

The interacting effects of diversity and propagule pressure on early colonization and population size

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Summary

1. We are now beginning to understand the role of intraspecific diversity on fundamental ecological phenomena. There exists a paucity of knowledge, however, regarding how intraspecific, or genetic diversity, may covary with other important factors such as propagule pressure.

2. A combination of theoretical modelling and experimentation was used to explore the way propagule pressure and genetic richness may interact. We compare colonization rates of the Australian bivalve *Saccostrea glomerata* (Gould 1885). We cross propagule size and genetic richness in a factorial design in order to examine the generalities of our theoretical model.

3. Modelling showed that diversity and propagule pressure should generally interact synergistically when positive feedbacks occur (e.g. aggregation). The strength of genotype effects depended on propagule size, or the numerical abundance of arriving individuals. When propagule size was very small (<4 individuals), however, greater genetic richness unexpectedly reduced colonization.

4. The probability of *S. glomerata* colonization was 76% in genetically rich, larger propagules, almost 39 percentage points higher than in genetically poor propagules of similar size. This pattern was not observed in less dense, smaller propagules. We predict that density-dependent interactions between larvae in the water column may explain this pattern.

Key-words: biodiversity, bivalve, community ecology, ecosystem function, genetic variance, marine invertebrate, oyster, rocky reef

Introduction

Diversity is most often considered at the species level; however, there is growing evidence to suggest that genetic diversity can play a central role in mediating a raft of ecological and evolutionary processes (Hughes *et al.* 2008; for review). Many studies have examined genetic diversity effects in terrestrial grassland and animal communities (Tsutsui *et al.* 2000; Crutsinger *et al.* 2006; Johnson, Lajeunesse & Agrawal 2006) and marine invertebrates (Gamfeldt *et al.* 2005; Aguirre *et al.* 2012) but only in isolation. The genetic diversity of colonizing organisms is likely to covary with the number of individuals arriving, but this relationship will be temporally and spatially variable. Indeed, the intraspecific diversity of propagules and

the density at which they arrive could both influence the relative success of that most fundamental of ecological processes, colonization.

Propagule pressure relates the numerical size (or the abundance of arriving individual organisms) and arrival rates of propagules, which are in turn comprised of individual organisms (Lockwood, Cassey & Blackburn 2005, 2009). We would argue that this term also encompasses the diversity and quality of arriving propagules (Hedge, O'Connor & Johnston 2012). Numerous studies have now demonstrated the strong effects of individual quality on settlement probability and postsettlement fitness (Pechenik, Wendt & Jarrett 1998), and effects resulting from the diversity of arriving propagules should not be ignored (Lockwood, Cassey & Blackburn 2005). Where empirical studies have been conducted, however, the relationship between propagule pressure and colonization is usually

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expressed as a simple dose-response function relating propagule size with the subsequent number of individuals surviving until census (Ruiz *et al.* 2000; Clark & Johnston 2005, 2009; but see Hedge, O'Connor & Johnston 2012). Further, despite the intensity of study that interspecific diversity has engendered over the past few decades (Loreau *et al.* 2001; Hooper *et al.* 2005; and references therein), we have a limited understanding of the role of a genetic and phenotypic diversity in altering ecological processes such as colonization. Recent experiments have manipulated the genetic diversity of colonizers to assess survival (Gamfeldt *et al.* 2005; Crawford & Whitney 2010; Burgess & Marshall 2011; Ellers *et al.* 2011) but again none have explicitly considered the propagule size of arriving organisms in combination with manipulation of genetic diversity.

Effects may result from complex interactions between genotypes, or more simply from 'sampling' type processes. 'Sampling effects', or the additive effects of single-genotype inclusions, may arise due to the inherent genetic variability within propagules (Ellers *et al.* 2011). Different genotypes within a propagule may be better colonizers that settle more readily (Gamfeldt *et al.* 2005). When there is a distribution of genotypes in the larval population, including more individuals (increasing propagule size) will lead to higher chance of including one of these 'fit' individuals. Propagule size and genetic diversity may, however, interact in a non-additive, more complex manner. Nonadditive, intraspecific facilitative effects (or 'complementarity' sensu Loreau *et al.* 2001) can alter postcolonization processes in grassland and other terrestrial plant systems (Johnson, Lajeunesse & Agrawal 2006; Cardinale *et al.* 2007; Ellers *et al.* 2011), but the causal mechanisms of such effects are likely to vary between taxa and systems (Mulder, Uliassi & Doak 2001). In marine systems, for example, the interaction of propagule size and diversity may be particularly strong due to the significant role of species-specific chemical signalling on colonization behaviour across a wide variety of taxa (Tamburri, Zimmer & Zimmer 2007; Tamburri *et al.* 2008). That is, larvae may recognize genetically dissimilar or similar kin and alter settlement behaviour accordingly. Facilitative, aggregative behaviour may enhance settlement of marine invertebrates, leading to predicted increases in colonization. Alternatively, there may be a fitness advantage of settling among kin, reducing settlement probability in response to increasing diversity. Increasing propagule pressure would arguably increase the strength of this chemical signalling in the environment; however, this would be difficult to test.

