

FEEDING ECOLOGY OF THE SEAGRASS-GRAZING NERITE
SMARAGDIA SOUVERBIANA (MONTROUZIER, 1863) IN
SUBTROPICAL SEAGRASS BEDS OF EASTERN AUSTRALIA

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(Received 19 June 2013; accepted 24 December 2013)

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 damage seagrass directly, the gastropod genus *Smaragdia* has adaptations and ecology that suggests it could be a specialized seagrass-feeding group, of which at least two species are known preferentially to consume seagrass. This paper investigated the dietary associations of *Smaragdia souverbiana*, one of the most widely distributed but least studied species of the genus, in the subtropical eastern Australian part of its range. Using field-based assessments of grazing damage and targeted laboratory feeding trials, we assessed the dietary associations, digestive ability and feeding preferences of *S. souverbiana* with local seagrasses (*Halophila ovalis*, *Zostera capricorni* and *Cymodocea serrulata*). We found that this species consumed and damaged all available species, but showed a strong preference for the most abundant and moderately digestible *Z. capricorni*. Although it avoided seagrass bearing a high epiphyte load 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 was preferred when both were available. This study adds to the growing body of literature demonstrating that *S. souverbiana*—and potentially many other grazing invertebrates—cause considerable damage to seagrasses directly, rather than targeting epiphytes.

INTRODUCTION

Grazing influences the health of seagrass ecosystems, but the classical model used to describe trophic links may be overlooking the functional complexity of the grazing guild (Valentine & Heck, 1999; Duffy, 2006; Valentine & 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 invertebrate grazers that target the epiphytes that colonize seagrass blades (Valentine & 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 (Hays, 2005; Tomas, Turon & 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 & Sommer, 2010). Several grazing invertebrate species have been documented to

damage seagrasses. This may occur inadvertently while grazing on epiphytes (Fredriksen, Christie & Bostrom, 2004; Marco-Mendez *et al.*, 2012), seagrass may be harvested to build shelter (van Tussenbroek & Brearley, 1998; Brearley, Kendrick & Walker, 2008) or invertebrates may feed directly on seagrass tissues (Wassenberg, 1990; Guidetti, 2000; Jormalainen, Honkanen & Heikkila, 2001; Hickman, 2005; Rueda & Salas, 2007; Brearley, Kendrick & Walker, 2008; Rueda *et al.*, 2009; Unabia, 2011; Reynolds, Carr & Boyer, 2012). Although the guild of epiphyte-grazing invertebrates associated with seagrass proposed by Valentine and Duffy (2006) undoubtedly benefits the seagrass, the distinct role of those that cause negative effects needs to be experimentally studied and theoretically acknowledged.

Organisms that directly feed on 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, Kohrs & Alberte, 1996; Brearley, Kendrick & Walker, 2008; Rueda *et al.*, 2009; Rueda, Salas & Gofas, 2011;

Holzer, Rueda & McGlathery, 2011a, b; Reynolds, Carr & Boyer, 2012), often from sensitive parts of the plant such as reproductive tissues (Wassenberg, 1990; Hickman, 2005; Nakaoka, 2005; Reynolds, Carr & Boyer, 2012) or young leaves (Rueda *et al.*, 2009, 2011), causing manifold damage to seagrass health by reducing fecundity and photosynthetic activity or increasing susceptibility to infection (Zimmerman, Kohrs & Alberte, 1996; Holzer, Rueda & McGlathery, 2011b). Seasonal pulses in the abundance of these grazers have been associated with peaks in damage to seagrass affecting over 80% of leaves, with up to 40% of the leaf surface removed (Zimmerman, Kohrs & Alberte, 1996; Bendell, 2006; Brearley, Kendrick & Walker, 2008; Rueda *et al.*, 2009; Unabia, 2011). By consuming seagrass directly, these grazers are not only having a direct 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 together with epiphyte feeders in a general epiphyte-grazing guild we are overlooking their specialized role.

