COMMUNITY ECOLOGY - ORIGINAL PAPER

Biotic resistance to invasion along an estuarine gradient

Brian S. Cheng · Kevin A. Hovel

Abstract Biotic resistance is the ability of native communities to repel the establishment of invasive species. Predation by native species may confer biotic resistance to communities, but the environmental context under which this form of biotic resistance occurs is not well understood. We evaluated several factors that influence the distribution of invasive Asian mussels (*Musculista senhousia*) in Mission Bay, a southern California estuary containing an extensive eelgrass (*Zostera marina*) habitat. Asian mussels exhibit a distinct spatial pattern of invasion, with extremely high densities towards the back of Mission Bay (up to 4,000 m^{-2}) in contrast with near-complete absence at sites towards the front of the bay. We established that recruits arrived at sites where adult mussels were absent and found that dense eelgrass does not appear to preclude Asian mussel growth and survival. Mussel survival and growth were high in predator-exclusion plots throughout the bay, but mussel survival was low in the front of the bay when plots were open to predators. Additional experiments revealed that consumption by spiny lobsters (*Panulirus interruptus*) and a gastropod (*Pteropurpura festiva*) likely

are the primary factors responsible for resistance to Asian mussel invasion. However, biotic resistance was dependent on location within the estuary (for both species) and also on the availability of a hard substratum (for *P. festiva*). Our findings indicate that biotic resistance in the form of predation may be conferred by higher order predators, but that the strength of resistance may strongly vary across estuarine gradients and depend on the nature of the locally available habitat.

Keywords Asian mussel · Enemy release · Generalist · Invasibility · Predation

Introduction

Biotic resistance is an important characteristic that allows native communities to resist invasion (Elton 1958). Initial studies of biotic resistance have highlighted the competitive effect that a diverse suite of native species can have on the invasibility of a community (Elton 1958; Kennedy et al. 2002; Stachowicz et al. 2002). Alternatively, native consumers may halt the establishment and spread of invaders through predation (Robinson and Wellborn 1988; Baltz and Moyle 1993; Reusch 1998; Byers 2002, 2005; Parker et al. 2006; de Rivera et al. 2005). However, the environmental context under which biotic resistance may be an important force in shaping patterns of invasion is not well understood. For example, little is known of how environmental gradients may interact with biotic resistance to determine invasion success (but see de Rivera et al. 2005). Consumer stress models predict that increasing stress along environmental gradients may affect consumers to a greater extent than their prey (Menge and Sutherland 1987; Menge and Olson 1990), with subsequent creation of predator refuges

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that may contribute to spatial variability in invasion success. Also, characteristics of a habitat (e.g., habitat attributes or habitat type) may modify biotic resistance by altering prey susceptibility (Byers 2002) or if predators are associated with a particular habitat type in a heterogeneous environment (Rilov and Schiel 2006).

In this study, we investigate the factors that control the spatial distribution of invasion for a non-indigenous bivalve, the Asian mussel *Musculista senhousia*, in a southern California estuary. Estuaries are ideal systems for testing how changes in environmental conditions affect invasive species and members of the recipient community because they often contain steep environmental gradients in physical properties (e.g., salinity and temperature) which may rapidly change. Attributes of habitat structure also often vary among sites within estuaries, in part due to human addition of structures, such as jetties, piers, and revetments. *Musculista senhousia* is a highly successful invader that is found in estuaries throughout the west coast of North America, Australia, New Zealand, the eastern Mediterranean, and the south of France (reviewed in Crooks 1996). Asian mussels are ecosystem engineers that form mats of byssal threads that can alter community composition in soft-sediment systems that typically lack habitat structure (Crooks 1998; Crooks and Khim 1999; Mistri 2002). They are approximately 100-fold more abundant than native bivalves in southern California, reaching densities of up to 10,000 m^{-2} (Dexter and Crooks 2000; Crooks 2001).

Within southern California estuaries, biotic resistance (Reusch 1998; Kushner and Hovel 2006) and strong gradients in Asian mussel density (Dexter and Crooks 2000) may result from predation by many native predators, such as fishes, crustaceans, birds, and gastropods, that consume Asian mussels (Crooks 2002). However, competitive interactions between Asian mussels and habitat-forming eelgrass (*Zostera marina*) may attenuate planktonic food supplies for mussels (Allen and Williams 2003) and prevent the establishment of mussel populations where eelgrass is dense (Reusch and Williams 1998, 1999; Williams et al. 2005). Alternatively, gradients of physiological stress due to abiotic factors or spatial variability in larval delivery and recruitment due to hydrodynamic patterns may be primarily responsible for variability in Asian mussel density within estuaries.

