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Differences in architecture between native and non-indigenous macroalgae influence associations with epifauna

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Abstract

Non-indigenous invaders may play ecologically similar roles to native species, and this may be reflected in the abundance, richness and composition of associated species assemblages. We investigated whether associations of epifauna with their macroalgal hosts differed between the non-indigenous Codium fragile ssp. fragile and native, congeneric C. fragile on three rocky shores in southeast Australia. Of the 38 taxa we recorded, 13 were unique to the native Codium and four to non-indigenous individuals. Holdfasts of non-indigenous Codium had double the taxon richness of epifauna compared to native holdfasts, and epifaunal abundances showed a similar but non-significant difference. Patterns of abundance and richness of epifaunal taxa on thalli of native and non-indigenous Codium varied depending on whether these measures were expressed per individual alga, thallus area or number of branches. The composition of epifaunal assemblages between native and non-indigenous Codium were significantly different, but differences among rocky shores were as great as those between macroalgal species. On all shores, two taxa, the gastropod Alaba opiniosa and gammarid amphipods, contributed most to compositional differences between native and non-indigenous Codium, and their abundances were influenced by branch number and associated epiphyte load. Host choice experiments manipulating the complexity and subspecies of Codium revealed that amphipods were more strongly influenced by branch number adjusted for epiphyte load than the identity of Codium. Our results highlight the importance of habitat features, such as structural complexity and associated epiphyte load, in determining whether native and non-indigenous species provide functionally equivalent habitats for associated assemblages.

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DIFFERENCES IN ARCHITECTURE BETWEEN NATIVE AND NON-INDIGENOUS MACROALGAE INFLUENCE ASSOCIATIONS WITH EPIFAUNA

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ABSTRACT: Non-indigenous invaders may play ecologically similar roles to native species, and this may be reflected in the abundance, richness and composition of associated species assemblages. We investigated whether associations of epifauna with their macroalgal hosts differed between the non-indigenous *Codium fragile* ssp. *fragile* and native, congeneric *C. fragile* on three rocky shores in southeast Australia. Of the 38 taxa we recorded, 13 were unique to the native *Codium* and four to non-indigenous individuals. Holdfasts of non-indigenous *Codium* had double the taxon richness of epifauna compared to native holdfasts, and epifaunal abundances showed a similar but non-significant difference. Patterns of abundance and richness of epifaunal taxa on thalli of native and non-indigenous *Codium* varied depending on whether these measures were expressed per individual alga, thallus area or number of branches. The composition of epifaunal assemblages between native and non-indigenous *Codium* were significantly different, but differences among rocky shores were as great as those between macroalgal species. On all shores, two taxa, the gastropod *Alaba opiniosa* and gammarid amphipods, contributed most to compositional differences between native and non-indigenous *Codium*, and their abundances were influenced by branch number and associated epiphyte load. Host choice experiments manipulating the complexity and subspecies of *Codium* revealed that amphipods were more strongly influenced by branch number adjusted for epiphyte load than the identity of *Codium*. Our results highlight the importance of habitat features, such as structural complexity and associated epiphyte load, in determining whether native and non-indigenous species provide functionally equivalent habitats for associated assemblages.

KEY WORDS: *Codium* · Habitat structure · Invasive species · Host preference · Rocky intertidal shores

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1. Introduction

Marine macroalgae provide important habitat for many organisms, and epibiosis is a common strategy employed by a large range of algal and sessile invertebrate species (Crooks, 2002; Kumagai, 2008). Invasion by non-indigenous macroalgae has the potential to modify significantly the structure of the habitat and associated patterns of abundance and composition of native epibiota, in turn altering native biodiversity and ecosystem processes (Wikstrom and Kautsky, 2004; Drouin et al., 2011; Arnold et al., 2016). To what extent invasive macroalgae can modify native assemblages of epibiota depends largely on the ability of the epibionts to colonise the invader, specificity of the epibiont organisms for hosts, as well as the ability of the invader to sustain and protect epibionts through increased habitat complexity (Wikstrom and Kautsky, 2004; Buschbaum et al., 2006).

It is generally thought that most marine epibionts are largely non-specific in their choice of host and that the vast majority can associate with a range of macroalgal species (Hay and Fenical, 1988; Taylor and Brown, 2006). Such generalist epibionts may be less influenced by invasion of non-indigenous macroalgae compared with specialist epibionts, because they can choose hosts that afford the greatest protection or habitat value at a particular place or time (Wikstrom and Kautsky, 2004; Buschbaum et al., 2006; Bates and DeWreede, 2007). Moreover, similarities in size, morphological complexity or chemical composition, for example, between native and non-indigenous algal hosts is also likely to influence host choice in native epibiota (Buschbaum et al., 2006; Lyons et al., 2007; Veiga et al. 2014).

Evidence of impacts of non-indigenous macroalgae on native epibiont communities is conflicting (Wikstrom and Kautsky, 2004). Although some studies have found that invasive macroalgae have only limited impact on species richness and abundance of epibionts (e.g., Viejo, 1999;

Wernberg et al., 2004), others have found that species assemblages of epibionts vary significantly between native host algae and non-indigenous species (e.g., Bellan-Santini et al., 1996; Wikstrom and Kautsky, 2004; Buschbaum et al., 2006; Schmidt and Scheibling, 2006; Gestoso et al., 2010; Guerra-García et al., 2012; Veiga et al., 2014; Dijkstra et al., 2017).

The non-indigenous green macroalga *Codium fragile* ssp. *fragile* (Suringar) Hariot (formerly *C. fragile* ssp. *tomentosoides*, Provan et al., 2008), which is thought to be native to Japan and the north Pacific region, has been ranked among the top five macroalgae worldwide with the greatest risk of becoming invasive (Silva, 1955; Trowbridge, 1998; Nyberg and Wallentinus, 2005; Provan et al., 2008). This subspecies has spread to many shores worldwide during the last century and is recognised as a significant ecosystem engineer (Bégin and Scheibling, 2003). Indeed, subspecies *fragile* is considered a nuisance species across much of its invaded range, primarily because of its high biomass, ability to outcompete resident algal species and tendency to attach to other organisms, such as commercially important shellfish (Trowbridge, 1999; Garbary et al., 2004).

Although the impacts of *C. fragile* ssp. *fragile* have been documented across rocky shores worldwide (Trowbridge, 1999), it is only considered to be a major economic and environmental pest in the northwest Atlantic Ocean and on southern shores of Australia (Chapman, 1999; Lutz et al., 2010). In Australia, where *C. fragile* ssp. *fragile* was first recorded in 1995, two native conspecific subspecies, *C. fragile* ssp. *tasmanicum* and *C. fragile* ssp. *novae-zelandiae*, and numerous native congeners (e.g., *C. harveyi*, *C. spinescens* and *C. australicum*) coexist along much of the temperate coastline (Silva and Womersley, 1956). Despite these close associations between native and non-indigenous *C. fragile* subspecies, and thus potential for impacts of invasion, there has been remarkably little investigation of the influence of non-indigenous *C. fragile* ssp. *fragile* on native species assemblages (Trowbridge, 1999; Lutz et al., 2010).

Here we investigated through detailed field sampling whether the invasion of *C. fragile* ssp. *fragile* has the potential to modify assemblages of associated epifauna compared to those on native *C. fragile* subspecies. Our investigation focussed on rocky intertidal shores in the Illawarra region of New South Wales, Australia. We also tested the prediction that compositional differences in epifaunal species assemblages were related to differences in the physical structure between the native and non-indigenous *C. fragile* subspecies, and the key epifaunal taxa contributing strongly to compositional differences were also identified. Finally, based on these findings, we used laboratory experiments to determine whether host choice of gammarid amphipods, which are commonly associated with *C. fragile* subspecies, was strongly influenced by macroalgal thalli with different levels of structural complexity.

