RESEARCH ARTICLE

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Ecological gradients and relative abundance of native (*Mytilus trossulus*) and invasive (*Mytilus galloprovincialis*) blue mussels in the California hybrid zone

Abstract Marine communities are experiencing unprecedented rates of species homogenization due to the increasing success of invasive species, but little is known about the mechanisms that allow a species to invade and persist in a new habitat. In central California, native (*Mytilus trossulus* Gould 1850) and invasive (*Mytilus galloprovincialis* Lamarck 1819) blue mussels and their hybrids co-exist, providing an opportunity to analyze the mechanisms that determine the distributions of these taxa. Spatial and temporal variation in temperature and salinity and the relative frequencies of these mussel taxa were examined between 2000 and 2004 at four sites in San Francisco Bay and four in Monterey Bay, which were chosen for their different positions along inferred estuarine/oceanic gradients in the hybrid zone. Mussels were genetically identified as the parent species or hybrids by amplifying regions of two species-specific loci: the adhesive byssal thread protein (*Glu-5′*) and the internal transcribed spacer region of ribosomal DNA (*ITS 1*). The proportion of *M. trossulus* at the eight hybrid zone sites correlated negatively with average salinity ($R^2 = 0.60$) and positively with maximal temperature ($R^2 \geq 0.72$), a somewhat unexpected result given what is known about the phylogeography of this species. The proportion of *M. galloprovincialis* showed the opposite pattern. The proportion of hybrids was correlated neither with habitat temperature nor salinity. Genotypes of mussel populations at an additional 13 sites from Coos Bay, Oregon (latitude 43.35°N) to Long Beach, California (latitude 33.72°N), sampled at various intervals between 2000 and 2004, were also determined. This survey confirmed previous reports that the hybrid zone lies between Monterey and the Cape Mendocino region (latitudes 36.63°N–40.5°N). Within Monterey and San Francisco Bays, however, the temporal comparisons (1990s vs. 2000s) revealed abrupt changes in the proportions of the two parent species and their hybrids on annual and decadal time scales. These changes indicate that the blue mussel populations are in a highly dynamic state. The survey also showed that, regardless of habitat, *M. trossulus* is consistently of smaller average size than either *M. galloprovincialis* or hybrids.

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Introduction

Quantification of spatio-temporal patterns of species' distributions and their causal link to abiotic and biotic environmental variables is important for understanding community structure and the mechanisms regulating that structure (Menge and Olson 1990). In studies of temperate marine intertidal communities, mussels in the genus *Mytilus* are important because they commonly are one of the most abundant invertebrates and they play pivotal roles in governing overall species composition (Ricketts et al. 1962). *Mytilus* spp. has a broad global distribution and there is a rich literature on their biology and ecology. McDonald and Koehn (1988) revealed that there is a significant genetic structure in populations of the cosmopolitan blue mussel (*M. edulis* species complex), which was long regarded as a single species. In fact, these populations comprise three separate genotypes, currently classified as *Mytilus trossulus*, *Mytilus edulis*, and *Mytilus galloprovincialis*. Following the genetic identification of three distinct species, hybrid zones have been characterized at several sites and are thought to occur in all regions where blue mussel distributions overlap. Additionally, of the three blue mussel species, only *M. galloprovincialis* has successfully invaded new locations, making our understanding of its invasion

success a potential key for understanding general mechanisms behind marine invasions.[1]

Mytilus spp. hybrid zones exist in many places, and can extend over hundreds of kilometers. Hybrid zones between *M. galloprovincialis* and *M. trossulus* occur in the North Pacific in Japan (Inoue et al. 1995, 1997; Suchanek et al. 1997), California (McDonald and Koehn 1988; Sarver and Foltz 1993; Rawson and Hilbish 1995; Suchanek et al. 1997; Rawson et al. 1999), and perhaps in East Asia, although this remains uncertain (Gosling 1991; Seed 1992; Zhuang 1992). A hybrid zone involving all three species of blue mussels exists in Puget Sound, as a result of aquaculture introductions (Heath et al. 1995; Anderson et al. 2002; Wonham 2004). The best-studied hybrid zone is between *M. edulis* and *M. galloprovincialis* in the N.E. Atlantic, particularly in England (Gardner 1994; Wilhelm and Hilbish 1998; Gilg and Hilbish 2000, 2003a, b, c; Hilbish et al. 2002) and France (Viard et al. 1994; Comesaña and Sanjuan 1997; Bierne et al. 2002a, b, 2003a, b). Finally, there are also hybrid zones between *M. edulis* and *M. trossulus* in the western Atlantic (Comesaña et al. 1999; Gardner and Thompson 2001; Rawson et al. 2003, 2004) and the Baltic Sea (Riginos et al. 2002; Bierne et al. 2003c). While the hybrid populations in southern England and continental Europe are the result of secondary contact over geological time scales, the hybrid zone in California is relatively new (decadal scale). Therefore, the latitudinal position of the California hybrid zone might show evidence of dynamic change (Geller 1999), although its current stability is undetermined (Rawson et al. 1999). This is a fundamental difference between long-established and recently established hybrid zones, limiting the comparative value of research between the two types for determining the drivers of distributional patterns.

Habitat characteristics of blue mussels vary widely from region to region, and include wave-exposed rocky intertidal habitats and quiet estuarine sloughs and bays (hereafter, referred to as protected sites). In the Northeastern Pacific and the Atlantic, blue mussels are abundant in intertidal habitats, where they commonly form dense beds. In California, although *M. trossulus* and *M. galloprovincialis* have been collected at rocky intertidal sites (Sarver and Foltz 1993; Rawson et al. 1996b, 1999; Suchanek et al. 1997), populations of these two congeners are sparse in the intertidal zone (Suchanek 1978). Low abundance in these intertidal sites is likely due in part to preferential predation by the abundant whelks *Nucella* spp., which exhibit a strong preference for blue mussels over ribbed mussels (*Mytilus californianus*) (Danner 1999), and partly due to low recruitment rates in California relative to Oregon (Connolly et al. 2001). However, aggregations of blue mussels are commonly found in California in bays and estuaries, which exhibit a variety of temperature and salinity microhabitats due to different influences of oceanic and estuarine conditions. Variation in the relative abundances of *M. trossulus* and *M. galloprovincialis* from site to site has led to the hypothesis that temperature and salinity drive the observed differences in distribution patterns among the blue mussel species (for example, see Sarver and Foltz 1993; Gardner 1994; Rawson et al. 1999), particularly in regions where distributions overlap.

Current biogeographic patterning of blue mussels thus is thought to be a function of the evolutionary history of each of the congeners, particularly differences in the oceanographic characteristics of the ocean basins in which the three blue mussel congeners evolved. *Mytilus trossulus* is native to the North Pacific, an ocean that has relatively low nearshore salinity, large diurnal tidal fluctuation, cold and variable surface water temperatures, and distinct oceanic seasons. Within the last 3.5–5.0 million years, a series of migrations and subsequent isolation events led to the speciation of *M. edulis* in the North Atlantic and *M. galloprovincialis* in the Mediterranean (Barsotti and Meluzzi 1968; Vermeij 1991; Seed 1992). During the last 3 million years, the Mediterranean has had higher salinity, less tidal fluctuation, warmer water temperatures, and less seasonal variation in temperature and salinity than the North Pacific. Thus, *M. trossulus* and *M. galloprovincialis* evolved under different selective pressures and are potentially adapted for quite different environments. Based on their diverse evolutionary histories with respect to environmental temperatures and salinities, several authors have suggested that these two environmental variables may play a key role in determining blue mussel distribution patterns in hybrid zones, including those in the Canadian Maritimes (Gardner and Thompson 2001), Europe (Bierne et al. 2002b), Britain (Hilbish et al. 2003) and California (Sarver and Foltz 1993). Furthermore, the patchwork distribution pattern of *M. trossulus*, *M. galloprovincialis*, and their hybrids in California has been described using the mosaic hybrid zone model (Harrison and Rand 1989), which hypothesizes that habitat-specific selection and physiological adaptation interact to shape the observed distribution pattern. Given that the California populations of blue mussels are distributed along an estuarine-oceanic gradient, temperature and salinity could affect physiological performance and thus their distribution within the hybrid zone. However, despite conjectures about the roles of temperature and salinity in shaping the mosaic hybrid zone, no existing studies provide definitive information on which of these two variables is of greater importance.

