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Microhabitat partitioning by seagrass mesograzers - Effects of predation, trait variation and species interactions

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ABSTRACT / ZUSAMMENFASSUNG

Community assembly and ecosystem function in diverse communities frequently depend on how ecologically similar species vary in their use of key resources. In ecosystems with high structural complexity microhabitat partitioning may reduce competition and facilitate species coexistence. While factors like predation, traits and species interactions are known to affect individual habitat choices, little is know how these factors are affecting habitat partitioning. In this study we used seagrass infauna from Bodega Harbor, CA, USA, to test if species with similar ecological function separate their habitat use. To do this we determined the microhabitat preferences of eight common seagrass mesograzers with a range of different traits. We then tested how these preferences varied with the presence of (a) predators, (b) traits, and (c) competitors that are either similar or dissimilar in habitat selection and traits. The distinctly different habitat choices we recorded for eight crustacean seagrass mesograzers indicate that these species partition their microhabitat use. Traits had only little effect on microhabitat partitioning: the use of only one out of four available microhabitats was predicted by traits. While microhabitat preferences were completely unaffected by the presence of predators, habitat shifts as a result of competitor presence were relatively small to overall separation of habitat use. These results suggest that microhabitat partitioning is fixed and flexibility is not favored in the presence of either predators or competitors. Therefore, if overlapping habitat use results in performance or fitness consequences, the function of habitat partitioning as a stabilizing mechanism for coexistence depends on species composition.

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Die Zusammensetzung einer Gemeinschaft und das Funktionieren eines Ökosystems hängen häufig davon ab, wie sich die Ressourcennutzung von

ähnlichen Spezies unterscheidet. In Ökosystem mit hoher struktureller Komplexität kann Mikrohabitat-Partitionierung Kompetition reduzieren und somit Koexistenz ermöglichen. In diesem Kontext ist bekannt das Einflüsse durch Räuber, Traits und Wechselwirkungen mit anderen Spezies die individuelle Mikrohabitatwahl bestimmen können. Man weiß jedoch nur wenig darüber ob diese Faktoren Mikrohabitat-Partitionierung beeinflussen. In dieser Studie verwenden wir Infauna aus einer Seegraswiese in Bodega Harbor, CA, USA, um zu testen wie Spezies mit ähnlichen ökologischen Funktionen ihre Habitat Nutzung aufteilen. Dazu haben wir die Mikrohabitat-Präferenz von acht verbreiteten Mesograzer Arten mit verschiedenen Traits ermittelt. Wir testeten dann ob die individuelle Habitatwahl von (a) Räuberpräsenz, (b) Traits, oder (c) anderen Arten abhängt, die ähnlich oder verschieden in Habitatpräferenz oder Traits sind. Die deutlich verschiedenen Habitatpräferenzen lassen Mikrohabitat Partitionierung zwischen den acht Arten vermuten. Traits hatten einen nur sehr geringen Einfluss auf die Partitionierung: nur eins von vier verfügbaren Habitaten wies ein überproportionales Aufkommen einer Traitgruppe auf. Während Räuberpräsenz keinerlei Auswirkungen auf Habitat Partitionierung hatte, waren die Veränderungen in Habitatwahl durch die Präsenz anderer Spezies relativ klein im Verglich zur generellen Partitionierung. Dies lässt vermuten dass Mikrohabitat Partitionierung statisch ist und Flexibilität unter Räuber- oder Kompetitorpräsenz nicht bevorzugt wird. Falls sich überschneidende Habitatpräferenzen nachteilig auf Fitness auswirken, dann hängt also die Rolle von Mikrohabitat Partitionierung als Koexistenz-Mechanismus davon ab welche Spezies in einer Gemeinschaft anzutreffen sind.

INTRODUCTION

Both community assembly and ecosystem function in diverse communities frequently depend on the way species that appear to be occupying the same niche vary in their use of key resources (Chesson, 2000; Cardinale et al., 2006). Seagrass ecosystems worldwide provide habitats for a great number of marine organisms, including both epiphytic algae and animals that live directly on seagrass blades (Hemminga MA and Duarte, 2000; Duarte, 2002; Orth et al., 2006), and that are seemly equivalent in trophic position and function (Duffy, 2006). At high population densities, infaunal grazers (mesograzers) can greatly reduce overgrowth by epiphytes, which considered detrimental to seagrass (McGlathery, 2001), and thereby enhance seagrass growth and survival (Jernakoff and Nielsen, 1997; Duffy and Harvilicz, 2001). Considerable research indicates that a more diverse assemblage of grazers is more efficient in removing epiphytes, and improves seagrass ecosystem functioning (e.g. Duffy, 2006; France and Duffy, 2006; Blake and Duffy, 2010). However, grazer diversity varies widely among natural seagrass beds and it remains unclear what factors exactly allow the coexistence of multiple grazer species.

