Summer 2018

# Shifting Species Boundaries: Mytilus Spp. on the Pacific Coast

Melinda Kathleen Wheelock
*California State University, Monterey Bay*

# SHIFTING SPECIES BOUNDARIES: *MYTILUS* SPP. ON THE PACIFIC COAST

---

A Thesis

Presented to the

Faculty of

Moss Landing Marine Laboratories

California State University Monterey Bay

---

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

in

Marine Science

---

by

Melinda Kathleen Wheelock

Summer 2018

**CALIFORNIA STATE UNIVERSITY MONTEREY BAY**

The Undersigned Faculty Committee Approves the

Thesis of Melinda Kathleen Wheelock:

SHIFTING SPECIES BOUNDARIES: *MYTILUS* SPP. ON THE PACIFIC COAST

_____

Jonathan B. Geller, Chair
Moss Landing Marine Laboratories

_____

Brigitte McDonald
Moss Landing Marine Laboratories

_____

James T. Carlton
Williams College

Digitally signed by Kris Roney
Date: 2018.08.16 07:11:49 -08'00'

_____

Kris Roney, Dean
Associate VP for Academic Programs and Dean of Undergraduate and Graduate Studies

_____

Approval Date

# DEDICATION

This work is dedicated, first and foremost, to Annette and Bill Wheelock. Your support and love keep me going. To Alyssa and Daniella, you inspire me every day. To all my friends and family who kept me sane when I thought the dissections would never end, thank you.

In loving memory of Kathleen Barron Wheelock and Bienvenido Santa Ana Jabson.

For as the shore configuration changes in the flow of time, the pattern of life changes, never static, never quite the same from year to year. Whenever the sea builds a new coast, waves of living creatures surge against it, seeking a foothold, establishing their colonies. And so we come to perceive life as a force as tangible as any of the physical realities of the sea, a force strong and purposeful, as incapable of being crushed or diverted from its ends as the rising tide.

Rachel Carson, *The Edge of the Sea*

# ABSTRACT

Shifting species boundaries: *Mytilus* spp. on the Pacific coast
by
Melinda K. Wheelock
Master of Science in Marine Science
California State University Monterey Bay, 2018

The two species of bay mussel present on the Pacific coast of North America, *Mytilus trossulus* and *M. galloprovincialis,* are morphologically very similar and typically difficult to distinguish by external characters. *Mytilus trossulus* is native to the eastern Pacific, and occurs in bays, estuaries and the outer coast from central California to Alaska. Its introduced counterpart, *M. galloprovincialis*, has replaced *M. trossulus* in southern California, and is found as far north as Humboldt Bay. Previous studies have shown that these sibling species co-occur and form genetic hybrids in central California between Monterey Bay and Humboldt Bay, though the exact hybrid zone is not well understood. Additionally, large numbers of adult *M. galloprovincialis* have drifted with tsunami debris from Japan to the US since 2012. The goal of this project is to further clarify the region of overlap in the species ranges of these mussels in the eastern Pacific.

Mussel populations in harbors and marinas between San Diego, CA and Newport, OR were sampled between 2013 – 2015, and tsunami debris was sampled between 2012 – 2014. Mussels were identified using a PCR assay for a nuclear marker which varies in size for each species. *Mytilus galloprovincialis* is now present in higher abundances in northern California, and *M. trossulus* is diminishing in abundance in its southern range. *Mytilus* galloprovincialis was the most abundant mussel on tsunami debris, and the Asian mussel *Mytilus coruscus* and a bivalve-inhabiting hydrozoan (*Eutima japonica*) were found on debris items. Tsunami debris has not resulted in detectable populations of *M. galloprovincialis* in Oregon.

# TABLE OF CONTENTS

PAGE

ABSTRACT ..... vi
LIST OF TABLES ..... vii
LIST OF FIGURES ..... ix
ACKNOWLEDGEMENTS ..... x
CHAPTER
1 SURVEY OF *MYTILUS* SPP. POPULATIONS IN CALIFORNIA AND OREGON ..... 1
Introduction ..... 1
Methods ..... 5
Sample Collection ..... 5
Species Identification ..... 8
Historical Samples ..... 9
Statistical Analysis ..... 9
Results ..... 9
Size and Genotype Distributions ..... 9
Genotype Distributions by Region ..... 11
Spatial Variation Within the Hybrid Zone ..... 13
Comparison to Previous Surveys ..... 14
Temporal Variation at One Location ..... 17
Discussion ..... 18
2 *MYTILUS* SPP. ON JAPANESE TSUNAMI MARINE DEBRIS ..... 23
Introduction ..... 23
Methods ..... 25
Results ..... 28
Discussion ..... 33
REFERENCES ..... 36

