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ORIGINAL PAPER

Individuals from non-native populations are stronger and bigger than individuals from native populations of a widespread seaweed

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Abstract Many species introduced to non-native regions undergo profound phenotypic change, but conflicting evidence remains on the frequency of such trait differentiation. Here, we describe two phenotype categories—biomechanical material properties and organismal size—that differ between and within native Japanese and non-native North America and Europe shorelines of the macroalga *Gracilaria vermiculophylla*. Biomechanical traits represent capacity to withstand wave energy and disperse by fragmentation, while body size in algae can reflect capacity for reproduction. Field-collected thalli from 43 populations were assayed for peak force (breaking force), peak stress (strength), breaking energy (strain energy storage), and modulus (stiffness). Thalli attached to hard substratum were approximately 27% stronger than thalli that were drifting, and non-native thalli were approximately 50% stronger and approximately 23% stiffer than native thalli. Non-native thalli had 28% more surface area than did native thalli. Interestingly, the population means of thallus strength and size were not tightly correlated; this suggests that their expression, evolution, or both is not tightly coupled. Stronger thalli were found in areas with cooler sea surface temperature, while the largest thalli were found in the cooler, northern European latitudes. This study stands alongside a surprisingly small number of studies that directly compare material properties between native versus non-native populations within a species, despite the potential importance of material properties in mediating invasion success. Future work would benefit from estimates of fitness in the field and address whether biogeographic shifts in material phenotypes reflect adaptive or non-adaptive processes and are either driving or reflecting invasion success.

Keywords Algae · *Gracilaria vermiculophylla* · *Agarophyton vermiculophyllum* · Haplodiplontic life cycle · Organismal body size · Biomechanical material properties · Rhodophyta

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Introduction

The global movement of non-native species homogenizes the Earth's biota and substantially alters local community processes and ecosystem function (Lockwood et al. 2007; Vila et al. 2011; Maggi et al. 2015). The successful establishment of non-native populations can be facilitated by microevolutionary shifts in adaptive phenotypes (Cox 2004; Colautti and Lau 2015; Hodgins et al. 2018), including shifts that occur at the site of initial introduction (Lombaert et al. 2010) or local adaptation that occurs post-establishment (Lee 2015). Shifts in traits can lower susceptibility to local consumers (Heger and Jeschke 2014, Keane and Crawley 2002; but see Lind and Parker 2010; Felker-Quinn et al 2013), increase tolerance of local abiotic stresses (Lee 2015), and increase resource efficiency (Hodgins et al 2018).

However, not all invasions are accompanied by variation among introduced propagules or adaptive phenotypic shifts. For many species, no phenotypic shifts have occurred (Ordonez 2014) and both plastic and canalized (i.e., fixed) phenotypes can allow successful establishment in non-native habitats (Parker et al. 2003; Schrieber et al. 2017). Moreover, genetic bottleneck effects during invasion may change the frequencies of phenotypes that have no fitness effects (i.e., are non-adaptive) in the non-native range (Hodgins et al. 2018). Thus, there is conflicting evidence over the frequency by which trait differentiation occurs.

The red macroalga *Gracilaria vermiculophylla* (Lyra et al. 2021; hereafter *Gracilaria*) is native to the northwestern Pacific Ocean but is now common to estuaries of northwestern Africa, Europe, and both coastlines of North America (Kim et al. 2010; Krueger-Hadfield et al. 2017). *Gracilaria* is an ecosystem engineer that can cover 80–100% of local soft-sediment habitats, outcompete native macroalgae, and alter community structure, species interactions, trophic pathways, and nutrient cycling (Thomsen et al. 2009; Byers et al 2012; Gonzalez et al. 2013; Kollars et al. 2016).

There are several documented shifts in ecologically-relevant traits between native and non-native regions in which *Gracilaria* is found. Relative to native populations, thalli (i.e., the photosynthetic free-living body) from non-native populations have greater tolerance of extreme heat, cold, and low salinity stress (Hammann et al. 2016; Sotka et al. 2018) and lower palatability toward *Littorina* snails and the isopod *Idotea baltica* (Nylund et al. 2011; Hammann et al. 2013) but not for the amphipod *Ampithoe valida* (Bippus et al. 2018; see also Nejrup et al. 2012). In addition, non-native thalli also have stronger chemical defenses against algal and bacterial epiphytes (Wang et al. 2017; Saha and Weinberger 2019; Bonthond et al. 2021). Some of these trait shifts are potentially adaptive. For example, greater tolerance of heat stress helps to explain persistence in the warmer climates of the non-native range and greater tolerance of low salinities helps explain expansion into oligohaline estuaries (Abreu et al. 2011; Sotka et al. 2018).

