

Phenotypic and phylogenetic evidence for the role of food and habitat in the assembly of communities of marine amphipods

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Abstract. The study of community assembly processes currently involves (a) long-standing questions about the relative importance of environmental filtering vs. niche partitioning in a wide range of ecosystems, and (b) more recent questions about methodology. The rapidly growing field of community phylogenetics has generated debate about the choice between functional traits and phylogenetic relationships for understanding species similarities, and has raised additional questions about the contribution of experimental vs. observational approaches to understanding evolutionary constraints on community assembly. In this study, we use traits, a phylogeny, and field surveys to identify the forces structuring communities of herbivorous marine amphipods and isopods living in adjacent seagrass and macroalgae. In addition, we compare our field results to a recently published mesocosm experiment that tested the effects of both trait and phylogenetic diversity on coexistence using the same species and system. With respect to community assembly processes, we found that environmental filtering was the dominant process in macroalgae habitats, that niche partitioning was the dominant process in seagrass habitats, and that the strength of these assembly mechanisms varied with seasonal fluctuations in environmental conditions and resource availability. These patterns are indicated by both phylogenetic relationships and trait distances, but the type of resources being partitioned in seagrass habitats can only be deciphered using trait data. Species coexisting in seagrass in the field differed not in their feeding niche but in traits related to microhabitat use, providing novel evidence of the relative importance of competition for food vs. habitat in structuring communities of phytophagous invertebrates. With respect to methodology, the results for seagrass habitats conflict with those obtained in mesocosms, where feeding trait diversity did promote coexistence and phylogenetic diversity had no effect. This contrast arises because a greater range of traits (some of which have much stronger phylogenetic signal than feeding traits) contribute to community assembly in the field. This highlights a mismatch between the processes that drive community assembly in the field and the processes we isolated in experimental tests, and illustrates that using phylogeny as a single proxy in both contexts may impede the synthesis of observational and experimental results.

Key words: amphipod; coexistence; community phylogenetics; competition; grazer; habitat partitioning; phylogenetic signal; relatedness within communities; seagrass; trait structure.

INTRODUCTION

Interpreting the processes underlying patterns of community composition relies on an understanding of the ways species' similarities and differences govern their co-occurrence. Hypotheses about community assembly involve one or both of (a) environmental filtering, in which species sharing certain environmental tolerances or habitat requirements co-occur in locations meeting those criteria, and (b) resource partitioning, in which species with sufficiently different feeding or habitat niches avoid competitive exclusion (MacArthur and Levins 1967, Weiher and Keddy 1995, Webb et al. 2006, Cornwell and Ackerly 2009). As measurements of similarities and differences, ecologists use functional

traits thought to affect species' interactions with each other and their environment, and increasingly, phylogenetic relationships between those species as a potential proxy for overall ecological differentiation (Webb et al. 2006, Emerson and Gillespie 2008, Cavender-Bares et al. 2009, Vamosi et al. 2009, Burns and Strauss 2011).

However, the amount of information about patterns and mechanisms of community assembly that is contained in data on specific traits vs. phylogenetic relationships remains unclear. On the one hand, phylogenetic relationships are potentially less biased than trait data; the phylogeny does not depend on our preconceptions about the types of functional traits that might matter in community assembly or how those traits should be quantified. In addition, phylogenetic relationships might integrate overall ecological similarity that stems from multiple traits (Kraft et al. 2007, Cadotte et al. 2008). On the other hand, phylogenetic relationships may tell us nothing about ecological similarity between

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species if the traits with the greatest influence on assembly processes are evolutionarily labile (Losos 2008, 2011). If we are interested in understanding the ecological mechanisms underlying community assembly, phylogenetic relatedness may also be harder to interpret than trait data if multiple potentially relevant traits show phylogenetic signal and all contribute to phylogenetic community structure.

In this study, we use both functional traits and phylogenetic relationships to investigate community assembly in a group of amphipod and isopod crustaceans in Bodega Harbor, California. These marine grazers use both seagrass beds and macroalgae on shallow mudflats for habitat and food, directly consuming the macrophytes and/or feeding on the microalgae that epiphytize seagrass leaves. We assess community composition across these two major habitat types and across seasonal variation in temperature and resource availability. We then test two contrasting hypotheses about community assembly in these species by comparing observed community structure (average phylogenetic and trait distances between co-occurring species) to that expected under random assembly from the harbor-wide species pool.

One hypothesis about the most important drivers of community assembly in one of our two habitat types (seagrass beds) comes from a recently completed mesocosm experiment, in which diversity in feeding traits promoted coexistence, and phylogenetic diversity had no effect on species interactions (Best et al. 2013). These results suggest that field communities should be more likely to contain species with divergent feeding niches, and may show no phylogenetic structure because feeding niche has little phylogenetic signal. However, despite the current focus on directly assessing the mechanistic links between phylogenetic relationships and ecological interactions using experimental approaches (Maherali and Klironomos 2007, Burns and Strauss 2011, Peay et al. 2011, Violle et al. 2011, Best et al. 2013), the relationship between experimental results and patterns of community assembly observed for the same species in the field remains unclear. As noted by Maherali and Klironomos (2007, 2012), field communities might be influenced by biotic and abiotic variables that vary over spatial or temporal scales greater than those in controlled experiments.

As an alternate hypothesis about the drivers of community assembly in the field, we note that previous work on niche specialization in both marine amphipods and phytophagous insects has shown that habitat choice to minimize predation risk or exposure to adverse environmental conditions may be more important than competition for food in structuring these communities (Strong et al. 1984, Bernays and Graham 1988, Hay et al. 1990, Duffy and Hay 1991, Hay and Steinberg 1992). This suggests that traits related to habitat choice may show more community structure than feeding traits. This may include both traits related to habitat use on a

macro-scale, such as water temperature tolerance along a depth gradient, or traits related to choice of microhabitat at a single location, such as body size or mechanisms for attaching to substrate. As several of these traits are correlated with phylogenetic distances in our species (i.e., have phylogenetic signal [Best and Stachowicz 2013]), field communities might plausibly show phylogenetic structure.