To elucidate causal relationships among temperature, salinity, and abundance of *M. trossulus*, *M. galloprovincialis*, and their hybrids, we determined the spatiotemporal variation in surface temperature and salinity and characterized the genotypes of adult blue mussels at

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[1]The term "invasive species" is used throughout this paper to mean a species that was transported by humans to a new location where it did not previously occur, and which has spread from its initial site of introduction to other sites, where it produces viable offspring (based on definition by Richardson et al. 2000).

eight Central California sites within San Francisco Bay and Monterey Bay. Because blue mussels are morphologically similar, each individual was genetically identified to the species level. We identified genotypes using previously reported PCR-based methods at two loci (Glu-5′ and ITS-1) and sampled sites each year during the study between 2000 and 2004. In addition, we measured temperature and salinity during the same period at frequencies that demonstrated the annual ranges (temperature and salinity) and daily ranges (temperature) in these variables. Our findings provide new evidence concerning the roles of temperature and salinity in establishing these species' distributions and illustrate the dynamic nature of species composition at different sites in the Central California hybrid zone.

Materials and methods

Sampling sites

We chose subtidal field sites from Coos Bay, Oregon (latitude 43.35°N) to Long Beach, California (latitude 33.72°N) for sampling mussels (M. trossulus Gould 1850 and M. galloprovincialis Lamarck 1819, and their hybrids) and sites in San Francisco and Monterey Bays for monitoring temperature and salinity (Fig. 1; Table 1). All sites were in harbors, on floating docks located closest to the harbor mouth where water flow was greatest. Because all of the sampled populations were continuously submerged, the temperature and salinity measurements reflect the environmental conditions the mussels encountered. Stress during periods of emersion was not a confounding variable.

Temperature and salinity monitoring

To characterize the physical habitat of Mytilus spp., we sampled water temperature and salinity at eight sites (four in Monterey Bay, four in San Francisco Bay) from January 2001 to February 2004. We used two temperature data loggers (iButton, Dallas Semiconductor; resolution ±0.5°C) at each site to collect high-frequency temperature data. Sampling periods at each site varied due to different deployment dates and accidental
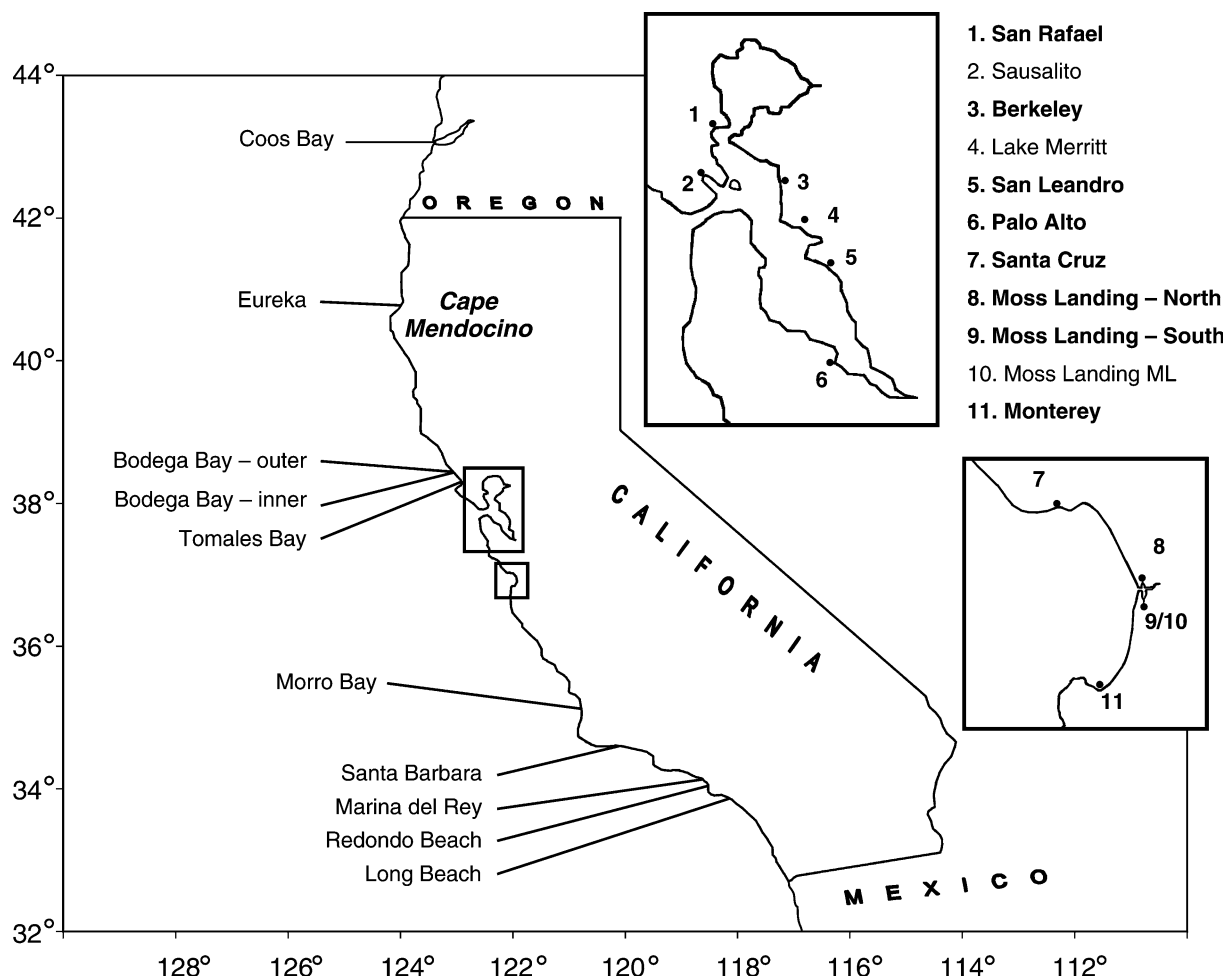

Fig. 1 Field sites—map of Pacific Coast, USA, with detailed insets of San Francisco and Monterey Bays. *Bold type* indicates primary field sites where temperature and salinity were monitored; plain type indicates sites where only mussel sampling occurred

Table 1 Field sites—symbols and specific locations

Site code	Sample number	City/region	Location
CB	93	Coos Bay	Charleston, Dock F
E	13	Eureka	Woodley Island Marina
BBC	34	Bodega Bay—outer	Coast Guard Dock, bay mouth
BBS	28	Bodega Bay—inner	Spud Marina, inner bay
TB	30	Tomales Bay	Miller Point, boat launch dock
SR	**79**	**San Rafael**	**Loch Lomond Marina, boat launch**
SA	23	Sausalito	Richardson Bay, Club Nautique dock
B	**121**	**Berkeley**	**Marina, slips O318-319**
LM	41	Oakland	Lake Merritt, Gondola Dock
SL	**83**	**San Leandro**	**Marina, slip A54**
PA	**77**	**Palo Alto**	**Sailing Station, Baylands Nature Preserve**
SC	**53**	**Santa Cruz**	**Harbor, public access dock at mouth**
MLN	**89**	**Moss Landing—North**	**North harbor, slip 63**
MLS	**142**	**Moss Landing—South**	**South harbor, slip B173**
MLML	26	Moss Landing ML	South harbor, Moss Landing Marine Laboratories dock
MM	**54**	**Monterey**	**Fisherman's Wharf, distal end**
MB	51	Morro Bay	Harbor, Embarcadero Road
SB	51	Santa Barbara	Harbor, floating dock near mouth
MDR	48	Marina del Rey	Marina, mouth of Basin A
RB	51	Redondo Beach	Harbor Master dock
LB	104	Long Beach	Long Beach Yacht Club
Total	1291		