There are dramatic shifts in ecological habitat and reproductive mode between populations in native versus non-native regions (Krueger-Hadfield et al. 2016). Native populations of *Gracilaria* are nearly always fixed to hard substrata (i.e., pebbles, rocks, and molluscan shells) in both estuarine and open coast shorelines. In contrast, many non-native populations occur on low-energy estuarine mudflats and drift as free-floating thalli or are glued to the tubes of decorator worms (Kollars et al 2016). The shift from primarily sessile to free-floating and drifting thalli is correlated with shifts from sexual to partially clonal reproduction. It also correlates with shift from the expected $\sqrt{2}$:1 ratio (i.e., haploid:diploid; when there are no fitness differences between the two ploidy stages, Destombe et al. 1989; Thornber and Gaines 2004) within native populations to overwhelmingly diploid dominated in the non-native range (> 90% on average, Krueger-Hadfield et al. 2016). We note that somatic development occurs in both the haploid gametophytic and diploid sporophytic stages, and that these ploidy stages are morphologically indistinguishable when vegetative (i.e., non-reproductive).

Here, we focus on two categories of thallus phenotypes—organismal body size and material properties—to test whether they correspond with shifts during invasion observed in animal or plant species. Broadly, non-native populations tend to have bigger individual sizes relative to native populations (Reed et al. 2012; Parker et al. 2013). The mechanism for this increase in size differs among species, but may be due to fewer enemies in non-native regions (i.e., parasites, consumers, competitors; Ehrlich 1989; Rodda and Tyrrell 2008; Reed et al. 2012; Heger and Jeschke

2014). To our knowledge, a direct comparison of thallus size between native and non-native populations has not been reported in *Gracilaria.*

A second category of phenotypes we explored were biomechanical material properties: peak force (breaking force), peak stress (strength), breaking energy (strain energy storage), and modulus (stiffness). These properties may reflect at least two ecological phenomena in marine macrophytes: dispersal by fragmentation and capacity to withstand wave energy (Thomsen 2004). For example, if fragmentation is an important mechanism by which the free-floating, non-native populations disperse and grow (as seen in other invasive macroalgae, Ceccherelli and Cinelli 1999; D'Amours and Scheibling 2007), then non-native populations may have a lower breaking force (i.e., less debranching resistance), thereby facilitating fragmentation. Moreover, as some native populations are more likely than non-native populations to be fixed to hard substrata (Krueger-Hadfield et al. 2016) and thus must resist waves rather than float or tumble with waves, thalli from native, fixed populations are predicted to be stronger (see Harder et al. 2006; Demes et al. 2011; Martone et al. 2012; Starko et al. 2015). Insights from contrasting native and non-native species at a single field site indicate that localized disturbances and substrate are important to consider for breakage and organism size (Thomsen 2004). There are relatively few studies that directly compare material properties between native versus non-native populations of the same species sampled across the range (Gribben and Byers 2020), despite the importance of material properties in mediating invasion success in their non-native range (e.g., peppers of Spector and Putz 2006; fouling invertebrates of Murray et al. 2012).

Methods

Sample collection

To explore patterns of thallus size and biomechanical properties, we collected thalli from 43 populations from across the extant range in the Northern Hemisphere. From May–October 2015, we sampled thalli from populations in native Japan (n = 15) and non-native continental shorelines of western North America (n = 6), eastern North America (n = 10) and Europe (n = 12) as part of a large collection effort (Krueger-Hadfield et al. 2017; Sotka et al. 2018; Table S1). At each site, we haphazardly collected 100 thalli that were at least 1 m apart, along a transect parallel to the shoreline. The water temperatures at the time of collection were statistically indistinguishable between native and non-native regions (range from 15 to 36 °C; average ~ 23 °C) and did not vary with latitude nor with January sea surface temperature (Pearson's correlation test $p > 0.6$; Sotka et al. 2018). The northeastern coastline of Japan is the geographic source for nearly all non-native introductions, with the exception of Pacific Northwest samples which are a mix of southern and northern Japanese samples (Krueger-Hadfield et al. 2017; Flanagan et al. 2021). For the purposes of this study, we are focusing on differences between native Japanese versus non-native populations. Due to the small number of source populations identified from our Japan-wide collection methods, we do not have enough replicates for a balanced test of differences between native source, native non-source, and non-native regions (sensu Sotka et al. 2018).

In the field, we recorded whether each thallus was fixed versus free-floating or drifting based on whether the holdfast of a thallus was fixed to hard substratum (bedrock, shell, pebble) or not. Thalli glued by the decorator worm *Diopatra cuprea* in southeastern estuaries of the United States (Kollars et al. 2016) and thalli buried in the mud were included in the drift category. Thalli glued to *Diopatra* tubes or buried drifted at some point in their lifespan because *Gracilaria* spores do not settle on soft sediment nor the tube. Thus, the cells of glued and buried thalli are more likely to have experienced forces similar to drifting thalli than to attached thalli which were attached with a holdfast to hard substrate during their entire lifespan. When material force and surface area variables were analyzed without the *Diopatra*-glued samples (analyses not shown), the statistical inference of the results was identical to when we included the *Diopatra*-glued samples as drift.