# LIST OF TABLES

PAGE

Table 1. Field sites and samples collected. .....7
Table 2. Proportion of *Mytilus* spp. at each site.....12
Table 3. Hybrid zone Chi-squared results.....16
Table 4. List of tsunami debris items sampled for *Mytilus* spp. .....26
Table 5. PCR primers used to identify *Mytilus* spp. .....28
Table 6. COIII identification of *Mytilus* spp. on Japanese tsunami debris .....29
Table 7. Occurrence of *Eutima japonica* in Japanese tsunami debris mussels.....31

# LIST OF FIGURES

PAGE

Figure 1. Map of sampling sites in California and Oregon for 2013-2015 *Mytilus* spp. survey. ....6

Figure 2. *Mytilus* spp. size composition within genotype. ....10

Figure 3. *Mytilus* spp. genotype frequency distributions by size class (shell length). ....10

Figure 4. *Mytilus* spp. distribution by bay region. ....13

Figure 5. *Mytilus* spp. genotype distribution by site within the hybrid zone. ....15

Figure 6. *Mytilus* spp. genotype distribution in Moss Landing, CA from 2002-2015 ....18

Figure 7. *Mytilus* spp. genotype distribution on Japanese tsunami debris landings from 2012-2014. ....30

Figure 8. Occurrence of the bivalve-inhabiting hydrozoan *Eutima japonica* in *Mytilus* spp. on Japanese tsunami debris landings from 2012-2014. ....32

# ACKNOWLEDGEMENTS

This work would not have been possible without the help and friendship of Becca Barnard. Thank you for spending countless hours driving around and collecting, measuring, and dissecting mussels with me. I would also like to thank my committee members for their support, guidance, and patience throughout my time at Moss Landing Marine Labs. I am very grateful to Gregory Ruiz for financial support during my sampling trips and for introducing me to Becca. Special thanks to Heather Fulton-Bennett, Cody Dawson, Robert San Miguel, Alyssa Wheelock, Annette Wheelock, Daniella Wheelock, and Angela Zepp for coming with me to collect mussels during the early stages of my thesis. Many thanks to Andrea Carpenter, Catherine Drake, Sarah Jeffries, Michelle Marraffini, and Emily Schmeltzer for allowing me to bounce ideas off of you and for being such kind friends. Last, but not least, thank you to the current and past members of the Invertebrate Zoology and Molecular Ecology Lab for your feedback and friendship over the years.

# CHAPTER 1

# SURVEY OF *MYTILUS* SPP. POPULATIONS IN CALIFORNIA AND OREGON

## INTRODUCTION

Increasing global travel and increased use of the coastal environment for commerce, shipping, and recreation have resulted in intentional and accidental transport of organisms around the world (Elton 1958; Carlton and Geller 1993; Bax et al. 2003; Ruiz et al. 2011). California's bays are heavily invaded marine regions, and San Francisco Bay in particular has one of the highest numbers of invasive species of any estuary in the world (Cohen and Carlton 1998; Ruiz et al. 2011). This high number of invasions, coupled with the existence of many large shipping ports and recreational harbors in the state, makes coastal California a model environment for studying range expansion of non-indigenous species.

Changes in climate – and thus, ambient water temperatures – also can have major impacts on species distribution and range expansion in marine organisms (Sagarin et al. 1999; Sorte, Williams, & Carlton 2010). Range expansion occurs when the distribution of a species extends past previously observed boundaries (Sorte, Williams, & Carlton 2010). The expansion of species ranges can occur naturally, where a species moves into suitable habitats at adjacent locations with similar community composition and physical characteristics (Sorte, Williams, & Carlton 2010; Sorte, Williams, & Zerebecki 2010). This expansion into new habitats is generally associated with native or introduced species that have a long history of establishment in areas surrounding the range margin, is rarely

seen outside the context of climate change, and should not be confused with the rapid colonization of new areas by a newly introduced species (Sorte, Williams, & Carlton 2010; J. Carlton, *pers. comm.*).