Theory predicts that within a given habitat the coexistence of competing species should be at least partly controlled by their differential use of available resources (MacArthur and Levins, 1964, 1967; Abrams, 1983; Chesson, 2000), of which microhabitat seems to be one of the most important (as reviewed by Schoener, 1974). In seagrass beds, architectural complexity limits the availability of microhabitats and the number of different niches (Warfe et al., 2008; Kovalenko et al., 2011). As overlap in microhabitat use makes exploitative competition for space between epifauna more likely (Schoener, 1983), distinct differences in microhabitat use could be an important way for mesograzer species to coexist. Previous work on arthropod epifauna from terrestrial (spiders: Butt and Tahir, 2010) and freshwater systems (amphipods: van Riel et al., 2007; Cothran et al., 2013; Dudgeon, 1989) has shown that microhabitat use can be partitioned to reduce territorial competition. In other words, if competition for space in seagrass mesograzers entails fitness consequences,

having distinct preferences for different microhabitats could reduce them. Mesograzers have to select their microhabitat in a system with high predation pressure (Duffy, 2006), and "enemy-free space" (Berdegue et al., 1996) has been observed to be a principal constraint for grazer habitat choice (Duffy and Hay, 1991; Boström and Mattila, 1999; Lasley-Rasher et al., 2011). The value of a microhabitat as shelter from predation is often defined by plant morphology (Martin-Smith, 1993; Kley et al., 2009), and grazer traits like size, shape and color that can determine successful habitat utilization. Furthermore, shelter-building abilities like the construction of silk tubes (Myers and Lowry, 2003) enable certain amphipod species to use certain parts of seagrass and macroalgae for protection from predation (Mancinelli and Rossi, 2001; Bartholomew and Shine, 2008; Warfe et al., 2008; McDonald and Bingham, 2010; Best and Stachowicz, 2012). Microhabitats with high value as shelter usually require a close matching with the grazer species' morphological features (Hacker and Steneck, 1990). Therefore, variability in grazer traits like size may translate to differences in habitat use (Korpinen and Westerbom, 2010).

While a close association with a microhabitat might increase fitness by avoiding predation, the interaction with stronger competitors might reduce fitness (Cothran et al., 2013). In this case, flexibility in microhabitat use, as shown by van Riel et al. (2007) for fresh water amphipods, and overall partitioning could reduce competition and facilitate species coexistence, but may result in increased predation susceptibility if species decide to leave their preferred microhabitat (Lasley-Rasher et al., 2011; Best and Stachowicz, 2012). The two most relevant questions in connection with this hypothesis are (i) if species with overlapping habitat use are actually competing for space and influence each other in their habitat selection, and (ii) if species can make context dependent decisions in the presence of predators or competitors, i.e. if predator presence or competition could cause grazers to deviate from microhabitat choices they would make in the absence of the same. Although habitat partitioning is considered an important stabilizing mechanism in many different terrestrial and aquatic systems, little is known about the factors that are

shaping it, or if it can be flexible at all.

To test if seagrass mesograzers have separated habitat use, and to investigate possible factors that drive habitat partitioning, we conducted a series of laboratory experiments using eight crustacean mesograzer species from a seagrass bed in Northern California, USA. First we examined whether species show different habitat choices in the absence of competitors, and whether those differences could be predicted from grazer traits. Then we tested whether habitat choice was dependent on the presence of predator cues, or the presence of a range of potential competitors varying in their niche similarity. Overall, our series of experiments tests for habitat partitioning in seagrass mesograzers and whether it depends on fixed differences between species, or if it is shaped by species interactions.

MATERIALS AND METHODS

Study system

We determined the microhabitat choices of 8 crustacean mesograzer species (7 amphipods and one isopod species (Table 1) from Bodega Harbor, California, USA (38° 19.110' N, 123° 04.294' W). These epifaunal mesograzers inhabit different above-ground parts of Zostera marina (hereafter: eelgrass) and *Ulva spp.* macroalgae (hereafter: *Ulva*). The species we included in this study are commonly found in eelgrass meadows of the northwestern USA and western Canada, and include species with a range of sizes and differences in their ability to build tubes (Table 1).

Table 1: Grazer species from Bodega Harbor that were used in this study (trait data from Best and Stachowicz (2013).

Species	Order	Biomass (mean dry weight in mg / species. ± SE)	Body size class	Species can build tubes
Idotea resecata	Isopoda	53.20 ± 9.29	large	no
Caprella californica	Amphipoda	4.10 ± 0.72	large	no
Ampithoe lacertosa	Amphipoda	17.00 ± 2.05	large	yes
Ampithoe sectimanus	Amphipoda	5.50 ± 0.45	large	yes
Allorchestes angusta	Amphipoda	1.17 ± 0.09	small	no
Pontogeneia rostrata	Amphipoda	1.32 ± 0.27	small	no
Aoroides columbiae	Amphipoda	1.48 ± 0.26	small	yes
Ischyroceros anguipes	Amphipoda	1.16 ± 0.28	small	yes

We collected the species between July and September 2013 from Bodega Harbor and transported them to the Bodega Marine Laboratory (BML), where all experiments of this study were conducted. We used only adult individuals in the experimental trials within two to five days after collection and only for a single trial. The large grazer species showed substantial size variation among adults, which was reflected in the experiments by establishing a similar size distribution and not changing it throughout all experiments performed with that species. All organisms were maintained in monoculture prior to experiments with identical water flow rates, 12:12 h light cycle and in compliance with the UC Davis Animal Care and Use protocol. We also performed all experiments at the same location and under similar conditions.

Microhabitat assessment

We recorded species specific microhabitat choice in laboratory experiments using one half of 38 l indoor glass aquaria (50 cm length x 25 cm width x 25 cm height). A fine mesh separated the two halves so that the effects of predator presence on habitat choice could be assessed in later experiments without actual predation (see below). The outer surface of the aquaria was covered with black plastic sheets to reduce influence of movement in the room on animal behavior. In the predator compartment an air stone was installed for water-circulation and oxygen supply.