We also noted the principal composition of the substrate: mudflat-mix (sites with soft sediment but varying levels of hard stratum such as shells or flotsam), mudflat (sites dominated by soft sediment and without abundant hard stratum), or rocky shore (cobble or bedrock abundant in the intertidal) at each site. We grouped mudflat and mudflat-mixed together for site-level analyses. Using dissecting microscopes, we noted reproductive state (i.e., reproductive tetrasporophyte [diploid], reproductive gametophyte [haploid male or haploid female], or non-reproductive [vegetative]) of each thallus.

Biomechanical properties dataset

Thalli without epiphytes or any morphological wounding or abrasion were shipped to Charleston in small polyethylene bags with seawater-moistened paper inside coolers with ice packs, and arrived within 2–3 days of collection. Thalli were then maintained in the dark at 15–20 °C for three to five days before assays began in Charleston. Thus, because these individuals were collected using identical protocols and with similar seasonal conditions, it is unlikely that population or biogeographic differences in phenotype were confounded by handling time, manipulation, or recent environmental variation.

We evaluated peak force (breaking force), peak stress (strength), breaking energy (strain energy storage), and modulus (stiffness) following methods as in Lees et al. (2018) on the main axis of the thallus. We employed a motorized vertical testing stand (Imada EMS-275, Northbrook IL USA) attached to a force gauge (Imada ZP-11, Northbrook IL USA) and a height gauge (Mitutoyo Digimatic height gauge, Aurora IL USA) for performing quasi-static uniaxial tensile tests to failure on thalli. Prior to initiating a tensile test, we clamped a thallus between the serrated grips on the testing stand. After clamping the top and bottom ends, we slowly pre-strained the thallus to its original length, in which the sample was vertically oriented and experiencing approximately 0.01 N of tensile force. Once in this position, we zeroed the height gauge and recorded the original length and diameter. Because of its cylindrical structure, we calculated each thallus' cross-sectional area (CSA) as $R^2 * \pi$, where R is one half the measured diameter. Thalli were strained at a rate of 1.5 mm/min. We recorded peak force based on the maximum force (N) just prior to failure (breakage of the thallus). This measure indicates the applied force needed to break the thallus. Thalli from 41 populations were tested and we did not collect enough material for two others (see Table S1 for population codes; elk, man).

Combining our morphometric data with the recorded force-distance data from tensile tests, we calculated stress and strain:

$$\mathrm{Stress} = \mathrm{F}/\mathrm{CSA}$$

where F is force in Newtons (N) just prior to failure, and CSA as above.

$$\mathrm{Strain} = \Delta \mathrm{L}/\mathrm{L}_0$$

where L_0 is the length of the initial algal thallus sample prior to initiating each tensile test, and ΔL was the difference between the initial length and the final length, which was measured incrementally until mechanical failure was achieved.

Using these stress–strain data, we evaluated three fundamental material properties. From stress–strain curves, we determined peak stress (i.e., breaking stress or strength) and peak strain (i.e., extensibility or strain at failure) for each individual thallus. We defined strength per individual as the maximum stress prior to failure and defined extensibility as the strain at breaking stress. On each stress–strain curve, we calculated the modulus (i.e., stiffness), which we defined as the stress/strain ratio (or slope) of the steepest linear portion on the curve. Modulus represents how much a material resists deforming, with lower modulus values indicating compliance and higher modulus values indicating more rigidness. Using the *auc* function in R::MESS (Ekstrøm 2019; R Core Team 2020), we calculated the area under the stress–strain curve to determine a sample's strain energy storage (a.k.a. breaking energy or toughness). Only samples that made it through a rigorous quality control protocol where no slippage was detected (such as samples coming loose from the instrument prior to breaking) were included in analyses.

Organismal size

Digital photographs were taken of individual thalli collected across a transect in each estuary using a haphazard sampling regime where samples were at least one meter apart. Thalli were placed in a large plastic dish, floated in seawater, and photographed using a camera stand together with a color card and scale bar as described in Lees et al. (2018). Images were analyzed by modifying methods of WinRhizo (Regent Instruments; Quebec Canada) as thallus structure in this species resembles terrestrial plant root traits with even diameters across the majority of the thallus. For each photograph, we retained surface area (cm^2). Light contrast in the photograph was enhanced using Photoshop (Adobe) as needed to ensure the entire thallus was considered. Epiphytes or soft substrate in images were set to background color. No difference in size was detected between enhanced and not-enhanced photographs (t-test $p > 0.05$), therefore all images were considered together. We also weighed wet biomass using a laboratory balance accurate to ± 0.01 g. Wet biomass and surface area were highly correlated (Pearson's correlation coefficient = 0.938; $p < 0.002$; n = 409 individuals) and we analyzed only surface area here. Thalli from 39 populations were photographed (see Table S1).