Methods

We performed all surveys and experiments in Mission Bay, CA, USA (Fig. 1), a shallow (depth 1–6 m) highly modified estuary that experiences seasonal hypersalinity (Largier et al. 1997). Physical properties, such as water temperature,
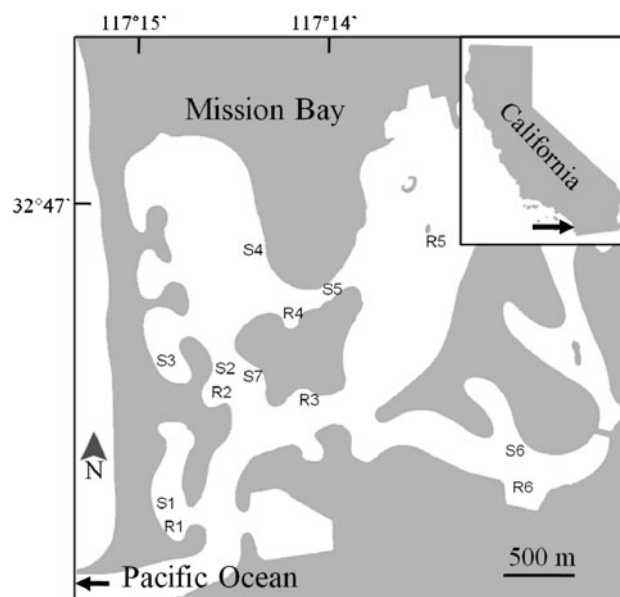

Fig. 1 Map of Mission Bay, CA, USA. Study sites are designated as having an adjacent rip-rap (*R1–6*) or soft-sediment (*S1–7*) habitat. Mouth of the bay is shown at the lower *left* and leads to the Pacific Ocean

salinity, residence time (Largier et al. 1997), and flow rate [Electronic Supplementary Material (ESM) 1], vary greatly among locations in Mission Bay. For example, in August 2007, mean water temperatures near the mouth of the bay differed from sites near the back of the bay by 3.3°C (Fig. 2). In the summer, salinity in the back of the bay can be greater than that in the front of the bay by as much as 2 PSU (Largier et al. 1997). Sediment characteristics also vary among locations, with sites near the front of the bay dominated by sand and those near the back of the bay dominated by mud (B. Cheng, unpublished data).

We conducted surveys and experiments to determine which combination of biotic and abiotic factors may be responsible for a persistent gradient in *M. senhousia* density, in which mussels densities are extremely high in the back bay, but nearly absent from the front bay (Dexter and Crooks 2000). In an effort to explain this pattern, we focused on spatial patterns of predation, recruitment, growth, and competition from eelgrass, but we did not focus on potential competition from native bivalves, such as *Chione fluctifraga* and *C. undatella*, because these species have little competitive effect on Asian mussels in Mission Bay, whereas Asian mussels are known to reduce the growth and survival of these natives (Crooks 2001).

Mussel and eelgrass sampling

To quantify spatial patterns of Asian mussel distribution and to determine whether Asian mussel abundance is related to seagrass habitat structure, we established 12 field

sites in the subtidal eelgrass habitat in Mission Bay (Fig. 1). Sites were selected that spanned the estuarine gradient and had >70% total eelgrass cover and a depth range of 1–2 m below mean lower low water. Although all 12 sites were located in eelgrass habitat, six were located adjacent to armored shoreline consisting of large boulders ("rip-rap") that had previously been placed in the intertidal zone to prevent erosion (hereafter "R sites"), whereas the remaining sites were located adjacent to soft-sediment shoreline that lacked rip-rap (hereafter "S sites"). We established the two site types because rip-rap may act as a source of potential predators of Asian mussels, particularly *Panulirus interruptus* and *Pteropurpura festiva* (Kushner and Hovel 2006). Based on preliminary evidence from 2006, in 2007 we established a new site (S7) in place of S3 (Fig. 1) to increase our sampling effort in a zone of high variability in terms of mussel survivorship. From 12 June to 15 July 2006 and from 4 June to 20 July 2007, we sampled for Asian mussels and eelgrass using a 12-cm-diameter sediment corer (area = 113 cm^2). At each site, we established three 30-m transects parallel to shore that were spaced 5 m apart. For each transect, we collected one sample at each of four random positions (at least 7 m apart) to a sediment depth of 2.0 cm (Williams et al. 2005) ($n = 12$ samples per site per sampling event × 12 sites × 2 sampling events = 288 cores). Cores were sieved through a 2.0-mm mesh screen in 2006; in 2007, we used a 1.0-mm mesh screen to facilitate greater detection of Asian mussel recruits. Samples were frozen and at a later date thawed in the laboratory before processing. For each core, we quantified Asian mussel density, shell height (SH), and eelgrass habitat characteristics (shoot density, mean shoot length, above-ground dry biomass, and below-ground dry biomass). One core from the 2006 and 2007 surveys, respectively, was lost during processing due to sample bag failures.

We used a principal components regression (Quinn and Keough 2002) to test for a relationship between eelgrass habitat characteristics and Asian mussel density. This approach is useful because it accounts for potential collinearity in the predictor variables and is unbiased in terms of variable selection (Graham 2003). For this analysis, we used normal probability plots to evaluate assumptions of normality and compared estimates against residuals to test for homogeneity of variances, transforming data when necessary (Underwood 1997). All statistical analyses were performed using Systat ver. 12 (Systat Software, San Jose, CA).

