjorndal, 1980; Preen, 1995; Kirsch, *et al.*, 2002; Lal, *et al.*, 2010; Christianen, *et al.*, 2012) whose propensity for movement leads to spatially dilute feeding effects (similar partitioning was found in coral reefs by Carpenter, 1986). Instead of investigating the diverse roles of the full suite of grazers, literature attention focuses on and solidifies the existing paradigm that invertebrates are predominantly epiphyte feeders that benefit seagrass indirectly (Heck and Valentine, 1999; Valentine and Duffy, 2006). Whilst functional grouping is vital to modelling the complex trophic dynamics of an ecosystem we must be wary of ignoring functional diversity in the quest for generalisation.

Fig.
7

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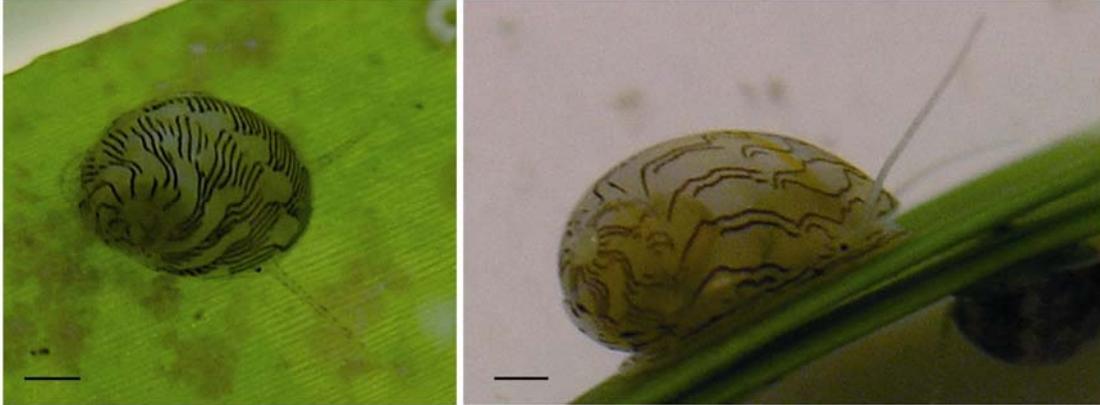CORRESPONDING AUTHOR: Renee Anne Rossini, r.rossini@uq.edu.au

Figure 1. Abapertural (left) and lateral (right) view of different live *Smaragdia souverbiana* specimens used in this study. Scale bar represents 1 mm.
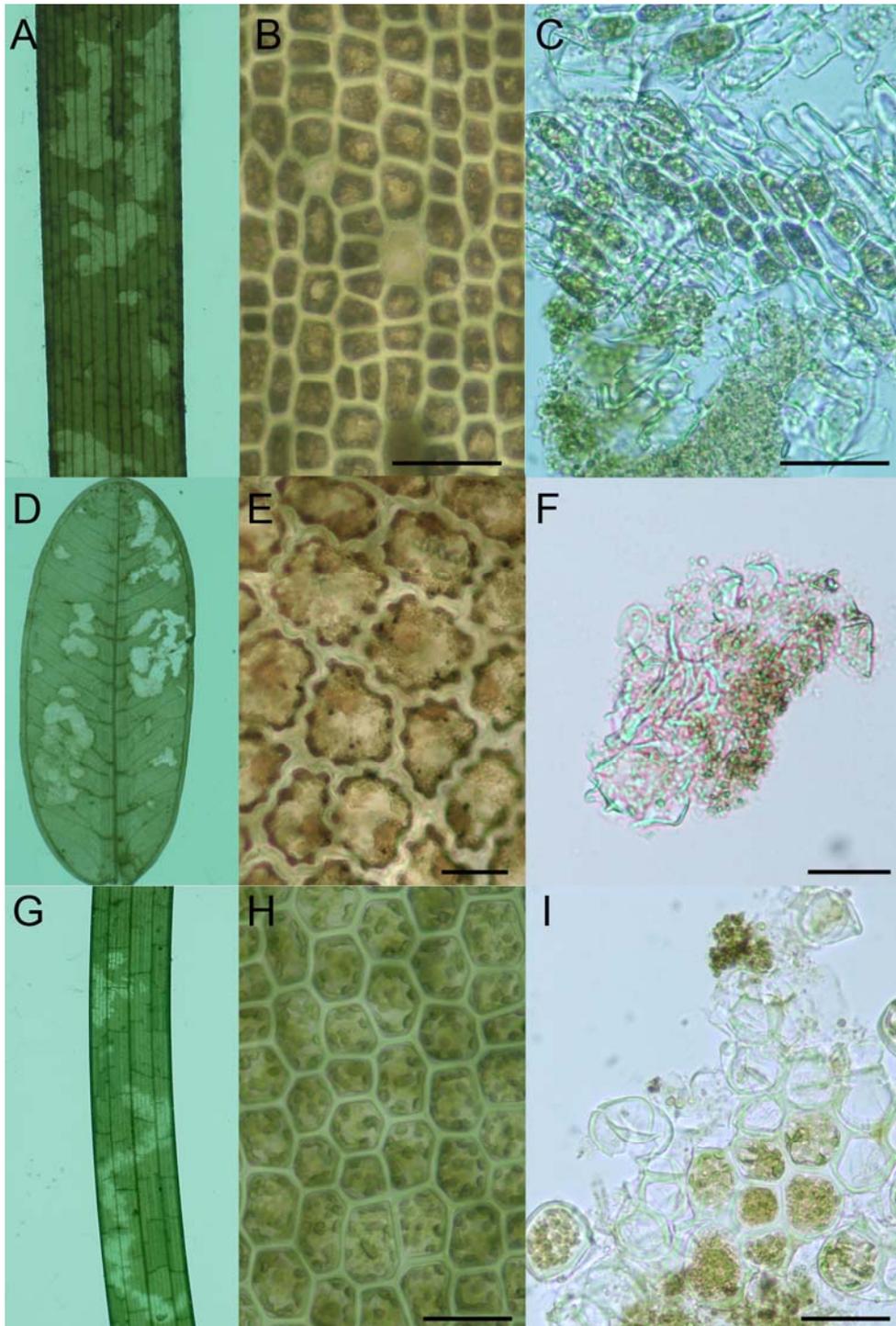
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Figure 2. Radular marks (A, D, G) and faeces (C, F, I) of *Smaragdia souverbiana* after grazing *Cymodocea serrulata* (A–C), *Halophila ovalis* (D-F), and *Zostera capricorni* (G-I). Unaltered cells of leaf tissues from *C. serrulata* (B), *H. ovalis* (E), and *Z. capricorni* (H) are shown for comparison. Scale bars represent 50 µm in all cases. *C. serrulata* leaf width: 6.4 mm; *H. ovalis* leaf width: 9.6 mm; *Z. capricorni* leaf width: 4.2 mm.