Statistical analyses

For the biomechanical dataset, our sample size per population (mean ± SD) was 17.1 ± 4.0 thalli across 41 populations and 11.9 ± 7.4 thalli for 40 populations for tetrasporophytes only (Table S1). This sample set (n = 700 total thalli and 474 tetrasporophytes) is not evenly split among fixation type (fixed vs. drift), region (native vs non-native), or shoreline (Fig. S1). Approximately 89% of 246 Japanese samples were fixed to hard-substrata, while only 10, 21, and 10% were fixed to hard-substrata in western North America (n = 101), eastern North America (n = 187) and Europe (n = 166), respectively (Fig S1A). This natural, ecological variation results in an unbalanced 2-way design; as such we have limited ability to detect interactions between fixation type and region (native vs non-native). However, we can test for these factors in isolation (i.e., is the mean fixation type or region distinct?) and whether they have additive effects. There were several soft- and hard-bottom locations within native and non-native regions (Fig S1B; Table S1), allowing us to assess interactions between region and habitat type.

The reproductive state (vegetative vs. reproductive tetrasporophyte) is not evenly distributed among sampled individuals from native and non-native regions (78 and 62% reproductive tetrasporophytes, respectively; Fig. S1A, B), which raises the possibility that region effects are biased by differences in material properties between gametophytes and tetrasporophytes (see Lees et al. 2018 for an example in one non-native population). We minimized the potential impacts of the ploidy differences by performing two sets of analyses: all samples together (vegetative thalli and reproductive tetrasporophytes) and a subset that includes only reproductive tetrasporophytes. Previous microsatellite surveys indicate that most vegetative thalli in these populations were also tetrasporophytes (Krueger-Hadfield et al. 2017; Sotka et al. 2018).

We estimated linear models fits by REML using R::lmerTest (Kuznetsova et al. 2017) to determine the effect of region (native vs. non-native), fixation type (fixed vs. drift) or site substratum (mudflat vs. rocky shore), and their interaction on phenotypes (biomechanical and size). We treated population as a random intercept effect and evaluated fixed effects using Satterthwaite's approximation. We observed residuals for model assumptions and used transformations when appropriate. We examined the potential for multicollinearity among biomechanical traits using variance inflation factor (VIF) and retained and report on all effects where VIF < 2. Using the full dataset, log-transformed AUC (area under the curve) was highly correlated with peak strain and peak stress (r = 0.86 and 0.90, respectively), but all other pairwise correlations were lower (r < 0.62 or less).

Linear models assessed the effects of continental shoreline (Japan, eastern North America, and Europe) and latitude using population as a random intercept. We did not include the western North American populations in this latitudinal analysis because populations clustered around two latitudes (i.e., three from northern region; three from southern region) rather than spread along the latitudinal extent as in the other shorelines. Instead, we present the data for western North America in Fig. S4-6. We examined model assumptions similarly as described for region (native vs. non-native) above. We also explored whether mean SST in January explains significant differences in traits with latitude. Mean SST for January was downloaded from BioOracle (Tyberghein et al. 2012; see Sotka et al. 2018 for details).

For the organismal size dataset, our sample size per population (mean ± SD) was 9.4 ± 1.4 thalli for 39 populations and 8.3 ± 2.3 thalli at 35 populations for tetrasporophytes only (Table S1). As with the biomechanics dataset, these samples (n = 366 total thalli and 289 tetrasporophytes) were not evenly split among thallus-specific fixation status, region, and shoreline (Fig S1C). We assessed the influence of fixation status (i.e., fixed vs. drift) using models as above. We note that there was no influence of water temperature at time of collection (one-way ANOVA; $F_{1,42} = 0.328$, $p = 0.570$). Organismal size phenotypes are not independent features; as such, we only present surface area.

We used two-way linear models to assess the relative impact of fixation status and region (native vs non-native) on total surface area, treating population as a random effect. We similarly analyzed the influence of latitude and shoreline, excluding western North America. A one-way linear model and treating population as a random effect indicated that there were no effects of the sea water temperature at time of collection on mean surface area per population ($p = 0.186$ for all thalli; $p = 0.428$ for tetrasporophytes; analyses not shown).

To assess whether surface area and material properties were correlated at the population-level, we generated population means of log-transformed material properties and projected surface area and evaluated their relationship in a series of Pearson correlation tests and assessed analogous correlations using non-transformed data and a series of Spearman rank correlation tests. Similarly, we examined the relationship between biomass and material properties or surface area.