Mussel recruitment

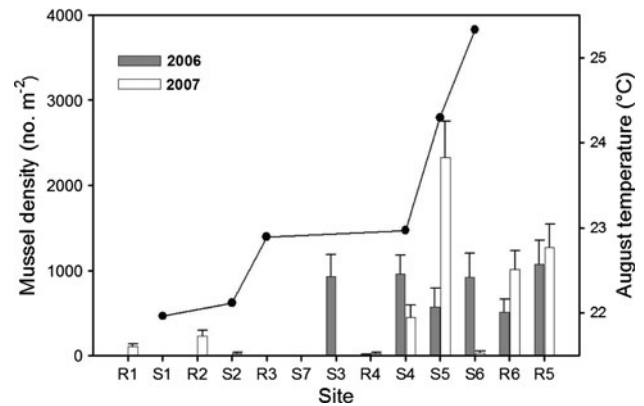In order to address temporal and spatial differences in Asian mussel recruitment, we conducted additional core

Fig. 2 Mean Asian mussel (*Musculista senhousia*) density (*bars*; *left* axis) in 2006 and 2007 and mean August water temperatures (*line*; *right* axis) in Mission Bay, CA. Sites are designated as having adjacent rip-rap (*R1–6*) or soft-sediment (*S1–7*) habitat and are listed from *left* to *right* with increasing distance from the bay mouth

sampling on 21 March and 15 May 2007 at six randomly selected sites (R1, R2, S2, R3, S4 and S6) to compliment the summer sampling. Cores were collected as described above. During sampling for Asian mussels and eelgrass in July 2007, we detected high densities of newly settled Asian mussels (SH < 5.0 mm) at R1 and R2, sites near the bay mouth where no sub-adult or adult mussels were detected in 2006 or June 2007 (Fig. 2). To help us determine how settlement and recruitment may influence Asian mussel abundance, particularly at these two sites with historically low levels of *M. senhousia*, we continued sampling once every week during August and at least once a month thereafter until mussels were no longer detected [September 2007 for R2 ($n = 83$ cores) and October 2007 for R1 ($n = 119$ cores)].

Abiotic effects

To test whether Asian mussel survival, growth, and gonad production varied among sites in Mission Bay in the absence of predation, we deployed mussels at sites within Mission Bay using artificial seagrass units (ASUs). The employment of ASUs allowed us to precisely control simulated eelgrass structural attributes and facilitated the deployment and recovery of mussels (Reusch 1998; Kushner and Hovel 2006). The ASUs consisted of a PVC ring (area 490 cm^2) with 2.0-mm mesh fabric clamped onto the bottom, plastic webbing fastened to the top to simulate eelgrass rhizome structure, and polypropylene ribbon tied to webbing to simulate eelgrass shoots. In each ASU, 15 mussels (2006 mean SH ± 1 SD = 22.1 ± 3.7 mm; 2007 = 20.5 ± 2.7 mm) were haphazardly placed within beach sand with the anterior margin at the sediment surface. ASUs were deployed along a transect parallel to the shoreline within eelgrass beds at distances of no less than

3 m apart. Mussels within ASUs were protected from predators with a cylindrical galvanized metal cage (0.64-cm^2 mesh, diameter 28 cm, height 24 cm). In January 2006, we deployed six ASUs for 4 weeks at each of four sites (S1, S2, S5 and S6); in January 2007, we deployed four ASUs at each of the same four sites for 8 weeks. We deployed mussels in the winter because our a priori hypothesis was that winter environmental conditions precluded Asian mussel establishment. Although we did not conduct this experiment during the summer, it is unlikely that summer conditions in the front bay reduce Asian mussel survival and performance because we observed recruitment and mussel growth during this period at R1 and R2 and because previous work in the front bay during the summer indicated that transplanted Asian mussels could live there for periods of at least 1 month (Kushner and Hovel 2006). Cages were monitored for damage and cleaned every 2 weeks.

At the end of each experiment, we calculated proportional survivorship for each ASU and eliminated from the analysis any ASU found to contain a predator (two and four cages in 2006 and 2007, respectively). For the 2007 experiment, we also removed gonad and somatic tissue from all living mussels and dried them at 80°C for 48 h. We calculated gonad indices (GI) as gonad dry weight divided by total dry weight. Mean proportional survivorship, mean GI, and mean total dry weight (DW) were analyzed as dependent variables in separate one-way analysis of variances (ANOVAs) to test for mean differences among sites. We graphically evaluated the normality of data and used Cochran's test to evaluate the assumption of homogeneous variances here and in all other ANOVAs. Data were arc-sine square root transformed when necessary to meet the assumptions of ANOVA (Underwood 1997). Differences in means were tested with Student–Newman–Keuls (SNK) post-hoc tests.

Predatory effects

We quantified Asian mussel predator-induced mortality among sites by exposing mussels to predators within ASUs (experiment 1). Within each ASU we placed 25 mussels (SH = 15–30 mm) and deployed six ASUs at each site for a period of 1 week between June and August 2006 ($n = 72$). ASUs were deployed 3 m apart along a transect parallel to the shoreline (depth 1–2 m). Four ASUs at each site were open to predators, while two were fully caged to evaluate handling effects and short-term abiotic stress on mussel mortality. We repeated the experiment from June to August 2007, except that at this time a total of ten ASUs were deployed at each site ($n = 120$), with six ASUs open to predators, two fully caged, and two enclosed within partial cages to evaluate potential caging artifacts. After 1 week, we recovered all plots and noted the presence of

any native predators and characterized mussels as: (1) alive, (2) dead with intact valves, (3) drilled, (4) crushed, or (5) missing. Drilled mussels are indicative of gastropod predation, whereas crushed mussels are a sign of crustacean predation (Reusch 1998). We attributed missing mussels to consumption by predators because survival in control cages was high and because fishes typically ingest mussels whole (Crooks 2002), whereas lobsters completely crush mussels (Reusch 1998).