2. Materials and Methods

The study was done in the austral summer and autumn (February to July) on rocky intertidal shores along the Illawarra coastline of New South Wales, Australia. The rocky intertidal shores of this region consist predominantly of shallow marine sandstone with wave-cut platforms and boulders. The intertidal region is generally exposed to medium to high levels of wave energy (Dakin, 1987) and experiences tidal ranges of up to 2 m.

2.1. Assemblages of epifauna on native and non-indigenous *Codium fragile* subspecies

To examine differences in assemblages of epifauna between native and non-indigenous *Codium fragile* subspecies, we collected samples at three rocky intertidal locations: Bulli (34º20′ 19.49″S, 150º55′ 34.73″E), Towradgi (34º23′ 10.35″S, 150º54′ 55.81″E) and Gerringong (34º45′ 02.64″S, 150º49′ 55.51″E). These locations were chosen because they had relatively high abundances of both native and non-indigenous *C. fragile* subspecies. For brevity we hereafter refer to native *C. fragile* ssp. *tasmanicum* and ssp. *novae-zelandiae* collectively as native *Codium*, and *C. fragile* ssp. *fragile* as non-indigenous *Codium*. Native subspecies had to be combined because they could not be readily distinguished in the field.

At each location we randomly selected *Codium* in the low to mid shore region where both native and non-indigenous *Codium* subspecies co-occurred. Due to the discontinuous nature of the rock platforms, we targeted three areas (2 m x 4 m) at each location to ensure we obtained samples from across the entire location. *Codium* was haphazardly collected from each site, and then eight individuals of each of the native and the non-indigenous subspecies were randomly chosen for comparison of epifauna at the three locations. Individuals were removed by cutting the thallus above the holdfast, and then prising the holdfast from the substratum. The thallus and holdfast of each individual were placed in separately labelled bags and immediately returned to the laboratory and placed in a freezer. After thawing, thalli and holdfasts were washed with freshwater over a 500µm sieve. Epifauna on thalli and holdfasts were examined separately because they are different morphological structures that are predicted to support different species assemblages (Schmidt and Scheibling, 2006; Lutz et al., 2010).

Epifauna were identified to the lowest possible taxon and the number of individuals for each taxon was recorded. The taxonomic richness (i.e. number of taxa) and abundance of epifauna on the thallus were standardised in three ways to determine how observed differences in the physical structure between native and non-indigenous *Codium* might influence species assemblages of epifauna. We present non-standardised outcomes and three standardised measures: thallus surface area (i.e. per 100cm^2), number of branches per thallus (i.e. per 100 branches) and biomass (g wet weight).

The total thallus surface area was calculated as the sum of the surface area of each branch and stipe (i.e. measured as the surface area of a cylinder: π × diameter × length). Due to the large number of branches per thallus it was impractical to measure surface area for each branch when calculating total thallus surface area. Therefore, a study was done to determine the minimum number of branches that could be measured while still obtaining an accurate estimate of thallus surface area. The results showed that the total thallus surface area could be estimated with minimal error (4.45% ± 1.45 for non-indigenous *Codium* and 8.13% ± 1.51 for native *Codium*) using a random subset of 30 branches. The surface area of each thallus was subsequently estimated as the mean surface area of 30 branches multiplied by the total number of branches per thallus.

Percentage cover of epiphytes on the thallus and holdfast was estimated as described by Lutz et al. (2010). The thallus was stratified into four sections: (1) the stipe; (2) <3 cm above the stipe; (3) 3-6 cm above the stipe; and (4) >6 cm above the stipe. A quadrat (1 cm x 1 cm transparency) was randomly placed over each of the four sections and the holdfast. The proportion of points overlying epiphytes or bare *Codium*, out of a total of 16 uniformly spaced points, was then recorded. Epiphyte cover on the entire stipe and holdfast were quantified using this approach.

For the larger sections (2, 3 and 4), five randomly placed replicate quadrats were sampled on either side of the thallus. A binocular microscope (Leica) at 6.4 x magnitude was used.

Two-factor, mixed model analysis of variance (ANOVA) was used to determine differences in the taxonomic richness and number of epifauna on the thallus and holdfast of native and non-indigenous *Codium* subspecies (fixed factor) at three locations (Bulli, Towradgi, Gerringong; random factor). Normality of the data was assessed visually and Cochran's C test was used to confirm homogeneity of variances. Data were transformed as necessary to conform to assumptions of normality and homogeneity of variance (Zar, 1999). If the transformation did not render variances homogeneous, analyses were done on untransformed data, and if variances were homogeneous at $P = 0.01$, but not at $P = 0.05$, then $P = 0.01$ was used as the probability level to assess significance in ANOVA (Underwood 1981). Likewise, if variances were not homogeneous at $P = 0.001$, but not at $P = 0.01$, then $P = 0.001$ was used as the probability level. Post-hoc pooling of factors was undertaken as necessary and where appropriate to increase the power of tests (Underwood, 1997). If significant differences were detected in ANOVA, post-hoc comparison among means was undertaken using Student-Newman-Keuls (SNK) tests. We used GMAV5 (University of Sydney) statistical software for these analyses. The effects of algal complexity and epiphyte cover on epifaunal taxa abundance and richness, the number of *Alaba opioniosa* and the number of gammarid amphipods on native and non-indigenous *Codium* across the study locations were investigated using correlation analysis.

Differences in species assemblages of epifauna between native and non-indigenous *Codium* and among locations were determined using PERMANOVA (Anderson, 2001). These contrasts were made with Bray-Curtis indices using untransformed data. Two-dimensional, non-metric multidimensional scaling (nMDS) was used to assess graphically these compositional differences. Similarity percentage analysis (SIMPER) was then used to assess the percentage contribution of epifaunal taxa to similarities within and dissimilarities between *Codium* subspecies. Average similarity (Sim/SD) and dissimilarity (Diss/SD) values identified taxa with large contributions to these similarities and dissimilarities; taxa with an average Diss/SD greater than 1.5 were considered to be good discriminators between native and non-indigenous *Codium* subspecies and among locations (Clarke, 1993; Clarke and Gorley, 2000). PERMANOVA was also done using presence/absence-transformed data to identify compositional differences due to rare or less abundant taxa.

2.2 Host choice experiments between native and non-indigenous *Codium fragile* subspecies

We investigated host preference of a common gammarid amphipod, genus *Hyale*, between native and non-indigenous *Codium* possessing thalli of different levels of structural complexity. *Hyale* spp. were used because surveys showed that they were abundant and commonly associated with *Codium* (Lutz unpublished data) and thus may be responsive to small-scale differences in thallus complexity and macroalgal identity. Preference trials were run in containers (48L) where individual amphipods were added to one of four experimental trials (n = 7 replicates per trial): (i) one piece each of native and non-indigenous *Codium*, both with low thallus complexity; (ii) one piece each of native and non-indigenous *Codium*, both with high thallus complexity; (iii) one piece each of native *Codium* with low or high thallus complexity; and (iv) one piece each of non-indigenous *Codium* with low or high thallus complexity. Trials (i) and (ii) investigated the effects of algal identity only on amphipod host preference, excluding the effects of algal thallus complexity, and trials (3) and (4) investigated the effects of algal thallus complexity on amphipod host preference, excluding the effects of algal identity.

A thallus with low complexity was defined as one with a surface area between 50 and 200cm^2, and a thallus with high complexity had a surface area between 250 and 400cm^2. The surface area of each piece of *Codium* was estimated from wet weight-surface area relationships, which confirmed that there was a strong correlation between these variables for both native ($R = 0.918$, $P < 0.0001$, $n = 24$ thalli) and non-indigenous ($R = 0.924$, $P < 0.0001$, $n = 24$ thalli) *Codium* (Lutz unpublished data). Surface area was therefore similar between pieces of native and non-indigenous *Codium* at the same level of complexity (i.e. trials 1 and 2). For trials with only native or non-indigenous *Codium* (i.e. trials 3 and 4), thalli were manipulated as necessary by adding or removing fronds and branches to match each complexity category. To ensure that wound formation created by removal from the field and frond and branch manipulations did not influence host choice, *Codium* thalli were placed in containers with seawater for 24 to 48 h to allow minor wounds on thalli to heal (Poore 2004).