Results

Biomechanical properties

Overall, the breaking force (i.e., strength) of a thallus depended on fixation status (drift vs. fixed), the habitat type in which the thallus was collected (mudflat vs. some hard substrata), and region (native vs. non-native). Thalli that were fixed to hard substrata required more force to break (29% and 24% more force in native and non-native ranges, respectively) relative to thalli that were free-floating or drifting when sampled (Fig. 1a). This was true when all thalli were analyzed ($p = 0.050$) or when only tetrasporophytes were analyzed ($p = 0.043$; Table 1a). Breakage force was greater among thalli collected from rocky shores (16% and 53% more force in native and the non-native ranges, respectively) than those collected from mudflats (Fig. 1b) regardless of whether all thalli ($p = 0.042$) or only reproductive thalli were analyzed ($p = 0.041$; Table 1B). Thalli collected in the non-native range required greater breaking force than did thalli collected in the native range in the model that included site-level substrata (47 and 53% more among attached and drifting thalli, respectively; Fig. 1b and Table 1B). This was a less of an effect when all thalli were analyzed ($p = 0.060$) than when only reproductive thalli were analyzed ($p = 0.048$).

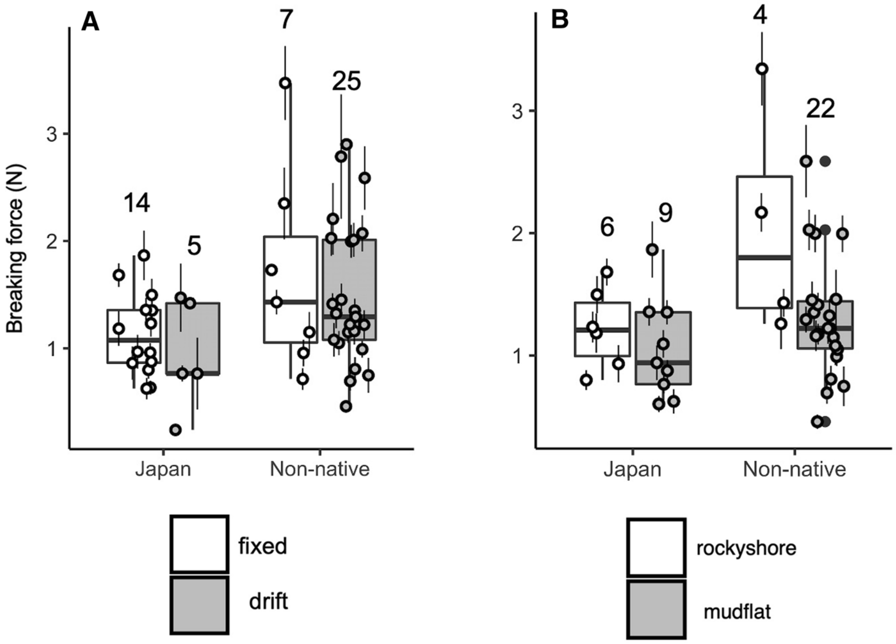

Fig. 1 Breaking force (N) between native and non-native populations, split by **a** whether the thallus is fixed or drifting, and **b** whether collected at mudflats or rocky substrata. Points and bars represent means and standard errors for individual populations. Numbers indicate number of populations. Box, line and whisker plots indicate mean, and the 5–95% and 25–75% quartiles for the group. See Table 1 for analysis

Table 1 Analyses of five biomechanical traits against (A) region and individual-level fixation status or (B) region and site-level substrata

We report the p-value from linear mixed models fit by REML on log-transformed data for either all thalli or tetrasporophytes only and using population as a random effect. For all thalli, the number of populations was 41 and the number of individuals per population ranged from 7–27. For tetrasporophytes only, the number of populations was 40 and the number of individuals per populations ranged from 1–27

ALL THALLI	Modulus	AUC	Peak Strain	Peak Stress	Breaking Force
A)					
(1|Population)	<0.001	<0.001	<0.001	0.860	0.596
Region (Native vs Non-native)	0.090	0.474	0.186	0.969	0.769
Attachment (Fixed vs Drift)	0.390	0.620	0.716	0.376	0.050
TETRASPOROPHYTES ONLY	Modulus	AUC	Peak Strain	Peak Stress	Breaking Force
(1|Population)	<0.001	<0.001	<0.001	0.928	0.597
Region (Native vs Non-native)	0.307	0.368	0.263	0.746	0.678
Attachment (Fixed vs Drift)	0.465	0.534	0.729	0.355	0.043
B)					
(1|Population)	<0.001	<0.001	<0.001	0.401	0.285
Region (Native vs Non-native)	0.043	0.9135	0.243	0.393	0.060
Site substrata (Mudflat vs Rocky)	0.396	0.095	0.110	0.346	0.046
TETRASPOROPHYTES ONLY	Modulus	AUC	Peak Strain	Peak Stress	Breaking Force
(1|Population)	<0.001	<0.001	<0.001	0.238	0.261
Region (Native vs Non-native)	0.139	0.784	0.300	0.601	0.048
Site substrata (Mudflat vs Rocky)	0.906	0.058	0.148	0.122	0.042