Sites in bold type are the primary field sites (monthly water sampling), while plain type indicates other locations for distributional survey, 2000–2004. Sample number indicates number of individuals genetically identified from that site

loss of equipment (Table 2). We measured temperature at 30-min intervals from January 2001 to August 2003, and at 1-h intervals from August 2003 to February 2004. From December 2000 to August 2003, we used two devices to measure salinity on a monthly basis at each site: a refractometer (January 2001 to January 2003: Reichert–Jung temperature compensated refractometer with special scale) and a hand held conductivity

Table 2 Temperature and salinity statistics, by site, 2001–2003

	San Francisco Bay				Monterey Bay			
	SR	B	SL	PA	SC	MLN	MLS	MM
Habitat category	E	I	I	E	O	I	I	O
Temperature (°C)								
Number of days sampled	966	966	966	963	240	783	932	961
Maximum	28.0	23.3	26.5	31.8	19.0	23.5	23.0	18.8
Minimum	6.8	3.5	5.8	5.3	10.3	7.5	7.5	9.5
Average	16.5	15.5	16.7	17.0	13.9	14.9	14.6	13.9
Standard deviation	4.3	3.3	4.4	4.9	1.5	2.2	2.1	1.4
Average rank	3	4	2	1	7	5	6	8
Range	21.2	19.8	20.7	26.5	8.7	16.0	15.5	9.3
Max 24-h range	7.0	3.8	4.5	12.8	2.8	8.0	7.5	4.5
Water/air R^2	0.73	0.70	0.73	0.70	0.55	0.60	0.58	0.56
Salinity								
Number of months sampled	35	35	35	34	10	27	34	34
Maximum	30.0	32.4	32.9	26.7	34.6	34.8	31.9	34.8
Minimum	11.1	17.7	19.1	11.5	26.7	16.6	8.2	23.4
Average	22.7	26.9	26.0	20.1	32.5	31.9	22.9	33.0
Standard deviation	6.0	4.0	4.2	4.2	2.3	3.6	6.6	2.0
Average rank	2	5	4	1	7	6	3	8
Range	18.9	14.7	13.8	15.2	7.9	18.2	23.7	11.4

Sites are arranged in North–South order: San Rafael (SR), Berkeley (B), San Leandro (SL), Palo Alto (PA), Santa Cruz (SC), Moss Landing—North (MLN), Moss Landing—South (MLS), and Monterey (MM). Habitat category distinguishes estuarine (E), oceanic (O) and intermediate (I) sites. The 24-h temperature range for each day at each site was calculated, and listed are the largest 24-h ranges. Average rank is rank order of sites, based on average temperature or salinity. Water/air temperature correlation is R^2 value for regression of daily maximum water temperature versus daily maximum air temperature (downtown San Jose, south San Francisco Bay) during monitoring. Air temperature data courtesy of the National Weather Service

sensor (January 2003 to August 2003: YSI 85 Oxygen, Conductivity, Salinity, Temperature hand-held sensor). In November 2003 and February 2004, we took additional salinity measurements with the YSI instrument. We deployed an oceanographic-grade conductivity temperature sensor (Sea-Bird Electronics, SBE 37 SM MicroCAT) both before and after large rain storms to obtain high-resolution information about the differences in the rate of change and the maximum daily range at two sites, Moss Landing—North (MLN) and Moss Landing—South (MLS). We used regression analysis to compare maximum daily water temperature to maximum daily air temperature (data courtesy of the National Weather Service) in downtown San Jose, South San Francisco Bay.

Mussel sampling and genetic identification

We initially collected mussels in 2000 at eight sites in San Francisco and Monterey Bay to identify populations for physiological experiments (Braby 2004) and also collected in various months each year between 2001 and 2004. We haphazardly collected mussels within 20 m of where temperature data loggers were deployed and monthly salinity samples were collected. We reached over the side of the dock and collected the first individual encountered, regardless of size. We then moved approximately 0.5 m and repeated the process. When we encountered a clump of mussels, we collected only a single individual. Using this method, mussels of all sizes qualified for collection. We measured the shell length (SL) of each individual, and then dissected and preserved gill tissue in 95% ethanol for later DNA extraction and genetic identification.

We used multiple DNA isolation protocols in these experiments, including membrane spin columns (Macherey-Nagel, Nucleospin® DNA extraction kit), guanidinium salt and silica bead extraction (Hoss and Paabo 1993), and Proteinase K tissue digestion (10 mM Tris–HCl pH 8, 1 mM EDTA, 0.3% Tween, 0.3% nonylphenyl-polyethyleneglycol [Sigma, IGEPAL CA-630], 0.03 U μl^{-1} Proteinase K; 55°C for 12 h, 98°C for 10 min). For all extraction methods, we used 1 μl of DNA template and polymerase chain reaction (PCR) methods to amplify two nuclear loci: the byssal thread protein locus (*Glu-5'*) (Rawson et al. 1996a), and the ribosomal internal transcribed spacer region (*ITS*) (Heath et al. 1995).

The *Glu-5'* locus amplifies a different sized fragment for *M. trossulus* (240 bp) and *M. galloprovincialis* (300 bp), because of an insertion in the *M. galloprovincialis* gene. An F1 hybrid between these two species amplifies both fragments (240 and 300 bp). We used the published primers (F-GTAGGAACAAAGCA TGAACCA; R-GGGGGGATAAGTTTTCTTAGG), slightly modified PCR chemistry (15 μl reaction volume with 1 μl DNA template, 0.1 U Taq polymerase,

1× Taq Buffer [50 mM KCl, 30 mM Tricine, pH 8.6], 200 nM dNTP's, 2 mM $MgCl_2$, 300 nM of each primer) and slightly modified cycling conditions (initial denaturation of 1.5 min at 94°C; 35 cycles of 94°C for 20 s, 53°C for 30 s and 72°C for 45 s; final extension of 2 min at 72°C).

The *ITS* locus amplifies a similar sized fragment in both species (~950 bp), but the species differ in the number of recognition sites for the *Hha*I restriction enzyme. After a restriction digest of the PCR products, there are several fragments but each species has a unique fragment (*M. trossulus* = 280 bp; *M. galloprovincialis* = 450 bp). An F1 hybrid between these two species exhibits both fragments (280 and 450 bp). We used the published primers (F-GTTTCCGTAGGTGAACCTG; R-CTCGTCTGATCTGAGGTCG), slightly modified PCR chemistry (same recipe as for *Glu-5'*, described above) and slightly modified cycling conditions (initial denaturation of 1.5 min at 94°C; 30 cycles of 94°C for 20 s, 55°C for 30 s and 72°C for 1 min; final extension of 2 min at 72°C). We then added 0.2 μl *Hha*I enzyme (New England Biolabs), 1.5 μl 10× buffer (New England Biolabs—Buffer 4) and 0.15 μl bovine serum albumin (100 μg ml^{-1}) to each PCR reaction and digested for 4 h at 37°C.