Native and non-indigenous *Codium* and *Hyale* spp. used in the experiment were collected from Gerringong. *Hyale* spp. were maintained in aerated seawater with fragments of native and non-indigenous *Codium* to ensure acclimation to laboratory conditions. While *Hyale* spp. were obtained by shaking *Codium* vigorously in seawater, other epifauna were removed by submerging *Codium* thalli in fresh water for up to 60 seconds, followed by a short submersion in seawater, and then another 60 seconds in fresh water (Roberts and Poore, 2005). This ensured that re-colonisation would not be affected by the presence of epifauna. Epiphyte load might also influence host choice, but epiphytes could not be removed without substantial damage to the macroalgae and, therefore, the cover of epiphytes was recorded for each *Codium.*

Trials were run in large plastic tubs (48L) to ensure that *Codium* fragments did not touch each other or the sides of the containers, both of which may affect the behaviour of *Hyale* spp. Using a pipette, individual amphipods were placed in the water column at the centre of containers and equal distances from the weighted *Codium* pieces. Only single *Hyale* spp. (9-19mm in length) were added to each replicate in a trial because the addition of many individuals could confound host choice. After one night (approximately 15 to 20 h), *Codium* pieces were removed from containers and plunged into fresh water to remove *Hyale* spp. A chi-square test was used to determine differences in amphipod colonisation between native and non-indigenous *Codium* and levels of thallus complexity, with Yates' correction for continuity applied because expected frequencies were less than five.

3. Results

3.1 Richness and abundance of epifaunal taxa

A total of 6042 epifaunal individuals across 38 taxa were recorded on thalli and holdfasts of 48 native and non-indigenous *Codium* individuals (Appendix 1). Of these 38 taxa, 13 were unique to the native *Codium* subspecies (e.g. the gastropod *Patelloida mufria*) and four to the non-indigenous individuals (e.g. the gastropod *Bembicium nanum*). Thirty-one taxa were found on thalli, 27 on native and 24 on non-indigenous *Codium*, and 19 taxa were found on holdfasts, 17 on native and 13 on non-indigenous *Codium*. Furthermore, 19 and seven taxa were restricted to the thalli and holdfasts, respectively. For example, the bivalve *Irus crenatus* and the gastropod *Thais orbita* were restricted to holdfasts, whereas the gastropod *Cantharidella picturata* and the decapod *Hippolyte australiensis* were exclusive to thalli. Most taxa of epifauna were present at all three locations, but 21 were restricted to single locations; for example, the gastropod *Bembicium nanum* and the bivalve *Notirus* sp. were only found at Bulli and Towradgi, respectively.

Patterns of taxon richness and abundance of epifauna on native and non-indigenous *Codium* at each location differed depending on whether richness or abundance was expressed as a function of non-standardised, thallus area, wet weight or number of branches (Fig. 1). The number of epifaunal taxa per thallus area did not vary significantly between *Codium* subspecies, but it did among locations, with Bulli and Towradgi both having approximately twice the taxon richness as *Codium* at Gerringong (Fig. 1c, Table 1, SNK tests). In contrast, epifaunal taxon richness per branch was significantly greater on native than non-indigenous *Codium* at both Towradgi and Gerringong, but not at Bulli (Fig. 1e, Table 1, SNK tests).

The number of epifauna per thallus area varied significantly between native and non-indigenous *Codium*, but this difference varied among locations, with non-indigenous thalli having more than twice the number of individuals than native thalli at Bulli only (Fig. 1d, Table 1, SNK tests). Similarly, the number of epifaunal taxa per branch was about two times higher on non-indigenous *Codium* thalli than native thalli at Bulli, whereas the opposite trend was observed for Gerringong, where epifaunal density was approximately seven times higher on native than non-indigenous thalli (Fig. 1f, Table 1, SNK tests).

On holdfasts of *Codium* epifaunal taxon richness was significantly different between native and non-indigenous *Codium*, with non-indigenous holdfasts having twice the taxon richness of native holdfasts (Fig. 1i, Table 1). Density of epifauna on *Codium* holdfasts show a similar pattern, but did not vary significantly between native and non-indigenous subspecies or among locations (Fig 1h, j, Table 1).

[Insert Table 1]

[Insert Fig. 1]

3.2 Effects of *Codium fragile* structure on epifaunal taxon richness and abundance

The effect of branch number on both taxon richness and number of epifauna varied significantly between native and non-indigenous *Codium* across locations (taxon richness: $t_{1,44} = 2.73$, $P < 0.05$, n = 24 thalli; density: $t_{1,44} = 2.59$, $P < 0.05$, n = 24 thalli) (Fig. 2a, c). The number of epifaunal taxa was not significantly correlated to branch number on non-indigenous *Codium*, whereas it increased significantly with branch number for native *Codium* (non-indigenous: R = 0.305, $P = 0.148$, n = 24 thalli; native: R = 0.445, $P = 0.030$, n = 24 thalli) (Fig. 2a). Similarly, the number of epifauna was not significantly correlated with branch number on non-indigenous *Codium*, but increased significantly with increasing branch number on native *Codium* (non-indigenous: R = 0.171, *P* = 0.426, n = 24 thalli; native: R = 0.586, *P* = 0.003, n = 24 thalli) (Fig. 2d).

[Insert Fig. 2]

3.3 Composition of epifaunal species assemblages

There were significant differences in the composition of epifaunal species assemblages between native and non-indigenous *Codium*, and among locations. This was the case for the assemblages on the thallus ($F_{2,\,42}$ = 3.75, P = 0.001), and the entire alga (thallus and holdfast combined) ($F_{2,\,42}$ = 3.42, P = 0.001), but not for the holdfast ($F_{2,\,42}$ = 1.53, P = 0.105) (Fig. 3; PERMANOVA analyses). The greatest dissimilarity in composition of epifauna between native and non-indigenous *Codium* was at Bulli (62.94%), followed by Towradgi (59.44%) and Gerringong (44. 73%) (Table 2). The composition of epifaunal species varied among locations as least as much as among native and non-indigenous *Codium*, with assemblages at Gerringong less similar to those at the other locations. Given the substantial differences between native and non-indigenous *Codium* among locations, separate SIMPER analyses were done between native and non-indigenous *Codium* at each location to determine which epiphyte species contributed to the compositional differences.

[Insert Fig. 3]

[Insert Table 2]

The gastropod *Alaba opiniosa* and gammarid amphipods were the most common and abundant epifauna and contributed strongly to compositional dissimilarities between native and non-indigenous *Codium* at all three locations (Table 2; SIMPER analyses). The abundance of *Alaba opiniosa* and gammarid amphipods varied significantly between native and non-indigenous *Codium* subspecies, but the direction of these differences varied between taxa and among locations (Fig. 4; Table 2).

3.4 Abundance of common epifauna

Alaba opiniosa and gammarid amphipods were identified as the best discriminators of epifaunal composition between native and non-indigenous *Codium* (Table 2; SIMPER analyses). Abundances of both *A. opiniosa* and gammarid amphipod varied significantly between native and non-indigenous *Codium* thalli, but this difference varied among locations (Table 2, Fig. 4). *Alaba opiniosa* was more than three times as abundant on the non-indigenous *Codium* as on native *Codium* at Bulli, whereas there was no significant difference at Towradgi and Gerringong where *A. opiniosa* was not abundant. In contrast, the abundance of gammarid amphipods at Gerringong was approximately seven times greater on native than on non-indigenous *Codium*, whereas there was no significant difference at Bulli or Towradgi where there were fewer amphipods.