The grazers could choose between the upper and lower half of single eelgrass shoots (in the analysis denoted as "Eelgrass canopy" and "Eelgrass stem"), *Ulva* macroalgae and the rest of the Aquarium (denoted as "Water"). For every aquarium we used four fully grown healthy eelgrass shoots without epiphytes that were collected in Bodega Harbor max. two days before an experiment. We removed all organisms and standardized the shoots by cutting off the rhizome at the first node and trimming them to 70 cm total length. Coated steel wire wrapped around the lower end of the shoots and magnets attached to the other side of the aquarium bottom held the 4 shoots at the center of the habitat compartment. In a water depth of 25 cm, this created an upright "stem" section in the lower 20 cm and a floating "canopy" section in the top five cm of the water column. Branching leaves created a larger volume in the canopy than below. We cleaned *Ulva* leaves of epiphytes, cut them to approx. 15 cm x 15 cm size, and added two pieces to each aquarium. Although total habitat area likely differed among the choices, the proportions of different habitats available was realistic based on their relative abundance in the field. Furthermore, the relative amount of different habitats available was the same across all experiments, allowing us to compare habitat choices among species.

To measure species-specific microhabitat choice we placed 12 individuals of a single grazer species in the habitat compartment (N=3 tanks for each species). After 24 h we disassembled the setup by gently removing the eelgrass shoots, spreading them out on a dry, white plastic surface and simultaneously removing the *Ulva* pieces and putting them into a separate

container. We then counted all individuals on the upper and lower half of the eelgrass shoots, on *Ulva*, and the remaining ones in the aquarium. We counted the number of living individuals recovered in each habitat type, and transformed these numbers to proportional abundances by dividing them by the total number of living individuals recovered from the tank. Death during the experiment was uncommon (usually zero, and less than one individual per replicate tank on average [0.3 ± 0.05]), as was the number of individuals that could not be located at the end of a trial ranged (average per tank 0.89 ± 0.11).

Predator presence

We assessed the effect of predator presence on microhabitat choices, by conducting an additional three replicates to which we added two predatory fish to the section of the tank without crustaceans present (N=3 tanks for each predator treatment). As predators we used one individual of each of the two most common crustacean predators in local eelgrass beds (Gamble et al., 2013) rockfish (*Sebastes* spp.) and cabezon (*Scorpaenichthys marmoratus*). We used juveniles with a body size of 6 ± 1 cm that were collected by seining from Bodega Harbor in the same locations as the crustaceans. To maximize predation cues we fed the fish pieces of the amphipod A. lacertosa one hour after the experiment started (Wisenden et al., 1999). We assessed species-specific microhabitat choice for six of the eight grazers as described above (*I. resecata* and *A. sectimanus* were only tested in predator free tanks due to insufficient abundances in the field at the time experiments were conducted).

Competitor presence

In a separate experiment, we tested whether the presence of competitors influenced crustacean habitat use by pairing each species with potential competitors that that varied in their potential habitat overlap. We maintained a density of 12 individuals of each species in each a tank and recorded their respective use of the four microhabitats as described above

(regardless of whether these species were actually shown to compete, we will hereafter refer to a second grazer species as "competitor" by convention). We selected 17 out of 28 possible pair-wise combinations from among the eight study species to assess the effect of overlap in habitat preferences and other traits (body size and tube-building ability) on habitat selection by each species (N=3 tanks per combination). Experiments with two species had twice the overall animal density as monocultures, so we tested for an effect of density on grazer microhabitat choice by conducting additional experiments with 24 individuals of a single species in a tank (N=3 tanks per species in high density). The experiments conducted and number of replicates performed are summarized in Table 2.

Table 2: Overview of all experiments, which were conducted with three replicate tanks per species or species combination in polyculture. Depending on field abundances, species were differently represented in polyculture trials. (*Number of polycultures using each species: *A. angusta* = 5, *A. columbiae* = 5, *A. lacertosa* = 6, *A. sectimanus* = 2, *C. californica* = 5, *I. anguipes* = 5, *I. resecata* = 4, *P. rostrata* = 2)

Diversity	Predators	Density	Species tested	Replicate tanks
Monoculture	no	low (12)	8	3
Monoculture	yes	low (12)	6	3
Monoculture	no	high (24)	8	3
Polyculture (2 species)	no	high (2 x 12)	8 in 17 combinations *	3 (per combination)

Analysis

To test if seagrass mesograzers differ in their habitat use, and if that habitat use is affected by the presence of predators or competitors, we used both multivariate and univariate approaches. For the core analyses, we used a series of PERMANOVA models (permutational analysis of variance Anderson, 2001). In each case, the response was a species' multivariate proportional abundances across the four habitats available in a tank. To visualize differences in and effects on habitat use, we used non-metric multidimensional scaling (MDS: Kruskal, 1964), which also allowed us to extract distances between species (hereafter referred to as "habitat separation", Fig. 2). Finally, to assess relationships between species traits

and responses to competition we also used univariate general linear mixed models (GLMM). Below we describe the specific models used for each question.

Habitat use in monocultures

To analyze if microhabitat use is different between species we used a PERMANOVA model with habitat use in low-density monocultures as response variable and grazer species as main effect. To capture the magnitude of these differences between habitat choices, we used the same data in an MDS analysis, and extracted pairwise euclidean distances between the centroids (which represent average multivariate habitat use for each species). We then tested whether habitat use in monocultures was influenced by either the density of individuals or the presence of predators. To test if the density (12 or 24 individuals per tank) affected microhabitat use we used a PERMANOVA model with species and density as main and interactive effects. To test if predator cues affected microhabitat use we used a PERMANOVA model species and predator presence as the main and interactive effects (using only low-density monocultures).