We visualized PCR products for both loci using gel electrophoresis on a 2% agarose gel stained with ethidium bromide. To arrive at a final genotype designation, we scored individuals at each locus separately and then scored across both loci. While two loci may not be enough to distinguish among the many types of backcrossed hybrids (Boecklen and Howard 1997), two loci are useful in a hybrid zone with little introgression (Rawson et al. 1999) and when coarsely categorizing individuals into the categories of parentals and hybrids (Boecklen and Howard 1997), as we have done here. These final designations are: *M. trossulus*, *M. galloprovincialis*, and hybrid (includes both F1 and backcrossed genotypes). We used the genetic identifications to calculate allelic frequency and Hardy-Weinberg equilibrium for each population, using the software BIOSYS (Swofford and Selander 1981). These analyses characterize the level of hybridization occurring at the sites, and provide an estimate of mixing between populations.

Meta-analysis of *Mytilus* spp. distribution

Extensive sampling of *Mytilus* spp. in the California hybrid zone from 1990 to 1995 provides a historical view of what the genotype distribution has been at certain sites. For this analysis of spatio-temporal variation, we extracted data from four publications (Sarver and Foltz 1993; Rawson et al. 1996b, 1999; Suchanek et al. 1997) Each study used a different set of molecular tools to genotype individuals, including both DNA- and allozyme-based methods. We used data from sites that were

Fig. 2 Sea surface temperature at Monterey (MM) and Palo Alto (PA) sites, 2001–2003. Seasonal and daily temperature variation was greater at PA than MM
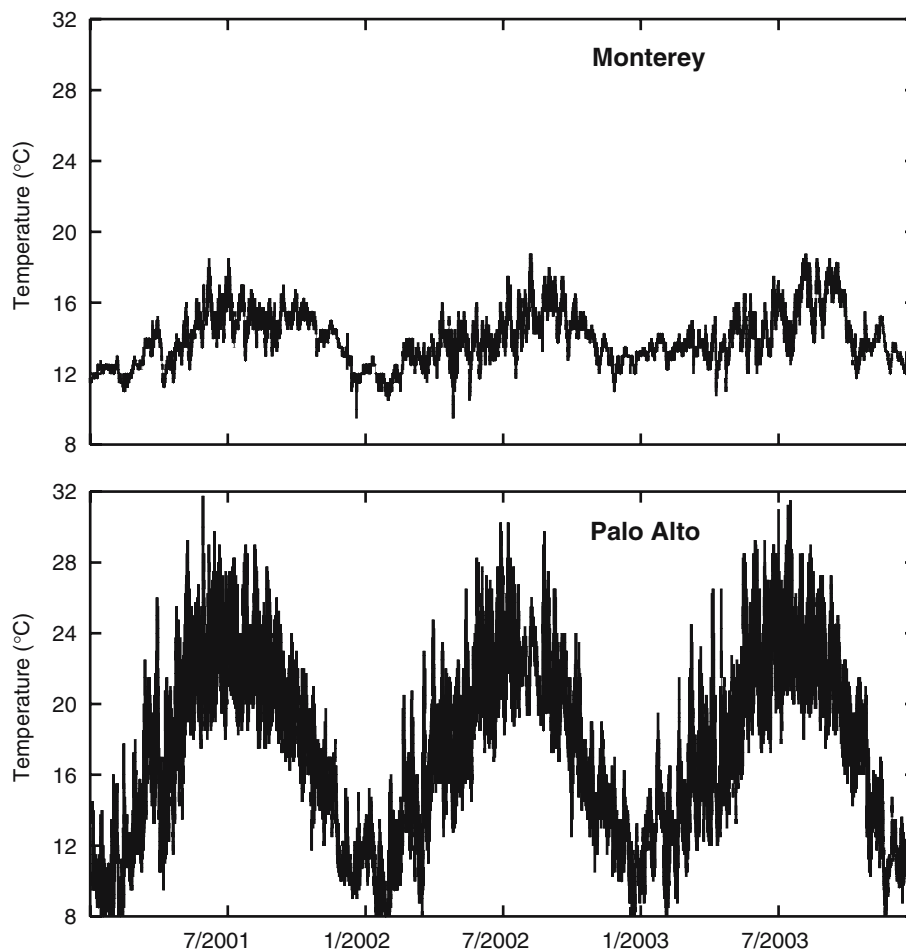
close to our own sites, but from both intertidal and subtidal locations.

Results

Habitat temperature and salinity

Sea surface temperature in protected habitats varied widely daily and seasonally, and the amount of variation was site-dependent (Fig. 2). Water temperature sometimes increased rapidly, as seen in Palo Alto (PA) during the summer when temperature changed by as much as $1.6°C\ h^{-1}$. Over the course of the 3-year monitoring period, sites in San Francisco Bay exhibited a greater range in temperature and higher average temperature than sites in Monterey Bay (Table 2). San Francisco Bay sites were consistently warmer than Monterey Bay sites from March through October, but slightly cooler during December and January.

Comparing maximal daily water temperature to maximal daily air temperature in downtown San Jose (at the southern end of San Francisco Bay), there was a strong correlation between air temperature and water temperature at all eight sites (R^2 values ranged from 0.55 to 0.73) (Table 2), although the correlation was stronger for San Francisco Bay sites. In addition to between-bay differences in the magnitude and range of temperature, there was also variation within each bay system. In San Francisco Bay, Berkeley (B) had the lowest temperatures (both average and maximum), San Rafael (SR) and San Leandro (SL) were intermediate, and PA had the highest temperature. This pattern was correlated with distance from the Golden Gate, the narrow channel connecting San Francisco Bay to the Pacific Ocean.

Like temperature, salinity varied daily (at the two sites surveyed) and seasonally (at all sites) and the variation was site-dependent (Table 2). When measured at a high frequency (two sites), salinity sometimes decreased rapidly, at a rate of over 6 ppt h^{-1} (from 29 to 6 in 4.3 h), during periods of both spring tides and heavy runoff (Fig. 3). Unlike temperature, there were fewer similarities among sites within a bay system, indicating that the salinity regime is driven by smaller spatial scale influences than is temperature.

Variability between sites at small spatial scales was most dramatically demonstrated at two sites, MLS in Monterey Bay and PA in San Francisco Bay. The salinity at MLS is strongly influenced by the Old Salinas River channel, as evidenced by MLS's low average salinity (relative to other Monterey Bay sites) and wide salinity range (Fig. 3; Table 2). The neighboring site,
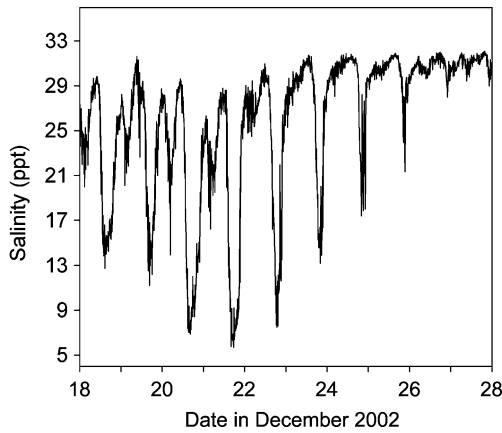
Fig. 3 Moss Landing—South (MLS), salinity December 18–28, 2002, during spring tides and a period of heavy precipitation during a winter storm. Salinity fluctuated as the tide shifted the location of the estuarine salt-wedge

MLN, is separated from MLS by only 1 km and yet had drastically different salinities (average, maximum, minimum, and range; Table 2), which were similar to other Monterey Bay sites [Santa Cruz (SC) and Monterey (MM)]. In contrast, these same traits suggested that MLS is more similar to sites in San Francisco Bay than neighboring sites in Monterey Bay. Within San Francisco Bay, PA had distinctly low salinity but the range was less than that of SR in the north. The greater salinity range at SR was likely attributable to the seasonality and dominance of the nearby Sacramento/San Joaquin River discharge. PA experiences very little natural freshwater discharge (San Francisquito Creek), but receives a stable year-round wastewater effluent from the municipal treatment center for the city of Palo Alto, keeping the salinity low.