The modulus (stiffness) was approximately 23% higher in thalli from non-native versus native regions. In the model that includes fixation status (fixed vs. drift; Figure S3; Table 1B), we detected a trend when examining all thalli ($p = 0.090$); this trend disappeared when only reproductive thalli were analyzed ($p = 0.307$). In the model that includes site-level substrata (Figure S4; Table 2B), the pattern was significant when all thalli ($p = 0.047$) were considered together and disappeared when only reproductive thalli were analyzed ($p = 0.137$). Beyond population differentiation, there were no effects of habitat type, region, or fixation type that explained variation in toughness (i.e., area under the curve, or AUC), peak strain nor peak stress (Figure S3-S4; Table 1). We present these results split by attachment type, habitat and continent in Figure S5-S6.

Multiple biomechanical properties varied with latitude in both the native and non-native range. Higher latitudes tended to have thalli that were stronger (Breakage force; Fig. 2a), more extensible (Max strain; Fig. 2b), and more pliant (Slope; Fig. 2c; see analyses in Table 2A). In contrast, there was no significant effect of latitude on AUC nor peak stress. There was also a significant effect of shoreline (Japan, eastern USA, and Europe) on these same traits (Table 2A). Patterns were generally consistent whether all thalli or only reproductive thalli were included. We note that the slope of regressions from western North America populations was in the opposite direction from the slopes among populations in Japan, eastern North America and Europe (Figure S7). Unfortunately, with our limited sampling along this coastline, we were unable to robustly test for an interaction between coastline and latitude on material properties which included western North America.

Latitudinal patterns were reflected in regressions of January mean sea surface temperature against material properties (Figure S8; Table 2B). Thalli from areas with cooler temperatures had higher breakage force, higher peak stress, and lower stress–strain slope (modulus).

Organismal size

Non-native thalli had greater size than did native Japanese thalli (Fig. 3; Table 3). Non-native thalli were approximately 28% bigger by projected surface area when all thalli were analyzed (58.8 vs 45.1 cm^2 for non-native and Japanese thalli, respectively; $p = 0.039$) and 80% bigger when only tetrasporophytes were considered in the analysis (58.7 vs 32.2 cm^2, respectively; $p = 0.007$; Figure S9). There were no effects of thallus fixation status (i.e., fixed vs drift). All analyses indicated a significant effect of population identity, a random effect in these models.

There was a significant effect of an interaction between January SST (a proxy for latitude) and

Table 2 Analyses of five biomechanical traits against (A) shoreline and latitude or (B) shoreline and January sea-surface temperature (SST)

ALL THALLI	Modulus	AUC	Peak Strain	Peak Stress	Breaking Force
A)					
(1|Population)	**<0.001**	**0.008**	**<0.001**	0.193	**<0.001**
Shoreline (Japan vs eNA vs Eur)	**<0.001**	0.754	**0.027**	0.382	**0.002**
Latitude	**<0.001**	0.727	**0.028**	0.207	**<0.001**
TETRASPOROPHYTES ONLY	Modulus	AUC	Peak Strain	Peak Stress	Breaking Force
(1|Population)	**<0.001**	**0.003**	**<0.001**	0.557	**<0.001**
Shoreline (Japan vs eNA vs Eur)	**<0.001**	0.578	**0.016**	0.507	**0.001**
Latitude	**0.004**	0.417	**0.012**	0.575	**<0.001**
B)					
(1|Population)	**<0.001**	**<0.001**	**0.001**	0.363	**0.001**
Shoreline (Japan vs eNA vs Eur)	**<0.001**	0.752	0.081	0.669	0.915
Jan SST	**<0.001**	0.721	**0.037**	0.207	**<0.001**
TETRASPOROPHYTES ONLY	Modulus	AUC	Peak Strain	Peak Stress	Breaking Force
(1|Population)	**<0.001**	**<0.001**	**<0.001**	0.647	**<0.001**
Shoreline (Japan vs eNA vs Eur)	**0.002**	0.651	0.072	0.555	0.912
Jan SST	**0.003**	0.452	**0.021**	0.515	**<0.001**

We report the p-value from linear mixed models fit by REML on log-transformed data for either all thalli or tetrasporophytes only and using population as a random effect. For all thalli, the number of populations was 41 and the number of individuals per population ranged from 7–27. For tetrasporophytes only, the number of populations was 40 and the number of individuals per populations ranged from 1–27

continental shoreline (excluding western NA) on surface area (Table S2). In particular, thalli from colder sites tended to have greater size than did those from warmer sites along the European coastline; in contrast there were few changes in size along either Japanese or eastern NA shorelines (Figure S10). These patterns held whether we analyzed all thalli or just tetrasporophytes (Table S2).

