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Zoogeographic Distributions of the Sibling Species *Mytilus galloprovincialis* and *M. trossulus* (Bivalvia: Mytilidae) and Their Hybrids in the North Pacific

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Abstract. Diagnostic length differences in a PCR amplified fragment of the gene for byssal adhesive protein were used to study the zoogeographic distribution of *Mytilus galloprovincialis* and *M. trossulus* along the west coast of North America and in Japan. The distributions of *M. galloprovincialis* and *M. trossulus* are patchy, although an overall geographic pattern emerges. *M. galloprovincialis* was the only species found on either Kyushu or Honshu, and it was the most abundant mussel from Tomales Bay to San Diego, California. *M. trossulus* was the only bay mussel found on Hokkaido and in Alaska, and it was by far the most abundant mussel along the coasts of Washington and Oregon.

Mytilus galloprovincialis and *M. trossulus* are sympatric and hybridize near Whidbey Island, Washington, in San Francisco Bay, and in San Diego Bay. A second diagnostic anonymous nuclear PCR marker was used to examine the extent of hybridization at Palo Alto, California. At this site, genotypes appeared to be a mixture of *M. galloprovincialis,* F_1 hybrids between *M. galloprovincialis* and *M. trossulus,* and backcrosses between the F_1's and *M. galloprovincialis.*

The discontinuity between the zoogeographic distributions of these two species at about 40°–41°N latitude in both the eastern and western Pacific suggests that temperature is a factor in determining their present distribution and limiting their dispersal to other regions.

Introduction

Biochemical and molecular systematics have dramatically altered our understanding of the ecology, evolution, and biogeography of bay or blue mussels of the genus *Mytilus* (McDonald and Koehn, 1988; Koehn, 1991; Seed, 1992; Rawson and Hilbish, 1995). The taxonomy of the *Mytilus edulis* complex has been confused (see Seed, 1992, for a full discussion of species definitions in this genus) as a result of considerable morphological similarity among species. For example, each species varies considerably in size and shape both within and among populations (Seed, 1968). Today, multivariate analyses of morphological shape (McDonald *et al.,* 1991) and analyses of molecular genetic variation (see below) have led to the recognition of three sibling species: *M. edulis* Linnaeus 1758, *M. galloprovincialis* Lamarck 1819, and *M. trossulus* Gould 1850. Early work suggested a simple geographic distribution in the northern hemisphere, with *M. edulis* being found in the temperate regions of the Atlantic and Pacific Oceans, and *M. galloprovincialis* restricted to the Mediterranean Sea. General recognition of *M. trossulus* resulted from surveys of allozyme variation in populations at higher latitudes in both the Atlantic and Pacific Oceans (Koehn *et al.,* 1984; McDonald and Koehn, 1988; Varvio *et al.,* 1988).

The discovery of hybridization among members of the *Mytilus edulis* complex blurred the tidy distribution patterns mentioned above. The first indication that the distributions were more complex than initially proposed was provided by electrophoretic surveys of genetic variation of mussels in the British Isles (Skibinski and Beardmore, 1979; Skibinski, 1983; Koehn, 1991, p. 134), which revealed the presence of *M. galloprovincialis,* and hybridization between *M. edulis* and *M. galloprovincialis* (Skibinski and Beardmore, 1979; Gosling and Wilkins, 1981; Skibinski, 1983; Skibinski *et al.,* 1978; 1980; 1983). Stud-

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ies also revealed that the bay mussels on the Pacific coast of North America were not *M. edulis,* nor were they a single species. McDonald and Koehn (1988), using allozyme markers, identified *M. galloprovincialis* in southern California, *M. trossulus* in northern California and further north, with a hybrid zone in and around San Francisco Bay. This pattern has been verified with studies using markers from mtDNA (Geller, 1994; Geller *et al.,* 1994; Rawson and Hilbish, 1995).