To analyze Asian mussel proportional mortality data, we conducted two analyses of covariance (ANCOVA; one for each year) that included the caging treatment (open vs. closed in 2006; open vs. closed vs. partial in 2007) as well as distance from the bay mouth (km) and habitat (rip-rap vs. soft-sediment) and all possible interactions in the model. We conducted analyses separately for each year because the partial cage treatment was not used in 2006. Distance from the bay mouth was calculated from a fixed point at the bay mouth to each site as the water flows. Because the initial analyses suggested no caging artifact and that a habitat-specific effect may be swamped by low mortality at back bay sites we conducted a final ANCOVA on data from open plots only and at sites <3.5 km from the bay mouth with distance, habitat, and their interaction in the model.

In order to partition the effects of different predator types on Asian mussel mortality, we conducted an additional caging experiment (experiment 2) using differently sized access ports (Micheli 1997). In October 2007, we deployed 18 ASUs at each of two sites, R1 and S1. Each ASU contained 25 mussels and was placed into one of four caging treatments: open ($n = 5$), large partial ($n = 5$), small partial ($n = 5$), and full ($n = 3$). Open ASUs had no cage, full ASUs had a full cage, large partial cages had two large access ports (14 × 14 cm), and small partial cages had four small access ports (6 × 8 cm). Large partial cages prevented large fishes from accessing plots but allowed entry by lobsters, crabs (e.g., *Portunus xantusii*, *Cancer* spp.), and *P. festiva*. Small partial cages excluded fishes, lobsters, and large crustaceans, allowing only small gastropods and small crustaceans into the plots. We used a two-way ANOVA to test whether proportional mussel mortality differed between sites and caging treatment. Three plots with upended cages were excluded from the analysis.

To assess the spatial distribution of benthic native predators, we quantified gastropod, crustacean, and fish densities using visual belt transects. For gastropods, we enumerated all *P. festiva* and other predatory whelks, such as *Roperia poulsoni* and *Ceratostoma nuttalli*, within 30 × 1-m transects at each of the 13 sites using SCUBA gear. Each site was visited during the summers of 2006 and 2007 and the spring of 2008 ($n = 36$). We also quantified densities of lobster and other mobile predators, such as the

round ray (*Urobatis halleri*) and crabs (*Cancer* spp. and *Portunus xantusii*). Mobile predator surveys were conducted at night, when lobsters and other nocturnal predators are actively foraging, with three 50×2-m belt transects parallel to the shoreline and no less than 7 m apart. A subset of eight sites were randomly selected and visited three times ($n = 72$ transects) from August to October of 2007.

Results

Mussel and eelgrass sampling

Asian mussel abundance varied substantially along the estuarine gradient. In 2006, mussels were prevalent at sites in the back of the bay but were completely absent from sites at the front of the bay (Fig. 2). In 2007, we detected the same general pattern, with the exception of recruitment events at R1 and R2. Relative to 2006, in 2007, we detected greater densities of Asian mussels at S5, R5, and R6 and a far lower density at S6. For both years, Asian mussel densities at the back of the bay exhibited high variability within and among sites (Fig. 2).

Asian mussel density was generally positively correlated with eelgrass structure. In both years, all measures of habitat structure loaded positively on PC1 (Table 1). Below-ground biomass and shoot counts negatively loaded onto PC2 in 2006 and 2007 (Table 1). In 2006 and 2007, these two principal components combined explained 90.8% (PC1 = 67.4%, PC2 = 23.4%) and 87.5% (PC1 = 62.5%, PC2 = 25.0%) of the variation in eelgrass habitat structure, respectively. In 2006, we found a positive relationship between PC1 and Asian mussel density and a negative relationship between PC2 and Asian mussel density (Table 1; ESM 2; Adjusted $r^2 = 0.196$). In 2007, the pattern was weaker, with only PC2 as a significant predictor of Asian mussel density and marginal evidence of an interactive effect of PC1 and PC2 (Table 1; ESM 2; Adjusted $r^2 = 0.157$).

Mussel recruitment

We did not detect any recruits in the front of the bay (sites <3.5 km from the bay mouth) in 2006, but recruits were detected at back bay sites (R3, S4, and S6) in both years. In 2007, we detected two recruitment pulses at R1 and R2, possibly from the change in protocol to a 1-mm^2 sieve size. Densities of newly settled Asian mussels at R1 and R2 peaked on 6 August 2007 and gradually declined thereafter (ESM 3). Mean mussel SH increased from approximately 3.5 mm just prior to the peak of mussel detection to approximately 6.2 mm on 22 August 2007, and decreased

Table 1 Principal components regression results for the relationship between eelgrass habitat structure and Asian mussel (*Musculista senhousia*) density