To test whether species' microhabitat use could be predicted by their traits (body size and tube building ability [Table 1]), we used habitat-specific GLMMs We used a separate model for each habitat, which had the proportional abundance in that microhabitat as the response variable, crustacean body size and tube building ability as main and interactive effects, and species identity as a random effect.

Habitat use in polycultures

We assessed the effects of competitor presence on microhabitat use by comparing the low-density monocultures to the polycultures. This contrasts the habitat choices a species might make if it colonizes a habitat that either is or is not already occupied by a second species, holding its own density constant. We approached this question with two different analyses: First we tested whether competitor presence caused species to change their microhabitat preferences. We used a single PERMANOVA model with

main and interactive effects of grazer species and competitor presence (monoculture vs. polyculture) to test for a species specific response. To investigate the individual responses of every species to competitor presence we then used individual PERMANOVA models for each species separately, again with competitor presence as the main effect. To determine whether the identity of the competitor affected microhabitat choice (as opposed to the simple presence or absence of any additional species) we used a second PERMANOVA model focusing only on habitat choices in polycultures and using competitor species identity and focal species identity as the main and interactive effects. This tested whether habitat choice is similarly influenced across all focal species paired with a given competitor. For this second model we again followed the overall model with individual models for each focal species separately.

Second we examined whether habitat separation between competitors is fixed, and determined only by their independent habitat choices we observed in monoculture, or flexible, so that it can be adjusted or increased when in the presence of a competitor. This was necessary to test if niche spaces are affected by competitor presence and therefore, if monoculture microhabitat use can be used to determine spatial niche overlap. To do this we used a GLMM with habitat separation in polyculture (habitat separation between the two species in a single polyculture tank) as the response variable, habitat separation in monoculture as the fixed effect and species combination as the random effect.

All statistics were done with the R programming language (v 3.1.0, R Core Team, 2013). We used the packages vegan for PERMANOVA (using Bray-Curtis distance matrices with 999 permutations) and MDS ordination, and *lme4* with *lmerTest* for GLMMs.

RESULTS

Microhabitat choices in monoculture

We found highly significant differences in monoculture microhabitat use among species in the absence of predators and competitors (PERMANOVA; grazer species effect, $F_{7,16}$=15.172, p=0.001). Individuals of *A. columbiae*, *A. lacertosa* and *A. sectimanus* were most abundant on *Ulva*, while *I. anguipes* and *I. resecata* were most abundant on eelgrass. *A. angusta*, *C. californica* and *P. rostrata* occurred most often in water. (Fig. 1, left panel). This same pattern of distinct habitat choices is evident in the MDS ordination plot, which shows that some species overlap strongly in microhabitat use, whereas others do not (Fig. 3). We found no effect of grazer density (12 or 24 individuals per tank) on species specific microhabitat choice in monoculture (PERMANOVA; grazer species X density, $F_{7,32}$=1.4557, p=0.151; density F = 0.961, p = 0.391).
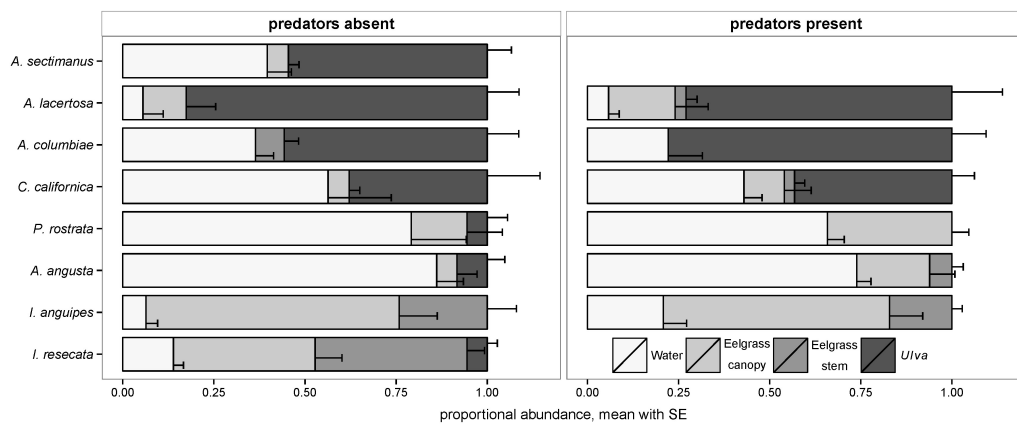