Complexity and patchiness in the distribution patterns of *Mytilus* species have been found not only on a broad geographic scale but also on a microgeographic scale. For example, ecological genetic studies have revealed variation associated with environmental conditions. In the British Isles, the abundances of *M. edulis* and *M. galloprovincialis* vary with both salinity and wave exposure: *M. galloprovincialis* is relatively more common in areas with high salinity and high wave exposure (Skibinski *et al.,* 1983). In a hybrid zone at the salinity gradient between the North Sea and the Baltic Sea, *M. edulis* alleles give way to *M. trossulus* alleles as salinity decreases (Väinölä and Hvilsom, 1991).

In the past, taxonomic work on *Mytilus* was made difficult by the lack of an easy and complete means of identifying species. Although identification of *M. edulis, M. galloprovincialis,* and *M. trossulus* is possible with electrophoretic surveys of proteins (Koehn *et al.,* 1984; Grant and Cherry, 1985; McDonald and Koehn, 1988; Väinölä and Hvilsom, 1991), it is now easier to identify species and their hybrids with DNA markers (Geller and Powers, 1994; Geller *et al.,* 1994; Inoue *et al.,* 1995; Rawson and Hilbish, 1995). For example, diagnostic mtDNA markers were used by Rawson and Hilbish (1995) to describe the distributions of *M. galloprovincialis* and *M. trossulus* from San Diego to Seattle. Identification of species with mtDNA is, however, more difficult in mussels than in other groups of species, for salt (Mytilid) and freshwater (Unionid) species have both maternally and paternally inherited mtDNA systems (Skibinski *et al.,* 1994; Zouros *et al.,* 1992; 1994; Geller, 1996; Hoeh *et al.,* 1996; Liu *et al.,* 1996). The difficulties of using mtDNA as a diagnostic marker have been overcome by the use of a nuclear, codominant diagnostic marker. Inoue *et al.* (1995) described a diagnostic length difference in a gene that codes an adhesive protein found in byssal threads. The fragment may be amplified readily from genomic DNA, and the three species have distinctly different fragment sizes, making it simple to identify all three species and their hybrids, in which two fragments are coamplified.

The primary objectives of this study are to describe the macrogeographic and microhabitat distribution of three species (*M. edulis, M. trossulus,* and *M. galloprovincialis*) and the occurrence of their hybrids. Our sampling is more extensive than in previous studies, with multiple sites at some localities and a greater geographic extent of sample sites. Finally, we have used a codominant, nuclear marker to identify the three species and their hybrids, and employed a second diagnostic marker to test for the presence of backcross genotypes within a hybrid zone.

Materials and Methods

Sample sites

Collection sites are shown in Figure 1 and described in Table I. Mussels from Tillamook Bay, Coos Bay (1991), San Francisco Bay, Elkhorn Slough, Morro Bay, and San Diego Bay were the same as those reported upon in Geller *et al.* (1994). Additional mussels were obtained from Whidbey Island in the Puget Sound and shipped live to Wilmington, North Carolina, whereas mussels from Yokohama, Japan, were preserved in ethanol. DNA extractions and amplifications of these samples were conducted by JBG. The remaining samples were handled by BRK and JBM. Samples were shipped, either alive or frozen, to Boulder, Colorado, and held in an ultracold freezer (−80°C) until DNA was extracted.

DNA markers

DNA was extracted from the mantle tissue of each mussel by following the protocol of Geller *et al.* (1994).