Component loadings of eelgrass habitat structure on PC1 and PC2			2006		2007	
			PC1	PC2	PC1	PC2
Variable						
Above ground biomass (g)			0.913	0.217	0.883	0.278
Below ground biomass (g)			0.845	−0.404	0.837	−0.370
Shoots (no. core^{-1})			0.888	−0.360	0.792	−0.500
Mean shoot length (cm)			0.600	0.771	0.628	0.733
Source	SS	df	MS		F ratio	P
ANOVA table for the effects of eelgrass principal components on Asian mussel density in 2006						
PC1	8.487	1	8.487		5.260	0.023
PC2	7.250	1	7.250		4.493	0.036
PC1 × PC2	2.431	1	2.431		1.507	0.222
Error	224.273	139	1.613			
ANOVA table for the effects of eelgrass principal components on Asian mussel density in 2007						
PC1	0.296	1	0.296		0.147	0.702
PC2	32.159	1	32.159		15.984	<0.001
PC1 × PC2	6.134	1	6.134		3.049	0.083
Error	279.667	139	2.012			

PC Principal component, *ANOVA* analysis of variance

thereafter. We also detected a gradually increasing number of drilled mussels over the duration of the recruitment pulse. In the last recruitment survey, there were more drilled mussels than live mussels, and the SH of the drilled mussels was greater than that of the live mussels, potentially indicating that small newly settled mussels escape detection by predators. Overall, size frequency distributions at both sites during this census were highly skewed to the right (ESM 4), with only 1.5% of mussels having a SH >10.0 mm.

Abiotic effects

Abiotic factors did not appear to limit Asian mussel survival and performance at sites near the front of the bay. In the 2006 outplant experiment, we recovered 22 of 24 ASUs containing 314 of 330 mussels (95.2%). There was no difference between mean mussel survivorship between sites along the gradient ($F_{3,18} = 0.98$, $P = 0.424$; ESM 5A). In the 8-week outplant experiment in 2007, we excluded S2 from the analysis because 22 of the 60 mussels collected possessed micro-holes, indicating predation by

small gastropods that were able to penetrate the 0.64-cm^2 mesh cages. Of the remaining 12 ASUs, we recovered 168 of 180 mussels (93.3%). As in 2006, there was no difference between mean proportional mussel survival among sites along the gradient ($F_{2,9} = 1.30$, $P = 0.319$; ESM 5B). However, there was a difference in mean dry weight (DW; $F_{2,9} = 38.04$, $P < 0.001$) and mean GI ($F_{2,9} = 10.89$, $P = 0.004$) among sites (ESM 5C, 5D), with mussels at S6 having a lower DW and GI than mussels at S1 and S5. The lower performance of Asian mussels at S6 was coincident with lower mussel densities detected on the 2007 surveys.

Predatory effects

For experiment 1, in both 2006 and 2007 there was a significant interactive effect of distance from the bay mouth and cage type on Asian mussel proportional mortality (Table 2). Mussels within control cages survived well at all sites [mean survival ± 1 standard error (SE) 88.1 ± 1.4%], but mussels placed in partial cage (cage-control) plots and open plots had high mortality at sites near the

Table 2 ANOVA table for experiment 1 that examined the influence of distance from the bay mouth (km), cage (open vs. partial vs. closed), and habitat (rip-rap vs. soft-sediment) on Asian mussel proportional mortality in 2006 and 2007 and for experiment 2 that examined the partition of predator effects (cage type) and site (R1 vs. S1) on Asian mussel proportional mortality

Source	SS	df	MS	F ratio	P
Asian mussel proportional mortality in 2006					
Distance	2.045	1	2.045	23.442	<0.001
Habitat	0.009	1	0.009	0.106	0.746
Cage	2.599	1	2.599	29.765	<0.001
Distance × habitat	0.001	1	0.001	0.01	0.919
Distance × cage	1.364	1	1.364	15.643	<0.001
Habitat × cage	0.099	1	0.099	1.129	0.292
Distance × habitat × cage	0.034	1	0.034	0.385	0.537
Error	5.234	60	0.087		
Asian mussel proportional mortality in 2007					
Distance	6.717	1	6.717	99.433	<0.001
Habitat	0.130	1	0.130	1.919	0.169
Cage	5.439	2	2.719	40.259	<0.001
Distance × habitat	0.050	1	0.050	0.745	0.390
Distance × cage	3.241	2	1.621	23.991	<0.001
Habitat × cage	0.073	2	0.037	0.541	0.584
Distance × habitat × cage	0.041	2	0.021	0.307	0.736
Error	7.295	108	0.068		
ANOVA table for experiment 2 to partition predator effects (cage type) and site (R1 vs. S1) on Asian mussel proportional mortality					
Site	0.148	1	0.148	2.533	0.124
Cage	8.546	3	2.849	48.806	<0.001
Site × cage	0.799	3	0.266	4.561	0.011
Error	1.459	25	0.058		

front bay and low mortality near the back of the bay. *Musculista senhousia* proportional mortality was high near the bay mouth, decreasing linearly towards the back of the bay at both rip-rap and soft-sediment sites (Table 2; Fig. 3). Near the bay mouth, ASUs always contained crushed mussels, indicative of crustacean predation (Reusch 1998) and of rip-rap sites (R1–3). *P. festiva* were found actively drilling mussels, and an average of 28% (±5.6% SE) of mussels possessed drill holes from recent predation. In the analysis of open plots at sites <3.5 km from the bay mouth, we found a significant habitat by distance interaction on Asian mussel proportional mortality, with the effect of habitat greatest at distances 2.5–3.0 km from the bay mouth. Proportional mortality was high near the bay mouth regardless of habitat type, but at increasing distance, rip-rap sites exhibited higher proportional mussel mortality than soft-sediment sites (ESM 7; Fig. 3a (sites left of reference line); interaction $P < 0.001$).