Here we explore the consequences of genetic diversity and propagule size on two common metrics of colonization: (i) the probability of at least one individual settling into a new area ('colonization probability') and (ii) incipient population size (or the number of individuals settling and surviving until census). We use the term genetic richness, as opposed to diversity, to highlight the fact that we use quantitative methods, not molecular, to infer changes

in genetic variability (sensu Hughes & Stachowicz 2004). We also use proportional settlement, rather than colonizer abundance, to more clearly highlight differences in settlement between different propagule sizes. We examine the role of aggregative behaviour as a mechanism by which the genetic richness/propagule size relationship may operate. Higher colonization with greater propagule richness and size in the absence of aggregative behaviour would suggest that the propagule size/genetic richness relationship may result simply from a sampling effect. We first model a theoretical system of N individuals introduced into a new environment and explore the consequences of altering aggregative behaviour under differing propagule size/genetic richness regimes. We then use a model organism, the common Australian oyster *Saccostrea glomerata* (Gould 1850), to experimentally test the hypothesis that increasing propagule size will alter the effects of intraspecific richness on colonization and early population size.

Materials and methods

MODEL FORMULATION

We constructed a model simulating the following system; N individuals are introduced to a new environment (propagule size), during which time they may colonize a new location within T time intervals. Individuals (i) differ in their genotype (g), which determines their probability of colonization. Importantly, we can vary the number of genotypes (G) and explore the consequences of diversity and N on colonization and incipient population size. We use (p_{gi}) to denote the colonization probability of a given individual i of genotype g , in a given time interval. Finally, individuals die at each time interval with a probability m .

For each genotype within the pool, G , values were chosen from a beta distribution, which ranges from zero to one. The beta distribution has two parameters ($a1$ and $a2$) that determine the shape, mean and variance. We chose $a2$ such that the genotypic distribution was subject to the following constraints; the mean of the beta distribution, \bar{p} , was set $\bar{p} = [a1/(a1 + a2)]$, a parameter value that we controlled and modified. The distribution was not U shaped (i.e. $a1 \geq 1$, $a2 \geq 1$), and variance was maximized given those constraints (i.e. we set $a1 = 1$; as parameter values increase, variance decreases).

We modelled intraspecific interactions, whereby previously established individuals affect the probability of establishment (aggregation) or survival of other individuals, using an aggregation coefficient b . Thus, the probability of colonization was a function of an individual's genotype, and the number of previous colonizers, given by;

$$q_{gi} = 1 - p_{gi} \quad \text{eqn 1}$$

$$p_{i,t+1} = 1 - q_{gi}^{1+bN_t} \quad \text{eqn 2}$$

Where $P_{i,t+1}$ is the probability of a given individual, i , colonizing in time $t + 1$, q is the baseline level that it does not establish, N_t is the number of other individuals that have already established at time, i , and b is the aggregation coefficient. If $b = 0$, there is no aggregation and all individuals act independently.

We simulated T time intervals; therefore, we scaled parameters m and a_2 so that overall mortality (m) and the average probability of colonization (\bar{q}) were corrected for the length of the time interval considered, to isolate the effect of each. The number of time intervals was considered because it could influence the opportunities for aggregative effects – the probability of colonization for each individual in time $t + 1$ was dependent on the established population at time t . This discretization was done for computational logistical reasons. Thus;

$$m_T = 1 - (1 - m)^T \quad \text{eqn 3}$$

$$\bar{p}_T = 1 - (1 - \bar{p})^T, \quad \text{eqn 4}$$

where \bar{p}_T and m_T were the probabilities by time T of colonization and mortality, respectively. We specified a desired level of \bar{p}_i and m_T , and calculated the appropriate parameter values m and n , such that the cumulative probability after some time T was the desired specified level.