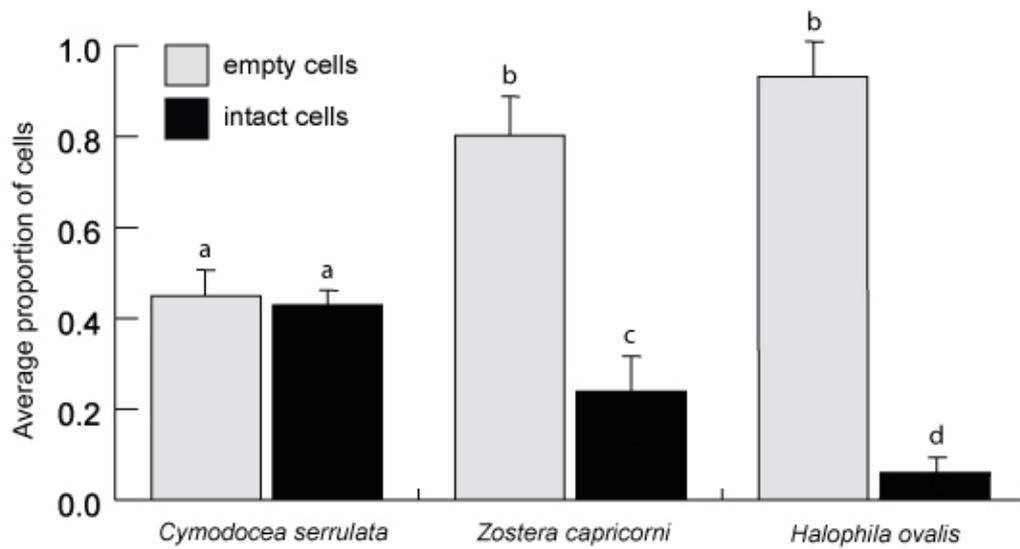CORRESPONDING AUTHOR: Renee Anne Rossini, r.rossini@uq.edu.au

Figure 3. Average proportions of digested (empty cells, grey) and undigested (intact cells, black) cells of each of the three seagrass species found in the faeces of *S. souverbiana* fed on a single species diet. Error bars indicate standard error. Values ranging from 0 to 1. Bars with the same letter are not significantly different (at p<0.01 alpha, Multi-factor ANOVA, Tukey post-hoc test)