Ultimately, our investigation of community assembly yields two major findings. First, we find that the relative importance of environmental filtering and niche partitioning varies between the two major habitats in this system, and between seasons. Second, for the seagrass habitats in our system, we find that community assembly in the field is indeed influenced by a different set of traits than those determining species interactions in experimental mesocosms, and that this has a profound impact on the probability that phylogenetic relationships predict community assembly patterns. These findings provide new insight into the drivers of between- and within-habitat community assembly in marine arthropods, as well as urging caution when using experimental manipulation of trait and phylogenetic diversity to mechanistically test conclusions from observational studies in the field.

METHODS

Study system

Invertebrate mesograzers in our system (Bodega Harbor, California: 38°19.110' N, 123°04.294' W) include 11 common amphipod species and two isopods (see Fig. 1 for names; Appendix A: Table A1 for additional details). These species are epifaunal, inhabiting extensive mudflats covered with beds of macroalgae (*Ulva* spp., hereafter *Ulva*), beds of *Zostera marina* (hereafter eelgrass) which also contain variable amounts of *Ulva* (Olyarnik and Stachowicz 2012), and the encrusting invertebrates and macroalgae (again, *Ulva*) growing on floating docks and pilings. Because eelgrass beds are deeper (range from 3 to 0 m below mean lower low water [MLLW]), than *Ulva* beds on mudflats (0 to 0.7 m above MLLW), *Ulva* beds reach higher water temperatures than eelgrass beds at low tide.

These amphipods and isopods feed on a variety of primary producers, including *Ulva*, eelgrass, eelgrass detritus, and epiphytic microalgae growing on the eelgrass blades. While one of the few non-crustacean grazers in this system (the sea hare *Phyllaplysia taylorii*) can also be an important grazer of epiphytic microalgae (Hughes et al. 2010), it is more patchily distributed and lower in abundance. The species included in this study therefore represent the vast majority of the grazers present in this system. Finally, of the 13 species in this study, the 9 most abundant amphipods were used in a corresponding mesocosm experiment that examined the effect of trait and phylogenetic diversity on competitive coexistence in eelgrass beds (Best et al. 2013).

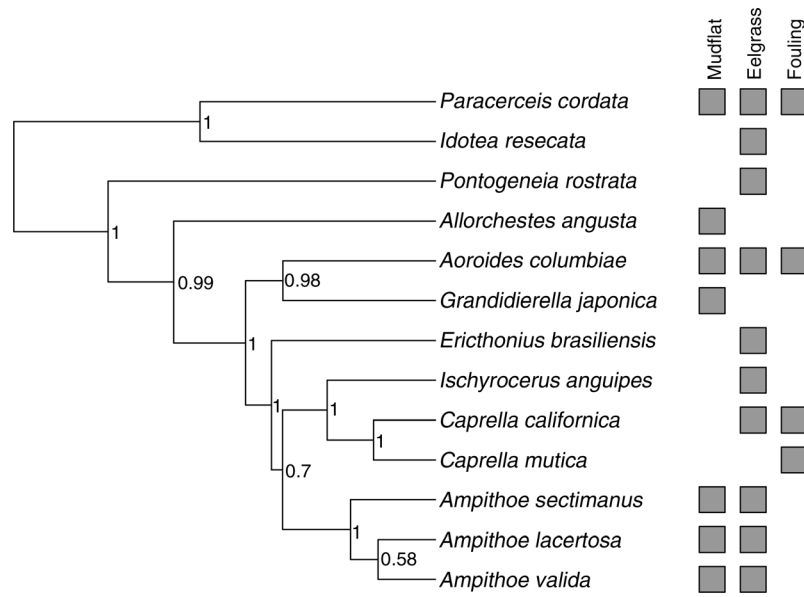


FIG. 1. Maximum clade credibility ultrametric phylogeny, with branch lengths in uncalibrated (relative) time units and nodes labeled with posterior probabilities. Isopods (*Paracerceis cordata* and *Idotea resecata*) are the outgroup. Boxes indicate the most common habitats for each species.

Field surveys

We measured abundance of all grazer species found within Bodega Harbor during seven survey periods between July 2009 and September 2010 (specifically, July, September, December, March, May, July, and September). This sampling scheme gave greater resolution to the summer and fall, the period of maximum population growth and abundance for most species in our system. At each survey period we collected community samples at each of seven different sites in Bodega Harbor, including five eelgrass meadows and two mudflats, with sites separated from each other by 500 m to 3 km. At each site, five samples were taken 10 m apart along a transect when the site was under ~ 0.6 m of water. To sample, we collected a vertical core of the water column using a fine-mesh bag ($< 500 \mu\text{m}$) affixed to a flexible 30 cm diameter hoop, capturing all vegetation and animals. This 30 cm diameter water column is a scale at which we expected the sampled species to interact, and is our definition of a community in this system.

In addition, to ensure that we fully characterized the grazer species pool available for community assembly, we also surveyed five fouling communities at a floating dock site. We used fouling plates the same size as the other samples, hung them vertically in a transect along the floating dock, and left them to accumulate *Ulva* and animals for two months prior to each collection. We did not attempt to test community assembly in floating dock communities because they tended to contain a variety of rare and nonherbivorous species, but we did include the common herbivores found in these communities in the species pool for null models of community assembly (as

detailed in *Community phylogenetic and phenotypic structure*). Only one of these species (*Caprella mutica*) was found exclusively in dock communities.