SIMULATIONS

To determine the general behaviour of the model, we created 1000 ‘realities’. Each reality mimicked our experimental set-up (see below), but allowed us to explore more broadly the ramifications of differences in propagule size and genetic diversity. Each reality consisted of a pool of 16 genotypes, which we subsampled to obtain different levels of diversity, G (1, 2, 4, 8, 16). For each level of diversity, N individuals were randomly chosen. We simulated different levels of N up to 256 (1, 2, 4, 8... 256) to examine the interactive effects of diversity and propagule pressure. For each combination of genotype and propagule pressure, we generated 300 replicates.

Thus, for each reality, we could examine whether the probability of colonization and/or the per capita number of colonizers (population size) differed depending on genetic richness and propagule size. The probability of colonization was the fraction of replicates where at least one individual colonized; thus, for the per capita number of colonizers, each replicate yielded a single estimate. To determine whether genotype had an effect, we compared $G = 1$ (all individuals had the same genotype) versus each other G (2, 4, 8, 16) at each given propagule size N . To test whether genotypic richness affected probability of colonization, we compared frequency of replicates that had established using a chi-square contingency table. We kept track of the directionality of relations and assigned a negative chi-square value whenever the number of establishments was greater for $G = 1$. To test whether genotypic richness affected number of colonizers (i.e. initial population size), we used a t -test.

We conducted sensitivity analysis with our 1000 realities using Latin Hypercube sampling (Blower & Dowlatabadi 1994) to determine which factors were most important. We varied $0.05 \leq \bar{p}_T \leq 0.3$, $0 \leq m_T \leq 0.3$, $3 \leq T \leq 10$ and $1 \leq b \leq 30$. These values were chosen to provide a range of values with enough range to illustrate any effects of aggregation in a theoretical context, remembering that $b = 1$ represented no aggregation behaviour. We used multiple regression to determine which factor most strongly affected the relation between genetic richness and probability of colonization and incipient population size [i.e. we regressed the parameter values against chi-square value and t -values, respectively (see above)]. Additionally, simulating a

range of parameter values allowed us to examine the generality of the consequences of differences in propagule pressure (N) and genetic richness (G). Within each combination of N and G , we calculated the probability of colonization and the average number of colonizers, estimated from 1000 realities.

EXPERIMENTAL METHODS

Using an experimental system and design that closely matched our theoretical modelling, we explored colonization of the Australian native bivalve, *S. glomerata*. The probability of settlement, and incipient population sizes, between offspring from single-mated pairs or mixed offspring from several mated pairs was compared.

SPAWNING AND REARING OF LARVAE

Experiments were conducted from April to August 2011; timed to coincide with the end of the *S. glomerata* spawning season in south-east Australia. Adult *S. glomerata* were obtained from wild stock collected from commercial leases in the Shoalhaven and Port Stephens estuaries, NSW, Australia. Strip-spawning techniques were used to create larval cohorts from eight separate mated pairs. Eggs from eight haphazardly chosen female adult oysters were transferred directly from the female gonad via pipette into individual 500-mL containers, then suspended in 400 mL of $1 \mu\text{L}^{-1}$ filtered sea water (FSW). Sperm were strip spawned from the male oysters and suspended in FSW. A 0.1 mL sample of each sperm suspension was examined using a haemocytometer to confirm motility and to estimate concentration. Sperm solution from a single male was added to an egg suspension from a single female, such that sperm concentration was $c. 10^5$ sperm mL^{-1} . Each sperm/egg suspension was observed microscopically, and more sperm solution added if less than two or three sperm were observed around the periphery of each egg. Once eggs from each mated pair showed signs of cell cleavage, the newly fertilized larval cohorts were transferred to individual 200-L tanks of FSW at 23 ± 1 °C for the duration of the larval rearing period. Larval cohorts were initially stocked at a density of 10 larvae mL^{-1} for the first 24 h, before being reduced to $c. 5$ larvae mL^{-1} until competent to settle. The rearing period for the larval cohorts was $c. 20$ day. During this time, water was changed every second day with new FSW and the larvae were fed *ad libitum* the micro algae *Pavlova lutheri*, *Chaetoceros muelleri*, *Chaetoceros calcitrans* and *Tahitian Isochrysis aff. galbana*. Larvae were deemed competent to settle when (i) larval size was $c. 330$ – $350 \mu\text{m}$, (ii) eyed pediveliger larvae were observed to be actively searching for substrate using their protruding foot and (iii) some larvae were observed to have settled to the sides and bottom of the tank.