In the caging experiment to separate predator effects (experiment 2), mussels placed in ASUs without a cage or in large partial cages had high mortality, but mussels placed in partial cages with small holes had high mortality only at the rip-rap site (Table 2; Fig. 3b). In the large partial caged plots at S1, 9.5% of the mussels were drilled while the remaining 90.5% were crushed. No mussels were found with drill holes at S1. Of the mussels in small partial cages, 21.1 and 19.7% were recovered with crushed or drilled shells, respectively. Asian mussels placed within full cages suffered low mortality (Fig. 3).

Lobster and *P. festiva* distributions were consistent with patterns of predation on Asian mussels. Both species had the greatest densities near the mouth of the bay, decreasing towards the back (Fig. 4). Lobsters were never observed past S2 and were more common at rip-rap sites, while *P. festiva* and the other muricids were essentially restricted to rip-rap sites, a possible requirement for egg mass deposition (B. Cheng, personal observation). Round rays were sparse compared to lobsters and were distributed somewhat evenly among sites, with highest densities at R6 (Fig. 4). The same pattern was exhibited by muricids other than *P. festiva*. We observed only two *Cancer* spp. and zero *Portunus xantusii* on the surveys.

Discussion

We found strong evidence that native predators confer biotic resistance that limits the distribution of an invasive species along a steep environmental gradient. Asian mussels were abundant in the back of Mission Bay and were absent from sites near the front of the bay. At sites near the front of the bay, nearly all experimental mussel assemblages that were exposed to predators were completely
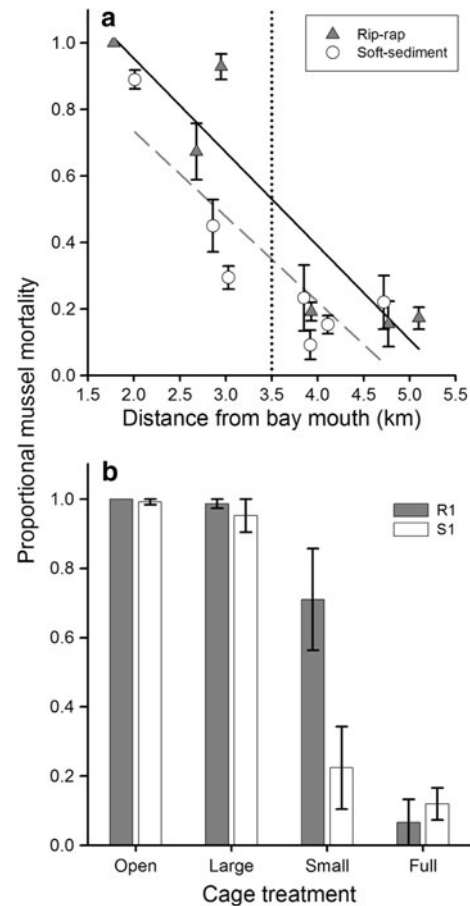

Fig. 3 a Proportional Asian mussel mortality (mean ± standard error) at rip-rap (*shaded triangles*) and soft-sediment (*open circles*) sites after 7 days vs. distance from the mouth of Mission Bay, CA (experiment 1). Data are graphed for open plot treatments and pooled across years for clarity and brevity. See ESM 8 for plot of mussel mortality in all of the cage treatments. *Solid black line* Linear best fit for rip-rap sites, *gray dashed line* best fit for soft sediment sites. *Vertical dotted reference line* marks 3.5 km from the bay mouth. **b** Proportional Asian mussel mortality (mean ± standard error) after 7 days in experiment 2. Open cages are subject to predation by all predators; large cages allow the entry of larger predators, such as spiny lobsters; small cages allow the entry of smaller predators, such as predatory whelks; full cages prevent access for all predators