While studying the introduction of an organism to a completely new environment can be a matter of recording presence and extent of the introduction, studying the range shifts of native and long-introduced species at very local scales is difficult because species' micro-range boundaries are dynamic and often fluctuate with local conditions (e.g., temperature or salinity) and community-level processes (such as recruitment and predation) (Sagarin et al. 1999; Parmesan 2006; Burrows et al. 2011). The variable nature of species boundaries requires careful consideration and monitoring before reaching the conclusion that the range is shifting outside of the current accepted boundary, as defined by physical conditions or community-level processes. However, the main determinant of the occurrence of a range shift or expansion is the record of an established, reproductive population outside of the historic range of a species (J. Carlton, *pers. comm.*).

Over long time scales, climate change has the potential to modify ranges of long-established introduced species (Sagarin et al. 1999; Parmesan 2006; Sorte, Williams, & Zerebecki 2010; HilleRisLambers et al. 2013). A review of range expansion related to climate change found that about 75% of range shifts examined were in a poleward direction, which is predicted by warming temperatures associated with climate change (Sorte, Williams, & Carlton 2010). It is thought that many introduced species may be better adapted to a wide range of environmental factors (such as temperature, salinity, and dissolved oxygen), and their expansion may be facilitated by the projected warming effects of climate change (Sorte, Williams, & Carlton 2010; Sorte, Williams, & Zerebecki

2010). This has important implications for marine community structures—shifting ranges of both native and introduced species can affect a species' potential for survival and growth and can thus dramatically change interactions within marine communities.

Mussels in the genus *Mytilus* are important members of many marine communities. Four species—the bay mussels *Mytilus edulis, M. trossulus,* and *M. galloprovincialis,* and the larger California mussel *M. californianus*—are presently found in North America. The *M. edulis* species complex is composed of three species of bay mussel which are morphologically very similar and typically difficult to distinguish by external characters alone. The blue mussel, *M. edulis* is native to the north Atlantic Ocean (McDonald et al. 1991). The Mediterranean mussel, *M. galloprovincialis*, is considered native to the Mediterranean and southern Europe, and is found in South Africa, East Asia (including Japan), Chile, Australia, and the west coast of North America (Wilkins et al. 1983; Grant and Cherry 1985; McDonald and Koehn 1988; Varvio et al. 1988; Branch and Steffani 2004; Wonham 2004). *M. trossulus* is considered native to the North Pacific, the northeast coast of North America, and the North Baltic Sea (Varvio et al. 1988; McDonald et al. 1991, J. Carlton, *pers. comm.*). In the Eastern Pacific, it is important to look at the respective distributions of *M. galloprovincialis* and *M. trossulus* to understand the potential for range expansion in *M. galloprovincialis*.

Genetic testing is the most reliable way to distinguish *M. trossulus* from *M. galloprovincialis*. Before genetic criteria confirmed the existence of multiple species, it was widely accepted that *M. edulis* was cosmopolitan in temperate areas. In 1988, McDonald and Koehn used enzyme electrophoresis to look at the genetic structure of Pacific coast *Mytilus*, and discovered the absence of *M. edulis*, and the presence of two

different species in this genus: *Mytilus trossulus* and *M. galloprovincialis*. The native *Mytilus trossulus* occurred from northern California to Alaska, and can also be found in the Baltic Sea and eastern Canada, while the introduced *M. galloprovincialis* was found in central and southern California (Koehn et al. 1984; McDonald and Koehn 1988; McDonald et al. 1991; Geller 1999). McDonald and Koehn also reported that *M. galloprovincialis* and *M. trossulus* appear to hybridize in central California (McDonald and Koehn 1988).

Subsequent studies conducted in California and parts of Oregon have confirmed the presence of both *M. trossulus* and *M. galloprovincialis* on the Pacific coast. Analysis of 15 gene loci in mussels from 18 sites in California and 1 site in Alaska, based upon 1990-1991 collections, found that genetic hybridization between these mussels is rare in the areas of distribution overlap in California (Sarver and Foltz 1993). Suchanek et al. (1997) observed a latitudinal "transition zone" between the distributions of *Mytilus* spp. that lay between 40° and 41° latitude on both sides of the North Pacific. They also identified *Mytilus* spp. in the North Pacific using an adhesive protein gene (described in Inoue et al. 1995), and found genetic hybrids of *M. trossulus* and *M. galloprovincialis* in both San Francisco and, on rare occasion, San Diego (Inoue et al. 1995; Suchanek et al. 1997). This study also reported low frequencies of *M. galloprovincialis* in Coos Bay, OR and at Cape Flattery, WA, though subsequent sampling at those sites has not confirmed the establishment of *M. galloprovincialis* (Carlton and Geller 1993; Suchanek et al. 1997; Wonham 2004). Rawson et al. (1999) confirmed the presence of a "hybrid" zone in central California between Cape Mendocino and Monterey Bay, but found (using 5 gene markers) that hybridization only occurred in about 22% of samples within this region of