Gastropods are a diverse and abundant component of the invertebrate fauna within seagrass habitats and members of at least four different family and higher groups (Neritidae, Lottidae, Nacellidae and Plakobranchoidea) have been documented to feed directly on live seagrass tissues (see Holzer, Rueda & McGlathery, 2011b for review). Studies pertaining to these confirmed seagrass feeders are limited and manipulative experiments in nature are rare (Holzer, Rueda & McGlathery, 2011b). Of those that have been studied, the neritid genus *Smaragdia* seems to be exclusively associated with seagrasses, and seagrass feeding has been documented in 2 of the 10 currently accepted species (*Smaragdia viridis* and *S. bryanae*), but very little peer-reviewed ecological information is available on most *Smaragdia* species. These neritids are found globally in tropical and subtropical regions, although the majority of studies of their feeding ecology have been focused on species occurring in the Caribbean (Holzer, Rueda & McGlathery, 2011a, b), Hawaii (Unabia, 2011) and the Mediterranean (Rueda & Salas, 2007; Rueda *et al.*, 2009, 2011), where all have been shown to ingest a range of available seagrass species and actively to avoid macroalgae and epiphytes. Their small size and radula morphology makes them well adapted to a seagrass-specific diet (Rueda & Salas, 2007; Rueda *et al.*, 2011; Unabia, 2011). The few and strong cusps on the lateral teeth rasp open multiple columns of cell walls with each stroke, ensuring that cell contents are directly ingested. Some species feed on a range of available seagrasses (Rueda & Salas, 2007; Holzer, Rueda & McGlathery, 2011a) or preferentially upon those with greater digestibility (Rueda *et al.*, 2011), while others associate specifically with just one seagrass species (Unabia, 2011). Those with broad distributions across ocean basins (e.g. *S. viridis*) appear to have populations connected by larval dispersal (Scheltema, 1971), but nevertheless show location-specific associations with different seagrass genera (Rueda & Salas, 2007; Holzer, Rueda & McGlathery, 2011a). Despite this, dietary preference for one seagrass species seems, so far, to be common.

Smaragdia souverbiana is one of the most frequently encountered and widely distributed species of the genus, but very few published accounts of its association with seagrasses exist to date anywhere within its broad Indo-Pacific range, which includes Japan (Higo, Callomon & Goto, 1999), New Caledonia (Heros *et al.*, 2007), eastern Africa (Chelazzi & Vannini, 1980; de Boer & Prins, 2002), Arabia (Dekker, 2000) and Australia (Barnes, 2010). Australian populations of *S. souverbiana* have been documented to associate with *Halophila ovalis*, *Halodule uninervis* and *Zostera capricorni* (synonym *Nanozostera muelleri capricorni*) throughout tropical and subtropical areas (Bendell, 2006; Barnes, 2010), but existing studies provide no evidence regarding their feeding ecology. Like some northern-hemisphere

Smaragdia species, it is likely that *S. souverbiana* consumes 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 possible that it may show local associations with different seagrasses. Dietary observations of *S. souverbiana* will aid in assessing the seagrass associations of the genus *Smaragdia* as a whole, as well as determining if (as observed for *S. viridis*) the feeding and ecological role of this broadly distributed member varies across its extensive range.

This study documents the abundance and feeding ecology of *S. souverbiana* inhabiting the seagrass beds of Moreton Bay in subtropical eastern Australia, with the aims to record its diet, ability to damage live seagrass tissues and dietary preferences.

MATERIAL AND METHODS

Study site

This study was carried out in the eastern part of Moreton Bay, Queensland (Australia), at three sites on the western coast of North Stradbroke Island: Amity Point (27°24'42.2"S, 153°26'14.2"E), Dunwich (27°29'37.9"S, 153°23'52.8"E) and Myora (27°27'59.4"S, 153°25'18.5"E). Samples were collected in intertidal seagrass beds during the winter (August) of 2009 and autumn (May) and spring (October) of 2012. The seagrass beds were predominantly composed of *Zostera capricorni* (synonyms *Nanozostera muelleri capricorni* and *Zostera muelleri*; see Les *et al.*, 2002), with a coverage at Dunwich of $73.51 \pm 4.57\%$ and at Myora of $75.99 \pm 3.17\%$, and of *Halophila ovalis*, with a coverage at Dunwich of $0.76 \pm 0.5\%$ and at Myora of $4.74 \pm 1.4\%$. Shoot density was not measured, but previous studies at Dunwich and Myora recorded an average of 200–500 shoots per m², remaining stable across seasons and years (Preen, 1992). 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 estimates of *S. souverbiana* density and seagrass damage

Density was estimated using two methods. The standard coring methodology was not used, because preliminary observations found *S. souverbiana* individuals to be patchily distributed, so that core extraction would be highly destructive for the seagrass bed. Instead, we employed visual census and epibenthic-harvesting methodologies. Quadrats of 1 m² (divided into 100 10 × 10-cm quadrants) were placed haphazardly throughout the seagrass bed in the autumn and spring 2012 sampling periods (a total of 20 1-m² quadrats sampled in each site and sampling period). Visual search lasted for 10 min, removing any *S. souverbiana* that could be seen in direct searches of all quadrants. Although small (most individuals are *c.* 2 mm aperture length), *S. souverbiana* could be found perched upon blades. In the spring sampling, in addition to visual census, abundance was estimated by collecting epibenthic harvests of seagrass blades from five randomly selected 10-cm² quadrants within each quadrat. All blades with sheath within the five quadrants were detached from the rhizome and placed immediately into a plastic 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 20 quadrats. For each quadrat, percentage cover of each seagrass species was assessed using the point-intercept method (i.e. 1 × 1 m quadrats with 100 points). The proportion of damaged leaves was assessed by haphazardly sampling ten blades of each