decimated within 1 week. This pattern of biotic resistance was strongly aligned with high densities of native predators (both crustacean and gastropod) at front bay sites. When the analysis was restricted to the front bay (sites <3.5 km), we found that Asian mussel mortality at rip-rap sites (2.5–3.0 km) was greater than that at soft-sediment sites in the same region. This higher mortality at the former sites was most likely due to high densities of *P. festiva* on adjacent hard substrate and declining lobster densities at soft-sediment sites in this region. The results of caging experiments combined with differing predation signatures (crushed vs. drilled shells) suggest that lobsters were an important consumer of Asian mussels at both R1 and S1 because of
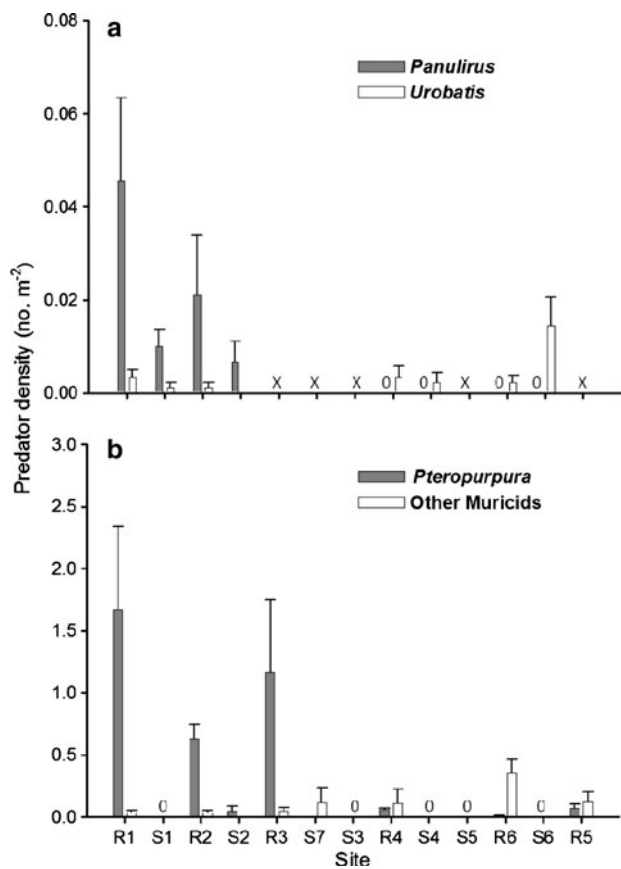
Fig. 4 Predator densities (mean number per m^2 + standard error) along the estuarine gradient in Mission Bay, CA for *Panulirus* (spiny lobster) and *Urobatis* (round ray) (**a**) and *Pteropurpura festiva* and other muricid gastropods (**b**). Sites are listed from *left* to *right* by increasing distance from bay mouth. *X* sites where surveys were not conducted, *0* surveys where no predators were observed

their higher rate of consumption and greater presence relative to gastropods at soft-sediment sites.

Our results suggest that the eelgrass habitat structure does not competitively limit the survival of Asian mussels in Mission Bay. In contrast to our findings, Reusch and Williams (1999), working in both San Diego and Mission Bay, found a disjunct distribution between Asian mussels and eelgrass, likely due to attenuation of food delivery by eelgrass structure and inhibition of eelgrass growth by dense mussel mats (Reusch and Williams 1998, 1999; Allen and Williams 2003). It is possible that we did not observe similar patterns because all of our sites were within Mission Bay, which likely has differing hydrodynamic patterns due to its convoluted shoreline relative to San Diego Bay. Additionally, phytoplankton concentrations in Mission Bay may be sufficient to maintain mussel populations within dense eelgrass patches, although we have not tested this hypothesis. Recruitment limitation apparently does not act to restrict Asian mussels from the front of Mission Bay, although recruitment rates may be lower

there compared to areas in the back bay with dense mussel populations. During the 2007 sampling period, we detected a significant recruitment pulse of Asian mussels to two front bay sites that was sustained over several months, and we thereafter observed the growth of mussels until a peak in mean SH of 6.2 mm. Moreover, Williams et al. (2005) detected Asian mussel recruits (density = 2,340 m^{-2}) in Mariner's Basin, the same area as R1 and S1. At front bay sites, the overall size structure of Asian mussels was skewed to the right, and over time the detection of Asian mussels decreased to almost zero (ESM 3 and 4), indicative of low survivorship at larger size classes rather than recruitment limitation which should exhibit a sawtooth distribution caused by sporadic propagule supply (Lewis et al. 1982; Kendall 1987). We note, however, that we only performed extended sampling at two sites in the front bay and that other factors, such as sediment instability, may play a role in limiting Asian mussels from this area. We found no evidence that abiotic conditions at sites near the front bay prevented establishment by Asian mussels: survival, DW, and GI were the same or greater there than at sites near the back of the bay. This is not surprising, given that Asian mussels are distributed across much of the west coast of North America and many other parts of the world. However, our experiments did not allow us to determine if summer conditions or chronic exposure (>8 weeks) to the front bay environment could significantly affect Asian mussel performance. Hypoxic events would be most likely to occur during the summer when high temperatures, a stratified water column, and long retention times could facilitate hypoxia (Justic et al. 1993). Hypoxia likely resulted in the 95% mortality of a cohort of Asian mussels in the Northern Adriatic Sea (Mistri 2002), and in Narragansett Bay, Rhode Island, hypoxia caused a bay-wide extirpation of the confamilial *Mytilus edulis* (Altieri and Witman 2006). Although hypoxia clearly can have strong effects on bivalve distribution and abundance, it is unlikely to explain the spatial Asian mussel density gradient in Mission Bay because mussels were abundant in the back of the bay where hypoxia is most likely, and they were absent from the front bay where tidal forcing should keep water well oxygenated.

We suspect that spiny lobsters were an important source of Asian mussel mortality in Mission Bay for several reasons. The consumption rate of predators that crush mussels was much greater than that of gastropods in experiment 2 where 100% of mussels in fully exposed plots were consumed. Although gastropods were allowed access to these plots, none of the recovered mussel shells were drilled, suggesting that crustacean predation preceded a gastropod response. In contrast, 29% of mussels in small caged ASUs survived, suggesting that the rate of gastropod predation is lower than that of larger predators, such as lobsters.