overlap and observed little indication of introgression. Braby and Somero (2006) used similar techniques to confirm the presence of a hybrid zone between Eureka and Monterey Bay; this study also found evidence for genetic hybridization at different sites within this zone. Whether *M. trossulus* and *M. galloprovincialis* form genetic hybrids capable of introgression, there is a zone of co-occurrence where both species are found in the same region; most studies agree that this zone lies in central California, although the boundaries are still not well understood (Sarver and Foltz 1993; Suchanek et al. 1997; Geller 1999; Rawson et al. 1999; Braby and Somero 2006a; Shinen and Morgan 2009; Lockwood and Somero 2011). More recent work has shown that more complex hybridization and co-occurrence patterns occur in the Puget Sound in Washington state and in British Columbia, but no persistent populations of *Mytilus galloprovincialis* have been found at open coast sites north of Crescent City, CA (Wonham 2004).

This project aims to further clarify the region of overlap in the species ranges of the native mussel *Mytilus trossulus* and the introduced mussel *M. galloprovincialis* in California and Oregon, using more extensive sampling and larger sample sizes than previous studies within the same system.

## METHODS

### Sample Collection

To examine the current distribution of *Mytilus* spp. on the Pacific coast 2,233 mussels were collected from the harbors in California and Oregon (Table 1, Figure 1). When possible, individuals were kept alive for up to 48 hours on ice and wrapped in seawater-dampened material (rather than multiple individuals placed together in water, in order to prevent post-collection transfer of symbionts) until dissection and tissue

collection. If mussels died before processing, they were frozen at -20°C and thawed before dissection.

Mussels were measured (shell length and width), opened, visually inspected for commensals or parasites, and the entire gill tissue was dissected out and either placed directly into a DNA extraction buffer or placed in 90% ethanol for later extraction.