In the study by Inoue *et al.* (1995) Me-15 and Me-16 PCR primers for a portion of an adhesive protein gene produced species-specific length variants of 180, 168, and 126 bp for *Mytilus edulis, M. trossulus,* and *M. galloprovincialis* respectively. Amplifications by BRK and JBM were conducted in a total volume of 25 μl using 10 m*M* Tris (pH = 8.3), 50 m*M* KCl, 0.01% gelatin, 200 μM dNTPs, 4 m*M* $MgCl_2$, 1.5 units Taq DNA polymerase, 2 pmol of each primer, 0.75 μl DNA template, and autoclaved ultrapure water. Cycling conditions consisted of an initial denaturing step of 94°C for 45 s. This was followed by 35 cycles of 94°C for 45 s, 56°C for 30 s, 70°C for 90 s and concluded with a final extension step of 70°C for 3 min. Amplifications conducted by JBG used 10 m*M* Tris (pH = 8.3), 1.5 m*M* $MgCl_2$, 50 m*M* KCl, 0.01% Triton-X 100, 0.01% gelatin, 0.01% NP-40, 200 μM dNTP's, 1.5 m*M* $MgCl_2$, 0.3 units Taq DNA polymerase, 25 pmol of each primer, 0.5 μl DNA template, and autoclaved ultrapure water in a volume of 25 μl. Cycling parameters were 30 s at 98°C followed by 30 cycles of 10 s at 95°C, 30 s at 54°C, and 30 s at 72°C. For all amplifications, 10 μl of the PCR product were run on 3% agarose gels, stained with ethidium bromide, and visualized under UV light.

A pair of primers for a single copy anonymous nuclear DNA locus (Cmg-93) in a deep-sea hydrothermal vent vesicomyid clam, *Calyptogenea magnifica* (Karl *et al.,* 1996), was found to amplify a single band of about 750 bp in *M. trossulus* but produced no product in either *M.*