The strong negative correlation between average temperature and average salinity ($R^2 = 0.59$) suggests that the sites can be classified on an oceanic-estuarine gradient (Table 2). More specifically, oceanic sites (MM and SC) are characterized by stable low temperature (average temperature $< 15°C$; 3-year temperature range $<10°C$) and high average salinity (average salinity > 30). Estuarine sites (SR and PA) are characterized by extreme and variable temperatures (3-year temperature range $> 20°C$) and by low and variable salinity (average salinity < 27). Intermediate sites (B, SL, MLN, and MLS) share characteristics of both estuarine and oceanic sites.

Mussel size and genotype distribution

We collected 1,291 mussels from 21 sites in California and Oregon between January 2000 and May 2004. Combining the genotype results from all 21 sites, we partitioned the mussels into 10-mm SL bins from 20 to 90 mm (excluding individuals with SL < 20 mm due to possible undersampling of the smallest mussels). *Mytilus trossulus* was abundant in the size classes < 50 mm, and

nearly absent from size classes > 60 mm (Fig. 4). Hybrids and *M. galloprovincialis* were present in all size classes sampled. Mussels with hybrid and *M. galloprovincialis* genotypes were more evenly distributed among size classes, compared to *M. trossulus*, which had a strong peak from 30 to 50 mm (mussels with SL from 30.0 to 49.9 mm). To demonstrate the likelihood of collecting a particular genotype given it is of a particular size, we graphed the same data set by showing the frequency of genotypes within each size bin (Fig. 4b). The presentation emphasizes that *M. galloprovincialis* is more abundant than *M. trossulus* in the hybrid zone, and again shows the distinctly different size distributions of the congeners and their hybrids. Although the sizes of the two species are clearly different, the size range and average size of all mussels (all taxa combined) collected within a site were similar among sites when averaged over all collection dates, suggesting that there were no
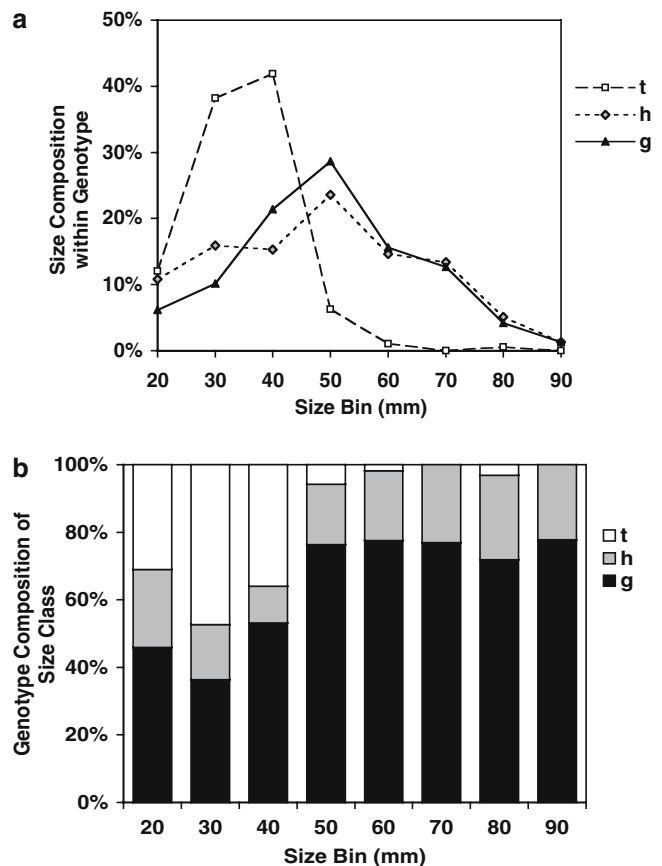

Fig. 4 *Mytilus* spp. Size distribution of mussel genotypes—all sites combined. **a** Area under each curve includes 100% of the individuals of that genotype throughout the size range shown (20–100 mm). Individuals were binned into 10-mm size classes (shell length), e.g., the size-bin "20" includes individuals from 20.0 to 29.9 mm SL. **b** Frequency (%) of genotypes within each size class. *M. trossulus* is abundant in the small size classes (< 50 mm) but *M. galloprovincialis* is more abundant in most size classes. *M. trossulus* (t) $n = 191$; *Mytilus galloprovincialis* (g) $n = 550$; hybrid (h) $n = 156$

site-specific patterns in population size structure or sampling biases at a particular site (analysis not shown).

The genotype distributions among sites exhibited distinct temporal and spatial patterns. Figure 5a shows a panel for each collection year (except 2004, in which two discrete collections are shown), with each bar displaying the genotype composition of that particular collection. The most thorough temporal sampling was at

Fig. 5 *Mytilus* spp. **a** Mussel genotype distribution by site and year of collection. **b** Cumulative genotype distribution from present study ("2000s") and from previously published studies ("1990s" synthesis of: Sarver and Foltz 1993; Rawson et al. 1996b, 1999; Suchanek et al. 1997). Meta-analysis includes mussels collected 1990 to 1995 ($n = 25$–85 individuals site^{-1}). Sites are listed in north–south order; *dashed lines* indicate major biogeographic regions: Oregon, northern California, San Francisco Bay, Monterey Bay, and southern California. *Columns* represent 100% of individuals sampled at that time point and site, with percentage composition by genotype indicated by the shading: *white* = *M. trossulus* (t), *gray* = hybrid (h), and *black* = *M. galloprovincialis* (g)
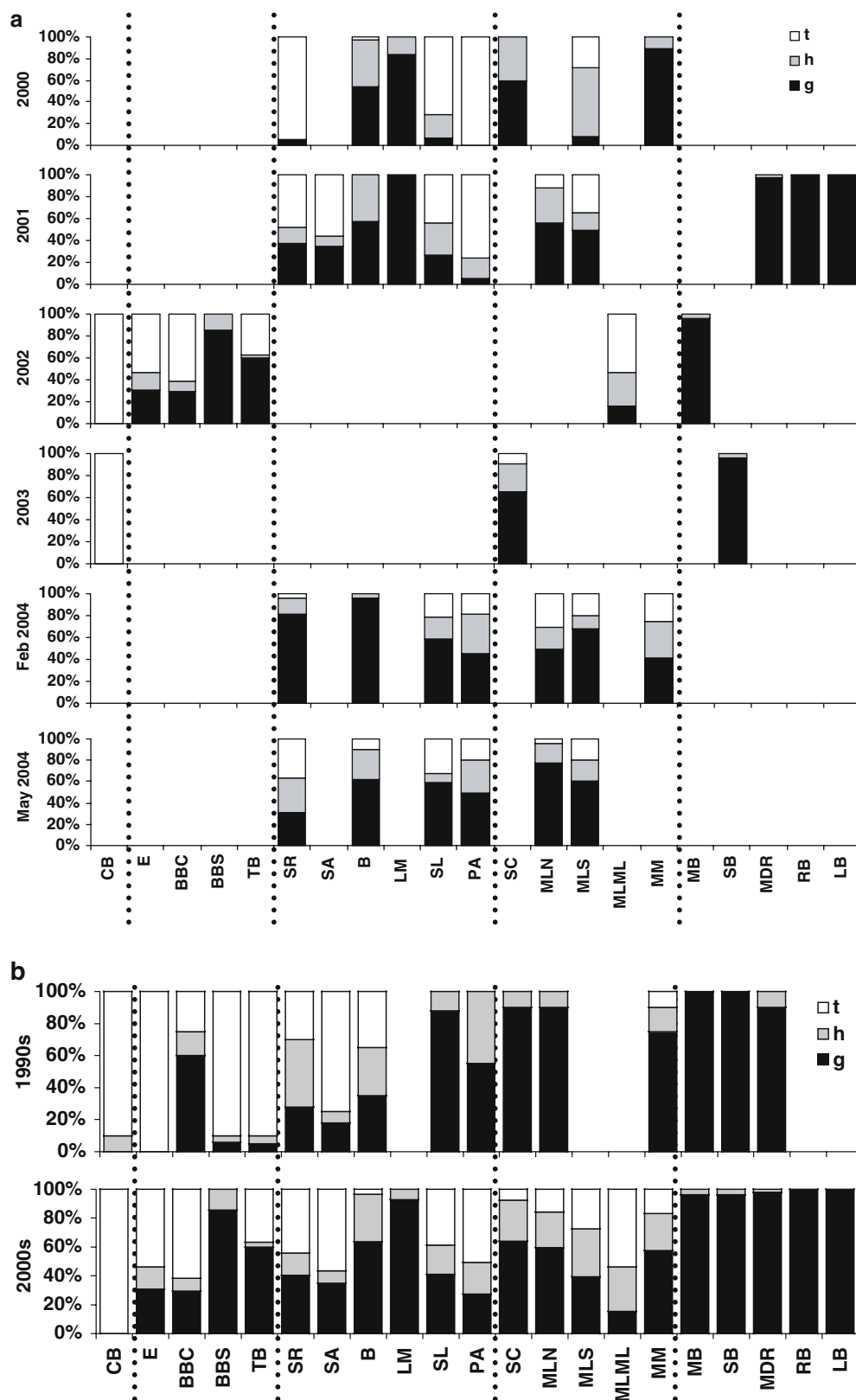
Table 3 *Mytilus* spp. Correlation of genotype distribution with site characteristics