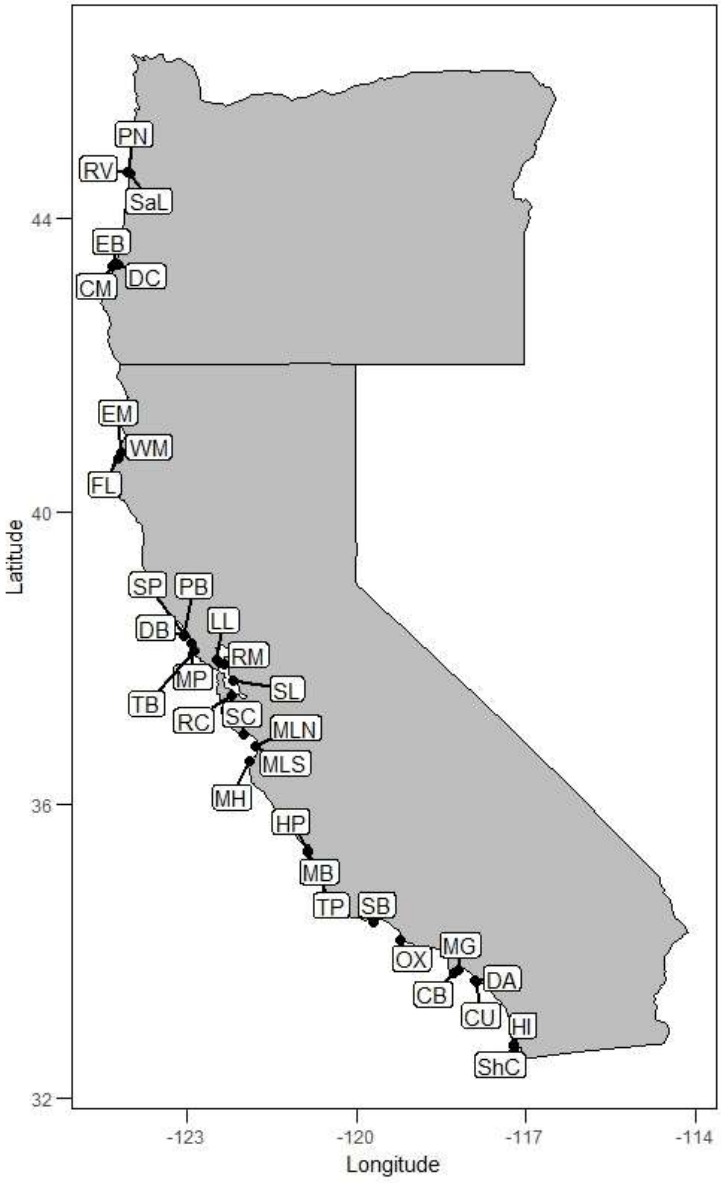


**Figure 1. Map of sampling sites in California and Oregon for 2013-2015 *Mytilus* spp. survey.**

**Table 1. Field sites and samples collected.**

| Bay Region | Site Code | Sample Number | Sampling date | Site Description |
|---|---|---|---|---|
| Yaquina | PN | 87 | 26-Sep-14 | Port of Newport |
| | RV | 72 | 26-Sep-14 | RV Park Marina |
| | SaL | 81 | 26-Sep-14 | Sawyer's Landing Dock |
| Coos | CM | 127 | 30-Sep-14 | Charleston Marina |
| | DC | 51 | 29-Sep-14 | Downtown Coos Bay |
| | EB | 52 | 29-Sep-14 | Empire Boat Launch |
| Humboldt | EM | 81 | 3-Oct-14 | Eureka Marina |
| | FL | 76 | 4-Oct-14 | Fields Landing Boat Launch |
| | WM | 88 | 5-Oct-14 | Woodley Marina |
| Bodega | DB | 34 | 7-Oct-14 | Doran Boat Launch |
| | PB | 41 | 7-Oct-14 | Porto Bodega Marina |
| | SP | 50 | 7-Oct-14 | Spud Point Marina |
| Tomales | MP | 37 | 7-Oct-14 | Miller Park Boat Launch |
| | TB | 65 | 7-Oct-14 | Tomales Bay Resort Marina |
| San Francisco | LL | 102 | 16-Nov-15 | Loch Lomond Marina |
| | RC | 67 | 13-Oct-14 | Redwood City Yacht Club |
| | RM | 97 | 16-Nov-15 | Richmond Marina |
| | SL | 61 | 13-Oct-14 | San Leandro Marina |
| Monterey | SC | 50 | 16-Nov-15 | Santa Cruz Harbor Boat Launch |
| | MLN | 49 | 12-Nov-15 | Moss Landing – North Boat Launch |
| | MLS | 48 | 12-Nov-15 | Moss Landing Harbor – South |
| | MH | 51 | 16-Nov-15 | Monterey Harbor |
| Morro | HP | 54 | 10-Nov-15 | Harbor Patrol Dock |
| | MB | 54 | 10-Nov-15 | Morro Bay Marina |
| | TP | 51 | 10-Nov-15 | Tidelands Park |
| Santa Barbara | SB | 84 | 7-Nov-15 | Santa Barbara Marina |
| Oxnard | OX | 71 | 7-Nov-15 | Channel Islands Harbor Patrol |
| Long Beach | CB | 74 | 7-Nov-15 | Cabrillo Boat Launch |
| | MG | 50 | 7-Nov-15 | Marina Green Boat Launch |
| Newport | DA | 48 | 16-Sep-15 | Dunes Aquatic Marina |
| | CU | 43 | 16-Sep-15 | Condo Unit Private Dock |
| San Diego | HI | 130 | 7-Aug-13 | Harbor Island Marina |
| | ShC | 107 | 8-Aug-13 | Shelter Cove Marina |
| **Total** | | **2233** | | |

Samples collected for distributional survey, 2013-15. Sample sites are shaded by bay region. Sample number indicates number of individuals genetically identified from that site.

## Species Identification

DNA was extracted using the MagJET Genomic kit that uses magnetic beads to purify DNA from animal tissues (© 2013 Thermo Fisher Scientific). Up to 200 mg of mussel gill tissue was placed in the digestion solution provided, and after a 12-16-hour incubation at 56º, the extraction continued, following the manufacturer's protocol for tissue extraction but at one-fourth of the recommended volume (J. Geller, *pers comm.*). Extractions were carried out in 96-well plates, with two no-template controls (NTCs) to check for well-to-well contamination: well D05 was reserved as the extraction control, while A01 served as a control for subsequent amplification (described below).