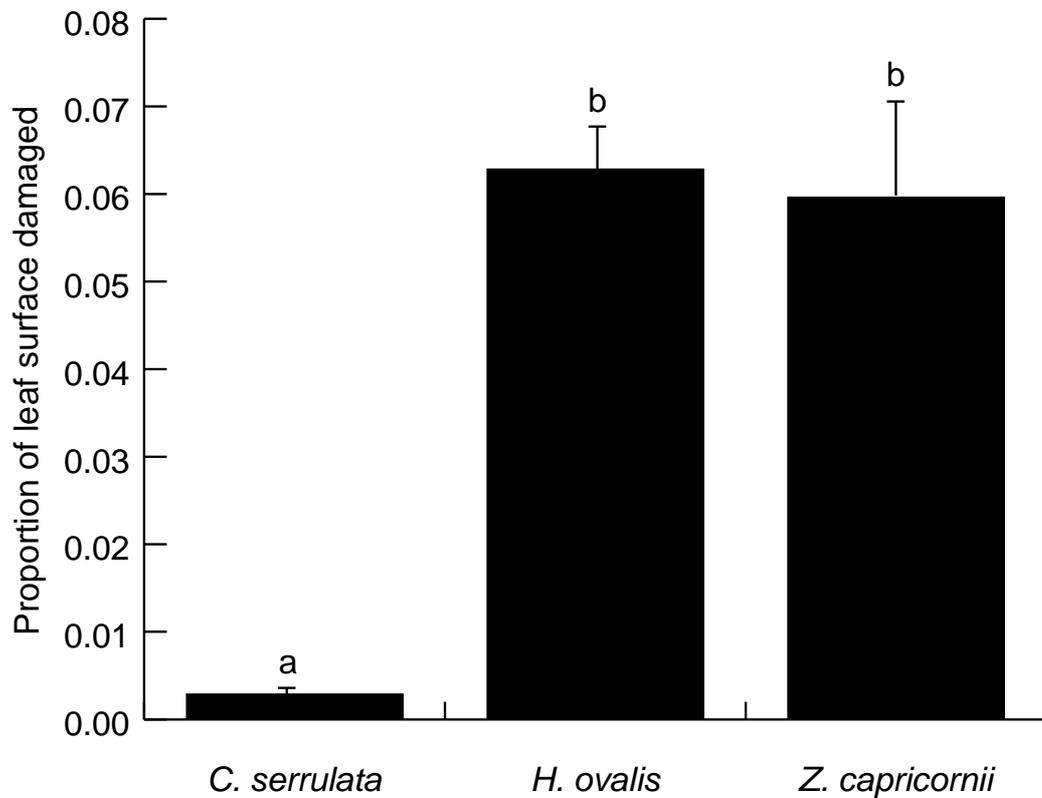CORRESPONDING AUTHOR: Renee Anne Rossini, r.rossini@uq.edu.au

Figure 4. The proportion of the leaf surface damaged after being exposed to *S. souverbiana* for 48hrs in a single seagrass species treatment (n=6). Error bars indicate standard error. Values ranging from 0 to 1. Bars with the same letter are not significantly different (at p<0.05 alpha, Single-factor ANOVA, Tukey post-hoc test).

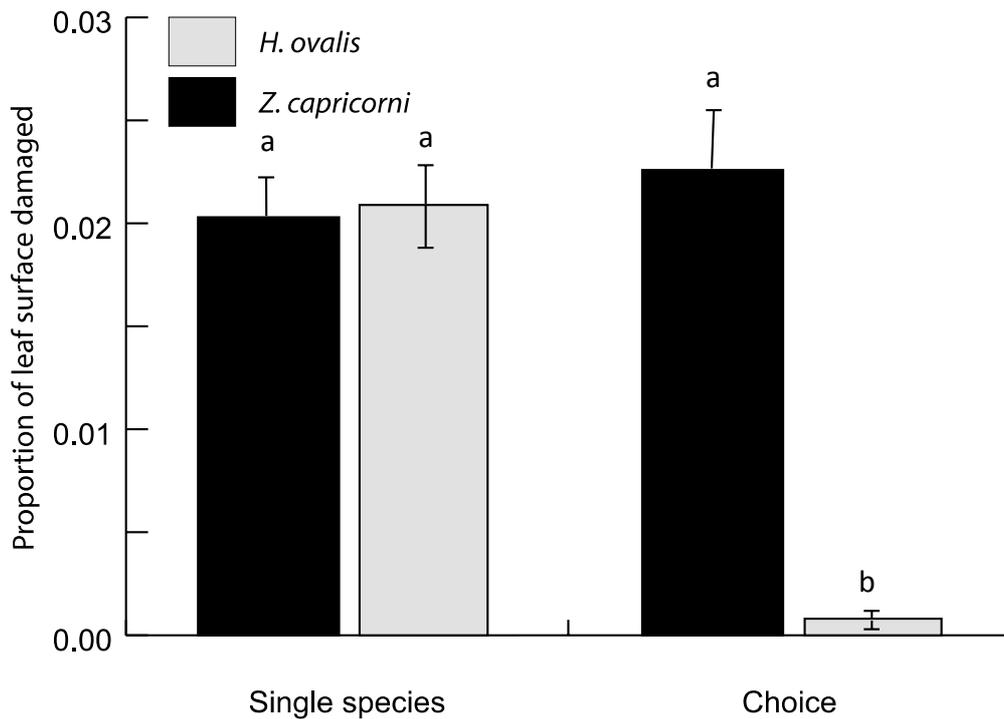CORRESPONDING AUTHOR: Renee Anne Rossini, r.rossini@uq.edu.au