LARVAL INOCULATIONS

After the larval rearing period, competent larvae from each of the eight mated pairs, henceforth referred to as monocultures, were transferred into individual 1-L containers of FSW (low richness, single crosses). A ninth mixed culture was constructed by combining equal amounts of larvae from each monoculture in a separate 1-L container (our high-richness treatment). In order to test the effects of propagule size and genetic variability on settlement, eight (low propagule size) or 60 (high propagule size) larvae from either the monocultures or the mixed culture were

added to a 40-mL petri dishes in a fully balanced factorial design with 300 replicates per treatment combination (total $n = 1200$). Larval densities within the Port Stephens estuary are generally around 0–0.2 late-stage larvae per mL. In some areas, densities as high as 2.5 larvae per mL are found. We therefore manipulated propagule density to both normal, and slightly raised, levels in our experimental system (details below). We were also able to test for differences in proportional settlement between individual monocultures by selecting equal numbers of petri dishes in the monoculture treatment from each cross (Stratified Random Assignment). Larval suspensions were gently mixed in order to fully resuspend any larvae that were not actively swimming and had dropped to the bottom of the container. Individual larvae were counted as they were drawn into a pipette and transferred to a petri dish. While this method resulted in haphazard sampling of larvae in the mixed cohort, there was no way of knowing the brood identity of individual larvae, and larval suspensions were thoroughly homogenized prior to pipetting larvae. We were therefore confident of sampling eight or 60 larvae from the mixed cohort without bias towards a particular genotype. The sea water/larvae suspension in each petri dish was made up to 40 mL with the addition of extra FSW. Petri dishes also received 2 mL of algal food solution (see above), enough to satiate them for several days. Petri dishes were arranged randomly on a bench in a constant temperature room at 15 °C. Petri dishes were left in darkness for 4 day before census.

CENSUS AND STATISTICAL ANALYSIS

After 4 days, petri dishes were gently shaken to ascertain how many larvae had settled to the substratum. Generalized linear modelling (GLM) was used to test for effects of richness (two levels, monoculture and mixed culture) and propagule size (two levels, High and Low) on (i) the proportional settlement rates within each treatment combination (akin to incipient size) and (ii) the probability of at least one larva settling within each treatment combination. A scale parameter, θ , was used to compensate for over dispersion (Zuur *et al.* 2009). We tested for interactive effects by first including all parameters, then removing the interaction term and conducting a log-likelihood test using a chi-square distribution (Zuur *et al.* 2009) comparing the residual and null deviances. Contrast estimates from the model were used to compare the treatment means for significance.

We analysed differences in proportional settlement between the individual larval families in the same way as above; remembering there were eight separate families reared during this experiment. We replaced the β (Diversity_{*i*}) term in our GLM with β (GenotypeID_{*i*}) representing the genotype identity of the oysters settling on petri dish (8 levels, G1–G8 categorical), where *i* represent the individual monocultures.

ADDITIVE OR NONADDITIVE EFFECTS

Given that we found an effect of richness on settlement of *S. glomerata*, we examined whether this result was a sampling effect or a biological interaction between the monocultures in the high-diversity treatment. We modified a bootstrap method outlined in Johnson, Lajeunesse & Agrawal (2006) and Mulder, Uliassi & Doak (2001). We compared the proportional settlement data we observed during the experiment in the mixed cultures, to data we ‘expected’ to find if there was no interactive effects (or ‘complementarity’ between

the larval broods; sensu Loreau & Hector 2001). For this system, we had the impediment of not knowing the family identity of individual settled oysters within the mixed cultures. The proportional settlement for the ‘null’ population was therefore created by summing the number of recruits from eight random petri dishes in the monoculture treatments (giving each of the eight monocultures equal probability of being included) and creating a proportional settlement measure by dividing by 480 (remembering that each petri dish in the high propagule pressure treatment had 60 larvae). This random selection of recruit data from the low diversity treatments was done without replacement, such that a total of 37 measures of summed proportional recruitment could be obtained (amount of petri dishes (300) divided by the number of petri dishes in each bootstrapped sample (8) = 37.5). The mean proportional recruitment from these 37 measures was obtained, and the process repeated 10 000 times (Johnson, Lajeunesse & Agrawal 2006). If the mean proportional settlement of our constructed population fell outside the 95% confidence limits of our observed population, then they were significantly different at the $P < 0.05$ level, providing evidence for nonadditive effects of richness. This bootstrap resampling method and the GLM models used above were coded in the R statistical environment (R Development Core Team 2011).