The species of all samples was identified using polymerase chain reaction (PCR) to amplify a region of the nuclear adhesive protein gene (Me15/16) that differs in length for each species in the *Mytilus* genus (Inoue et al. 1995). This gene is also able to detect genetic hybridization among the three bay mussel species (Inoue et al. 1997). PCR was conducted using a three-step program (3-minute initialization at 94°C, and 30 cycles of the following: (1) 1-minute denaturation at 94°C, (2) 2-minute annealing at 47°C, (3): 45-second extension at 72°C). PCR products were analyzed by gel electrophoresis on a 2.0% MetaPhor (© 2007 Lonza) agarose gel made with 1X Tris-Borate-EDTA buffer and stained with ethidium bromide. Samples were scored by product size as *M. galloprovincialis* (126-bp product), *M. trossulus* (168-bp product), or as hybrids with both products (visualized as bands at different points on the gel, see Inoue et al. 1995).

## Historical Samples

Historical survey data were compiled from Braby and Somero (2006) and from a mussel monitoring program conducted in Moss Landing Harbor (S. Bartl and H. Kibak, *pers. comm.*).

## Statistical Analysis

Data analysis was conducted using the program R Studio (© CRAN, R version 3.3.2). The California-Oregon distribution data were examined using a Chi-squared analysis comparing expected and observed proportions of *M. galloprovincialis* and *M. trossulus* at each sampling site. Expected values were estimated from data reported by Braby and Somero (2006), and the null hypothesis is that there will be no difference between population frequencies from 2006 to 2013-15.

# RESULTS

## Size and Genotype Distributions

A total of 2,233 mussels from 10 bay regions in California and Oregon were collected between September 2013 and November 2015 and successfully amplified using the Me15/16 primers for the size-variable region of the nuclear adhesive protein gene region. Genotype results from all sites (in and outside the hybrid zone) were combined and partitioned into 10-mm shell length bins (excepting the largest mussels, binned as >100 mm), and were visualized as the percent of each species (genotype) in each size bin (Figure 2) and the genetic makeup of each size bin (Figure 3). *Mytilus trossulus* (n = 780) shows a peak in abundance in the smaller size classes (1-50 mm), and few individuals larger than 50mm were identified as *M. trossulus*. *Mytilus galloprovincialis* (n = 1,414) was more prevalent in the larger size classes. The largest specimens collected (>80 mm

shell length) were identified as *M. galloprovincialis.* Few hybrids (n = 39) were found overall but occurred in larger size classes than *M. trossulus* (>70 mm).