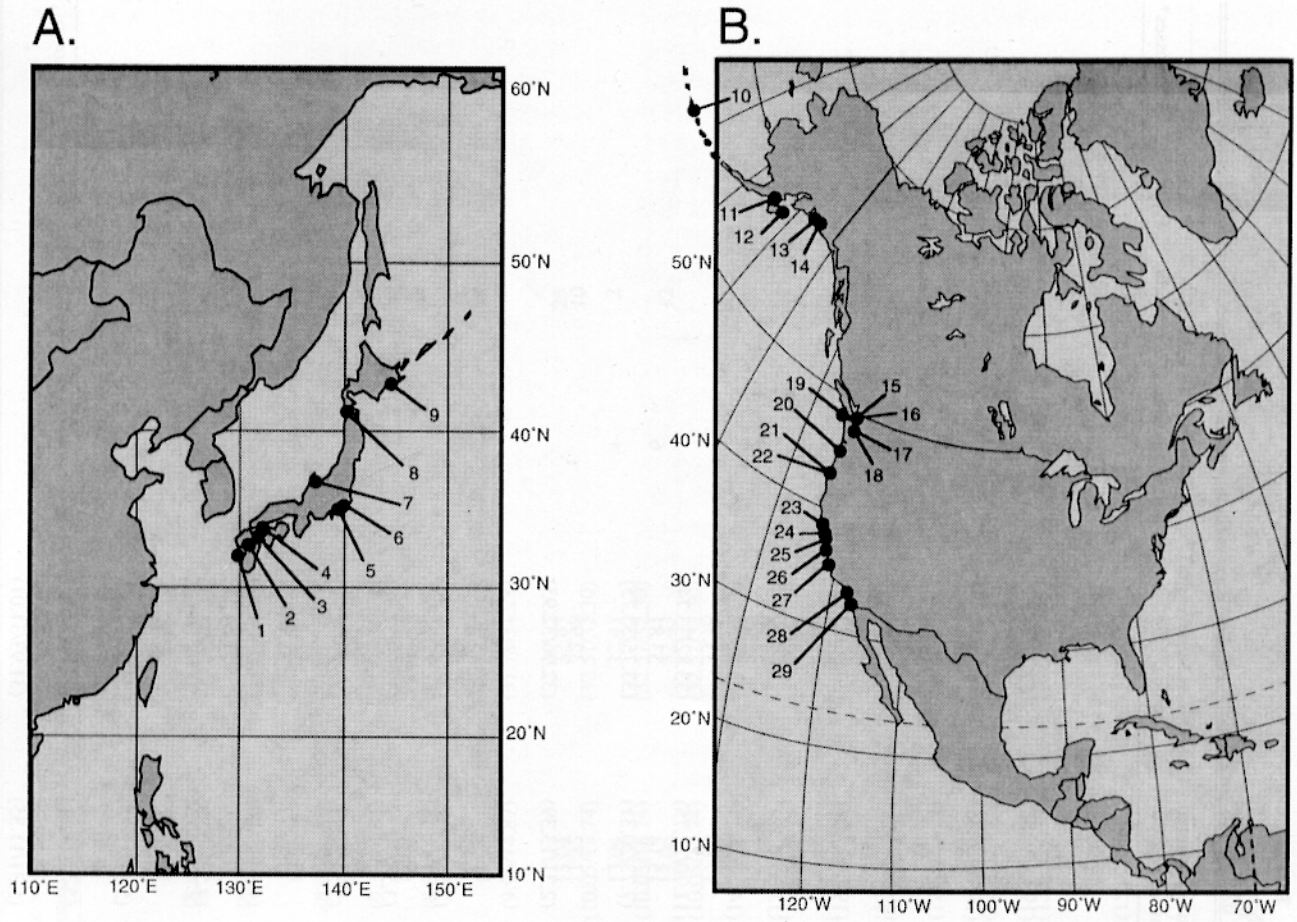

Figure 1. *Mytilus* collection sites in Japan (A) and North America (B).

edulis or *M. galloprovincialis*. This marker, in conjunction with the diagnostic marker of Inoue *et al.* (1995), permitted a more thorough analysis of genotypes in a zone of hybridization between *M. trossulus* and *M. galloprovincialis* at Palo Alto, California. F_1 hybrids between *M. galloprovincialis* and *M. trossulus* would have two bands at the Inoue marker, and a product for the Cmg-93 locus. An individual with two bands at the Inoue marker and no Cmg-93 product is one of several genotypes produced by backcrossing an F_1 hybrid with a specimen of *M. galloprovincialis*. The presence of this genotype clearly indicates that hybridization has extended beyond the F_1 level.

Amplifications of Cmg-93 were conducted by BRK and JBM in a total volume of 25 μl using 10 m*M* Tris (pH = 8.3), 0.01% gelatin, 50 m*M* KCl, 300 μ*M* dNTPs, 2.5 m*M* $MgCl_2$, 1.5 units Taq polymerase, 6.25 pmol each of primer, bovine serum albumin (0.1 μg/μl), 0.75 μl DNA template, and autoclaved ultrapure water. Cycling conditions consisted of an initial denaturing step of 94°C for 60 s. This was followed by 35 cycles of 94°C for 60 s, 50°C for 60 s, 70°C for 60 s and concluded with a 70°C 2-min extension step. Ten microliters of the PCR product were run on 1% agarose gels, stained with ethidium bromide, and visualized under UV light.

Annual and monthly (January and July) means for temperature and salinity for each site were taken from the NOAA World Ocean Atlas (1994). Data were obtained from the nearest point for each site from a 1° × 1° grid of the North Pacific Ocean. In addition, some indication of the degree of wave exposure was recorded for most of the collection sites.

Results

On both sides of the North Pacific Ocean, *M. galloprovincialis* predominates in the lower latitudes, and *M. trossulus* predominates in the higher latitudes (Table I, Fig. 1). In Japan, we found *M. galloprovincialis* alone at all sites on the southernmost island, Kyushu, and on Honshu, whereas only *M. trossulus* was found on the northern island of Hokkaido. *M. galloprovincialis* is most abundant in southern California, but only *M. trossulus* is found in Alaska. We did not detect *M. edulis* in any of our population samples.