Results

SIMULATION AND ANALYTIC MODELS

The mechanisms of colonization differed when measuring incipient size (i.e. the number of colonizers) or the probability of colonization (the probability of at least one individual colonizing within a system). The probability of

Table 1. Sensitivity analysis of simulation modelling examining the relationship between genetic richness and propagule pressure under differing amounts of aggregative behaviour

	Incipient population size	Colonisation probability
Intercept	−1.928** (0.586)	0.565 (3.574)
<i>N</i>	0.015*** (0.001)	0.211*** (0.020)
Number of genotypes	0.164*** (0.013)	1.077*** (0.080)
Mortality	−2.544 (3.452)	−41.200 (21.686)
Max t	−0.048 (0.042)	−0.845** (0.260)
Prob. Estab.	22.412*** (5.628)	95.503** (34.326)
Prob. Estab ²	−58.651*** (16.173)	−289.825*** (99.250)
Aggregation	0.033*** (0.009)	−0.055 (0.058)
<i>N</i> ²		−0.001*** (0.000)
<i>R</i> -square	0.326	0.253
<i>N</i>	992	941

Model specifications are found in eqns 1 and 2. (*) <0.05, (**) <0.01, (***) <0.001.

colonization was independent of the aggregation coefficient (b , eqn 2, Table 1); however, aggregation was important in determining the incipient population size (Table 1). Incipient population sizes increased with greater genetic richness and propagule pressure (Fig. 1a). This increase was not observed without the inclusion of the aggregation parameter (Table 1). Moreover, the strength of the genotype effect depended on propagule size (Table 1, Fig. 1a). This pattern was similar when modelling the probability of colonization (at least one larvae settling), albeit with no link to aggregation (Fig. 1b). Further, when propagule pressure was reduced below a

low threshold ($c. 4$ individuals), we found that greater genetic richness unexpectedly led to a reduction in colonizer abundance (Fig. 1b). These formed the theoretical expectations for the relation and interactions between propagule pressure, genetic diversity and intraspecific facilitative effects (e.g. aggregation) in determining the probability of colonization and incipient population size.

To examine these phenomena in greater detail, we constructed a simplified analytically tractable model, with only two genotypes, two individuals and two time intervals. The full working of this model can be found in the Supporting Information.