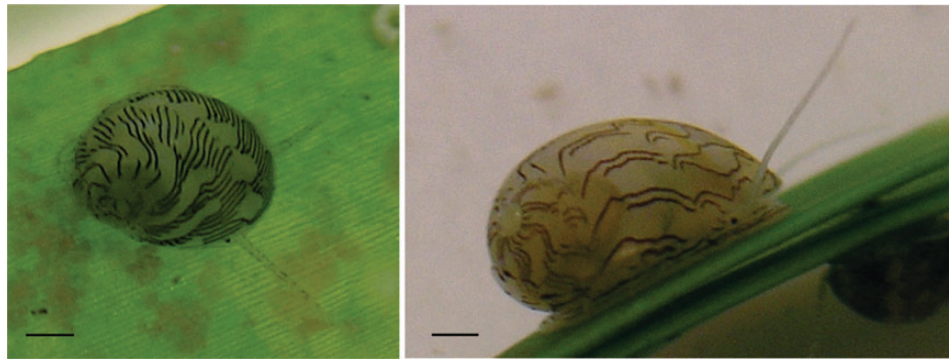


Figure 1. Abapertural (left) and lateral (right) views of different live *Smaragdia souverbiana* specimens used in this study. Scale bar represents 1 mm.

species in each quadrat. Leaves were transported to the laboratory and immediately inspected under the dissection microscope. The number of leaves with signs of grazing damage was counted and all damaged leaves were photographed to assess the proportion of the surface area damaged (see below). As the damage to each species is not independent within a quadrat, damage across species was analysed by subsampling from quadrats (giving $n = 10$ per site) and then 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 experiments on different seagrass species

Feeding trials were established in order to characterize the morphology of the radular marks on each seagrass species, to determine whether epidermal tissues of seagrasses were ingested preferentially from particular areas of the shoot and to evaluate the damage caused to each seagrass species. Thirty individuals of *S. souverbiana* with aperture lengths ranging from 1 to 3 mm were collected during all sampling intervals at Dunwich and Amity Point.

The first set of experiments was conducted in the winter of 2009 and used 12 of these individuals. After a 24-h starvation period, individuals were placed in 2.5-l plastic trays containing filtered seawater and shoots of different seagrass species that did not display any grazing marks on their leaves (four snails with shoots of *Z. capricorni*, four with *H. ovalis* and four with *C. serrulata*). At the end of each experiment, seagrass blades were examined for radular marks using a dissection microscope and areas with grazing damage were photographed and fixed in Lugol. The epiphyte load on the leaf surface adjacent to the grazed area was estimated using ranked categories (1: low epiphyte load with <10% of leaf surface area covered by epiphytes; 2: intermediate epiphyte load with *c.* 50% cover; 3: high epiphyte load with >90% cover). The proportion of leaves from each epiphyte category was not manipulated and represented the natural epiphyte load at the sampling time, with categories 2 and 3 being dominant (>70% of leaves). The experiments were repeated three times and the snails returned to the field.

The second set of experiments was carried out during the winter 2009 and 2012 sampling intervals using 18 individuals under more controlled conditions, to ascertain if differing amounts of damage were done to the three common seagrass species. Single individuals of *S. souverbiana* were placed in 50-ml jars perforated with 1-mm holes, suspended in an aerated and circulating 1000-l tank in an outdoor Perspex-roofed aquarium and left to starve and acclimatize for 24 h. Six replicate treatments of control (seagrass blade with no snail, in identical perforated jars) and treatment (seagrass and snail) were established

by fastening *c.* 4 g of blot-dried green leaf tissue, lacking epiphytes, of the three most common seagrass species (*Z. capricorni*, *H. ovalis* and *C. serrulata*) in each jar using a small cable tie (giving a total of 36 jars, 18 with snails, 18 without). After 48 h seagrass was removed and the leaves photographed for damage assessment (see below). Leaves in control treatments showed no damage during the experiment and were removed from the analysis. The amount of damage to each seagrass species was analysed by a one-factor ANOVA with seagrass species as a fixed factor. Data for the proportion of the leaf surface damaged failed the Cochran C test for heterogeneity of variance, which was remedied by arcsin transformation.

Faecal contents analyses

In order to contrast the digestibility of the different seagrass species, *S. souverbiana* was 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 24 h. Faeces were collected using a Pasteur pipette and fixed in Lugol for microscopic analysis. Empty seagrass cells (without cytoplasm and chloroplasts) and intact cells (with cytoplasm and chloroplasts) were counted in 10 samples from all individuals that fed on each seagrass species combined. The potential assimilation of the three seagrass species was then estimated as the proportion of empty and intact seagrass cells. Data were analysed as the average proportion of empty to total (empty and intact) cells across all ten replicates, in a one-factor ANOVA with seagrass species as the fixed factor.