	M. trossulus		Hybrid		*M. galloprovincialis*	
	R^2	Slope	R^2	Slope	R^2	Slope
Temperature						
Average	**0.60**	10.69	0.37	−2.99	**0.50**	−7.71
Maximum	**0.72**	3.36	0.33	−0.80	**0.67**	−2.56
Minimum	0.07	−2.09	0.00	0.06	0.11	2.04
Range	**0.50**	2.03	0.18	−0.43	**0.51**	−1.61
Salinity						
Average	**0.60**	−2.74	0.07	0.32	**0.76**	2.42
Maximum	**0.60**	−4.89	0.13	0.79	**0.69**	4.09
Minimum	0.38	−1.70	0.02	0.13	**0.52**	1.57
Range	0.13	1.30	0.00	0.03	0.22	−1.33

Site characteristics are the same as in Table 2 and genotypes are the same as those in Fig. 5b (2000s). Correlations with R^2 of 0.5 or more are bold type

four San Francisco Bay sites: SR, B, SL, and PA. From 2000 to 2004, there appeared to be a shift from *M. trossulus* to *M. galloprovincialis*, particularly at SR, SL, and PA; these three sites are categorized as intermediate or estuarine sites (Table 2) and are furthest into the San Francisco Bay estuary. However, there was an unexplained variation among collections at short time intervals (Fig. 5a: compare February 2004 and May 2004 genotype composition at SR).

The population structure at the sites indicates that *M. trossulus* and *M. galloprovincialis* co-occur at sites but do not frequently interbreed. The two loci (*Glu-5′* and *ITS*) exhibited similar allele frequencies for the *M. galloprovincialis* allele across all hybrid zone sites and

heterozygote deficiency at most of the hybrid zone sites (Table 4). These two calculations indicate that the species-specific alleles are more tightly linked in the hybrid zone (which could be due to linkage disequilibrium or to a physical linkage) and that hybridization events are rare.

Meta-analysis of published *Mytilus* spp. distributions from 1990 to 1995 (Sarver and Foltz 1993; Rawson et al. 1996b, 1999; Suchanek et al. 1997) provides comparison with the data presented here and insight into how quickly the genotype composition of *Mytilus* spp. populations can change (Fig. 5b). At Monterey Bay sites, *M. trossulus* comprised a greater percentage of the population in the 2000s (cumulative genotype distribu-

Table 4 *Mytilus* spp. Estimates of allelic frequency (for *M. galloprovincialis* allele), Hardy–Weinberg heterozygote deficiency (observed, expected, F_{IS} or inbreeding coefficient), and the probability of deviation from Hardy–Weinberg equilibrium for each locus

	Allelic Frequency		Hardy-Weinberg				
			Heterozygote deficiency			Deviation (P)	
Site	Glu-5′	ITS	Obs	Exp	F_{IS}	Glu-5′	ITS
CB	0	0	0	0	–	–	–
E	0.385	0.385	0.154	0.492	0.687	0.010	0.010
BBC	0.338	0.338	0.088	0.454	0.806	0	0
BBS	0.931	0.931	0.138	0.131	−0.053	0.732	0.732
TB	0.613	0.597	0.016	0.486	0.967	0	0
SR	0.468	0.481	0.139	0.502	0.723	0	0
SA	0.391	0.391	0.087	0.487	0.821	0	0
BM	0.828	0.807	0.283	0.299	0.054	0.352	0.747
LM	0.963	0.963	0.073	0.071	−0.028	0.844	0.844
SL	0.554	0.488	0.139	0.500	0.722	0	0
PA	0.387	0.369	0.181	0.473	0.617	0	0
SC	0.802	0.764	0.226	0.342	0.339	0.009	0.016
MLN	0.747	0.680	0.180	0.409	0.560	0	0
MLS	0.569	0.510	0.286	0.497	0.425	0	0
MLML	0.345	0.345	0.241	0.460	0.476	0.002	0.028
MM	0.731	0.704	0.231	0.409	0.435	0	0.004
MB	0.982	0.973	0.009	0.044	0.795	0	0
SB	1	0.981	0.019	0.019	0	1	0.922
MDR	1	0.990	0.010	0.010	0	1	1
RB	1	1	0	0	–	–	–
LB	1	1	0	0	–	–	–

Hardy–Weinberg heterozygote deficiency estimate was similar for both loci, so only the combined estimates are shown. Primary field sites are in bold type