After transporting all sample bags to the Bodega Marine Laboratory, we cleaned each blade of eelgrass and algae of grazers, collected the grazers on a 1-mm sieve, and counted adults of each species (defined on the basis of size and secondary sexual characters; egg production in females and, for amphipods, gnathopods [claws] in males, as detailed in Chapman 2007). We dried eelgrass and *Ulva* at 60°C and used dry mass as our measure of macrophyte abundance. We also determined the density of epiphytic microalgae on eelgrass blades by randomly selecting four full leaves from each eelgrass sample, scraping, filtering, drying, and weighing all epiphytes from these leaves, and dividing the dried mass by the leaf surface area. Finally, to record water temperature at each of our sites we deployed HOBO Pendant temperature loggers (Onset Computer Corporation, Pocasset, Massachusetts, USA). We anchored these loggers ~ 5 cm from the sediment surface, in order to measure temperatures within rather than above the surrounding eelgrass or macroalgae at all tidal depths. For sites with little variation in depth we used one logger, and for sites with steeper depth gradients we used two, with one at either end of the sampling transect. Loggers recorded temperature every 30 minutes; from these records we extracted the maximum temperature at each location in each month in order to compare the peak temperature stress experienced by grazers at that habitat. This is consistent with our measurement of grazer temperature tolerance as survival under maximum observed temperatures (see *Traits*). However, we

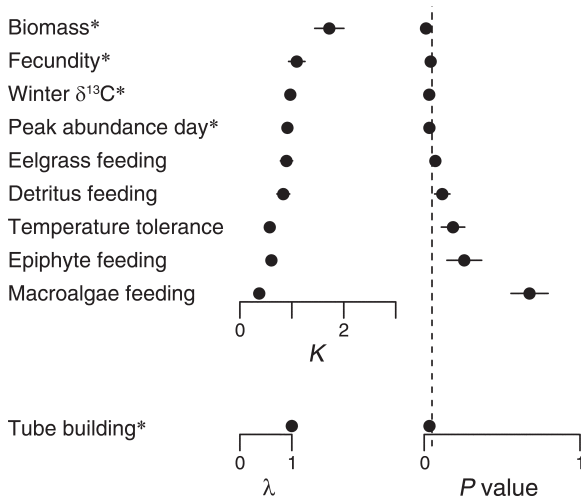


FIG. 2. Relative phylogenetic signal in traits. For continuous traits, points represent the mean estimate of K and the mean P value from the randomization test (\pm SD) across 1000 trees sampled from the posterior distribution of ultrametric trees. For the one discrete trait (tube building), the mean value of λ and the mean P value from the corresponding likelihood ratio test are given. The dashed lines indicate the $P = 0.05$ significance threshold and "*" indicates traits with average P value less than this threshold. Continuous traits are ordered top to bottom from most to least evidence for phylogenetic signal based on both the estimate of K and the P value. We emphasize the relative signal among traits rather than whether each trait does or does not show significant signal, because the power for these tests is known to be low for fewer than 20 species (Blomberg et al. 2003, Best and Stachowicz 2013).

also note that other summary metrics, such as mean temperature, indicate the same relative temperature differences among sites.

Traits

For each of the 13 species in our study, we used previously reported data for a variety of potential environmental filtering and resource use traits (Best and Stachowicz 2013). We also used phenology as a trait, as indicated by whether the period of peak abundance for each species was March, May, July, September, or December (see Appendix B: Fig. B1), and assigned each species a trait value equal to the median day of that month (e.g., March = day 75). Previously reported traits include body mass, temperature tolerance, tube building, stable isotope signatures, and feeding rates (Best and Stachowicz 2013). Briefly, we measured body mass as average individual dry mass, temperature tolerance as the reduction in survival time in water of elevated temperature (25°C, approaching the warmest temperature observed) compared to an ambient summer temperature control of 12°–15°C, and tube-building as the ability to build tubes on or in any substrate, conferred by the presence of silk-producing glands (Myers and Lowry 2003).

To represent the feeding niche for each species, we chose to measure the potential and realized components

of the diet rather than to rely on morphological characters, which we have not found to be adequate predictors of diet in our species (Best et al. 2013). These traits are previously reported in Best and Stachowicz (2013), but summarized here to aid interpretation. First, we used carbon stable isotope signatures ($\delta^{13}\text{C}$), measured in winter (December 2009), as a potential indicator of realized niche in the field (Best and Stachowicz 2013). Second, as a measure of fundamental niche we assessed feeding rates using no-choice feeding trials on each of the four commonly available foods (eelgrass, eelgrass detritus (hereafter "detritus"), *Ulva*, or epiphytic microalgae). Feeding rates (consumption per individual per day) were measured in terms of wet mass (for eelgrass, detritus, and macroalgae), or chlorophyll *a* (for epiphytic microalgae). We also considered the total number of foods each species can potentially consume (niche breadth), which ranges from 1 (almost all species eat epiphytic microalgae) to 4 (see Best and Stachowicz [2012] for more detail on feeding trial methods).

Phylogeny

To represent evolutionary relatedness between the species in our field communities we used a Bayesian phylogeny based on sequences of three genes: COI, 16S mitochondrial rRNA, and 18S nuclear rRNA (Best and Stachowicz 2013). The maximum clade credibility phylogeny obtained using BEAST (Drummond and Rambaut 2007) is given in Fig. 1. However, to incorporate uncertainty in topology and branch lengths, we sampled from the posterior distribution of ultrametric trees (with relative branch lengths) and used this set of trees in our analyses. We have previously reported the relative phylogenetic signal in all traits other than phenology (Best and Stachowicz 2013), but summarize the findings in Fig. 2, using Blomberg's K (Blomberg et al. 2003) for continuous traits, including the phenology trait measured in this study (day of peak abundance), and Pagel's λ (Pagel 1999) for discrete traits (in this case tube-building ability). Higher values of K or λ (up to a limit of 1 for λ) indicate greater correspondence between trait distances and phylogenetic distances, and P values < 0.05 indicate that the correspondence between trait and phylogeny is nonrandom. Uncertainty in the phylogeny did not affect the relative strength of signal in our traits (Fig. 2).

Community phylogenetic and phenotypic structure

For each community sample, we calculated the average trait value in that community at the species level (mean trait value across the species present) and at the individual level (the abundance-weighted mean across all individuals present). Both of these calculations assume that individuals in the field can be represented by the mean trait value for each species. We justify this assumption by noting that, while there is intraspecific

variation in all traits, this variation is much less than the variation between species (Best and Stachowicz 2013).

In addition to the average trait value in each community, we calculated the trait and phylogenetic distances between species as the Mean Pairwise Distance (MPD) and Mean Nearest Taxon Distance (MNTD) for each community (Webb et al. 2002). For the phylogenetic distances, these metrics are based on the branch lengths separating species in a community. To account for phylogenetic uncertainty, we calculated MPD and MNTD for 100 trees sampled from the posterior distribution of phylogenies, averaged the MPD and MNTD values over these 100 results, and used these averages in our analysis. However, when we repeated our analysis with each of the 100 individual trees from this sample we reached the same conclusions about phylogenetic structure in every case.