For studying the faecal content of *S. souverbiana* under field conditions, 10 individuals were collected randomly from the seagrass beds at the Dunwich and Myora sites during sampling (on two occasions: May and June 2012 giving $n = 40$ total) with the majority of individuals being found on *Z. capricorni*. Each individual was immediately isolated in a 5-ml jar filled with filtered seawater and left to defaecate for 24 h before being released. Faeces were immediately collected using a Pasteur pipette, then wet-mounted on slides for observation under a compound microscope. Faecal contents were compared with slides of available food sources collected fresh from the field at the same time (*H. ovalis*, *Z. capricorni*, *C. serrulata* and smears of epiphyte scrapes that contained macro- and microalgae, cyanobacteria, diatoms and a matrix of 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.

Seagrass preference experiments

Experiments on feeding preference used only *Z. capricorni* and *H. ovalis*, owing to the very low grazing rates on *C. serrulata* observed

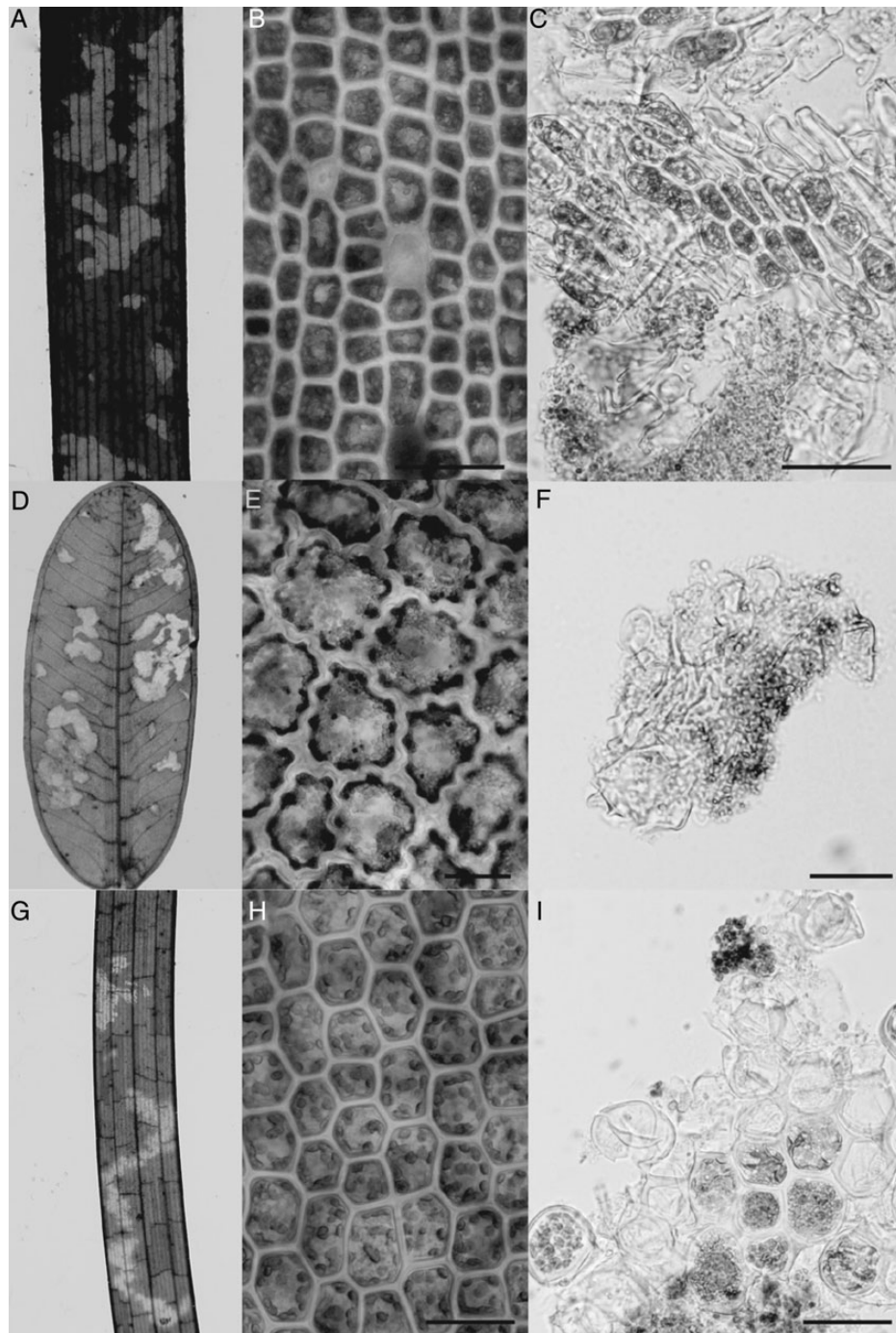


Figure 2. Radular marks (**A, D, G**) and faeces (**C, F, I**) of *Smaragdia souverbiana* after grazing *Cymodocea serrulata* (**A–C**), *Halophila ovalis* (**D–F**) and *Zostera capricorni* (**G–I**). Unaltered cells of leaf tissues from *C. serrulata* (**B**), *H. ovalis* (**E**) and *Z. capricorni* (**H**) are shown for comparison. Scale bars represent 50 μm in all cases. *Cymodocea serrulata* leaf width: 6.4 mm; *H. ovalis* leaf width: 9.6 mm; *Z. capricorni* leaf width: 4.2 mm.