Fig. 6 *M. galloprovincialis.* Correlations between genotype and environmental factors at the eight primary field sites. Correlation of mussel genotype (shown as % *M. galloprovincialis*) with **a** average temperature ($\pm$SD to show spread of data) and **b** average salinity ($\pm$SD to show spread of data). Genotype has standard error estimates around a mean, derived from multiple sampling events between 2000 and 2004. Correlations describe a significant portion of the variation at these sites (genotype vs. temperature $R^2 = 0.64$; genotype vs. salinity $R^2 = 0.71$). **c** Correlation of average temperature and average salinity ($\pm$SD) ($R^2 = 0.59$)
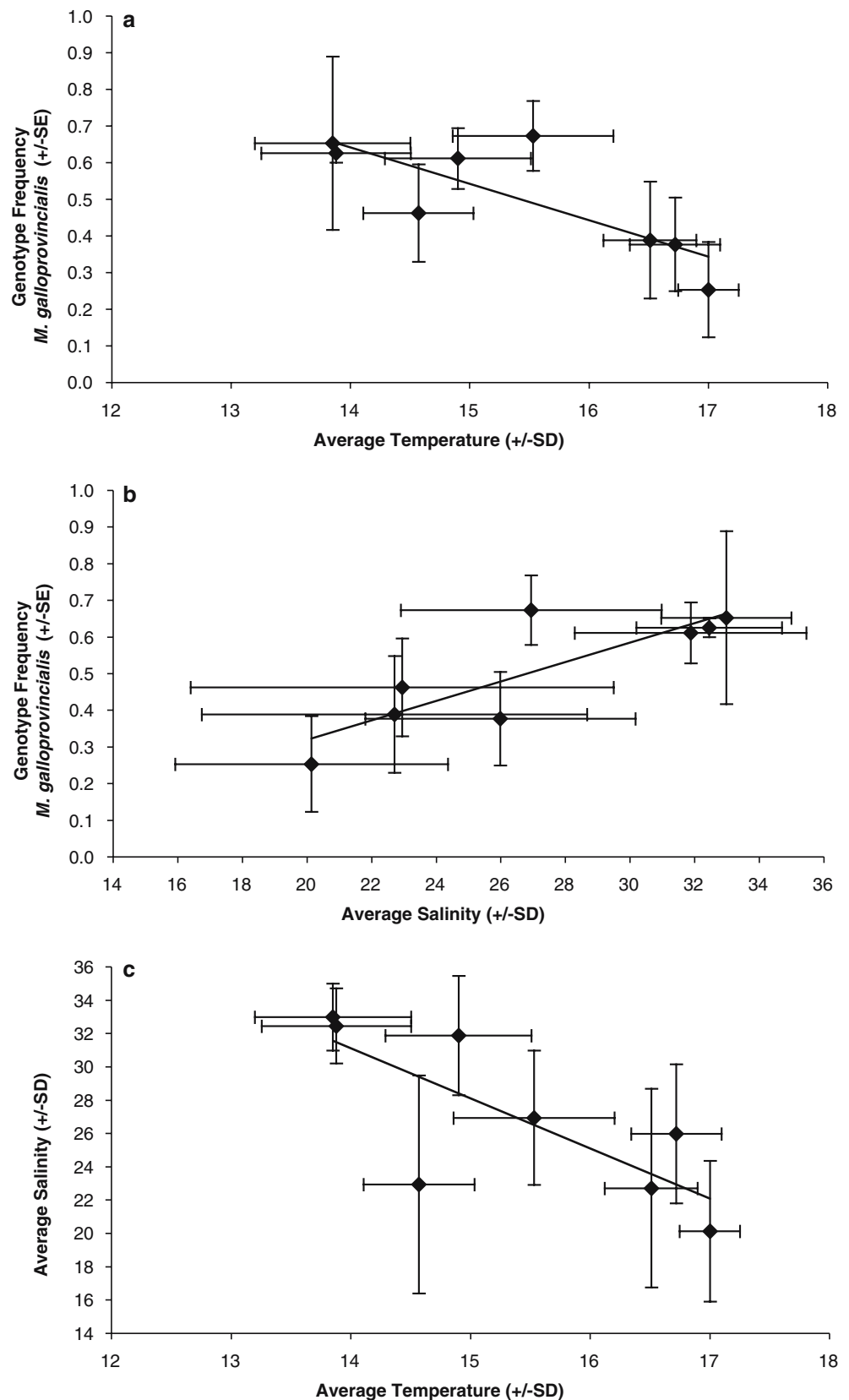
tion from all 5 years of sampling in this study) than it did in the early 1990s (cumulative genotype distribution from all four papers cited above). This is an unexpected result because *M. galloprovincialis* has moved north-wards since the beginning of its invasion of California (Geller 1999) and Monterey is at the southern end of the

hybrid zone. At San Francisco Bay sites, *M. trossulus* also constituted a greater percentage of the population than found previously, particularly in the south bay, at SL and PA. The trend changed north of San Francisco Bay, where we found fewer *M. trossulus* than in the 1990s studies at three of four sites.

Looking just at the cumulative 2000–2004 samples, there were several notable patterns in the spatial distribution of genotypes throughout California. As noted by previous authors, sites from Morro Bay south were dominated by *M. galloprovincialis* in 2001–2003, with occasional hybrid individuals but no *M. trossulus*. Coos Bay, Oregon, remained dominated by *M. trossulus*, indicating that a hybrid zone has not established in this area. It is also evident that within the hybrid zone (Eureka—Monterey) the genotype composition at a particular site is unpredictable based on latitude alone, as expected on the basis of the mosaic hybrid zone model for *Mytilus* spp. distributions. Looking at a smaller spatial scale, however, there were patterns within bay systems. In San Francisco Bay, SR, Sausalito (SA), SL, and PA all had significant *M. trossulus* populations and the sites are more estuarine than the intermediate B site. In Monterey Bay, there was a similar pattern with more *M. trossulus* at MLS and Moss Landing Marine Laboratories (MLML) (both in South Harbor and so influenced by the Old Salinas River Channel), compared to SC, MLN, and MM. Thus, there is evidence that *M. trossulus* was more abundant at sites with a stronger estuarine influence.

Correlating *Mytilus* spp. distribution with temperature and salinity

Several temperature and salinity characteristics were correlated with genotype distribution at the eight primary field sites. Average temperature at a site (over the 3-year monitoring period), was positively correlated with the percentage of *M. trossulus* found at that site ($R^2 = 0.60$) and negatively correlated with the percentage of *M. galloprovincialis* ($R^2 = 0.50$) (Table 3; Fig. 6a). Maximal temperature and temperature range were also positively correlated with *M. trossulus* abundance and negatively correlated with *M. galloprovincialis* abundance. There was a weak positive correlation between the proportion of *M. galloprovincialis* and the minimum temperature at a site. These patterns suggest that *M. trossulus* was more abundant at sites with warmer summer temperatures, cooler winter temperatures and at sites that were thermally variable. No thermal factors had a strong correlation with the abundance of hybrids at a site.

Salinity characteristics at a site were also correlated with genotype distribution. Average salinity (over the 3-year monitoring period) was negatively correlated with *M. trossulus* abundance ($R^2 = 0.60$) (Table 3; Fig. 6b) and positively correlated with increasing *M. galloprovincialis* abundance at a site ($R^2 = 0.76$).

Maximal and minimal salinity were also positively correlated with *M. galloprovincialis* abundance, indicating that *M. galloprovincialis* was found at more saline sites (both higher maximum and higher minimum salinity). The salinity range was weakly (although positively) correlated with *M. galloprovincialis* abundance, suggesting that this factor is less relevant than the extremes. Overall, these data suggest that *M. trossulus* is more abundant at sites with low salinity, whether measured as average, maximal, or minimal salinity. As with temperature, hybrid abundance at a site was not correlated with salinity.

Because habitat salinity and temperature were strongly correlated with each other ($R^2 = 0.59$) (Fig. 6c), it is not possible, on the basis of distribution data alone, to draw conclusions as to which of these two environmental variables is of greater importance in governing the distribution patterns of the different genotypes.

Discussion

Mytilus spp. distributions are clearly linked with the abiotic environment in central California, following an estuarine-to-oceanic gradient, which suggests that differential biological responses to habitat temperature and salinity by *M. trossulus* and *M. galloprovincialis* contribute to the observed pattern. This conclusion agrees in part with assessments by previous authors, that genotype correlates with both temperature and salinity within the California *Mytilus* spp. hybrid zone (Sarver and Foltz 1993; Gardner 1994; Rawson et al. 1999). Sarver and Foltz (1993) suggested that *M. trossulus'* distribution in the same central California region during the early 1990s supported the hypothesis that it is a euryhaline cool-water mussel. However, the patterns described here in relation to temperature tolerance per se do not match the predictions one would make based on the Sarver and Foltz study nor the evolutionary history of each species: the hypothetically cooler-adapted species (*M. trossulus*) was found at sites with the highest temperatures and lower salinities.