We calculated the trait versions of MPD and MNTD using the pairwise differences in species' trait values, with a few additional adjustments. First, to calculate distances for the phenology trait, we used circular statistics (implemented in R [R Development Core Team 2012] using the `dist.circular` function with the chord method in the `Circular` package [Agostinelli and Lund 2011]) to account for the possibility that the shortest distance between two months is either forward or backward in seasonal time. Second, we combined our feeding data to calculate multivariate feeding trait distances in two ways. We measured "feeding niche" distances using a binary distance matrix, where the distance between any two species is the proportion of foods on which they do not overlap. We also measured "feeding rate" distances using a Euclidean distance matrix based on each species' continuous feeding rates on each of the four foods. We used both distance matrices to calculate both MPD and MNTD for both null models.

To determine whether the sampled communities contained species more or less similar to a random draw from the total species pool, we standardized MPD and MNTD against two null models. The first null model randomized the community matrix (occurrence of each species in each community) while maintaining the species richness of each community. The second randomized the community matrix while maintaining both the species richness of each community and the overall frequency of each species (i.e., the number of communities in which each species occurs), using the independent swap algorithm (Gotelli 2000). For both approaches, we created 999 null communities for each of the seven time periods. We randomized species across all communities within a time period rather than between time periods to avoid confounding the effects of trait and phylogenetic distances with seasonality in grazer frequency. However, all but one species were present in at least one community at every time period. Therefore, only the independent swap null model, which maintains

grazer frequency, is strongly dependent on the time period.

Because differences in species frequency may or may not result from the community-structuring forces under examination (i.e., environmental or species-interaction effects), it is important to consider the results relative to both types of null models (Kembel and Hubbell 2006). However, it is also important to consider which null model might better represent the species pool for a particular system before the action of community assembly processes. For this study, we present the results for MPD relative to the independent swap null model because species in our system vary in their overall prevalence. As one example, the four exotic species in our system (Appendix A: Table A1) are generally lower in abundance than their native nearest relatives, possibly for historical rather than biological reasons. In each case, we note whether using different metrics or null models changes the result. All calculations for diversity metrics and null models were carried out in R (R Development Core Team 2012) using the `Picante` package (Kembel et al. 2010).

Analysis

To understand the forces structuring grazer communities in eelgrass vs. mudflat habitats, we used mixed models to test for differences in phylogenetic and phenotypic structure between habitats and seasons. We used habitat, month, and the habitat \times month interaction as the fixed effects, and to account for nonindependence of observations in space and time we included site (seven levels), sampling period (seven levels), and sampling location on the transect ($5 \times 7 = 35$ levels) as random effects. This random effects structure accounts for spatial autocorrelation in community distance measures, but it cannot account for the impact of spatial autocorrelation during the assembly of null models (Hardy 2008). Therefore, we also conducted all analyses at the "site" level by pooling all community samples within each site. Using this approach, our conclusions about phylogenetic and phenotypic structure were the same as our conclusions using all community samples separately. We present the results using each individual sample as the unit of replication (with the model previously described) because we think that these communities better reflect the spatial scale at which species interact.

RESULTS

We found clear differences in community phylogenetic structure between habitats, with communities in eelgrass habitats composed of more distantly related species than expected under random assembly (MPD $>$ 0), and communities in mudflat habitats composed of more closely related species (MPD $<$ 0; Fig. 3A; Appendix C: Table C1). This nonrandom structure was present throughout the year, and consistent across both metrics and both null models.