There was a significant difference in the relationship between the abundance of *Alaba opiniosa* and the number of branches between native and non-indigenous *Codium* ($t_{1,44} = 3.97$, $P < 0.05$, n = 24 thalli) (Fig. 2e). On native *Codium*, the abundance of *A. opiniosa* increased significantly with increasing number of branches, whereas on non-indigenous *Codium* the abundance of *A. opiniosa* decreased significantly with increasing branch number (native: R = 0.575, $P = 0.003$, n

= 24 thalli; non-indigenous: R = -0.513, *P* = 0.010, n = 24 thalli). The relationship between the abundance of gammarid amphipods and the number of branches did not vary significantly between native and non-indigenous *Codium* ($t_{1,44}$ = 0.29, *P* < 0.05, n = 24 thalli). Overall for both *Codium* subspecies, the abundance of gammarid amphipods increased significantly with increasing number of branches (R = 0.328, *P* = 0.023, n = 48 thalli).

Epiphyte cover had a marked effect on the abundance of *A. opiniosa* and gammarid amphipods (Fig. 2). There was a significant difference in the relationship between the abundance of *A. opiniosa* and epiphyte cover between native and non-indigenous *Codium* ($t_{1,44}$ = 4.18, *P* < 0.05, n = 24 thalli). The abundance of *A. opiniosa* increased significantly with increasing epiphyte cover on non-indigenous *Codium*, whereas the abundance decreased with increasing epiphyte cover on native *Codium* (non-indigenous: R = 0.601, *P* = 0.002, n = 24 thalli; native: R = 0.462, *P* = 0.023, n = 24 thalli). There was a significantly different relationship between gammarid amphipod abundance and epiphyte cover between native and non-indigenous *Codium* ($t_{1,44}$ = 3.46, *P* < 0.05, n = 24 thalli). Gammarid amphipod abundance increased significantly with increasing epiphyte cover on native *Codium*, but did not vary significantly with increasing epiphyte cover on non-indigenous *Codium* (native: R = 0.660, *P* = 0.001, n = 24 thalli; non-indigenous: R = 0.124, *P* = 0.564, n = 24 thalli).

3.5 Experimental examination of host choice by a common epifaunal taxon: Gammarid amphipods

Gammarid amphipods (*Hyale* spp.) showed no difference in its choice between native and non-indigenous *Codium*, regardless of whether thallus morphology was simple (χ^2 = 1.143, $P > 0.05$; proportion native = 0.714; proportion non-indigenous= 0.286) or complex (χ^2 =1.143, $P > 0.05$; proportion native = 0.286; proportion non-indigenous = 0.714). When exposed only to native *Codium*, individuals of *Hyale* spp. showed no significant preference for thalli with low or high complexity (χ^2 =1.143, $P > 0.05$; proportion low = 0.286, proportion high = 0.714).

In contrast, *Hyale* spp. settled significantly more often on non-indigenous *Codium* with high complexity than on non-indigenous *Codium* with low complexity (χ^2 = 4.571, $P < 0.05$; proportion low = 0.143; proportion high = 0.857). Furthermore, when pooling the data for native and non-indigenous *Codium*, individuals of *Hyale* ssp. showed a strong preference for thalli with high complexity over thalli with low complexity (χ^2 = 7, $P < 0.05$), but not between native and non-indigenous *Codium* (χ^2 = 0.143, $P > 0.05$). These results were independent of epiphyte load, because the cover of epiphytes did not vary significantly between native and non-indigenous *Codium* or between thalli with low or high complexity (Table 3).

[Insert Fig. 5]

[Insert Table 3]

4. Discussion

The present study shows that the composition of epifaunal assemblages did not only differ significantly between native and non-indigenous *Codium fragile* subspecies, but also varied substantially among rocky intertidal shores in southeastern Australia. Generally, the invasive *C. fragile* ssp. *fragile* supported substantially fewer epifaunal taxa but higher abundances of those that were present. This indicates that *C. fragile* ssp. *fragile* is not functionally equivalent as epifaunal habitat to native *C. fragile* subspecies. Consequently, the invader is likely to modify diversity and composition of epifauna on these shores, leading to the potential homogenisation of epifaunal assemblages. Furthermore, species richness and abundance varied with the architecture of the alga (such as branch number and surface area), which might at least partially explain the lack of functional equivalence between the subspecies (Harvey et al., 2010).

Our findings also emphasise the importance of spatial variation in epifaunal assemblages. The gastropod *Alaba opiniosa* and gammarid amphipods of *Hyale* spp. were the most abundant taxa and the main contributors to differences between *C. fragile* ssp. *fragile* and native *C. fragile* subspecies at all locations. *Alaba opiniosa* was substantially more abundant on *C. fragile* ssp. *fragile* at Bulli, whereas gammarid amphipods were more abundant on native subspecies at Gerringong. These outcomes argue strongly for the inclusion of multiple locations when examining potential impacts of non-indigenous species.

Worldwide, little is known about the effects of *C. fragile* ssp. *fragile* on assemblages of native epibiota and the mechanisms driving assemblage change. We found that *C. fragile* ssp. *fragile* on rocky intertidal shores supported fewer epifaunal taxa across all locations and generally greater gastropod abundance than native subspecies. In contrast, Schmidt and Scheibling (2006) reported a high diversity of epibiota on the invasive *C. fragile* ssp. *fragile* relative to native kelp in shallow subtidal waters off Nova Scotia, Canada. These differences likely reflect habitat selection by epifauna attributable to variation in chemical, structural and morphological characteristics between the algal species, whereas we compared very closely related taxa. Buschbaum et al. (2006), for example, found that a macroalgal invader and native macroalgae had similar epibiont communities on rocky shores, whereas the invader in sedimentary habitats supported a higher diversity of epibionts by providing additional habitat. Therefore, variations in habitat and geography are also likely to be important when examining the influence of non-indigenous *Codium* compared to native species. However, non-indigenous species may not only impact species richness and diversity, but they also have the potential to significantly alter trophic structures potentially leading to dramatic ecological changes in communities (Harvey et al., 2010).

Epiphyte load has been shown to vary spatially and substantially between native and non-indigenous *C. fragile* subspecies on rocky intertidal shores in Australia (Lutz et al., 2010). Greater epiphyte load may increase the structural heterogeneity of the surface of the host alga, in turn increasing the space available for epifaunal colonisation, as well as providing protection from predators (Christie et al., 2007). Indeed, both epifaunal abundance and epiphyte load were greater on non-indigenous *C. fragile* ssp. *fragile* than native subspecies at Bulli, whereas both epifaunal abundance and epiphyte load were greater on native *C. fragile* at Gerringong (see also Lutz et al., 2010). Moreover, the abundance of *A. opiniosa* was enhanced by epiphyte load on *C. fragile* ssp. *fragile*. These relationships provide evidence for the indirect role of epiphytes in structuring epifaunal assemblages on native and non-indigenous host algae. The variation in epiphyte load between *C. fragile* subspecies across locations might thus account for the extensive spatial variation in epifaunal assemblages, but this is yet to be substantiated by any empirical study (Lutz et al., 2010).

The structurally complex *C. fragile* ssp. *fragile* supported a greater abundance of epibionts than native subspecies at some locations, consistent with evidence for the role of host thallus complexity in structuring epibiont communities (Arrontes, 1999; Chemello and Milazzo, 2002; Schmidt and Scheibling, 2006; Veiga et al., 2014, 2016; Dijkstra et al., 2017). Similar facilitative effects of the complex structure of thalli on epibionts have been reported elsewhere (e.g., Arrontes, 1999; Chemello and Milazzo, 2002), including protection from predation, desiccation and amelioration of high wave energy.