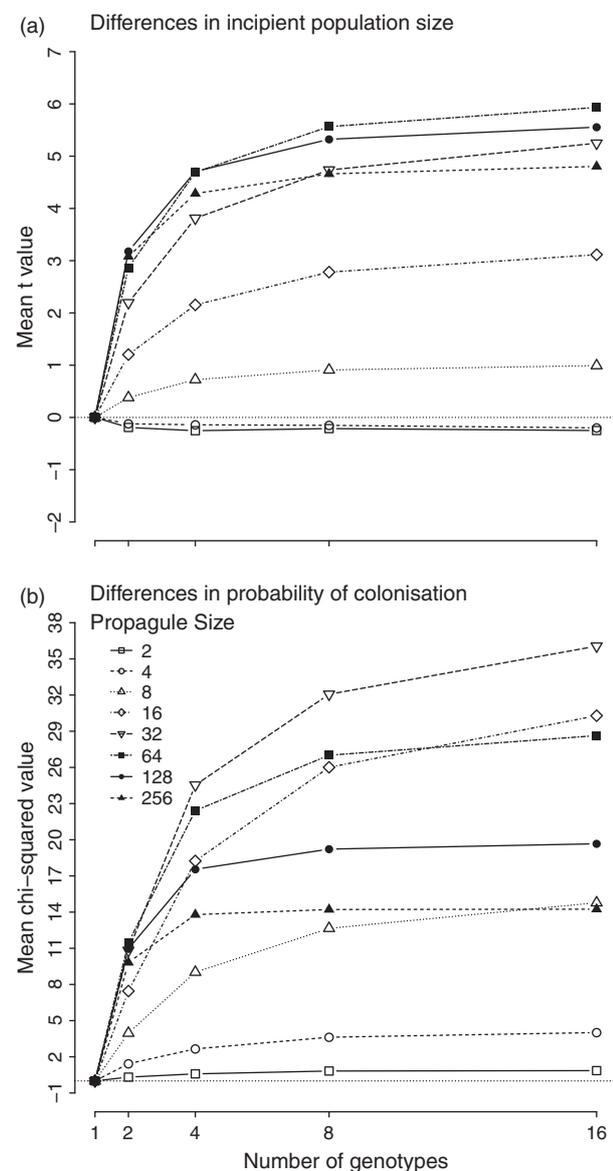


Fig. 1. Theoretical results from simulations of 1000 realities with different levels of genetic richness and propagule size. (a) t -values resulting from comparisons comparing $G = 1$ to each of the other levels and (b) chi-square values resulting from the comparisons of the frequency of replicates with at least one oyster settler in the $G = 1$ treatments compared to each of the other genetic richness treatments. The dotted horizontal line is at $y = 0$.

COLONIZATION EXPERIMENT

Probability of colonization

Probability of colonization increased interactively with greater propagule pressure and genetic richness (LR χ^2 (Gen \times PP)_{1,1206df} = 4.31, $P = 0.03$). The probability of at least one larvae settling was $c. 76\%$ in mixed cultures and large propagule sizes (probability of settlement = $1/[1 + \exp(1.175)]$; Table 2), almost 37% percentage points greater than in monocultures of the same propagule size (Table 2). This pattern was not observed in smaller propagules (Table 2).

Table 2. Parameter estimates (\pm Standard Error) from binomial generalized linear modelling of (i) incipient population size and (ii) probability of colonization

	Incipient population size	Probability of colonisation
Intercept [†]	-3.147*** (0.117)	1.175*** (0.136)
Gen. Richness [‡]	-0.363* (0.181)	-0.487** (0.182)
Prop. Press. [§]	0.665*** (0.146)	-1.722*** (0.181)
Gen. Rich \times Prop. Press. [¶]	0.327 (0.220)	0.516* (0.249)
N	1200	1200
Null deviance	164.16	1662.9
Residual deviance	153.693	1508.316

[†]The *intercept* is the parameter estimate for Prop. Press = High and Gen. Rich. = High.

[‡]Change in estimate from the *intercept*, relating to the Prop. Press = High. Cten. Rich = Low treatment.

[§]The change in estimate from the *intercept*, relating to the Prop. Press = Low, Gen. Rich = High treatment.

[¶]The interaction effect, or the change in estimate relating to the Prop. Press = Low and Gen Rich = Low Treatment.

Parameter estimates compare the difference in logits (log odds) between the large propagule size, mixed cultures (the intercept) and the three other treatments. That is, the difference in logits between the large propagule size, mixed cultures, treatment and the large propagule size, monoculture, treatment is -0.363 . (* < 0.05 , ** < 0.01 , *** < 0.001).

Incipient population size

The statistical evidence for an interaction between propagule pressure and genetic richness was weaker when measuring incipient population size (LR χ^2 (Gen \times PP)_{1,1206df} = 2.31, P = 0.13). There was, however, a difference in population sizes between treatments (Table 2, Fig. 2). Large, mixed cultures had predicted proportional settlement of around 4%, almost 40% greater than the proportional settlement in large monocultures (Table 2, Fig 2). This pattern was not observed in propagules of smaller size (Table 2) as expected theoretically.

Between cohort analysis

There was no interaction between cohort identity and propagule size when we tested for ‘between cohort’ variation in incipient population size (LR χ^2 (Gen \times PP)_{7,553df} = 2.08, P = 0.95, Fig. 2) or probability of colonization (LR χ^2 (Gen \times PP)_{7,553df} = 5.94, P = 0.54, Fig. 2). Only propagule pressure was found to be an adequate predictor of both colonization probability (LR χ^2 (PP)_{7,553df} = 53.80, P < 0.001) and incipient population size (proportional settlement; LR χ^2 (PP)_{7,553df} = 32.15, P < 0.001).

Additive vs. interactive effects on incipient population size

After 10 000 permutations of our bootstrapped samples, the mean proportional settlement of our null population was 0.029; less than the mean of our observed settlement in the high propagule pressure treatment, 0.041 (t_{300df} = 4.29; P < 0.001).