in the experiments described above. Using the same circulating-seawater system as in the feeding trials for assessing seagrass damage, 10 replicate jars of each treatment were set up, first placing a single *S. souverbiana* in each jar and leaving it to starve for 24 h. Seagrass was fixed into containers by fastening a total of *c.* 4 g of blot-dried pieces of the designated seagrass species (*Z. capricorni*, *H. ovalis* or both) with small cable ties in either control (seagrass, no snail) or treatment (seagrass and snail) jars (giving a total of 60 jars) and snails were left to feed for 24 h. Seagrass was removed from the jar, all leaves photographed and damage

inflicted assessed using the methods described below. Control treatments experienced some senescence but no epidermal damage and therefore were removed from the analysis. As the relative amounts of each seagrass species within choice treatments is not independent, direct comparison within the choice treatment between seagrass species cannot be made. These choice/no-choice experiments were loosely designed according to the simplest of the methodologies recommended by Underwood and Clarke (2005) for assessing preference (also used by Jackson & Underwood, 2007). Statistical comparisons

were made to determine 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's *t*-test.

Assessment of leaf damage

All leaves to be assessed for damage were wet-mounted between two microscope slides and photographed under a dissecting microscope by transmitted light and using a digital camera. The proportion of the leaf area suffering damage was then assessed using image analysis in Image J software by tracing the total planar 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 in the grazed component.

RESULTS

Smaragdia souverbiana were found at all study sites in all sampling periods (Fig. 1). Visual estimation within quadrats *in situ* underestimated abundance by 95% (mean \pm SE 0.85 ± 0.34 individuals m^{-2} for visual estimation, *vs* 16.92 ± 6.71 individuals m^{-2} for epibenthic-harvest method), so only the latter method was used. Abundance in the spring sampling interval was patchy, both within and between sites, with large variance in abundance per m^2 within a site. As a result of the high within-site variance there was no significant difference between abundance at each site, regardless of a higher mean at Dunwich (mean \pm SE 26.15 ± 8.59 individuals m^{-2}) than at Myora (7.69 ± 4.82 individuals m^{-2}) (one-factor ANOVA: $n = 13$, $F = 3.51$, $P = 0.73$) and despite 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 *Zostera capricorni*, *Halophila ovalis* and *Cymodocea serrulata* (Fig. 2). Grazing damage 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 epiphyte loads in *Z. capricorni* (category 1 epiphyte load on 77% of grazed areas), *H. ovalis* (category 1 on 60%) and *C. serrulata* (category 1 on 81%). The faeces of half of the 10 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 and filamentous algae). Of the seagrass portion, cells of *Z. capricorni* were found most frequently (0.7 individuals in spring and 0.55 individuals in winter), 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 are not equally digestible (single-factor ANOVA on empty cells, $n = 10$, $F = 76.33$, $P < 0.0001$). A significantly larger proportion of empty cells was found in the faeces of individuals fed on *H. ovalis*, followed by that of individuals fed on *Z. capricorni*, while individuals fed on *C. serrulata* had equal proportions of empty and intact cells (Fig. 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 of *Z. capricorni* and *H. ovalis*, but significantly less to *C. serrulata* (one-factor ANOVA: $n = 6$, $F = 12.48$, $P = 0.001$; Fig. 4). The same pattern occurred again when assessing preference in single-species treatments, but *S. souverbiana* showed a strong preference for *Z. capricorni* when offered a choice. They caused identical amounts of damage to *Z. capricorni* in choice and no choice situations (Student's *t*-test: $n = 10$, $F = 0.08$, $P = 0.77$) and significantly less damage to *H. ovalis* when *Z. capricorni* was present (Student's *t*-test: $n = 10$, $F = 21.7$, $P < 0.001$; Fig. 5).

Site-specific patterns of damage on seagrasses were found in the field (two-factor ANOVA: proportion of leaves damaged,

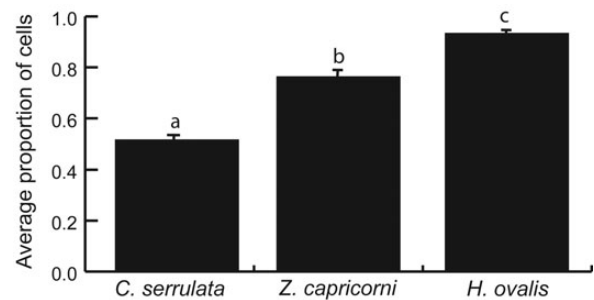


Figure 3. Average proportions of digested (empty) cells of each of the three seagrass species found in the faeces of *Smaragdia souverbiana* fed on a single-species diet. Error bars indicate standard error. Values range from 0 to 1. Bars with the same letter are not significantly different (at alpha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