Fig. 1: Species specific habitat choices for the studied species shown as proportional abundances of alive individuals that we found on a certain habitat after 24 hrs. (Lost or dead individuals are excluded). Left panel: proportional abundances from the experiments without fish in the predator compartment. Right panel: proportional abundances from the experiments with one rockfish and one cabezon in the predator compartment (predator-treatment was not applied for *A. sectimanus* and *I. resecata* due to insufficient abundances in the field)
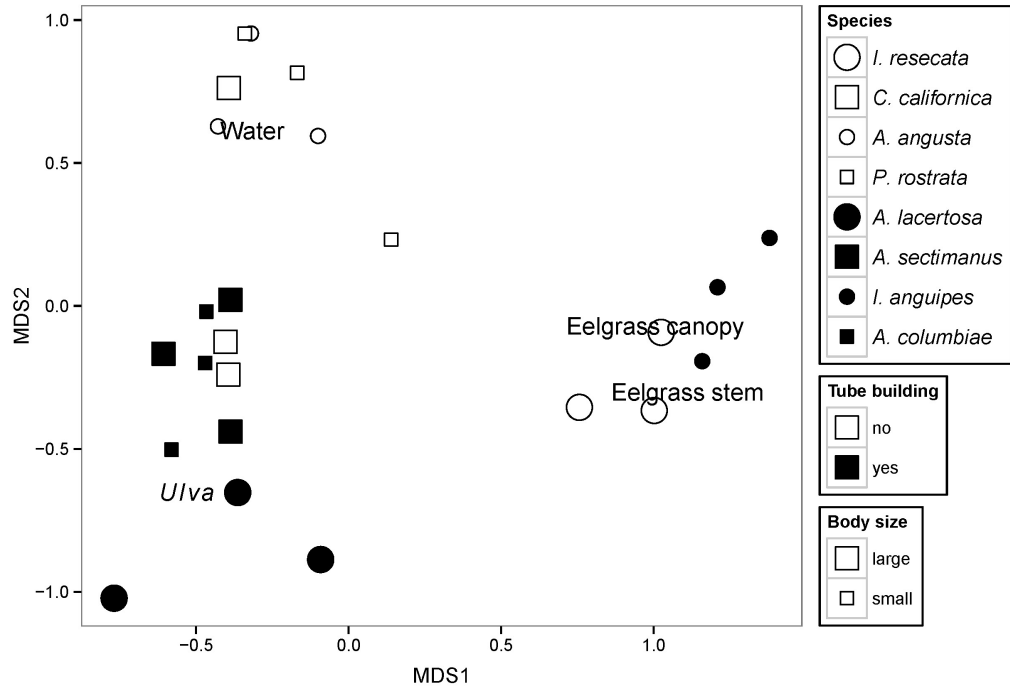
Fig. 2: MDS-plot of microhabitat choices of the studied species in monoculture with no predators. Pairwise centroid distances between species specific microhabitat use are denoted in the text as "habitat separation" in the text. Shape- and size-coding indicates species specific traits.

We found quite limited evidence that body size or tube building traits can predict habitat use. There was no clear effect of either trait individually on the proportional abundance in any individual habitat, except for a tendency for higher occurrence of non-tube-building grazers in water (GLMM, tube building ability effect, $F_{1, 5.7576} = 4.0162$, p= 0.094; random effect of species p = 0.04). It appears that in combination these traits may more clearly indicate one dimension of habitat use; species that are both small and non-tube-building were clearly more abundant in the water column than on any substrate, but since there are only two species per combination of traits this cannot be definitively distinguished from random species effects (Fig. 3).
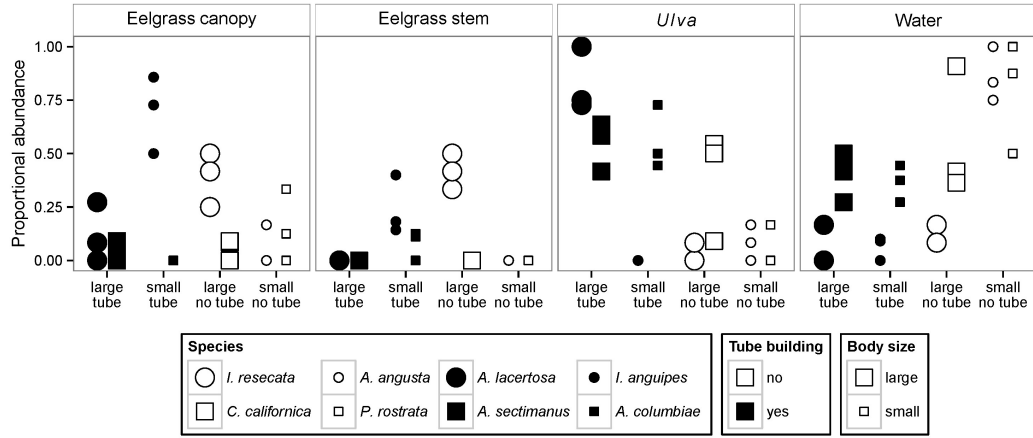
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Fig. 3: Proportional abundances per habitat as a function of traits (body size class and tube building ability). Each panel shows proportional abundances from one microhabitat, shape- and size-coding indicates species specific traits.

There was no effect predator presence on overall microhabitat use (PERMANOVA; predator presence effect, $F_{1,34}$=1.1516, p=0.661) or species specific microhabitat use (PERMANOVA; grazer species X predator presence, $F_{5,24}$=30.2785, p=0.321). Therefore we pooled monoculture habitat choice data from both predator and no-predator monocultures to increase the number of available replicates from three to six for all following analyses. Hereafter, whenever references to monoculture habitat choices are made they aim at this pooled dataset, unless stated otherwise.