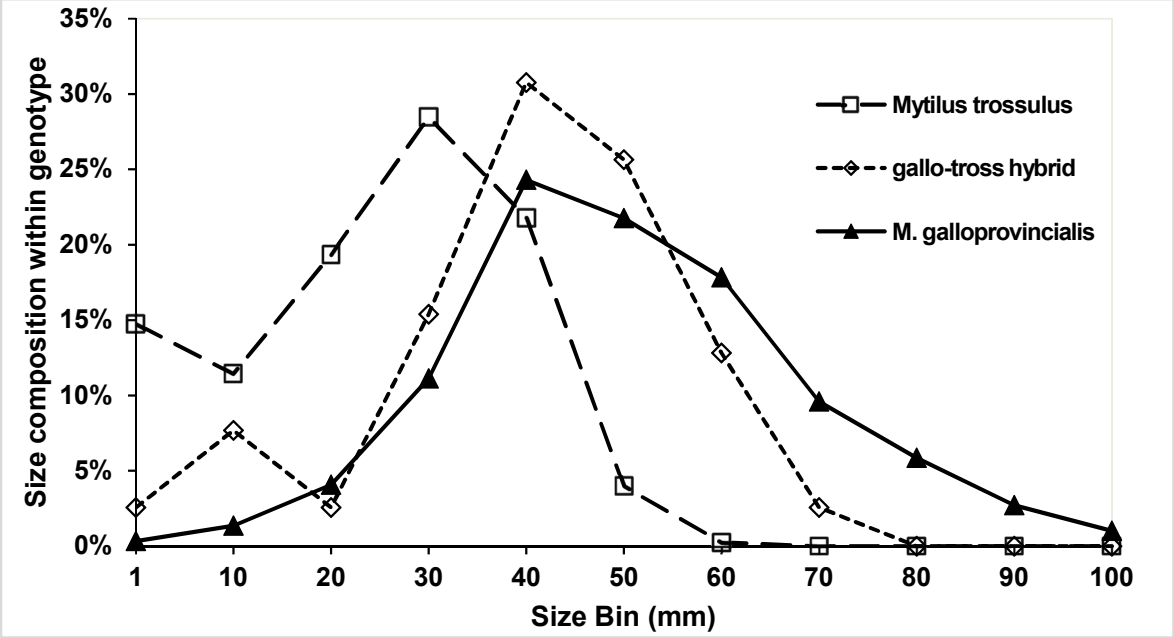


**Figure 2. *Mytilus* spp. size composition within genotype.**

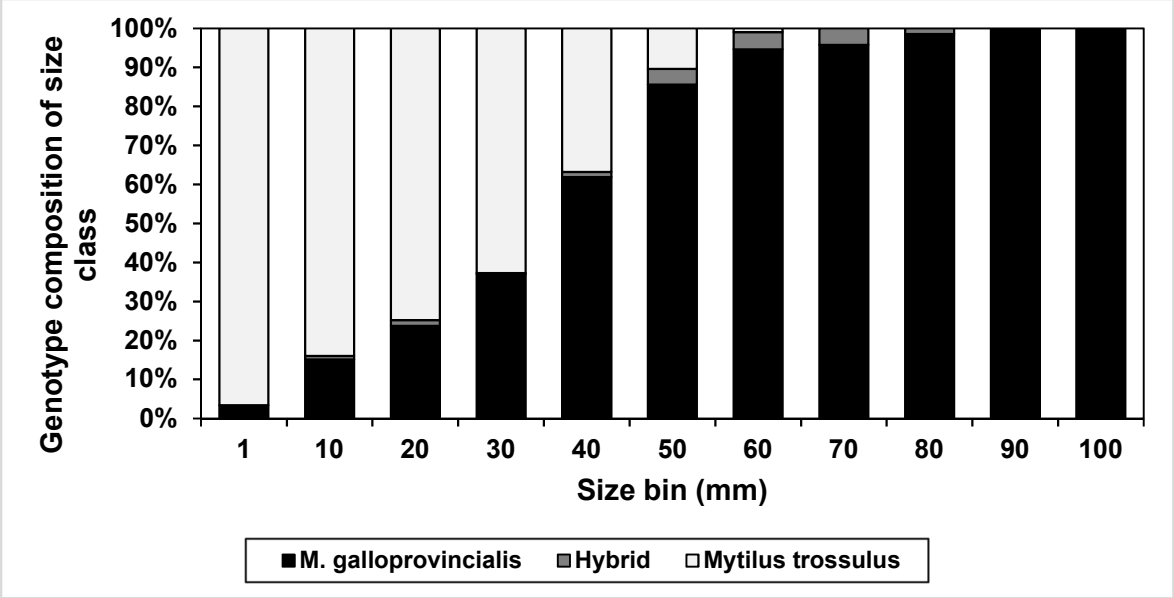


**Figure 3. *Mytilus* spp. genotype frequency distributions by size class (shell length).**

## Genotype Distributions by Region

The genotype distributions throughout the sampling range showed distinct spatial patterns of species distributions in bay mussel populations (Table 2, Figure 4). Populations in Oregon were comprised of only the native *Mytilus trossulus*. The proportion of *M. trossulus* in each California region declined progressively to the south. There appeared to be a sharp decline in *M. trossulus* south of Bodega Harbor, where the overall population was split evenly (though there is evidence of variation in genotype distributions within Bodega Harbor itself). Tomales Bay contained a persistent population of *M. trossulus*, but the overall population was predominantly comprised of *M. galloprovincialis*. This trend of increasing *M. galloprovincialis* genotypes holds in locations further south – sampling sites in San Francisco Bay showed no *M. trossulus* genotypes, and Monterey Bay showed very small persistent populations of *M. trossulus* only in Moss Landing. These results also suggest small hybrid genotype populations occurring in the previously established hybrid zone (Braby and Somero 2006a), though in smaller proportions than previously recorded for these regions. Southern California mussel populations are comprised only of *M. galloprovincialis* genotypes.