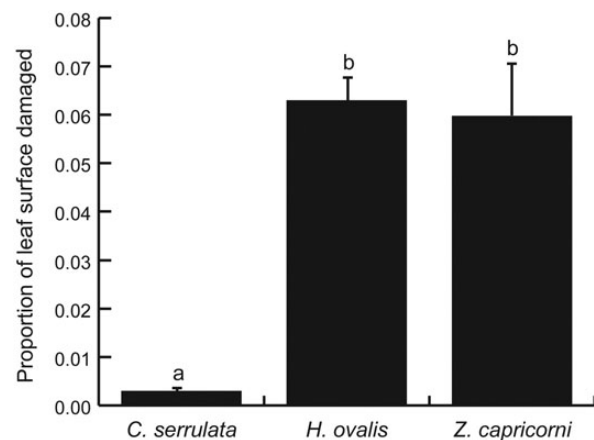


Figure 4. Average proportion of the leaf surface damaged after being exposed to *Smaragdia souverbiana* for 48 h in a single seagrass species treatment ($n = 6$). Error bars indicate standard error. Values range from 0 to 1. Bars with the same letter are not significantly different (at alpha $P < 0.05$,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

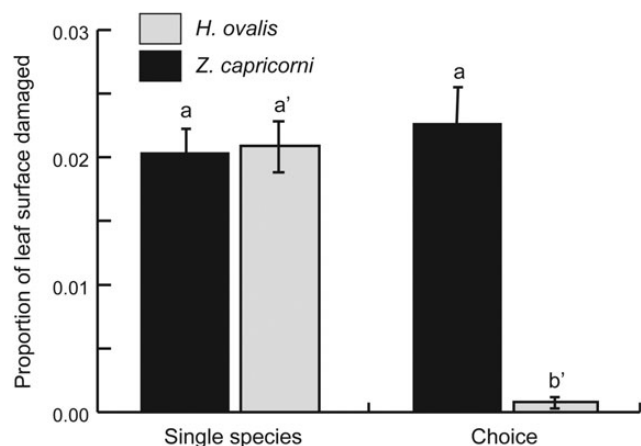


Figure 5. Proportion of leaf surface damaged after being exposed to *Smaragdia souverbiana* for 24 h in either a single seagrass species or choice treatment (both seagrass species available) ($n = 10$ per treatment). Error bars indicate standard error. Values range from 0 to 1. Bars with the same letter are not significantly different at alpha $P < 0.05$. Comparisons between seagrass species within treatment are not possible in this experimental design due to lack of independence.

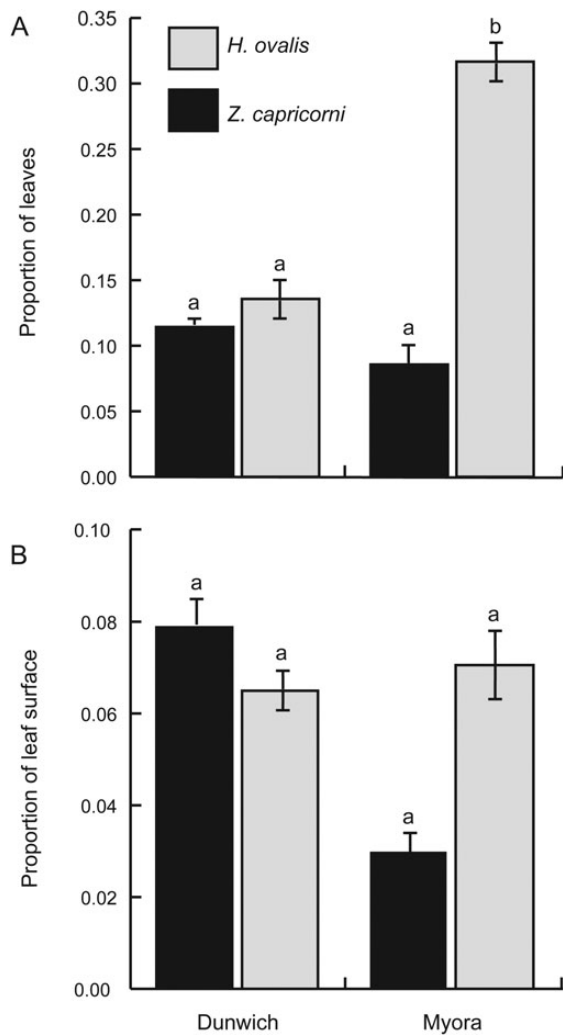


Figure 6. Proportion of leaves (of total leaf sample) and of leaf surface damaged for two seagrass species (light, *Halophila ovalis*; dark, *Zostera capricorni*) in the field at two sites shown as **A** the average proportion of sampled leaves showing signs of damage ($n = 10$ quadrats per site per species), and **B** the average proportion of leaf surface of damaged leaves that was grazed ($n = 5$ leaves per site per species). Error bars indicate standard error. Values range from 0 to 1. Bars with different letters within each graph are significantly different at alpha $P < 0.05$, ascertained using Tukey post-hoc test.