Figure 5. Proportion of leaf surface damaged after being exposed for 24h in either a single seagrass species or choice treatment (both seagrass species available) (n=10 per treatment). Error bars show standard error. Values ranging from 0 to 1. Bars with the same letter are not significantly different (at alpha p=<0.05), though comparison between seagrass species (i.e. proportion damaged of *H. ovalis* vs. *Z. caprinorni*) within the choice treatment is not possible in this experimental design due to lack of independence.
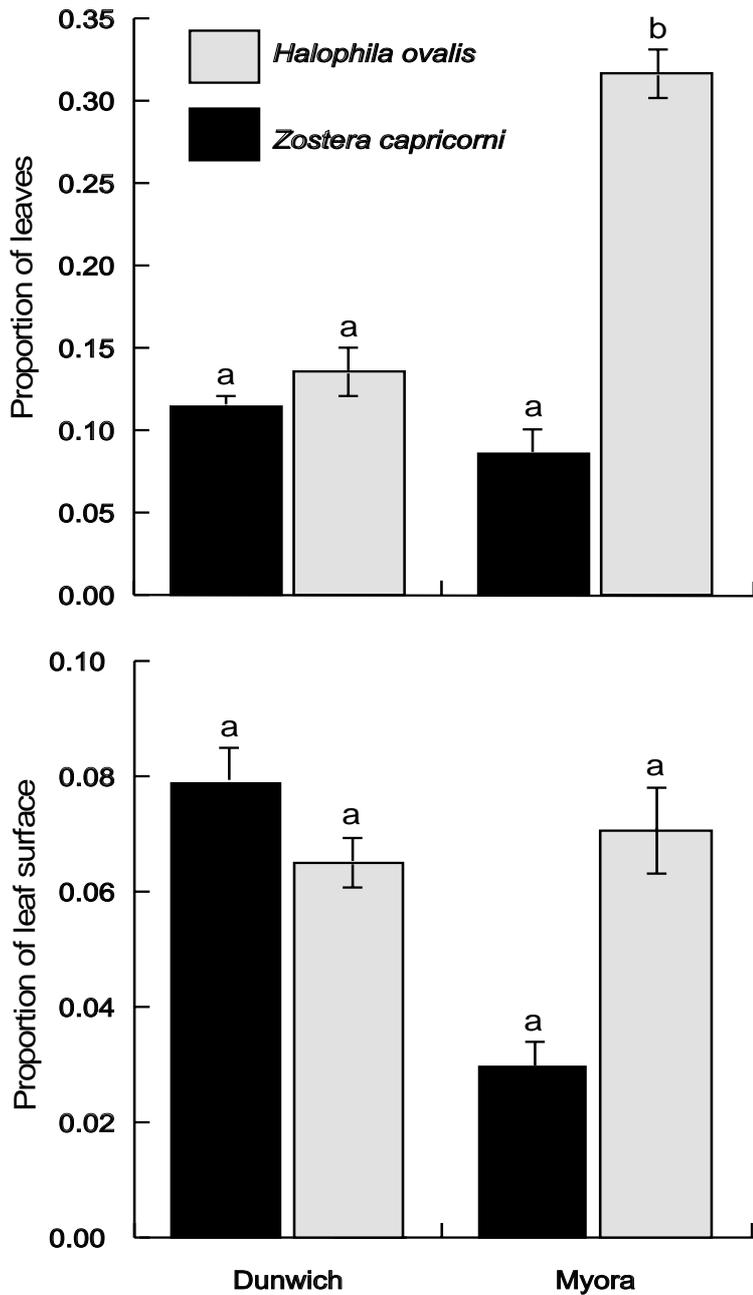
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Figure 6. Proportion of leaves (of total leaf sample) and of leaf surface damaged on two seagrass species (light – *Halophila* ovalis, dark- *Zostera capricorni*) in the field by *S. souverbiana* at two sites shown as a) the average proportion of sampled leaves showing signs of damage (n=10 quadrats per site per species), and b) the proportion of leaf surface of damaged leaves that were grazed (n=5 leaves per site per species). Error bars show standard error. Values ranging from 0 to 1. Bars with different letters within each graph are significantly different at p=<0.05, ascertained using Tukey post-hoc test.

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