Unexpectedly, for some common epifaunal taxa, such as *A. opiniosa*, there was a negative relationship between their abundance and thallus complexity (i.e. number of branches per thallus) of *C. fragile* spp. *fragile*, indicating that other factors, including epiphyte load, wave exposure, desiccation stress and perhaps chemical defence of the host alga, likely influence epifaunal assemblages. Drouin et al. (2011) found that while epifaunal abundance and taxon richness was positively linked to thallus biomass of *C. fragile* ssp. *fragile*, the abundance of invertebrates and grastropods was not affected by thallus complexity when the thallus structure was manipulated. Therefore, while structural complexity is an important influence on the composition of epibiont assemblages, it is unlikely to be the only factor responsible for differences in epibiont assemblages between native and non-indigenous *C. fragile* subspecies. Finally, *C. fragile* ssp. *fragile* has been shown to use secondary wound-activated metabolites as chemical defences against herbivory and that these chemicals reduce the incidence of herbivory by grazers (Lyons et al., 2007). Indeed, some studies have demonstrated that *C. fragile* ssp. *fragile* is avoided by generalist herbivores (e.g., Malinowski and Ramus, 1973; Hanisak, 1980;

Trowbridge, 1995). Although evidence for the use of chemical defences by *Codium* species is scant, it is possible that they account in some way for differences in epibiont diversity and composition observed here.

There is no evidence to suggest that differences in (or the absence of) epifaunal herbivores that might not be able to feed on non-indigenous *Codium* potentially facilitate its invasion. There was no difference in the presence of two common herbivorous slugs, *Placida dendritica* and *Elysia maoria*, for either native or non-indigenous *C. fragile* subspecies. Similarly, in Australia Trowbridge (2004) reported that the common sacoglossan slug *Placida aoteana* fed equally on the non-indigenous and native *Codium* subspecies.

The common epifaunal gammarid amphipod, *Hyale* spp., showed no preference for either the non-indigenous *C. fragile* ssp. *fragile* or native *C. fragile* subspecies as hosts. Rather, *Hyale* spp. preferred to take up residence on complex algal thalli, regardless of host identity. While the presence of epiphytes has been shown to influence habitat selection in some marine amphipods (e.g., Christie et al., 2007), it is unlikely that epiphytes influenced host choice of *Hyale* spp. since epiphyte cover did not vary between trials. Schmidt and Scheibling (2006) found that *C. fragile* ssp. *fragile*, which has a complex thallus architecture, and a native kelp, which has a relatively simple structure, supported distinct epifaunal assemblages. They suggested that differences in habitat selection by epifauna could most likely be attributed to variation in chemical, structural and morphological characteristics between the algal species. Using feeding assays, Cacabelos et al. (2010) compared the preferences of grazers for either native or non-indigenous seaweeds. Their results suggested that grazers prefer native seaweeds, but the results varied depending on the grazers, with one sea urchin showing no preference for either native or non-indigenous seaweed.

In addition to the dramatic differences in epibiont assemblages between native and non-indigenous *C. fragile*, there was extensive spatial variation in composition among rocky shore locations. For instance, the abundance of epifauna was greater on non-indigenous than native *C. fragile* at Bulli, while the opposite trend occurred at Gerringong. Similar spatial variation in epibiont assemblages has been found within and between numerous intertidal habitats worldwide (e.g., Harris and Jones, 2005; Buschbaum et al., 2006; Torres et al., 2015). For example, Harris and Jones (2005) investigated populations of *C. fragile* spp. *fragile* on the Isles of Shoals, USA, and reported that densities of epifauna varied between locations, with higher densities occurring at sheltered compared to wave exposed sites. Although we did not investigate wave exposure in relation to epibiont assemblages on native and non-indigenous *C. fragile*, it was most likely a minor factor because the three locations we sampled were exposed to moderate to strong wave action. In addition, *C. fragile* ssp. *fragile* has also been shown to vary seasonally between microhabitats (e.g. rock pools versus lower intertidal) and locations on rocky shores in Australia and New Zealand (Trowbridge, 1996, 1999; Campbell, 1999), but such effects would not account for differences here because all samples were collected from areas of emergent rock at the same time. What is responsible for these differences in our study is not known, but importantly we still could detect differences in epibiont assemblages between native and non-indigenous *Codium* despite this variation, indicating that substantially difference assemblages are responding in the same way.

In conclusion, *C. fragile* ssp. *fragile* supports different assemblages of epifauna than the native subspecies, with fewer epifaunal taxa but with more individuals, and this increase in the abundance of a few dominant taxa may elicit adverse effects on ecosystem structure and function through altered food webs and trophic interactions. Despite this, extensive spatial variation in epibiont assemblages also exists, making it difficult to predict impacts of *C. fragile* ssp. *fragile* on native communities in all invaded areas. This study has identified that host complexity and epiphyte load are important factors structuring epibiont assemblages, although other unstudied factors, such as seasonality and chemical structure of the host alga, as well as habitat type and wave exposure are also likely to be influential and are deserving of attention. The study validates concerns that the presence of the invasive *C. fragile* ssp. *fragile* causes changes in the native biota and is not likely to be functionally equivalent to native *Codium* subspecies, and strongly emphasises the need to investigate patterns of invasion and changes to associated assemblage structure across multiple locations.

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Appendix 1: Presence of various species of epifauna on the holdfast (HF) and thallus (TH) of native and non-indigenous (NI) *Codium fragile* at Bulli (B), Towradgi (T) and Gerringong (G).

Class Order Suborder	Family Taxa	Algal structure		Location	
		Native	NI	Native	NI
Gastropoda	Cingulopsidae				
	Eatonina sp.	HF, TH	HF, TH	B, T, G	B, T, G
	Columbellidae				
	Pseudamycla dermestoidea	TH	TH	T	B
	Species 1	TH	HF, TH	T, G	B
	Elysiidae				
	Elysia maoria	HF, TH	HF, TH	B, G	B, T, G
	Haminoeidae				
	Haminoea tenera		TH		B
	Limapontiidae				
	Placida dendritica	HF, TH	HF, TH	B, T, G	B, T, G
	Litiopidae				
	Alaba opiniosa	HF, TH	HF, TH	B, T, G	B, T, G
	Lottiidae				
	Patelloida latistrigata	TH		G	
	Patelloida mufria	TH		G	
	Littorinidae				
	Afrolittorina acutispira	TH		B	
	Bembicium nanum		TH		B
	Muricidae				
	Morula marginalba		TH		B
	Thais orbita	HF		T	
	Neritidae				
	Nerita atramentosa	TH		G	
	Triphoridae				

Class Order Suborder	Family Taxa	Algal structure		Location	
		Native	NI	Native	NI
	Species 2		TH		B
	Trochidae				
	Austrocochlea porcata	TH		G	
	Cantharidella picturata	TH	TH	B	B
	Cantharidella sp.	HF, TH	HF, TH	B, G	B, T
	Phasianotrochus eximius	TH	HF, TH	T	B, G
	Species 3	HF, TH	HF, TH	B, T, G	B, T, G
	Turbinidae				
	Tricolia sp.	TH		G	
Bivalvia	Veneridae				
	Irus crenatus	HF	HF	T	T
	Irus sp.	HF		T	
	Veneridae				
	Notirus sp.	HF		T	
	Mytilidae				
	Lasea cf. *australis*	HF, TH	HF, TH	B, T, G	B, T, G
	Musculus alganus	HF, TH	HF, TH	B, T, G	B, T, G
	Musculus sp.	TH		B	
	Neoleptonidae				
	Neolepton sp.	HF		B	
	Other				
	Species 4	TH	TH	G	T
Pycnogona		TH	TH	B	T
Insecta					
Hymenoptera		TH	TH	T, G	T

[Type text]

Class Order Suborder	Family Taxa	Algal structure		Location	
		Native	NI	Native	NI
Copepoda					
Harpacticoida		TH	TH	B, G	B, G
Malacostraca	Hippolytidae				
	Hippolyte australiensis	TH	TH	G	G
	Hymenosomatidae,				
	Halicarcinus ovatus	TH	TH	B	B, T, G
Malacostraca					
Amphipoda					
Gammaridea		HF, TH	HF, TH	B, T, G	B, T, G
Ostracoda	Species 5	HF		B	
Polychaeta					
Aciculata	Nereididae				
	Perinereis sp.	HF		T	
	Syllidae				
	Typosyllis sp.	HF, TH	HF, TH	B	B

Table 1: ANOVA analyses for differences in epifaunal diversity and abundance on native and non-indigenous *Codium fragile*. The number of taxa and number of individuals of epifauna on (a) the thallus standardised for area (100cm^{-2}) and per 100 branches and (b) the holdfast standardised by wet weight (g). Abundance of key taxa (c) was also standardised (100 branches^{-1}). *Codium* ssp. (fixed factor) at Bulli, Towradgi and Gerringong (Location: random factor) (n = 8). Data transformation in all analyses: ln (x + 1). For all variables, variances were homogeneous according to Cochran's test. * P < 0.05; ** P < 0.01; *** P < 0.001; NS = not significant, d.f. = degrees of freedom.