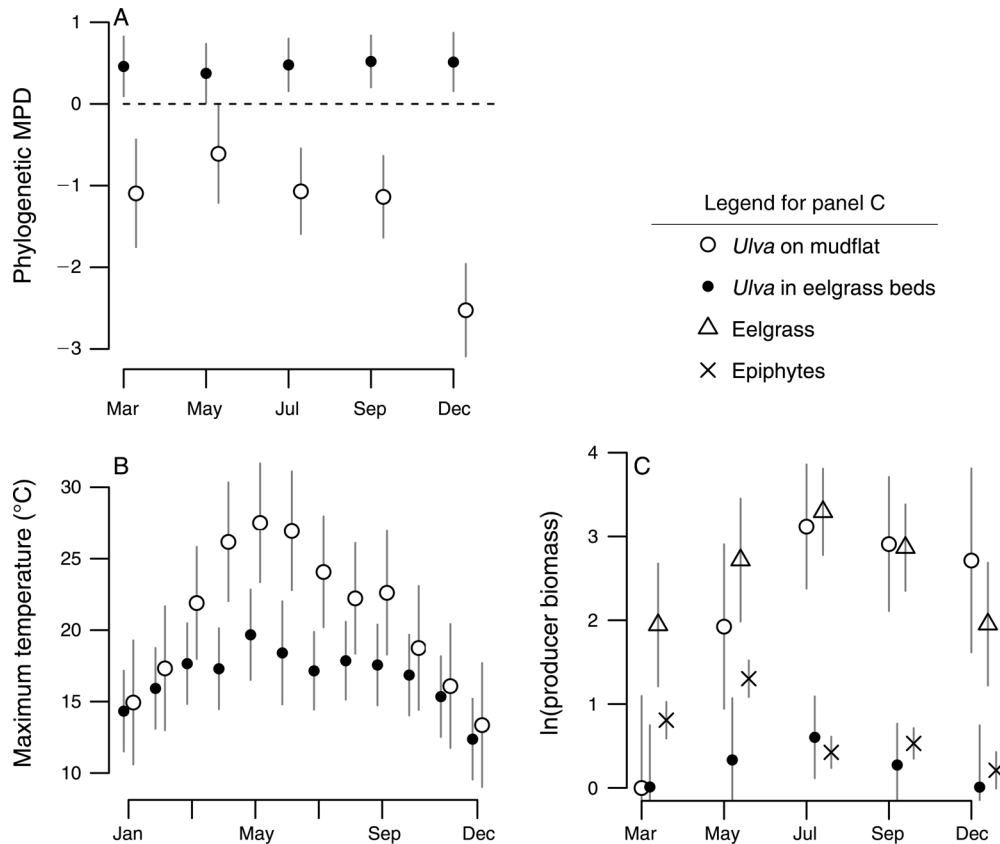


FIG. 3. Seasonal variation in (A) phylogenetic diversity, measured as the Mean Pairwise Distance (MPD) relative to the independent swap null model, (B) maximum water temperature, and (C) the natural log of primary producer abundance (biomass in grams of dry mass per community sample for *Ulva* and eelgrass, or in milligrams of dry mass per square centimeter of eelgrass leaf area for epiphytic microalgae). In panels (A) and (B) open circles represent mudflat habitats and solid circles represent eelgrass beds; in panel (C) the legend gives additional symbols for the eelgrass and epiphytic algae found in eelgrass beds. Error bars are 95% confidence intervals; in panel (A) points with error bars that do not cross 0 indicate significant structure.

In addition to phylogenetic structure, we also found differences in average trait values between habitats and nonrandom phenotypic structure for several traits. We found that average grazer size was smaller in communities in mudflat habitats than those in eelgrass habitats (Fig. 4A, with and without abundance-weighting; $P < 0.007$ in Appendix C: Table C2), and that grazers in mudflat communities were more similar in size than expected from random assembly (Fig. 4B). In contrast, in eelgrass communities, species were less similar in size (higher MPD) than in mudflat communities ($P < 0.02$ in Appendix C: Table C1), and less similar than expected by random assembly (Fig. 4B). This was consistent across all distance measures and null models (Appendix C: Table C1).

Communities on mudflats tended to have higher average temperature tolerance than those in eelgrass beds (Fig. 4C), but this difference was dependent on month (significant habitat \times month interaction; Appendix C: Table C2). The difference in average temperature tolerance tended to be greater early in the year (March to July; Fig. 4C), when the mudflats reached their

highest maximum temperatures due to midday low tides (Fig. 3B). Mudflat communities also had less variation in species' temperature tolerance than expected under random assembly (Fig. 4D), suggesting that high tolerance is necessary to survive in those habitats. In contrast, the variation in temperature tolerance among species in eelgrass communities was no different from random (Fig. 4D). This asymmetry is not surprising, given that species with or without tolerance of warm temperatures may be able to survive in the cooler, deeper waters of most eelgrass beds. The structure in mudflat communities was consistent across both metrics and null models, as was the lack of structure in eelgrass communities. However, the difference between the two habitats was clearer under the MPD metric than the MNTD metric (Appendix C: Table C1).

The average timing of maximum abundance was very similar in eelgrass and mudflat communities (Fig. 4E; $P > 0.5$ in Appendix C: Table C2). However, communities from the two habitats differed in the among-species variation in phenology ($P < 0.0001$ in Appendix C: Table C1). Communities in mudflat habitats contained