We did not find a correlation between population-level means of projected surface area and any of the five material properties (Pearson's correlation test for each correlation df = 39; $p > 0.15$). Spearman's Rank correlation tests were similarly not-significant ($p > 0.05$) with one exception: a negative relationship between surface area and AUC ($p = 0.038$) however, this relationship had less support when we tested within either native ($p = 0.237$) and non-native range ($p = 0.057$) independently.

Discussion

Overall, we find that (1) non-native thalli were bigger than native thalli, (2) non-native thalli were stronger (i.e., required greater breaking force) than are native thalli, (3) thalli fixed to hard substrata were stronger than drifting thalli, and (4) fixed and drifting thalli displayed no difference in overall thallus size. As these were field-collected individuals that were not reared in a common garden, habitat- and population-level differences reflect genetic and/or plastic responses to local environments. Below, we describe the drivers and implications of each of these patterns.

First, non-native thalli tended to be larger, and this is consistent with many other invasive species in which non-native individuals tend to be larger than native populations (Parker et al. 2013). The demographic mechanism by which this occurs (i.e.,

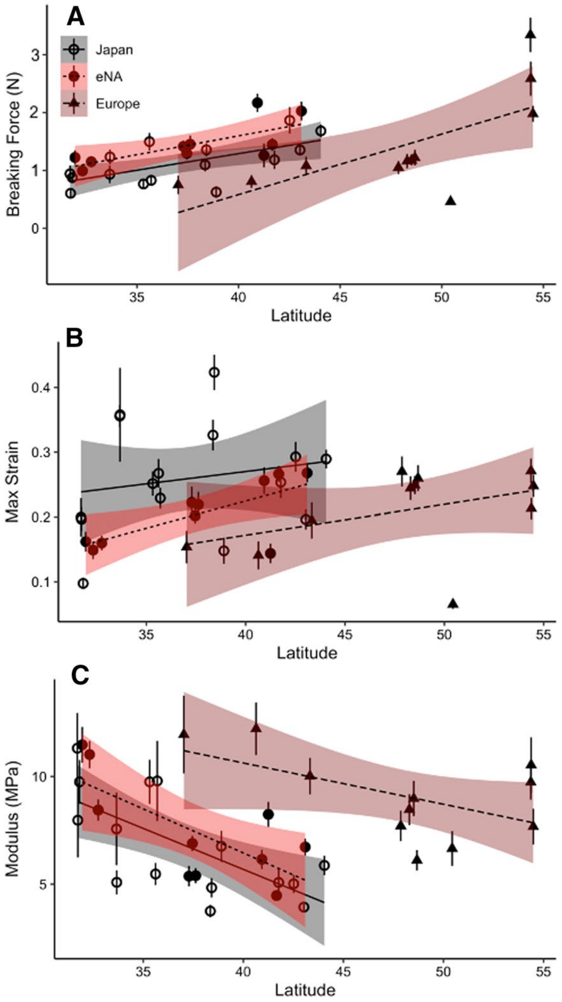

Fig. 2 Material properties regressed against latitude along shorelines of Japan, eastern North America (eNA), and Europe. **a** Peak breaking force, **b** Max strain and (C) Modulus (stress–strain slope). Points and bars represent means and standard errors for individual populations See Table 2 for analysis. Western NA (North America) data are included in Fig S6

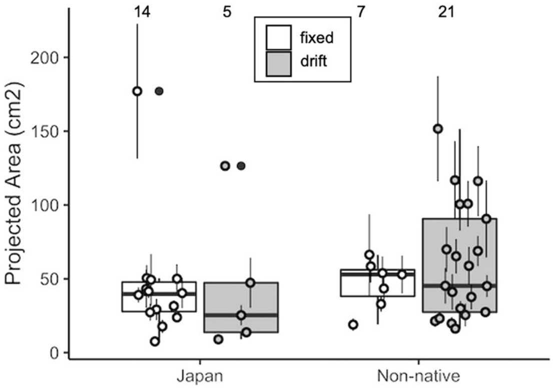

Fig. 3 Projected area (cm^2) of thalli between native and non-native populations, split by thallus fixation status (fixed versus drift). Numbers indicate population sample size. Non-native thalli were larger than were Japanese thalli ($p = 0.039$; Table 3). Points and bars represent means and standard errors for individual populations. Box, line and whisker plots indicate mean, and the 5–95% and 25–75% quartiles for the group. Data from tetrasporophytes and vegetative thalli combined were included

Table 3 Analyses of organismal size against region and individual-level fixation status

	Surface area
ALL THALLI	
(1|Population)	**< 0.001**
Region (Native vs Introduced)	**0.039**
Attachment (Fixed vs Drift)	0.301
TETRASPOROPHYTES ONLY	
(1|Population)	**< 0.001**
Region (Native vs Introduced)	**0.007**
Attachment (Fixed vs Drift)	0.590