Microhabitat choices in the presence of competitors

We found that species differed in their response to a potential competitor, as indicated by the highly significant interaction of grazer species and competitor presence (PERMANOVA: grazer species X competitor presence $F_{7,128}$= 3.126, p=0.001). Models for each species individually revealed that *A. angusta, A. columbiae, I. anguipes* and - to a lesser extent - *I. resecata* shifted habitat choices when competitors were added, whereas *A. lacertosa* and *C. californica* did not (see p-values in Fig. 4). As previously reported, the number of grazers per tank did not affect microhabitat choice in monoculture, so any response to competitors we observed were the result of species interactions rather than increased total density.
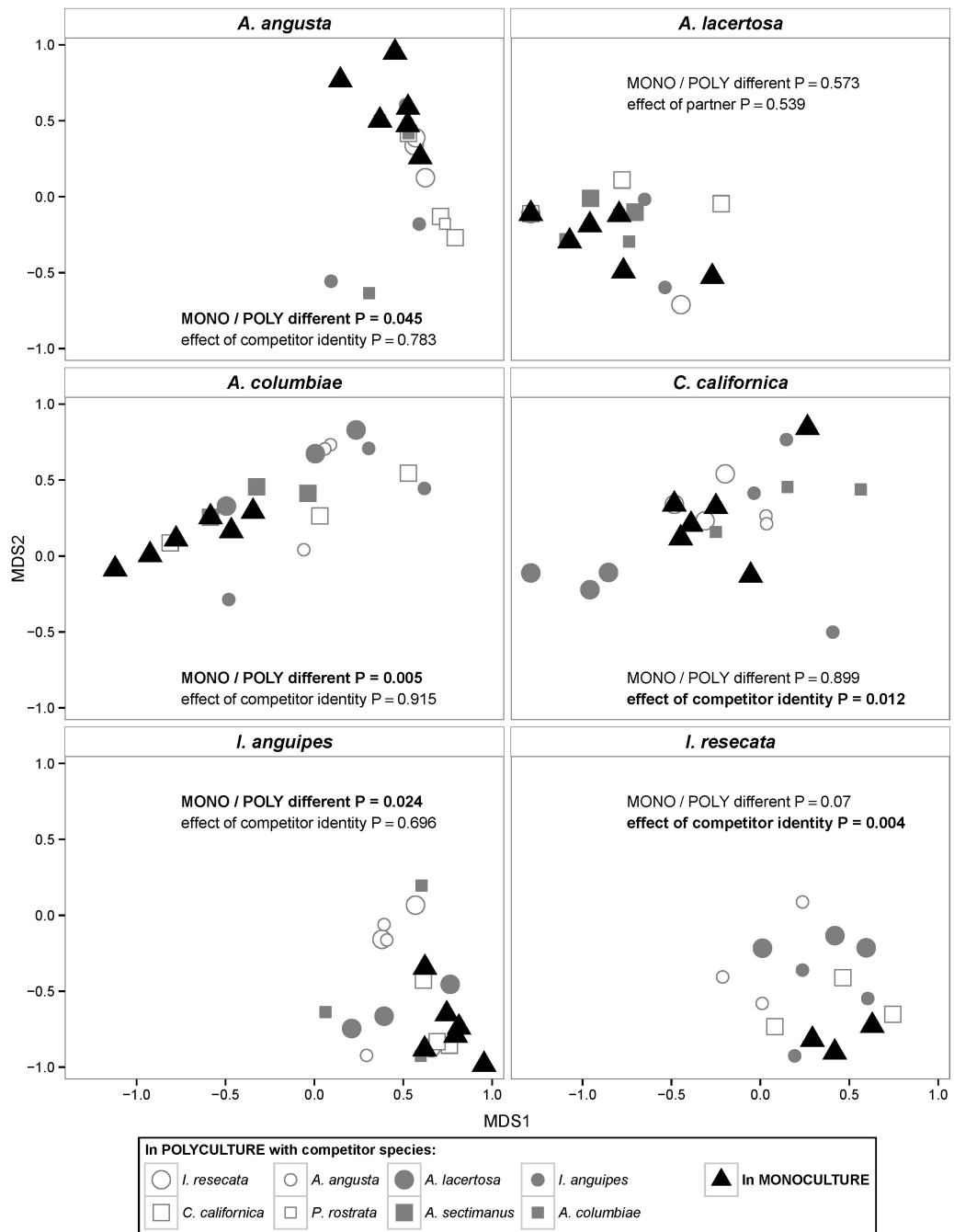
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Fig. 4: MDS-Ordination of microhabitat choices from all experiments (except high density); grouped by species for better view. Panels show habitat choices of one species each; P-values show results from two separate species specific PERMANOVA models that tested for (a) differences between monoculture and polyculture (effect of competitor presence) and (b) an effect of competitor identity on habitat choice within the polycultures. All points within a panel represent the habitat choice of a single species; habitat choice for that species in monoculture is indicated by black triangles and habitat choice in polycultures is indicated by shapes indicating the identity of the competitor (grey symbols), Habitat choices of *P. rostrata* and *A. sectimanus* are not shown, because only 2 polyculture experiments with these species were performed due to insufficient abundances in the field.

In our overall test of whether species in polyculture occupied different parts of the habitat in depending on the identity of their competitor, we did find a tendency for all species to make somewhat similar habitat choices in response to a given competitor (PERMANOVA: competitor identity effect, $F_{7,94}=1.6784$, p=0.06), although this was still secondary to underlying species differences in microhabitat use (PERMANOVA: grazer species effect, $F_{7,94}=23.2943$, p=0.001).