Source (d.f.)		*Codium* ssp.: C (d.f. = 1)		Location (d.f. = 2)		C × Location (d.f. = 2)		Residual (d.f. = 42)
		MS	*F*	MS	*F*	MS	*F*	MS
(a) Thallus	No. taxa (100cm^{-2})	0.049	0.55 NS	0.209	4.90*	0.0889	2.08 NS	0.0427
	No. taxa (100 branches^{-1})	2.418	4.83 NS	0.301	3.18 NS	0.5003	5.29**	0.0947
	No. individuals (100cm^{-2})	3.135	0.95 NS	6.659	19.60***	3.3061	9.73***	0.3397
	No. individuals (100 branches^{-1})	1.692	0.27 NS	7.363	21.17***	6.2086	17.86***	0.3477
(b) Holdfast	No. taxa (g^{-1})	0.936	4.93*	0.153	0.81 NS	0.1235	0.65 NS	0.1931
	No. individuals (g^{-1})	1.795	3.66 NS	0.026	0.06 NS	0.4900	1.06 NS	0.4606
(c) Taxon	*Alaba opiniosa* (100 branches^{-1})	0.038	0.01 NS	35.399	83.41***	3.970	9.35***	0.424
	Gammarid amphipods (100 branches^{-1})	2.695	0.59 NS	8.294	17.06***	4.530	9.32***	0.486

Table 2: Taxa contributing more than 5% to compositional dissimilarities (%) of epifauna between native and non-indigenous *Codium fragile* ssp. at Bulli, Towradgi and Gerringong (n = 8). The mean abundance (number of individuals) of taxa on entire (holdfast and thallus native (N) and non-indigenous (NI) *Codium* is provided. Taxa are listed in descending order of percentage contribution to average dissimilarities (%) for each location. Diss. /SD: average dissimilarity / standard deviation. Comp. Diss.: compositional dissimilarities.

Location	Average Diss. (%)	Epifaunal Taxa	Mean Abundance		Diss. / SD	Comp. Diss. (%)
			N	NI		
Bulli	62.94	*Alaba opiniosa*	58.13	323.00	2.04	76.91
		Gammarid amphipods	22.75	33.25	0.89	10.28
Towradgi	59.44	*Alaba opiniosa*	32.50	21.25	1.36	36.15
		Gammarid amphipods	6.75	20.13	0.99	23.12
		Lasea cf. *australis*	5.38	8.88	0.87	15.95
		Placida dentritica	1.63	5.50	1.26	6.18
		Trochidae Species 3	1.13	4.25	0.71	6.04
		Musculus alganus	4.13	0.75	0.74	5.34
Gerringong	44.73	Gammarid amphipods	72.13	54.13	1.61	79.09
		Eatonina sp.	3.63	2.63	1.08	6.25

Table 3: Analysis of differences in epiphyte load (% cover) on native and non-indigenous *Codium* (*Codium* ssp.: fixed factor) and between algae with low and high complexity (Complexity: fixed factor) (n = 7 individuals). For all variables, variances were homogeneous according to Cochran's test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS = not significant

	Codium ssp. (C)		Complexity		C × Complexity		Residual
	MS	*F*	MS	*F*	MS	*F*	MS
Epiphyte cover	0.007	0.02 NS	0.150	0.41 NS	0.005	0.01 NS	0.364