We report the p-value from linear mixed models fit by REML on log-transformed data for either all thalli or tetrasporophytes only and using population as a random effect. For all thalli, the number of populations was 39 and the number of individuals per population ranged from 5–12. For reproductive tetrasporophytes only, the number of populations was 35 and the number of individuals per population ranged from 3–12

Are individuals in non-native populations older? Do they grow faster?) and the ecological mechanisms that generate this (i.e., Are they freed from local enemies? Are they in greater nutrient resource environments?) are yet unknown. While we saw no statistical influence of fixation status (drift vs. fixed) on organismal size, and our sampling were representative of the fixation profiles across the populations (largely free-floating in the non-native range, more often fixed in the native range), it is possible that a more balanced sampling design to specifically examine intra-population and inter-population difference may uncover subtle differences. For many studied macroalgal species, larger thallus size indicates higher productivity and propagule production (Denny et al. 1985), suggesting that the fitness of thalli, particularly via vegetative growth, would also be greater in the non-native range. We note that any increases in spore production in soft sediment habitats may not result in increased recruitment rates because of the lack of hard substratum upon which spores can settle.

Second, the increase in strength among non-native thalli is somewhat counter-intuitive. We predicted that non-native thalli could be weaker because this could facilitate fragmentation, which is consistent with the greater clonality rates among non-native relative to non-native populations (Krueger-Hadfield et al. 2016). Fragmentation is an efficient mechanism for both long-distance dispersal and increased population growth rates of *Gracilaria* (Guillemin et al. 2008) and non-native populations often have greater frequency of phenotypes that increase dispersal capacity in the expanding front of an invasion (Blanchette et al. 2002; Wright 2005; Shine et al. 2011; Seale and Nakayama 2020). However, our prediction was not supported by these data; rather non-native populations were stronger, even after the effect of site-level substrata is considered (Table 2B). We note another study found that a non-native seaweed (*Codium fragile*) had the highest breakage force among multiple species that occurred in low-energy estuaries (Thomsen 2004). Taken together, we suggest that non-native species and populations in low energy estuarine systems may generally be stronger.

It is possible that plastic or genetic increases in strength positively influence local population growth rates, but this remains to be robustly tested. One limitation of our study is that we focused on the main thallus axis and did not measure material properties of the branching node, which may be additionally relevant for fragmentation (see Lees et al. 2018). Increases in strength may also be a pleiotropic consequence of other traits that are under selection (i.e., are selectively neutral), and that the trait on its own is non-adaptive.

Third, increases in strength for fixed thalli relative to free-floating and drifting thalli suggest that thalli are responding to increases in drag that occur when fixed, even in the low-energy estuaries in which we collected thalli. In one of the few published comparisons of material properties between free-floating thalli relative to fixed thalli, free-floating thalli of the brown alga *Turbinaria ornata* are weaker and more brittle (Stewart 2006). We still require studies on how interactions with other species (e.g., through grazing or epibionts see e.g., Burnett and Koehl 2019), and variation in tide forces or wind generated waves (e.g., Burnett and Koehl 2021) may influence algal material properties in response to substrate.

Interestingly, the population means of thallus strength and body size were not tightly correlated either in the native or non-native range. This suggests that their expression, their evolution, or both are not tightly coupled. Alternatively, other organismal traits that we did not measure (e.g., growth rates, age) may be a better predictor of thallus strength than size. Moreover, it is possible that at small, local spatial scales that we did not sample, there are genotypes adapted to microgeographic gradients in wave energy we have missed in our broad survey.

In conclusion, our study provides evidence that direct comparisons of material properties between native versus non-native populations of an invasive species may uncover differentiation that may contribute (or has already contributed) to invasion success, but which has largely been overlooked (but see also Spector and Putz 2006; Murray et al. 2012). Whether or not this phenotypic shift is adaptive or non-adaptive depends on future experiments. For example, because sampling for material properties is destructive, we were unable to measure relative fitness of alternative phenotypes (e.g., strong vs weak) under field conditions which would be required (Heger and Jaschke 2014; Hodgins et al. 2018). However, our results do suggest that biomechanical properties should be added to the growing list of phenotypic shifts in *Gracilaria* that may have facilitated its invasion, along with greater tolerance for abiotic stresses and resistance to enemies which has been shown to be important in invasion biology in other biomes (see Introduction).

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Author contributions CJM, SAKH, AC, and EES conceived the project; SAKH, BAF, and EES collected samples; CJM, SAKH, BAF, LEL, and EES collected data; CJM and EES analyzed data.

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Availability of data and material (Data transparency) The datasets generated during and/or analyzed during the current study are available at https://github.com/esotka/AgarVermBiomechanics.git.

Code availability Software application or custom code.

Declarations

Conflict of interest None.

Ethical approval None required.

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