It is apparent from our results that salinity, as well as temperature, must be considered when evaluating the role of abiotic factors in setting the distribution patterns of native and invasive blue mussels in hybrid zones. We observed wide fluctuations in sea surface temperature and salinity at central California sites, and these fluctuations varied on time scales and with magnitudes relevant to biological systems. Sea surface temperature at a site varies on hourly to seasonal temporal scales, is directly related to air temperature (daily warming), and is influenced regionally by the degree of protection at a site (i.e., estuarine sites along an estuarine-oceanic gradient see greater temperature fluctuation). Sea surface temperature is likely consistent within a region (e.g., San Francisco Bay) and in the present study air temperature explained up to 70% of the variation in sea surface

temperature. In contrast, salinity at a site can vary widely over a few hours when spring tides and heavy rainfall coincide, but the dominant pattern at most sites is seasonal: salinity is lower in winter, such as seen in SR (June–August 30 ppt, December–February 11 ppt). Furthermore, within a region, salinity does not follow consistent patterns among sites, suggesting that salinity is more variable locally than is temperature. For example, the salinity at MLS, especially as compared to its neighboring site MLN, indicates that it is more similar to San Francisco Bay sites, despite the fact that the temperatures are more similar to other Monterey Bay sites. Ultimately, this suggests that one would expect to see local differences in genotype distribution to be governed by the salinity characteristics of a site (e.g., distribution in the hybrid zone) and regional scale differences in genotype distribution to be governed by temperature characteristics (e.g., latitudinal distribution along NE Pacific coast).

While our study focuses on subtidal or a-tidal populations of *Mytilus* spp. (allowing temperature and salinity at the sea surface to be used as gauges of these two types of abiotic stress), we expect the temperature and salinity habitat of intertidal mussels to be even more extreme and to play a larger role in establishing distributional patterns. In addition, we have looked at temperature and salinity effects independently of anoxia and desiccation, two additional abiotic stressors that will add to stress effects in intertidal populations. By looking at temperature and salinity effects in isolation, however, we can be more confident of their role in observed distributional patterns.

The present study and the comparison of our data with previous data sets demonstrate that fluctuations in *Mytilus* spp. genotype distribution occur over short temporal and spatial scales in the Central California hybrid zone. This indicates that the hybrid zone is still in a dynamic state, and there is no indication of whether it is stationary (in a state of dynamic equilibrium) or if it is shifting northwards, as expected. The meta-analysis shows that there have been regional changes in the frequency of the two genotypes across much of the hybrid zone. In particular, there has been an increase in the frequency of *M. galloprovincialis* in the north, suggesting that it may be increasingly dominating the populations in the northern end of the hybrid zone. More interesting, in terms of invasion biology, is the increase in *M. trossulus* genotypes in the southern part of the range, particularly in Monterey Bay. As an example, Rawson et al. (1999) observed that the distribution pattern within San Francisco Bay from 1990 to 1995 mimicked the overall pattern throughout California: the southernmost site in their study, the San Mateo Bridge, had the highest abundance of *M. galloprovincialis*. In contrast, we found that the southern-most San Francisco Bay site (PA—a few kilometers south of the San Mateo Bridge), had a significant, *M. trossulus* population and that *M. trossulus* comprised a greater proportion of the population than when sampled 10 years ago. This suggests

two things: (1) that the San Mateo Bridge population is simply a very different population of mussels, perhaps due to habitat (especially if the sampling occurred in the center of the bridge, which is coincident with the main channel/strongest oceanic influence in South Bay); or (2) that the South Bay population has changed dramatically over 10 years towards a higher abundance of the native, *M. trossulus*. Regardless, the pattern found by Rawson et al. (1999) is not representative of the current structure of the San Francisco Bay population.

Limitations to our meta-analysis stem from differences in genetic identification techniques among the studies. Among the four studies, one used multiple allozyme loci and the other three studies used different combinations of nuclear and mitochondrial loci to make genotypic distinctions. In the meta-analysis, therefore, we are making comparisons across locus types, assuming that the loci give us equivalent data. While this may not be a safe assumption, the PCR-based approach used in the present study and in the three nonallozyme studies all demonstrated that the loci have similar genotype frequencies to each other, a result of heterozygote deficiency and presumed low rates of interbreeding. This concordance indicates that comparisons across loci and allozyme data sets in this particular hybrid zone are likely valid and allow us to analyze the temporal aspects of the genotype distribution.

The high variation among collection events (both within this study and between this study and those in the 1990s) could be partly a consequence of sample size. Our samples for each collection ranged from 13 to 104 and averaged 31 individuals. We cannot eliminate the possibility that larger sample sizes would have produced less variable data but the high variation we observed is consistent with the life histories of the species. While there are no aging techniques for mussels (with the possible exception of shell isotope analysis), it is likely that the generation time of these species is short—a year or less (see Toro et al. 2002, who demonstrated that *M. trossulus* has male and female gonad at SL < 10 mm). A short generation time, coupled with periodic recruitment of larvae with disparate genotypes could result in the observed rapid population changes.

Mytilus spp. size showed clear and consistent patterns across hundreds of kilometers of sampling, suggesting that there was a significant difference in maximal length between *M. trossulus* and *M. galloprovincialis* individuals. Blue mussel growth and maximal size are known to be highly plastic, being dependent on many factors including temperature, food availability, and exposure (Seed and Suchanek 1991). So, it is perhaps surprising that *M. trossulus* exhibits such a restricted size range (up to 50 mm) throughout the highly variable environments in California, while *M. galloprovincialis* and hybrids grow to much greater lengths throughout the range (up to 100+ mm). Because of the low frequency of *M. trossulus* with shell lengths > 50 mm, it is likely that this size distribution is due to genetic limits on *M. trossulus* growth, rather than plastic responses to differences in

diet or physical condition. This hypothesis is supported by the size distribution of *M. trossulus* in Oregon, which peaks at a SL of approximately 40 mm (Dr. Bruce Menge, Oregon State University, personal communication).

An analysis of the size distribution would be more meaningful if the age structure of these individuals was known. It is possible that either the age structure of the genotypes is similar (indicating that *M. trossulus* is simply a genetically smaller species) or that the age structure of *M. galloprovincialis* is right-shifted (indicating that *M. galloprovincialis* survives to an older age than *M. trossulus*). While we know that within a species of blue mussel size and fecundity are closely linked (Seed and Suchanek 1991) it is not necessarily appropriate to make comparisons of size structure and fecundity between species of mussels (Maloy et al. 2003). Thus, while the greater average size of *M. galloprovincialis* might allow a relatively higher fecundity, data to test this conjecture are lacking.

The role of abiotic factors also merits additional examination in the context of the future invasive success of *M. galloprovincialis*. Our finding that this invasive species is currently most prevalent in what we have termed oceanic habitats within the central California hybrid zone, where salinities are high and temperatures are low, needs to be interpreted in the broader context of this widely occurring species' biogeography. While distribution data per se do not allow strong conclusions to be drawn about the relative influences of temperature and salinity on *Mytilus* spp. distribution in the hybrid zone (because of covariation in these two environmental factors), the fact that *M. galloprovincialis* is known to tolerate higher temperatures than those measured in this study at other sites in its biogeographic range leads us to conjecture that low salinity may be of primary importance in determining its further expansion into more estuarine locations within the current hybrid zone. If lower salinity in estuaries is in fact limiting for this invasive species, then its spread into current estuarine habitats will depend on the future maintenance of an estuarine environment, which is intimately linked to water-use policies, and to the effects of global climate change. To further explain the patterning found in this and earlier studies, physiological traits and the supply of recruits of the genotypes to these sites will help us better understand whether causal relationships exist among environmental temperature, salinity, and mussel distribution, and to quantify the congeners' ability to persist and thrive under particular environmental conditions (Braby 2004).

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