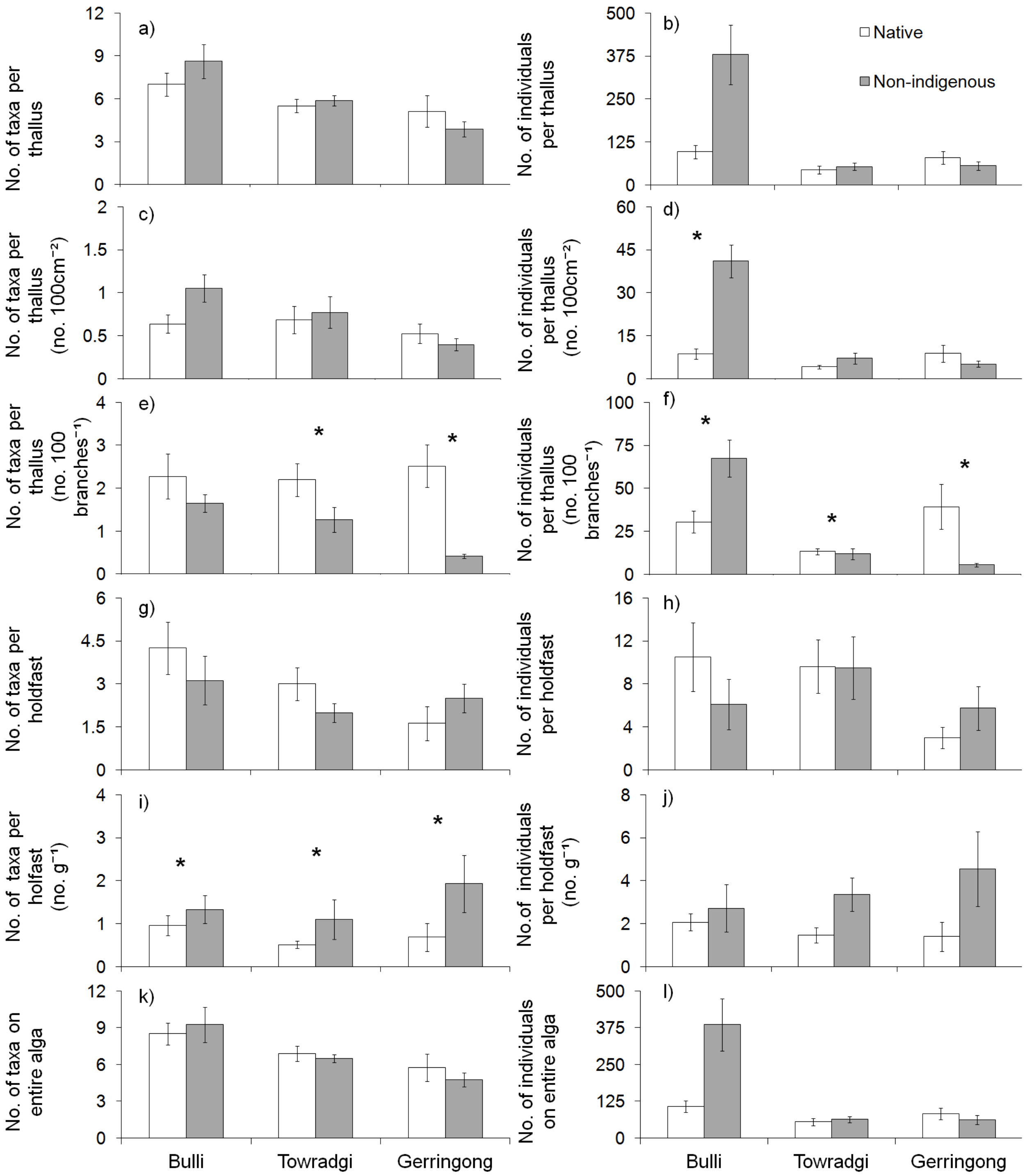
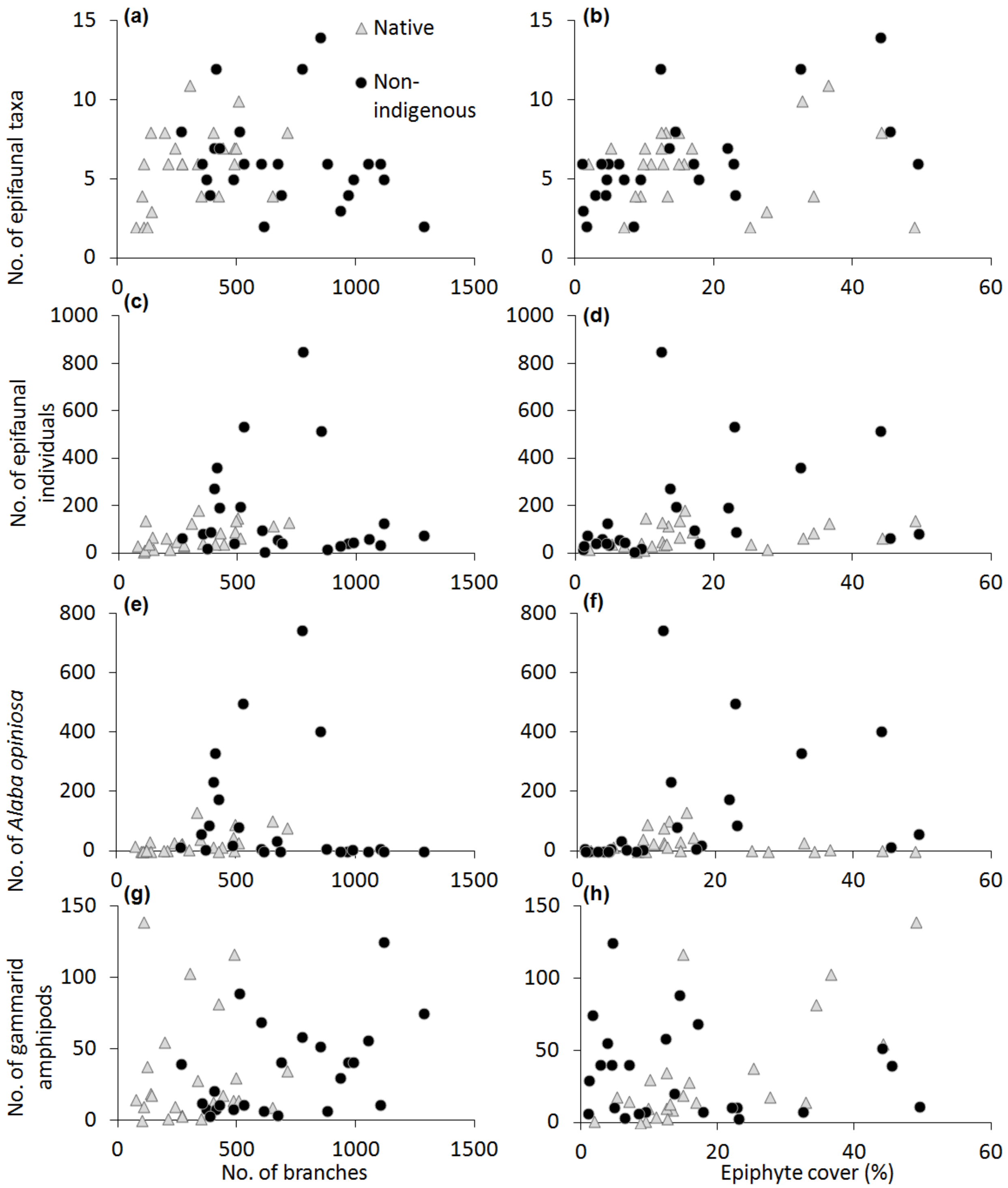


Figure 1. Mean (±SE) number of taxa on thalli (total (a), 100cm^{-2} of thallus (c), 100 branches^{-1} of thallus (e)), number of individuals on thalli (total (b), 100cm^{-2} of thallus (d), 100 branches^{-1} of thallus (f)), number of taxa on holdfasts (total (g), g^{-1} of holdfast (i)), number of individuals on holdfast (total (h), g^{-1} of holdfast (j)), number of taxa (k) and number of individuals (l) over

entire alga for epifauna on native (white bars) and non-indigenous (grey bars) *Codium fragile* subspecies at each of three locations: Bulli, Towradgi and Gerringong (n = 8 individuals). For each pair of bars with each graph, asterisks denote significant differences between native and non-indigenous *Codium* at a given location. Differences among locations are reported in the text. Note differences in scale among *y*-axes.



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636 **Figure 2.** Relationship between branch number and epiphyte cover (%) on (a, b) number of

637 epifaunal taxa, (c, d) number of epifauna, (e, f) number of *Alaba opiniosa*, and (g, h) number of

gammarid amphipods on native and non-indigenous *Codium* across the study site (n = 24 thalli). Note differences in scale between the *y*-axes.

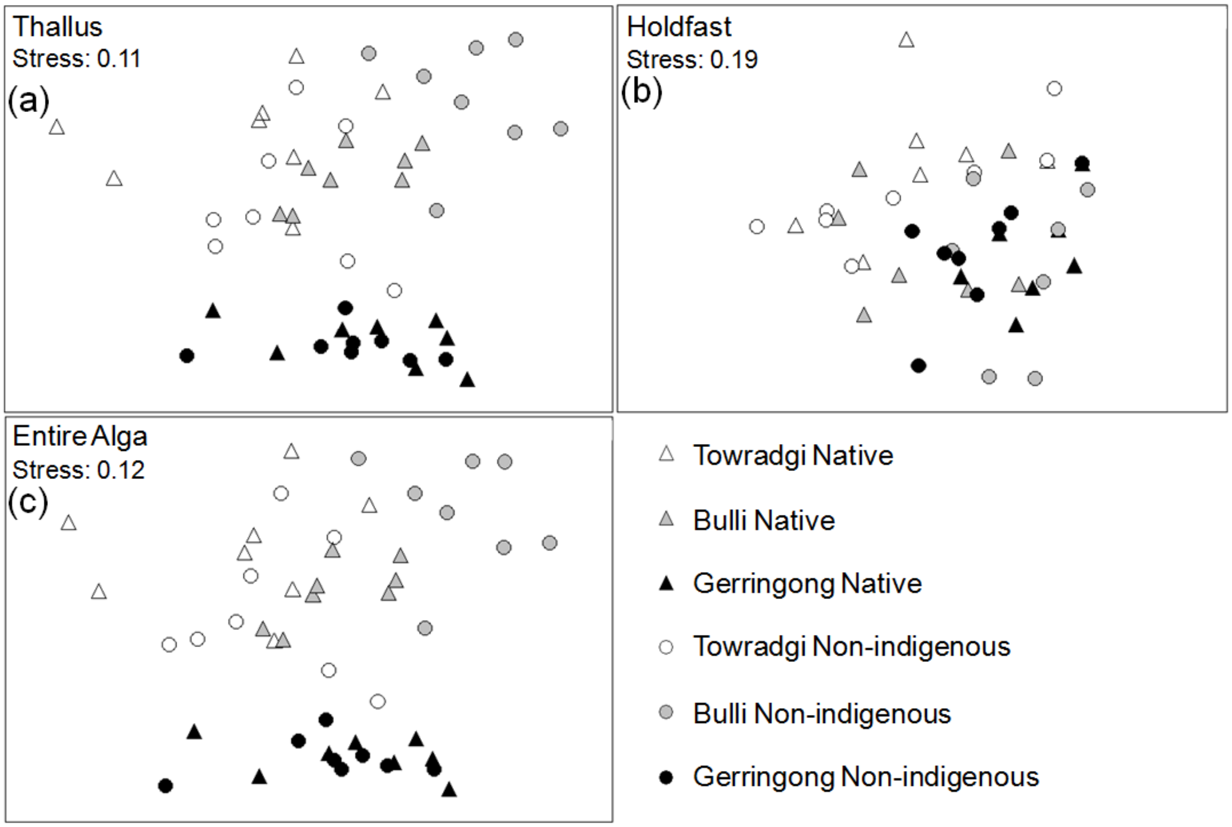

Figure 3. nMDS (non-metric multi-dimensional scaling) ordination of species composition of epifauna for (a) thalli, (b) holdfasts and (c) entire plants of native and non-indigenous *Codium* at Bulli, Towradgi and Gerringong (n = 8 individuals).