There were no interactions between the identity of the focal species and the identity of the competitor (PERMANOVA: grazer species X competitor identity, $F_{19,68}=1.123$, p=0.305). Species specific models revealed that only *I. resecata* and *C. californica* were influenced by competitor identity (their habitat use in the presence of each competitor species is distinct and not overlapping - see respective panels in Fig. 4 for details and P-values)

After testing for effects of competitor presence on microhabitat choice, we used an overall model to test if species differed in their habitat separation under competitor presence. In agreement with a strong effect of species identity in the previous models, we found that relative separation of microhabitat use between species remained constant from mono- to polyculture. The separation of habitat use in monoculture had a highly significant effect on distances between habitat use within a tank (GLMM: p=0.0074 - Fig. 5). This indicates that a shift of microhabitat use in response to a competitor was small compared to differences in microhabitat use observed in monoculture: microhabitat separation among species was mostly driven by fixed species differences that are independent of species interactions.
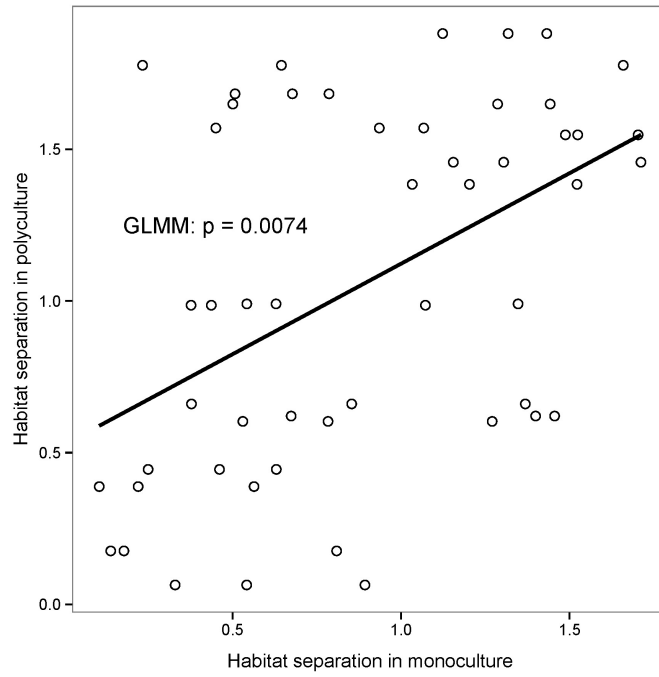
Fig. 5: Pairwise distances between habitat choices in polyculture within the same tank (Polyculture habitat separation) as a function of the corresponding habitat separation in monoculture. Polyculture habitat separation is correlated with monoculture habitat separation (GLMM: monoculture habitat separation=response variable, polyculture habitat separation = fixed effect, species combination = random factor, $F_{1,14.997}$=9.5579, p=0.0074), meaning that overall differences between species' habitat choices in the presence of competitors is not different from their microhabitat separation in monoculture (extracted from Fig. 3).

DISCUSSION

In our investigation of the potential for microhabitat partitioning among seagrass mesograzers, we found that species have distinct microhabitat preferences that are largely un-affected by the presence of both predators and competitors. This indicates that species have evolved individual close associations with particular habitats rather than flexible context-dependent habitat choices. In addition, we found that these distinct microhabitat choices are difficult to predict from traits that are commonly associated with microhabitat use and show significantly dispersed structure in field communities in this system (Best and Stachowicz, 2014). Below we compare our findings to other studies from this system, and discuss the observed microhabitat partitioning and the implications it may have for species coexistence.

Microhabitat partitioning among species from the same guild such as we have discussed here is associated with differences in various traits that species have developed to utilize specific segments of a habitat gradient. (Kovalenko et al., 2011; Warfe et al., 2008). We found that three out of four amphipods with the ability to build tubes were mainly found in *Ulva*, where the majority in fact had constructed tubes. *Ulva* is more flexible than eelgrass and has a large continuous surface, which might facilitate tube construction. We observed that even the largest specimen of tube builders could conceal themselves by folding an *Ulva* piece around their body. *I. anguipes* with its flattened body shape might not be large enough to use *Ulva* for effective camouflage, but can stay between the leaves in the eelgrass canopy or branching sections on the stem. This supports the hypothesis that concealment for tube building grazers is facilitated by choosing a habitat that matches body shape (Holmlund et al., 1990; Hacker and Madin, 1991; Norderhaug, 2004) and size (Hacker and Steneck, 1990; Bartholomew, 2002; Robson et al., 2005). As opposed to the relatively sessile and concealed tube building grazers, the two small non tube building amphipods were visible in the water column where they were present in highest abundance. We observed that - relative to other species - these small amphipods were swimming very fast when kept in containers

in the lab, which might allow them to both avoid detection and facilitate escape. Seagrass beds have often turbid waters and complex plant structures, therefore small body size and good maneuverability might be advantageous to avoid predation.

These observations and previous work suggest that host plant morphology and grazer species are closely matched via traits like body size and shape, and other specializations like the ability to build and hide in tubes that reduce the risk of predation (Hacker and Steneck, 1990) or match diet preferences (Best and Stachowicz, 2012; McDonald and Bingham, 2010). However, our findings could not directly underpin this hypothesis, as the use of a specific microhabitat could not be statistically predicted by the traits we included in this study, except for an association of non-tube building grazers with water: P=0.065, Fig 3). Grazer traits may still have value as a predictor of microhabitat use as there were some limitations to our approach. First, the analysis of a combination of traits has relatively low power, because only two species are available for each combination of traits. Second, we only assessed a small number of morphological traits, but the morphological variation among our species is greater than depicted by just size and tube building ability. This variation may be important when considering the mentioned close matching of plant and grazer morphology. Third, like many habitats, eelgrass beds contain relatively diverse microhabitats in a broad range of sizes and shapes, and although we probably included a substantial proportion of the microhabitats that are available in eelgrass beds, they might not be enough to display if a species' microhabitat choice is really distinct from another. Flowering shoots of eelgrass, epiphytic macroalgae or a branched rhizome area could be examples of other microhabitats that are possibly differentiated between species.