Discussion

The role of diversity as a key determinant of basic ecological processes is paradigmatic (Hooper *et al.* 2005). Similarly, propagule size, on some level, should always affect population success and persistence (Schiel 2004). Using theoretical simulation modelling and a laboratory-based settlement experiment, we show that these important factors may interact. Genetic richness effects are observed for both incipient population sizes and the probability of colonization only when propagule sizes are larger. There are, however differences in the mechanisms underlying each metric of colonization. Our modelling highlights the role of nonadditive, aggregative behaviour in increasing population sizes in larger propagules. Conversely, modelling failed to provide any evidence of nonadditive aggregative facilitation when measuring the probability of colonization (at least one individual settling). Our experimental manipulations of *S. glomerata* also show that population sizes, and the probability of colonization, increased with greater genetic richness and propagule size although the observed mechanisms by which this phenomena operates seem to differ from our theoretics.

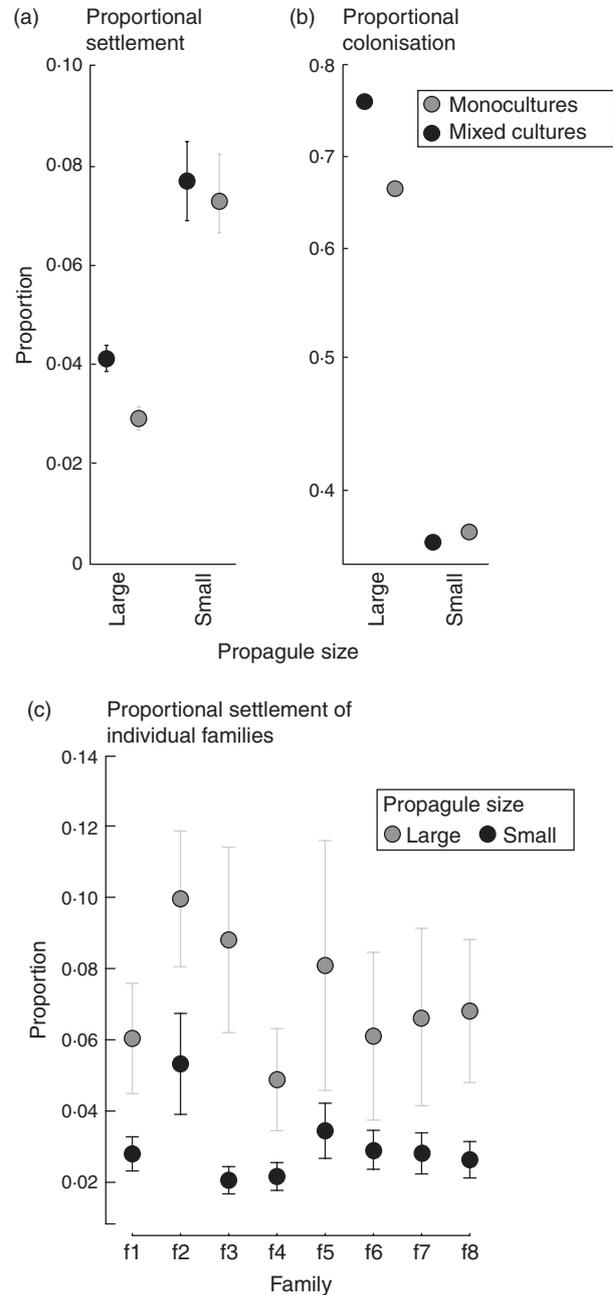


Fig. 2. (a) The percentage of petri dishes within each treatment combination that had at least one *S. glomerata* larvae settling to the substratum. Filled circles are the mixed cultures, and grey circles are the monoculture treatments. (b) The mean proportional settlement of *S. glomerata* in small and large propagules, in either monoculture or mixed culture. Filled circles are mixed culture treatments, and grey circles are the monocultures. Bars represent one standard error around the mean. (c) Mean proportional settlement of *S. glomerata* from each of the eight different mated pairs (monocultures). Bars represent the one standard error around the mean. Filled circles represent large propagules (60 larvae), and grey circles represent small propagules (eight larvae).