Hybridization was not detected in the samples from

Table I

The identity of individual Mytilus galloprovincialis, M. trossulus, *and their hybrids in the North Pacific*

Fig. 1 Ref. #	Location	Habitat type	Collected	Latitude/ longitude	Mean water temp (°C) annual (Jan/July)	Mean salinity (ppt) annual (Jan/July)	*Mytilus galloprovincialis*	*Mytilus trossulus*	Hybrids	Backcrosses*
	JAPAN									
	Kyushu									
1	Amakusa	protected	1992	32°26′N; 130°06′E	22.36 (19.13/26.88)	34.24 (34.79/33.27)	6			
2	Kumamoto	protected	1996	32°30′N; 130°40′E	22.36 (19.13/26.88)	34.24 (34.79/33.27)	5			
	Honshu									
3	Iwakuni	protected	1988	34°09′N; 132°11′E	22.90 (19.85/27.08)	34.49 (34.80/33.97)	28†			
4	Hiroshima	protected	1996	34°24′N; 132°27′E	22.90 (19.85/27.08)	34.49 (34.80/33.97)	3			
5	Yokohama	protected	1993	35°28′N; 139°42′E	20.78 (16.68/24.68)	33.98 (34.29/33.60)	3‡			
6	Chiba (Kominato)	protected	1992	35°36′N; 140°07′E	18.64 (14.17/23.17)	33.10 (33.51/32.61)	6			
7	Toyama	protected	1988	36°42′N; 137°14′E	17.69 (11.70/22.75)	33.76 (33.82/33.74)	7†			
8	Mutsu Bay (Aomori)	semi-exposed	1992	41°05′N; 140°55′E	15.14 (7.73/20.31)	33.81 (33.75/33.75)	6			
	Hokkaido									
9	Akkeshi Bay	protected	1992	43°18′N; 145°31′E	11.72 (2.21/13.39)	32.98 (32.96/32.93)		10		
	ALASKA									
	Aleutian Isl.									
10	Adak Isl.	very protected	1992	51°50′N; 176°40′W	6.80 (3.54/7.73)	32.93 (32.99/32.90)		2		
10	Adak Isl.	protected	1992	51°50′N; 176°40′W	6.80 (3.54/7.73)	32.93 (32.99/32.90)		4		
10	Adak Isl.	very exposed	1992	51°50′N; 176°40′W	6.80 (3.54/7.73)	32.93 (32.99/32.90)		2		
	Gulf of Alaska									
11	Kinak Bay	exposed	1992	58°06′N; 152°53′W	7.79 (4.40/10.26)	31.70 (32.27/31.62)		13		
12	Ouzinki Pass	protected	1992	57°55′N; 152°30′W	7.87 (4.53/10.38)	31.84 (32.26/31.71)		12		
	Prince William Sound									
13	Knight Isl. (Herring Pt.)		1992	60°29′N; 147°46′W	8.40 (5.31/11.35)	31.38 (31.93/31.01)		2		
13	Knight Isl (Herring Bay)		1992	60°27′N; 147°42′W	8.40 (5.31/11.35)	31.38 (31.93/31.01)		2		
14	Chenega Isl.		1992	60°23′N; 148°00′W	8.40 (5.31/11.35)	31.38 (31.93/31.01)		2		