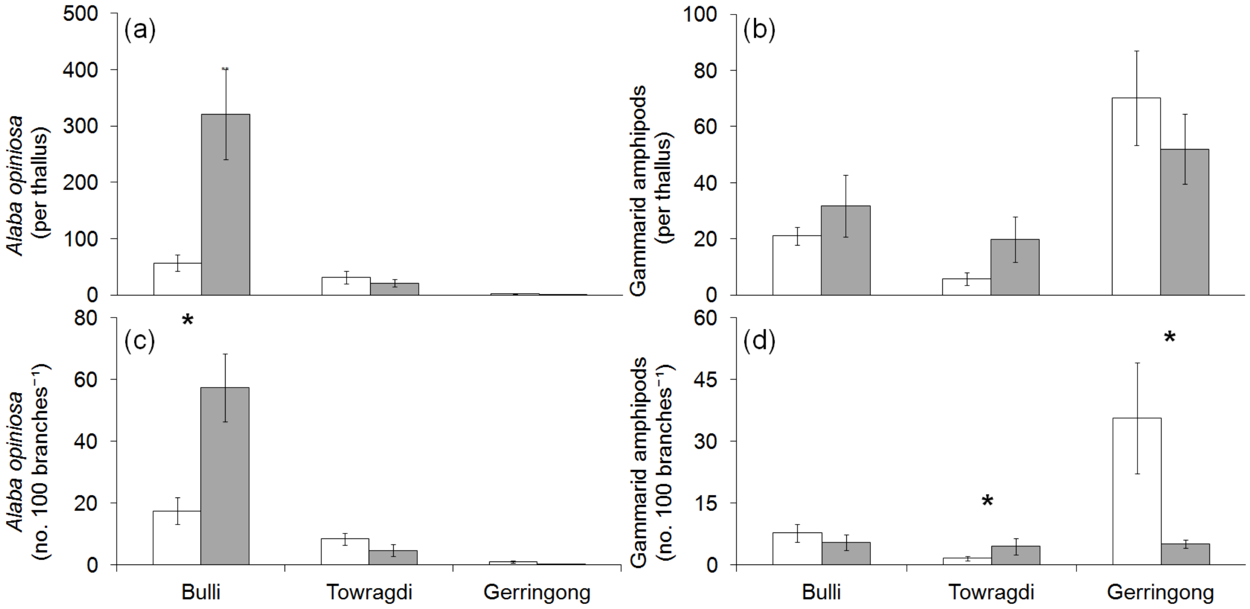

Figure 4. Mean (±SE) abundance of *Alaba opiniosa* per thallus (a) and per 100 branches (c), and abundance of gammarid amphipods per thallus (b) and per 100 branches (d) on native and non-indigenous *Codium* subspecies at each of three locations: Bulli, Towradgi and Gerringong (n = 8 algae). Asterisks denote significant differences between native and non-indigenous *Codium* at a given location, according to SNK tests. Note differences in scale among *y*-axes.

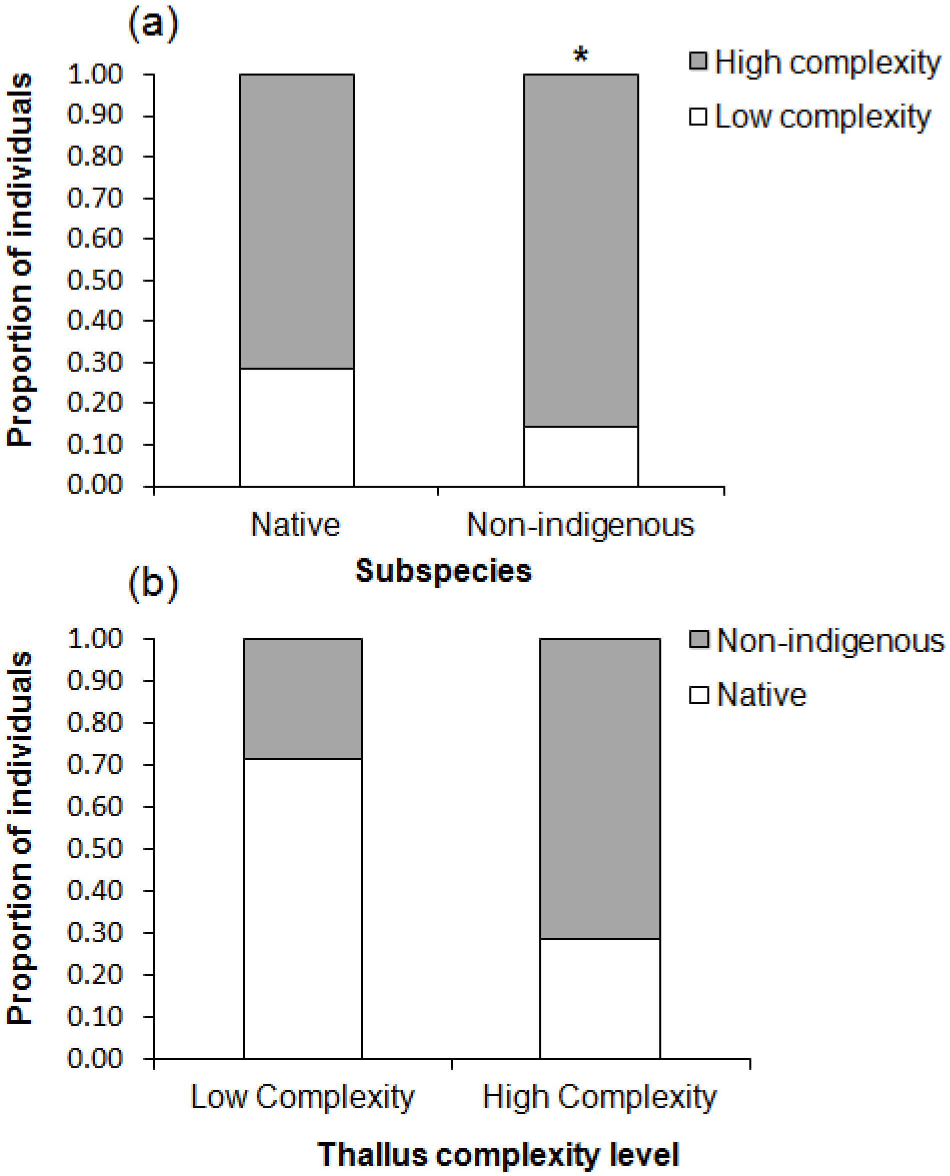

Figure 5. The proportion of individuals of *Hyale* spp. choosing (a) algae with high vs. low thalli complexity and (b) native vs. non-indigenous *Codium* when offered either both native and non-indigenous *Codium* or both thalli with high and low complexity (n = 7 animals per trial). Asterisk denotes significant differences at $P < 0.05$.