$n = 10$, $F = 9.8$, $P = 0.003$; 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 a significantly greater proportion of damaged leaves (Tukey HSD post-hoc: $n = 13$, $P = 0.008$). Differences in damage per leaf were not statistically significant (Fig. 6). No *C. serrulata* was encountered during sampling, so the damage occurring naturally to this species was not ascertained.

DISCUSSION

Smaragdia souverbiana were readily found at all sites and in all study periods, suggesting that they are a common element of seagrass assemblages in Moreton Bay. As has been shown in studies of other congeners (Rueda *et al.*, 2011; Unabia, 2011), *S. souverbiana* readily feeds on commonly available seagrasses, showing

a preference for the most locally abundant and most digestible species (*Zostera capricorni*). We argue that to include seagrass-feeding invertebrates such as *Smaragdia* with those feeding on epiphytes overlooks their functional individuality.

Unlike other representatives of the genus *Smaragdia* that feed mostly on seagrass, the diet of the majority of individuals of *S. souverbiana* assessed here included epiphytic material. Faecal analysis of field-caught individuals found that the epiphytic component of the faeces was generally equal to the seagrass component (Rossini & Rueda, pers. obs.). Unlike *S. viridis*, in which a seagrass-rich seam was directly visible in the faeces (Rueda & Salas, 2007), faeces of *S. souverbiana* were homogenous brown. *Smaragdia* will undoubtedly ingest epiphytes inadvertently when grazing seagrass, but the higher representation of this food type in *S. souverbiana* suggests that it may play a more important role in the diet of this particular species. In contrast to other *Smaragdia* species, *S. souverbiana* may have a two-fold influence on seagrasses, grazing on both epiphytes and on the seagrass itself.

Differences in methodology between this and previous studies mean that this study may have overestimated the contribution of epiphytes to the diet of *S. souverbiana* (Rueda *et al.*, 2009, 2011; Holzer, Rueda & McGlathery, 2011a, b; Unabia, 2011). While we measured the probability of an individual egesting each food type, others have given the proportion of the food type in the faeces of all sampled individuals. Epiphytic material was also found in the faeces of most *S. viridis* studied in the Mediterranean, although it represented a very small proportion of the faeces (Rueda, pers. obs.). In the present study, a small amount of epiphytes in the faeces of all individuals gave a high ranking to this food type, resulting in a potential overestimate of the importance of epiphytes. Furthermore, previous studies of *S. viridis* harvested seagrass and *Smaragdia* from deeper sites (2–7 m depth) (Rueda *et al.*, 2009, 2011; Holzer, Rueda & McGlathery, 2011a, b), where epiphytic assemblages are likely less developed due to light attenuation, compared with the intertidal seagrass beds studied here. Studies of *S. souverbiana* in equivalent habitats will aid in establishing if this higher representation of epiphytes in the diet of *S. souverbiana* is a reflection of its trophic ecology, habitat or this difference in methodology.

Smaragdia souverbiana damages and consumes two of the most common seagrasses it associates with, displaying a preference for *Z. capricorni*. Associations of other *Smaragdia* species with *Zostera* and *Halophila* have been described, such as that of *S. bryanae* (Unabia, 2011) and *S. rangiana* (Zuschin & Hohenegger, 1998; Zuschin, Janssen & Baal, 2009) with *Halophila*, and that of *S. viridis* with *Zostera* (Rueda & Salas, 2007, 2008; Rueda *et al.*, 2009), but this study is the first to find a simultaneous association with both, and adequately to demonstrate a preference for one of them. In the present study, preference was only observable when seagrasses were offered as a choice. Although *S. souverbiana* has a preference, its diet is flexible and it will consume equal amounts of less preferred food if its preferred food is unavailable, a pattern of flexibility similar to that observed in *S. viridis* in the Caribbean (Holzer, Rueda & McGlathery, 2011a) and the Alboran Sea (Rueda *et al.*, 2011). This represents an advantage for colonizing new areas and a safeguard against local extinction in areas where the availability of each seagrass is variable. Preference for *Z. marina* has been suggested in Mediterranean populations of *S. viridis* (Rueda *et al.*, 2011) and for *H. hawaiiiana* in Hawaiian populations of *S. bryanae* (Unabia, 2011) but these studies did not adequately test for preference in a choice/no-choice setup (see discussion by Underwood & Clarke, 2005). The results of experiments conducted here are testament to the importance of such design, because 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 is necessary if the assumption that seagrass herbivore