If colonization is measured as the simple probability of at least one individual arriving, settling, and surviving until census (‘probability of colonization’ in the current study), then our theoretics indicate that facilitative

interactions (e.g. aggregation) have little effect. This is logical; if our metric is a measure of the probability of at least one propagule colonizing, it is intuitively disconnected to how many individuals of the same propagule have already settled (the mechanism by which our model was constructed). Despite this, our manipulative experiment with *S. glomerata* showed evidence for non-additive interactions between propagule pressure and richness on the probability of at least one larvae settling. We posit that presettlement processes may also determine colonization probabilities, something that was not explicitly explored in our theoretics. This would explain the nonadditive increase in colonization probability in propagules of greater richness and size. It is often difficult to distinguish the root causes of non-additive mechanisms experimentally, for example niche differentiation or facilitation, and these mechanisms are generally grouped into the catch-all term ‘complementarity’ (Loreau & Hector 2001). Chemical signalling within the water column may induce a facilitative response, prior to any larvae actually settling. This would then increase the probability of at least one individual settling, that is, probability of colonization in the current study. This is subtly different to the current paradigm in the colonization literature that emphasizes facilitation between settled individuals and larvae, or between newly settled juveniles and adults (Tsutsui *et al.* 2000; Toonen & Pawlick 2001). Presettlement facilitative processes were not explored within our theoretical framework, nor was our experiment able to test for the mechanisms behind such effects. This would be a novel direction for future research.

While colonization probability is an intuitively important metric of colonization, measuring incipient population sizes is perhaps more important for biphasic, sessile marine animals (Connell 1961; Gamfeldt *et al.* 2005). We used proportional settlement as our metric for population size, given the different levels of propagule size. Our modelling highlights aggregative behaviour in determining early population sizes. The degree to which a species shows aggregative facilitation theoretically determines the strength of the genetic richness/propagule size relationship. Aggregative behaviour mediates this relationship unexpectedly when propagule size is small and individual probability of settlement is low (Supporting Information). If propagule sizes are small, then our modelling suggests that genetically similar propagules will settle in greater numbers when the benefits of aggregation are weak. Conversely, as propagule sizes increase, the aggregative effects grow in strength as more individuals settle. Thus, even individuals with low settlement probabilities are eventually induced to settle.

Larger populations of *S. glomerata* occurred when genetic diversity increased, but only in propagules of greater size. Our empirical evidence for interactive effects is, however, statistically weaker. The interaction term, while remaining in the model, explained little of the variation. So while our experiment clearly highlights the

role of diversity and propagule size in determining incipient populations of *S. glomerata*, the nonadditive nature of this interaction is questionable. We did, however, show clear effects of diversity. Additionally, our bootstrapping technique provided evidence that increasing diversity in this system increased incipient population sizes of oysters via a non-additive type method, perhaps via a chemical signalling pathway inducing settlement in areas of high genetic richness in the water column larvae. Indeed, both additive and nonadditive processes would invariably lead to increases in colonization in this system. Increased propagule sizes within our experimental apparatus may have led to a density-related, biologically stressful environment. Genotypes may respond differently to biotic stress, leading to variation in population performance when genotypes are mixed (Yachi & Loreau 1999; Mulder, Uliassi & Doak 2001) and propagule sizes increase.

There are many applied fields of biological research that might benefit from understanding interactions between genetic richness and propagule pressure. Environmental and demographic stochasticity are usually invoked to explain why small founder populations have much higher chances of extinction (Hughes *et al.* 2008). Many invasive species, however, establish and spread from very small amounts of propagule input (Simberloff, 2009). The disjunct between these two concepts raises the idea of an ‘invasion paradox’ (Simberloff, 2009). Multiple introductions, particularly from several locations, may partly resolve this paradox due to the subsequent predicted increase in genetic richness (Kolbe *et al.*, 2004; Simberloff, 2009). In marine systems, ballast water and hull fouling of commercial and recreational vessels are the main vectors of invasive spread (Ruiz *et al.* 2000). Logically, these vectors may entrain larvae from multiple source locations on their voyage creating a genetically rich larval population that is introduced into new areas. Here we provide some of the first evidence to show that increasing the genetic richness of a larval population may increase the colonization of marine organisms. Larger more diverse populations would be more protected from Allee effects or demographic and environmental stochasticity, leading to predicted increases in population viability.

Acknowledgements

We are extremely grateful to C. Bezic Alpenes for all her help in the laboratory, and K. Johnston and S. O'Connor for larval rearing. We also thank R. Smith, W. Brassil, K. Edge, C. Ietella, L. McPhan, J. Lavender and N. Rivero for added laboratory assistance. LHH was supported by an Australian Government Post Graduate Award, and an Australian Research Council Discovery Grant awarded to ELJ. We are unaware of any conflict of interest between any authors of this manuscript.

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Received 31 January 2013; accepted 9 July 2013

Handling Editor: William Gurney

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Data S1. Simplified, analytically tractable, model investigating propagule pressure and diversity with only two genotypes, two individuals and two time intervals.