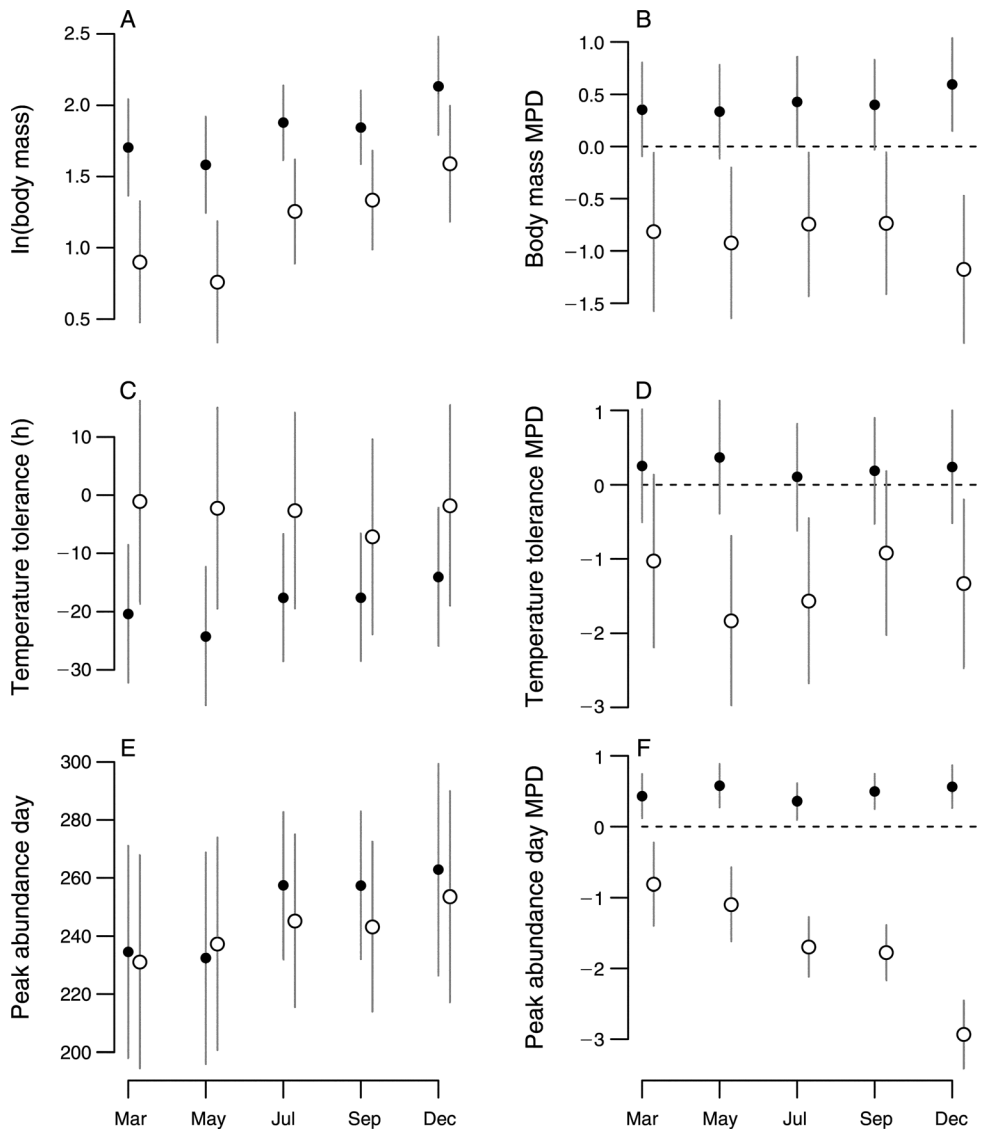


FIG. 4. Species-level average community trait values and trait diversity for (A, B) grazer body mass (mg dry mass per individual presented on a natural log scale); (C, D) temperature tolerance (change in survival time [in hours] under elevated temperature); and (E, F) timing of peak abundance (see *Traits*). In panels (B), (D), and (E), Mean Pairwise Distance (MPD) is relative to the independent swap null model. Error bars are 95% confidence intervals, so points with error bars that do not cross 0 indicate significant structure. Open circles are communities in mudflat habitats and solid circles are communities in eelgrass beds.

species that were more similar in their phenology than expected, especially later in the season (Fig. 4F). Given that *Ulva* is the primary food and habitat available in mudflat communities, and is the only primary producer to be unavailable in early spring (Fig. 3C), it is not surprising that communities on mudflats are more restricted in their phenology. Total grazer abundance in mudflat communities also appeared to follow *Ulva* abundance (see Appendix D: Fig. D1b). Diversity in phenology in eelgrass beds, on the other hand, was higher than expected (for MPD but not MNTD). Thus, communities in eelgrass beds at any given time contain species that are both near to and far from their peak

abundance, suggesting some differentiation in seasonal niches.

For the range of feeding traits we used in this study, both habitat type and seasonal variation in resources within those habitats affected community trait composition. For example, communities on mudflats tended toward higher average feeding rates on *Ulva* in the summer season (Fig. 5A; Appendix C: Table C2), when *Ulva* is available (Fig. 3C). However, average feeding rate on epiphytes was also higher in species found in mudflat communities in July and September (Fig. 5B; Appendix C: Table C2), even though epiphytic microalgae are much more available on eelgrass blades than

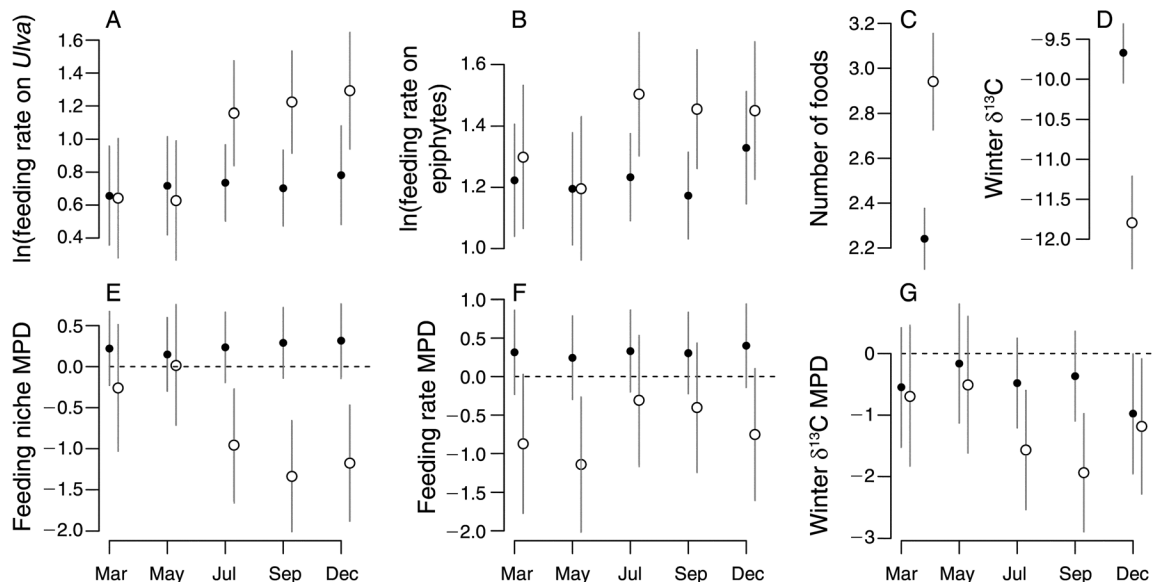


FIG. 5. Species-level average community trait values and trait diversity for feeding traits. Average feeding trait values are (A) feeding rate on *Ulva* macroalgae in milligrams of wet mass consumed per individual per 24 hours (log scale), (B) feeding rate on epiphytes in micrograms of chl *a* per individual per 24 hours (log scale), (C) number of foods consumed, and (D) the $\delta^{13}\text{C}$ signature (% difference from V-PDB) for each grazer measured in winter. Diversity in feeding traits is calculated as (E) feeding niche diversity (diversity in which foods are consumed), (F) feeding rate diversity (diversity in continuous feeding rates on all foods), and (G) diversity in winter $\delta^{13}\text{C}$ signatures (Note that to standardize the observed distances in $\delta^{13}\text{C}$ by those expected under the null models, it was necessary to exclude one species for which we did not have a winter $\delta^{13}\text{C}$ signature: *Caprella mutica*, which is present only in the fouling community of floating docks in the summer). In panels (E–G) the Mean Pairwise Distance is relative to the independent swap null model. Error bars are 95% confidence intervals, so points with error bars that do not cross 0 indicate significant structure. Open circles are communities in mudflat habitats and closed circles are communities in eelgrass beds.

Ulva blades. Our two additional univariate feeding traits (number of foods consumed and the winter $\delta^{13}\text{C}$ signature) were also consistently different between mudflat and eelgrass communities across seasons ($P < 0.03$ and $P < 0.0001$, respectively, in Appendix C: Table C2). The species in mudflat communities had on average greater potential feeding niche breadth than those in eelgrass communities (Fig. 5C), and were more depleted in ^{13}C (Fig. 5D). This difference in $\delta^{13}\text{C}$ is consistent with our previous finding that *Ulva* has a lower $\delta^{13}\text{C}$ value than eelgrass in winter (Best and Stachowicz 2013).