assemblages are predominantly generalist is to be challenged (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 for nontrophic reasons. The digestibility of seagrass tissues for *Smaragdia* is linked to their capability to break open cell walls while rasping and large-celled species appear easier to break and provide more cytoplasm per cell (Holzer, Rueda & McGlathery, 2011a; Rueda *et al.*, 2011). In the present study *Halophila 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, Zapato & Escobar, 1980). For small organisms, such as *Smaragdia*, food plants also serve as shelter (Reynolds, Carr & Boyer, 2012), providing protection from fish predators that have been shown to control populations of small grazing invertebrates (Lewis & Anderson, 2012). In our study area egg masses of *S. souverbiana* were only found on the base of sheaths of *Z. capricorni* (four observations, unpublished), indicating that associations with this seagrass species are multifaceted. As in terrestrial insect-grazing systems (and as demonstrated for *S. bryanae* by Unabia, 2011), suboptimal hosts can and will be used, but this can have major impacts on the survivorship or reproductive success of adults, and on fitness of the offspring (Thompson, 1988). Although it seems those species of *Smaragdia* studied to date can consume a suite of seagrass hosts, without adequate assessments of preference and studies of performance across the life cycle it is not known whether populations can be sustained on less preferred foods.

The ability of *S. souverbiana* to damage seagrass was highly variable in the field, reflecting the patchiness in populations and potential role of other seagrass grazers. High variability in the abundance of *Smaragdia* and functionally similar micrograzers occurs at both spatial (Zimmerman, Kohrs & Alberte, 1996; Rueda & Salas, 2008) and temporal scales (Bendell, 2006; Brearley, Kendrick & Walker, 2008; Rueda & Salas, 2008; Rueda *et al.*, 2009, 2011). This may result in highly variable patterns of damage to seagrass, often peaking during summer when abundance of dominant micrograzers reach maxima in some areas and habitat types (Rueda & Salas, 2008; Rueda *et al.*, 2009; Unabia, 2011). A more detailed study of the role of *S. souverbiana* would need to monitor grazing throughout the year, since both seasons addressed by the present study are likely to be outside the likely grazing peak in summer.

The genus *Smaragdia* is currently the subject of taxonomic revision, and it is possible that morphologically similar sympatric species of *Smaragdia* occupy similar seagrass habitats in eastern Australia, displaying local differences in their feeding ecology (Y. Kano & H. Fukumori, pers. comm.) and contributing to the variability of our field observations. 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.

Smaragdia souverbiana is not the only invertebrate feeding directly on seagrasses in this region; 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 obscured if they are combined into an epiphyte-only feeding guild, diverse functions within seagrass feeders should not be overlooked. It is often the complementarity of a diverse range of grazers, not the homogenous contribution of an identical set, that results in ecosystem-level effects (Duffy, Richardson & Canuel, 2003; Burkepile & Hay, 2008, 2011; Schmitz, 2008; Burkepile, 2013). During sampling we inadvertently collected small isopods and sacoglossans with seagrass (as mentioned in other studies, e.g. Bendell, 2006; Barnes, 2010; Barnes & Barnes,

2011), both of which have also been recorded to cause direct damage to seagrasses. The feeding ecology and population dynamics of the diverse representatives within the seagrass-feeding guild must be documented before their functional roles can be correctly classified.

Species of seagrass-feeding invertebrates like *S. souverbiana* have the potential to influence seagrass dynamics directly, differentiating them from epiphyte-controlling invertebrates in the same range of size and movement, and also from trophically similar but functionally distinct large seagrass grazers (Bjorndal, 1980; Preen, 1995; Kirsch, Valentine & Heck, 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). Existing literature focuses on and therefore reinforces the paradigm that invertebrates are predominantly epiphyte feeders that benefit seagrass indirectly (Valentine & Duffy, 2006), instead of investigating the diverse roles of the full suite of grazers. While functional grouping is vital for modelling the complex trophic dynamics of an ecosystem, we must be wary of ignoring functional diversity in the quest for generalization.

ACKNOWLEDGEMENTS

The authors would like to acknowledge a number of undergraduate volunteers from The University of Queensland and University of California Education Abroad Program (UCEAP) (Ian Jacobson, Amanda Heidt, Oliver Yuan, Lisa Cait McAlpine, Paula Ezcurra, Tiffany Takade, Jeffery Modlin, Chris Gow, Derek Smith and Dustin Ingagiola), whose passion, interest and advocacy for these miniscule snails is reflected in the quality of the present study. We thank Dr Dana Burfiend for her ongoing promotion and support of this project. We thank the staff of Moreton Bay Research Station for their tireless efforts and ingenuity. Comments from Lucy Hurrey, Yasunori Kano, David Reid and two anonymous reviewers greatly improved this manuscript. Voluntary financial and time contributions from J. Rueda and R. Rossini, and the University of Queensland's School of Biological Sciences International Programs Office provided all equipment and salaries supporting this study.

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