Table I *(Continued)*

Fig. 1 Ref. #	Location	Habitat type	Collected	Latitude/ longitude	Mean water temp (°C) annual (Jan/July)	Mean salinity (ppt) annual (Jan/July)	*Mytilus galloprovincialis*	*Mytilus trossulus*	Hybrids	Backcrosses*
	WASHINGTON									
	San Juan Isl.									
15	Eagle Cove	semi-exposed	1992	48°28′N; 123°02′W	12.06 (8.96/14.35)	31.01 (31.02/30.86)		6		
16	Argyle Creek	protected	1992	48°32′N; 123°01′W	12.06 (8.96/14.35)	31.01 (31.02/30.86)		6		
	Puget Sound									
17	Whidbey Isl. (Penn Cove)	protected	1994	48°13′N; 122°41′W	12.06 (8.96/14.35)	31.01 (31.02/30.86)	5	13	2	
18	Skookum Bay	protected	1992	47°09′N; 123°03′W	12.46 (9.38/14.81)	30.99 (31.06/30.77)		7		
19	**Tatoosh Isl.**	exposed	1996	48°23′N; 124°44′W	12.04 (8.84/14.33)	31.39 (31.47/31.22)		5	1	
	OREGON									
20	**Tillamook Bay (Marshall)**	protected	1991	45°23′N; 123°59′W	13.06 (9.96/14.87)	31.60 (31.78/31.28)		17		
	Coos Bay									
21	Isthmus Slough	protected	1991	43°22′N; 124°12′W	13.04 (10.05/13.74)	32.43 (32.51/32.30)		25		
21	Isthmus Slough	protected	1996	43°22′N; 124°12′W	13.04 (10.05/13.74)	32.43 (32.51/32.30)	4	2		
22	South Slough	protected	1992	43°20′N; 124°19′W	13.04 (10.05/13.74)	32.43 (32.51/32.30)		12		
	CALIFORNIA									
23	**Tomales Bay**	protected	1992	38°11′N; 122°54′W	13.81 (11.99/13.65)	32.92 (32.90/33.50)	9			
24	**San Francisco Bay (North Beach)**	protected	1992	37°49′N; 122°24′W	13.48 (11.71/13.05)	32.95 (32.96/33.12)	16	26	5	
25	**Palo Alto (Yacht Club)**	protected	1990	37°29′N; 122°06′W	13.98 (12.42/13.91)	33.06 (33.06/33.17)	10		2	2*
25	**Palo Alto (Yacht Club)**	protected	1996	37°29′N; 122°06′W	13.98 (12.42/13.91)	33.06 (33.06/33.17)	25		10	15*
26	**Elkhorn Slough**	protected	1992	36°35′N; 121°55′W	13.98 (12.42/13.91)	33.06 (33.06/33.17)	21		2	
27	**Morro Bay**	exposed	1992	35°45′N; 120°56′W	14.14 (12.71/14.39)	33.25 (33.25/33.34)	25		1	
	San Diego									
28	Scripps Pier	exposed		32°51′N; 117°17′W	16.02 (15.02/17.69)	33.52 (33.48/33.58)	10			
29	San Diego Bay	protected	1992	32°35′N; 117°08′W	16.02 (15.02/17.69)	33.52 (33.50/33.54)	18	3	11	

* The use of two sets of primers in the Palo Alto samples allowed discrimination of F1 hybrids and backcrosses. See text for further details.
† These individuals were cultured from ballast water from ships originating from these ports.
‡ Collected as drift from a protected beach.

Japan, but hybrids were common in San Diego Bay and San Francisco Bay. Both *M. galloprovincialis* and *M. trossulus* and their hybrids were detected at Whidbey Island, near a mussel farm that raises *M. galloprovincialis.* A single hybrid was found in a sample from Tatoosh Island, Washington.

The patchy distribution of species is apparent in and around the bays of California. For instance, only *M. galloprovincialis* was present on the outer coast at Scripps Pier in San Diego, but just a few kilometers away, *M. galloprovincialis, M. trossulus* and their hybrids were found in San Diego Bay. In San Francisco Bay, near the Golden Gate Bridge at North Beach, both species and hybrids were common (34% *M. galloprovincialis,* 11% *M. trossulus,* 55% hybrids). However, deeper in the bay, at Palo Alto, *M. trossulus* was apparently absent, but *M. galloprovincialis* and hybrids were common. The individuals collected from Palo Alto were analyzed with the two diagnostic markers to examine the extent of hybridization (Table I). In both 1990 and 1996, we identified genotypes consistent with F_1 hybrids (14% and 20%, respectively) and backcrosses (14% and 30%, respectively) of F_1 hybrids to *M. galloprovincialis.*

In Isthmus Slough and South Slough within Coos Bay, only *M. trossulus* was found in 1991. However, a sample taken from Isthmus Slough in 1996 contained both *M. galloprovincialis* and *M. trossulus.* It is not known whether the difference between the two samples taken from Isthmus Slough reveals a change over time or preexisting microgeographic variation.