As mentioned above, plant morphology is likely to define the value as shelter from predation and "enemy-free-space" (Berdegue et al., 1996; Brose, 2003), which can also be a principal constraint for grazer habitat choice by fixed animal-plant associations (Duffy and Hay, 1991; Boström and Mattila, 1999; Lasley-Rasher et al., 2011). This could be an

explanation for our finding that the presence of two fish predators did not influence grazers in their microhabitat choices, even though the predators were very active and attempted to feed on the grazers behind the mesh barrier. This is in agreement with previous laboratory experiments (e.g. Boström and Mattila, 1999; Moran et al., 2010; Lasley-Rasher et al., 2011) and suggests that predator presence does not directly influence grazer microhabitat preferences in the short term. Unlike colonization (Vonesh et al., 2009) and feeding rates (Duffy et al., 2005), choices for microhabitats might not be made as a consequence to predator presence, but likely represent an evolved response to consistently high predation risk (Duffy and Hay, 1991). Unpublished data shows that gammarid amphipods tethered in the field in Bodega Bay without access to preferred habitat are rapidly consumed (50-100 % consumed with in 24h; J. Stachowicz unpublished data). This is consistent with data for other epifauna which suffer reduced mortality from predation when provided with their preferred host plant (Holmlund et al., 1990; Duffy and Hay, 1991; Sotka, 2007; Moran et al., 2010; Lasley-Rasher et al., 2011).

As most arthropod communities with high predation pressure, seagrass mesograzers are competing for microhabitats that provide enemy-free space (Berdegue et al., 1996; Duffy and Hay, 1991). Species with overlap in microhabitat use or other dimensions of their niche are also more likely to compete with each other (Schoener, 1983). To reduce the potentially negative effects of territorial competition, some species are able to change their microhabitat use in the presence of stronger competitors. van Riel et al. (2007) have shown that freshwater amphipod species can use a different type of substrate as microhabitat when an invasive competitor with similar habitat use is present. In a different study, freshwater amphipods varied in abundance in their preferred microhabitat, depending on the identity of a present competitor (Cothran et al., 2013). In this study we tested if seagrass mesograzers can change their microhabitat use in the presence of other species with different levels of niche overlap. We found that the presence of competitors caused some species to change their specific microhabitat use: of six species that were individually tested

for differences between monoculture and polyculture, three species with small body size shifted significantly in habitat preference (*A. angusta*, *A. columbiae*, *I. anguipes*). One large species (*I. resecata*) showed a tendency for changed habitat preference, and two other large species (*A. lacertosa* and *C. californica*) did not change their microhabitat use at all in the presence of competitors (Fig. 4). Although not statistically evident by an effect of body size, large species seemed to respond less to the presence of competitors. An explanation for this could be that large species are easily detectable by predators and may commonly rely on protection from host plants to avoid being eliminated from the system (Duffy and Hay, 1991), while smaller species that are less susceptible to predation may persist outside of these refuge habitats *C. californica* had weak overall microhabitat preferences and did not respond to competitor presence. This could be due to the absence of epiphytic cover and as our unpublished data (work by Elena Huynh) suggests, food rather than competition for enemy-free space might be a main driver of abundance of this species.

In general these shifts were small compared to overall microhabitat separation, which was not altered under the presence of competitors. The cost of avoiding a competitor by choosing to leave a microhabitat, or of staying in the different microhabitat itself might be an increased predation susceptibility. Species that are adapted to a habitat which offers best protection from predation might not benefit from being flexible in their microhabitat choice (Lasley-Rasher et al., 2011) if predation susceptibility is still lower in a densely populated preferred microhabitat than in a less-populated alternative microhabitat. Furthermore, species specific differences seem to be maintained in the presence of competitors, as suggested by the highly significant main effects of focal species identity in the multivariate tests for effects of competitor presence and competitor identity. To further investigate if these effects translate to fixed habitat partitioning between species under competitor presence, we tested if habitat separation of species within the same tank was different from their habitat separation in monoculture. We found that overall relative monoculture and polyculture habitat separation are correlated with high

significance (Fig. 5). This is clear evidence that the separation of microhabitats we observed is unaffected by the presence of competitors. In other words, habitat partitioning in seagrass mesograzers is fixed and flexibility is not favored in the presence of either predators or competitors, at least within the environments provided in our lab experiment.

The distinctly different habitat choices we recorded for eight crustacean seagrass mesograzers indicates that these species partition their microhabitat use. However, we found only little correspondence between species-specific habitat use and two traits that are significantly dispersed among species coexisting in eelgrass communities in the field (Best and Stachowicz, 2014). The tendency for small, motile grazer species to be less benthic and more water-column associated stands for only one out of four dimensions of habitat use we tested, and offers limited power of inference given the number of species with particular trait combinations used in this study. Further investigations could encompass additional traits, species, and/or microhabitats to test animal-plant relationships with higher resolution. Even minor differences microhabitat use could allow the coexistence of highly specialized species, as it has been shown for butterflies that utilize different microhabitats along a temperature gradient (Kleckova et al., 2014), and spiders that can detect fine scale differences in plant morphology (De Omena and Romero, 2008). The niches of these species should be narrower, which, as shown by our results, may impede the ability of an organism to be flexible in the presence of predators and competitors (Butt and Tahir, 2010). The tradeoff between microhabitat specialization and flexibility in the presence of competitors could explain why observed shifts in microhabitat use were relatively small compared to relative overall microhabitat separation. If overlapping habitat use results in performance or fitness consequences, then the function of habitat partitioning as a stabilizing mechanism for coexistence depends on species composition because habitat partitioning is fixed, regardless of the presence of competitors or predators.

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