Evidence for community structure in feeding traits was also habitat and season specific. Community structure in feeding niche, feeding rates, and $\delta^{13}\text{C}$ signature was interactively affected by habitat and month, and sometimes depended on the null model (Appendix C: Table C1). In general, mudflat communities showed clear clustering in feeding traits in months in which *Ulva* was abundant (July to December; Fig. 3C), whereas feeding traits in eelgrass communities rarely differed from null expectations (Fig. 5E, F, G), providing little evidence for food resource partitioning in eelgrass habitats. However, under the richness null model eelgrass communities did show greater variation in feeding rates (but not feeding niche) than expected; observed Mean Pairwise Distance was significantly

higher than the null expectation under the richness model in four out of five months.

DISCUSSION

Despite equivalent species richness (Appendix D: Fig. D1a) and considerable overlap in species identity, we found that the relative importance of environmental filtering and niche partitioning varies substantially between the two major habitats in our system. Eelgrass beds, which provide a variety of food types and microhabitats year-round, hosted grazer communities composed of distantly related species with divergent phenologies and body sizes. In contrast, mudflats with only seasonally available *Ulva* to provide habitat, and higher abiotic stress in the form of higher water temperatures at low tide, hosted phylogenetically and phenotypically clustered grazer communities. Furthermore, the magnitude of this structure varied by season, with the strongest clustering coinciding with peaks in resource abundance and maximum water temperatures. This suggests that the diversity of available habitats or resources, as well as the level of abiotic stress (Graham et al. 2012), might constrain community structure in consumers.

In addition, whereas recent experimental results suggested that coexistence among grazers in these eelgrass beds is promoted by feeding trait diversity and unaffected by phylogenetic diversity (Best et al. 2013),

eelgrass communities in the field appear to be largely structured by traits that are unrelated to food niche. Instead it seems that traits potentially related to microhabitat niche, such as grazer body size and behavior, play a large role in structuring eelgrass communities. This appears consistent with much earlier work in both marine and terrestrial systems that suggests that arthropod species' niches may be driven by the availability of habitat (particularly enemy-free habitat) rather than the availability of food (Strong et al. 1984, Bernays and Graham 1988, Hay et al. 1990, Duffy and Hay 1991). Experimental studies of the effect of habitat availability and diversity on competitive outcomes in these species could be used to further test this specific hypothesis in the future. Below, we first examine the specific traits that appear to structure grazer communities in mudflat and eelgrass habitats. We then compare the interpretation of community assembly patterns and processes and the relative value of phylogenetic and trait information in the context of field studies vs. controlled experiments.

*Community assembly on mudflats:
environmental and habitat filtering*

We found that species in mudflat communities are phylogenetically clustered, or more closely related than expected under random assembly (Fig. 3A). In mudflat communities, grazers must withstand higher maximum water temperatures (Fig. 3B), and have access to a narrower range of habitats and foods (primarily *Ulva*). This is therefore consistent with a number of previous studies where greater phylogenetic clustering has indicated stronger environmental filtering in more stressful environments. These include bees (Hoiss et al. 2012) and hummingbirds (Graham et al. 2012) along elevation gradients, plants along gradients of fire frequency (Verdú and Pausas 2007) and fertility and rainfall (Anderson et al. 2011), and bacteria along gradients in oxygen availability (Bryant et al. 2012) and ammonia concentration (Horner-Devine and Bohannan 2006).

To look more closely at the types of environmental and habitat filters operating in mudflat communities, we examined phenotypic structure in individual traits. We found that grazer communities in mudflat habitats had lower average body mass, higher average temperature tolerance, higher average feeding rates on *Ulva* macroalgae, and higher average feeding niche breadth than communities in eelgrass habitats (Fig. 4A, C; Fig. 5A, C). In terms of variation around those mean trait values, we found that mudflat communities are more closely clustered than expected under random assembly with respect to body mass, temperature tolerance, phenology, and in summer and fall, feeding niche (Fig. 4B, D, F; Fig. 5E). Interestingly, even though species in mudflat communities appeared more closely spaced ecologically, these communities did not have lower species richness than eelgrass communities (Appendix D: Fig. D1a).

The phylogenetic clustering in mudflat communities is likely a reflection of the clustering by phenology and/or body mass, and independent of the clustering by high temperature tolerance, because the former traits have much stronger phylogenetic signal (Fig. 2). Environmental filtering of body size according to the size of available habitat spaces has previously been shown for both amphipods in algae (Hacker and Steneck 1990) and shrimp in sponges (Hultgren and Duffy 2012), and it is not surprising that species using the most seasonally restricted resource (*Ulva*, Fig. 3C) have a limited temporal niche. In addition to size and phenology, phylogenetic clustering in mudflat communities may be partly driven by shared strategies for predator avoidance, which can have a large effect on habitat choice in amphipods (Stoner 1980, Duffy and Hay 1991). Communities on mudflats had a higher proportion of species capable of building tubes in the mud and macroalgae; on average, 72% of species in a mudflat community were tube-builders, compared to 35% of species in an eelgrass community ($P = 0.019$ for the effect of habitat on this proportion using a mixed model to account for site and time as for other community-average trait values). Some of these species experience a substantial decrease in predation susceptibility when building tubes in *Ulva* relative to eelgrass (Best and Stachowicz 2012), and the ability to build tubes is a highly conserved trait (Myers and Lowry 2003, Best and Stachowicz 2013: Fig. 2).

*Community assembly in eelgrass beds:
habitat partitioning*

In contrast to mudflat communities, eelgrass communities were phylogenetically even, with community members more distantly related than expected under random assembly (Fig. 3A). This is frequently interpreted as evidence that competitive exclusion between close relatives is more important than environmental filtering. Even or random phylogenetic structure can also result from convergent evolution in traits that are involved in filtering rather than separating community members (Kluge and Kessler 2011, Savage and Cavender-Bares 2012), but in our system, no traits showed phenotypic clustering in eelgrass communities. These communities were random with respect to temperature tolerance (Fig. 4D), suggesting that there is no trade-off preventing species with tolerance of high temperatures from also inhabiting colder, deeper waters, and that eelgrass communities are indeed subject to weaker temperature filtering than mudflat communities. This is further substantiated by the fact that the total number of species ever found in eelgrass was higher (12 out of 13 species) than the number of species ever found at mudflat sites (9 out of 13 species). The relative abundance of each of these species in each habitat type is given in Appendix D: Fig. D2.