Although our survey identified microgeographic variation in species distributions, an environmental cause was not apparent. For our collection sites, neither wave exposure (Suchanek, 1978) nor salinity supplied any predictive power to explain the distribution of species.

Despite the lack of any explanative value in environmental conditions at a microgeographic scale, on a broader geographic scale the transitions between *M. galloprovincialis* and *M. trossulus* occur between 40° and 41° north latitude on both sides of the Pacific. Data in Table I allow a comparison of temperature regimes across the transition zones. At the break on the eastern side of the Pacific, there are only slight differences in mean temperatures (compare sites 22 and 23). The only appreciable difference is a drop of about 2°C in the mean January temperature at the northern site (site 22). However, a comparison of sites 8 and 9 in Japan reveals substantial differences in temperature across the transition zone. The northern site not only has a lower mean January temperature (by about 5.5°C), but also lower mean annual and mean July temperatures (by 3°C and 7°C, respectively). A comparison of the eastern and western sites at the southern boundary of pure *M. trossulus* (sites 9 and 22) reveals that mean January temperatures are quite different (2.21° and 10.5°C, respectively), but that annual mean temperatures (11.72° and 13.74°C, respectively) and mean July temperatures (13.39° and 13.74°C, respectively) are similar.

Discussion

In comparison to previous studies, our finer scale geographic sampling of *Mytilus* in California revealed a more patchy distribution of pure species and a greater geographical extent of hybrids. Whereas previous studies had suggested that pure *M. galloprovincialis* extended as far north as Point Conception, we found a population of pure *M. galloprovincialis* in Tomales Bay, 700 km further north. Furthermore, our studies of populations on both sides of the Pacific revealed that the transition zones are at similar latitudes, and at similar temperatures, suggesting that temperature influences the distributions of these species.

Hybridization and patchiness

The authors of a previous study (Rawson and Hilbish, 1995) concluded that hybridization of *M. galloprovincialis* and *M. trossulus* was rare in southern California. They sampled from the pier at the Scripps Institution of Oceanography, and they found only *M. galloprovincialis.* Similarly, our sample from the same site also yielded only *M. galloprovincialis* (Table I), but more extensive sampling inside San Diego Bay in a small-craft marina revealed both species and their hybrids.

Although earlier reports indicated that hybridization was evident in San Francisco Bay (McDonald and Koehn, 1988; Sarver and Foltz, 1993) and relatively infrequent elsewhere (Rawson and Hilbish, 1995), this survey revealed hybrids in San Diego Bay, Elkhorn Slough, two areas within San Francisco Bay, and two sites in Washington. San Francisco Bay and Elkhorn Slough are located near the southern boundary for continuous populations of *M. trossulus* and the northern boundary for continuous populations of *M. galloprovincialis,* so hybridization there is not surprising. Studies of mussels in San Francisco Harbor (Rawson *et al.,* 1996) and the present study (Table I) revealed that the proportions of pure species, F_1 hybrids, and backcrosses vary across the bay, with *M. galloprovincialis* becoming more common deeper in the bay. The studies of mussels from Palo Alto clearly indicate that hybridization and backcrossing are common, and that the mixing appears to be persistent over time. Presence of hybrids away from this contact zone around San Francisco requires further explanation. The San Diego Bay site is in a marina where small craft may have introduced adult or larval *M. trossulus,* initiating hybridization. *M. galloprovincialis* and hybrids at Whidbey Island may be progeny of cultured mussels in a nearby mariculture facility. A hybrid at Tatoosh Island is possibly an indication of transport of hybrid larvae from Puget Sound.