Additionally, where the adjacent habitat was soft-sediment, *P. festiva* was completely absent, whereas lobsters were observed at every front bay site surveyed (R1, 2 and S1, 2). Asian mussels were also a primary prey item in lobster guts from the front of Mission Bay (K. Hovel, unpublished data), and in pilot lab experiments, lobsters were capable of consuming dozens of mussels during a 12-h period (B. Cheng, unpublished data). High spiny lobster density on transects in the front of Mission Bay, coupled with few observations of round rays and crabs, suggest that spiny lobsters play a large role in restricting Asian mussels to the back of the bay. Although the ability of lobsters to consume other invasive species is unknown, our results suggest that further reductions in lobster abundance and size due to overfishing (Dayton et al. 1998) may render estuarine communities more susceptible to invasion by nonnative species, particularly if other native predators are unable to compensate.

Although we suspect lobsters are important Asian mussel consumers, we acknowledge the role of other predators in contributing to our observations of biotic resistance. Indeed, the resilience of invasion resistance may be enhanced by a diversity of generalist predators (Sax et al. 2007). *Panulirus interruptus* has a varied diet crossing several phyla (Diaz Arredondo and Guzman del Proo 1995), and *P. festiva* is known to feed on several other native bivalve species (Peterson 1982), although they do exhibit a preference for Asian mussels (Reusch 1998). Notably, *P. festiva* predation on Asian mussels was high at R3 (Mean drilled mussels $\pm$ SE 60.8 $\pm$ 9.9%), a site with the second highest density of *P. festiva*. Octopuses (*Octopus* spp.) were also observed at rip-rap sites and are likely capable of Asian mussel consumption by pulling apart the valves or by drilling a distinctive oval-shaped hole (although we did not observe this). A significant proportion (26%) of mussels in two small-caged ASUs (which lobsters could not enter) were crushed, suggesting that crabs (e.g., *Cancer* spp.) have the potential to consume Asian mussels in high quantities, although we surveyed few crabs within Mission Bay. Additionally, a local population of small unidentified whelks penetrated predator-exclusion cages and were able to prey on 33.3% of mussels at S2 (*Pteropurpura* leaves an average drill hole of 0.687 mm^2 compared to a hole area of approximately 0.126 mm^2). Combined, these native predators are therefore capable of limiting the proliferation success (sensu Bishop and Peterson 2006) of Asian mussels in the front bay; however, this invader's invasion success (i.e., establishment and potential to spread) remains high in the back bay.

Our results highlight the importance of estuarine gradients in altering biotic resistance generated by native communities. In Tomales Bay, northern California, the introduced whelk *Urosalpinx cinera* and European green

crab *Carcinus maenas* are restricted to the back of the bay, in part by native cancrid crab predation that is present at front bay sites (Kimbro et al. 2009). Cancrid crabs have also been implicated in the restriction of European green crabs to back bay sites of Yaquina Bay, Oregon (Hunt and Yamada 2003). Although the factors responsible for restricting predators to sites near the mouth of estuaries remain untested, these predators and those in our study may be unable to tolerate extreme environmental conditions relative to their invasive prey, as predicted by the consumer stress model (Menge and Sutherland 1987; Menge and Olson 1990). One component of this model suggests that consumers have a lower tolerance for stress because they are able to move to benign environmental conditions relative to their sessile prey that must cope with stress due to their inability to move. In our study, native predators may be restricted from back bay sites due to extreme high temperatures or fine sediment characteristics that inhibit locomotion or basic metabolic functions, such as respiration. Biotic resistance may therefore be an important process that restricts invader distributions under conditions of low environmental stress, whereas invasive prey may obtain a refuge from predation in more stressful and perturbed habitats where their predators are unable to persist. While it is known that habitat attributes can affect the susceptibility of invaders to predation (e.g., sediment grain size; Byers 2002), the type of adjacent habitat can also influence community structure by serving as a source of predation pressure (Rilov and Schiel 2006). Our study documented the effect of adjacent habitat on the abundance of native predators (*Pteropurpura* and *Panulirus*) and subsequent effects on Asian mussel mortality. The results suggest that characteristics of an adjacent habitat and potentially its configuration can have implications for biotic resistance and spatial patterns of invasion.

Investigations of biotic resistance have generally focused on the role of diversity in the invaded environment in shaping competitive interactions with invasive species. While competition clearly has the capacity to generate invasion resistance within a community, predation should be evaluated by also considering biotic resistance because the latter can strongly influence the spatial and temporal pattern of an invasion (Sax et al. 2007). The results of our study suggest that biotic resistance and the enemy release hypothesis may operate at different points along an environmental gradient to determine the distribution of an invasive species. Environmental gradients are common throughout terrestrial, freshwater, and marine ecosystems. Therefore, the mediation of biotic resistance and the enemy release hypothesis by abiotic factors may be a generalized phenomenon contributing to patterns of invasion. In coastal marine habitats and estuaries where invasions are prominent, the continued removal of predators (trophic skew

sensu Duffy 2003) may also erode the biotic resistance of native communities, potentially increasing their susceptibility to invasion.

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