Given that both traits and phylogeny indicate relatively weak environmental filtering in eelgrass

communities, we can then use trait data to evaluate whether phylogenetic evenness is the result of niche partitioning. In terms of partitioning of food resources, eelgrass communities were random with respect to most measures of feeding diversity (Fig. 5E, F, G, but not feeding rate diversity in comparison to a richness null model). Except for the $\delta^{13}\text{C}$ signature, feeding traits tended to have low phylogenetic signal (Fig. 2), so it is not surprising that their phenotypic structure does not match phylogenetic structure. However, these results are directly opposite from our experimental results in this system, where feeding trait diversity, and not phylogenetic diversity, promoted coexistence (Best et al. 2013).

In contrast, the trait data for field communities suggests that species in eelgrass beds may be partitioning microhabitat (lower proportion of species building tubes; even size structure; Fig. 4B). Given the difference in average species size between eelgrass beds and mudflats along with the even size structure within eelgrass habitats, size appears to be the kind of trait that can define both α niches (species' resource use within a community) and β niches (species' distribution along resource or environmental gradients between communities), as has been previously documented in terrestrial plants (Ackerly and Cornwell 2007, Cornwell and Ackerly 2009). Eelgrass habitats tend to have a higher diversity of microhabitats than *Ulva* beds, with the smallest species often building tubes higher in the canopy or swimming in the water column, and larger species clinging to eelgrass leaves using modified appendages, or building tubes either between the leaves where they branch from the sheath or in drift *Ulva*. An important alternate food and habitat when present, *Ulva* was available in 42% of eelgrass bed communities in the summer, although at a much lower abundance than on mudflats (Fig. 3C), and was included in the mesocosm experiment in this system (Best et al. 2013).

Microhabitat segregation by size has been previously reported for amphipods (Korpinen and Westerborg 2010), and size-based selection of microhabitats has been shown to reduce predation susceptibility in amphipods and shrimp (Nelson 1979, Main 1985). This suggests that microhabitat segregation within eelgrass beds could be due to competition for enemy-free space (Bernays and Graham 1988). However, greater than random size spacing in eelgrass beds could also be driven by other habitat requirements, such as avoiding dislodgement when flow rates are high. Either of these mechanisms for microhabitat partitioning could then drive the seasonal niche separation we observed (Fig. 4F). Although variation in phenology has been previously reported in amphipods as a mechanism for reducing sterile matings between closely related species (Kolding and Fenchel 1979), it could also be a mechanism for avoiding competition for the same microhabitat at the same time.

Observations vs. experiments: the scope and context of phylogenetic data

Although previous mesocosm results indicated that feeding niche diversity promotes coexistence (Best et al. 2013), we found little evidence of this in the field. Instead, resource partitioning appears to happen at larger spatial and temporal scales. While our mesocosms and field communities were similarly sized (either a bucket or a sampled water column ~ 30 cm in diameter), in the field many important assembly processes apparently take place between rather than within communities. Some species particularly well suited to utilize *Ulva* macroalgae for food and habitat are concentrated in mudflat communities, while grazers in eelgrass appear to achieve coexistence by differing in body size, temporal niche, and tube-building ability. Of course, it is still possible that feeding niche diversity may have some impact on competitive outcomes in the field, especially towards the beginning of winter, when resource abundance is low and grazer population density is high. However, our results suggest that food niche is less important than habitat niche at the community level, which is consistent with previous hypotheses that food choice is less important than habitat requirements for amphipod species individually (e.g., Duffy and Hay 1991, Sotka et al. 1999).

In addition to different conclusions about coexistence mechanisms, our experimental and field results produced different conclusions about the relative value of phylogenetic and trait data for understanding those mechanisms. Whereas phylogenetic diversity had no effect on species interactions in mesocosm communities, field communities were phylogenetically structured. If it is generally the case that a greater number of traits affect field communities than are relevant in any given controlled experiment, phylogeny may frequently be a more meaningful proxy in observational studies, although its mechanistic interpretation will still be specific to a particular clade or species pool. However, if our aim is to tackle questions of community assembly by synthesizing the observation of patterns and the experimental verification of component processes, using a single proxy for all relevant ecological variation may be problematic. Because traits vary in their phylogenetic signal, phylogenetic relatedness may not be able to simultaneously represent the different types of ecological variation that are relevant in each context and at each scale, and may make it difficult to reconcile contrasting results. With trait data, on the other hand, surveys and manipulative experiments work as complementary approaches that elucidate the specific ecological similarities and differences that promote coexistence under different conditions. In our system, mesocosm results showed that feeding niche diversity can promote coexistence in the absence of larger-scale environmental variation, and the absence of predators, whereas field results suggest a different set of traits may be more important for understanding assembly across divergent

habitats and seasons. Furthermore, as our field results illustrate, there are multiple community assembly processes that may be acting simultaneously in the field. With trait data, we can specifically design arrays of experiments that isolate and test the relative importance of each of these processes, rather than being limited to manipulating a single metric of ecological differentiation. In this way, the synthesis of observational and experimental trait-based approaches allows us to keep building on our understanding of assembly mechanisms across scales and across systems, rather than reducing results to a currency that cannot be easily translated across either.

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SUPPLEMENTAL MATERIAL

Appendix A

Taxonomic authorities, origin, and primary habitat type for species in the study ([Ecological Archives E095-063-A1](#)).

Appendix B

Timing of peak abundance for each species ([Ecological Archives E095-063-A2](#)).

Appendix C

Statistical results for effects of month and habitat type on trait and phylogenetic diversity, and on average trait values ([Ecological Archives E095-063-A3](#)).

Appendix D

Additional results for community richness, abundance, and composition ([Ecological Archives E095-063-A4](#)).