Sporadic episodes of transplantation by means of ships will cause the presence of *Mytilus* species and their relative abundances to fluctuate over time. Although the adults are effectively sedentary, the pelagic larvae are in the water column for 3 weeks or more; depending on the currents, they may move distances in excess of 200 km. But in addition to their natural dispersal, mussels are moved about extensively on the hulls of and in the ballast water of ships. Freighters moving between continents can inoculate a bay with many millions of larvae, and thus the distributions and the relative numbers of species of *Mytilus* might change from year to year (Carlton and Geller, 1993).

The absence of *M. edulis* in our samples, as in previous allozyme and mtDNA reports, remains enigmatic, for there are several reports that *M. edulis* has been transplanted to the west coast of North America. For example, hundreds of oyster epibionts, which include *M. edulis,* have been accidentally released in Pacific sites (Carlton, 1989). Furthermore, we suspect that individuals of *M. edulis* are introduced regularly to western ports by cargo ships discharging ballast water (Carlton, 1989; Carlton and Geller, 1993). *M. edulis* has subsequently been identified (D. Heath, pers. comm.) from a sample of alien mussels (Heath *et al.,* 1995) collected in the Georges Strait, north and west of Vancouver, near an area where *M. edulis* is farmed.

Transition zones

In California, the transition between *M. galloprovincialis* and *M. trossulus* is near 40° or 41°N latitude, approximately the Cape Mendocino region. Previous studies had suggested that the northern limit of pure *M. galloprovincialis* was at Point Conception or Morrow Bay (Sarver and Loudenslager, 1991; Rawson and Hilbish, 1995), but our study revealed pure *M. galloprovincialis* in Tomales Bay, nearly 700 km further north. This transition is far north of the biogeographic boundary at Point Conception, a recognized demarcation between cold-water and warm-water biotas (Valentine, 1966), where many molluscan species have a distributional limit.

Which variables control the latitudinal distributions of *M. galloprovincialis* and *M. trossulus?* Temperature is generally believed to control distributional boundaries of marine organisms (Valentine, 1966), and *Mytilus* is no exception (Seed, 1976). *M. edulis* is able to withstand freezing in ground ice at −20°C for 6 to 8 months of the year (Williams, 1970), but the northern distribution for *M. edulis* may be limited by the temperatures that permit breeding (*e.g.,* Stubbings, 1954; Barnes, 1957). Similarly, the southern limit of the range of *M. edulis* in the western North Atlantic is determined by summer temperatures, which are lethal for *M. edulis* on the Outer Banks of North Carolina (Wells and Gray, 1960).

In both the eastern and western Pacific, the transition zones between *M. galloprovincialis* and *M. trossulus* occur where the annual mean temperature is between 13° and 14°C. On both sides of the Pacific, the southernmost populations of pure *M. trossulus* (sites 9 and 22) experience mean July temperatures of 13.39° and 13.74°C, respectively. We hypothesize that summer temperatures warmer than 13°–14°C approach the upper thermal limit for *M. trossulus* and reduce the viabilities of larvae and adults. Although historically *M. trossulus* was established south of this latitude in California, it was probably restricted to cooler sites. Geller (unpubl. data) used mitochondrial 16S rRNA gene sequences to identify *M. trossulus* collected from Catalina Island in 1900 and from Monterey Bay in the 1870s. Both Catalina Island and Monterey Bay are cooler than the collection sites employed in the present study in San Diego Bay. We do not propose that the temperature of 14°C is an absolute barrier, for we sampled *M. trossulus* from San Diego Bay (mean annual temperature = 16.02°C, mean July temperature = 17.69°C). However, the presence of *M. trossulus* within San Diego Bay but not on the open coast, at the Scripps Pier, suggests that persistent reintroduction by means of intracoastal boating is necessary to maintain *M. trossulus* in the relatively warm localities in southern California. If our hypothesis is correct, *M. trossulus* would not persist in San Diego Bay without recurrent introductions by ships.

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