**Table 2. Proportion of *Mytilus* spp. at each site**

| Site | *M. galloprovincialis* | Hybrid | *M. trossulus* |
|---|---|---|---|
| PN | 0 | 0 | 1 |
| RV | 0 | 0 | 1 |
| SaL | 0 | 0 | 1 |
| CM | 0 | 0 | 1 |
| DC | 0 | 0 | 1 |
| EB | 0 | 0 | 1 |
| EM | 0 | 0.025 | 0.975 |
| FL | 0 | 0 | 1 |
| WM | 0.148 | 0.034 | 0.818 |
| DB | 0.206 | 0.088 | 0.706 |
| PB | 0.463 | 0.024 | 0.512 |
| SP | 0.580 | 0.060 | 0.360 |
| MP | 0.568 | 0.027 | 0.405 |
| TB | 0.985 | 0.015 | 0 |
| LL | 0.951 | 0.049 | 0 |
| RC | 1 | 0 | 0 |
| RM | 1 | 0 | 0 |
| SL | 1 | 0 | 0 |
| SC | 0.720 | 0.280 | 0 |
| MLN | 0.939 | 0.041 | 0.020 |
| MLS | 0.875 | 0.042 | 0.083 |
| MH | 0.961 | 0.039 | 0 |
| HP | 1 | 0 | 0 |
| MB | 1 | 0 | 0 |
| TP | 1 | 0 | 0 |
| SB | 1 | 0 | 0 |
| OX | 1 | 0 | 0 |
| CB | 1 | 0 | 0 |
| MG | 1 | 0 | 0 |
| DA | 1 | 0 | 0 |
| CU | 1 | 0 | 0 |
| HI | 1 | 0 | 0 |
| ShC | 1 | 0 | 0 |

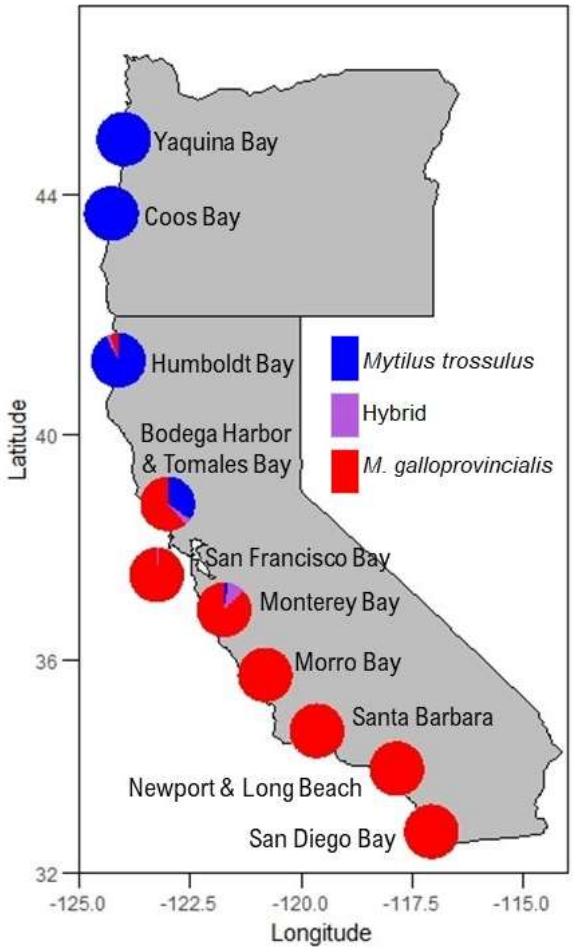


**Figure 4. *Mytilus* spp. distribution by bay region.**

## Spatial Variation Within the Hybrid Zone

Figure 5 shows relative proportions of *Mytilus trossulus, M. galloprovincialis,* and hybrids in each site sampled within the hybrid zone described by Braby and Somero (2006). In Humboldt Bay, *M. trossulus* genotypes were found in abundance at all sampling sites. Hybrid genotypes were found at Eureka and Woodley Marinas, and *M. galloprovincialis* was only found at Woodley Marina.

All Bodega Harbor sites contained all three genotypes. The highest frequency of *M. galloprovincialis* occurred at Spud Point Marina, and the highest frequencies of both *M. trossulus* and hybrid genotypes were observed at Doran Beach, which is closer to the

mouth of the harbor than either Spud Point and Porto Bodega. Similarly, all three genotypes were also found in Tomales Bay, but *M. trossulus* genotypes were only present at Miller Park. Hybrid genotypes were found at low frequency (<10%) in both sites sampled.

In San Francisco Bay, no pure *M. trossulus* genotypes were detected. Hybrids were only found at Loch Lomond Marina and made up <10% of the mussels sampled at that site (1.5% of total 327 samples). All other sites in San Francisco Bay were found to contain only *M. galloprovincialis*.

*Mytilus trossulus* was detected in Monterey Bay, although only in the two Moss Landing sampling sites, and made up about 2-8% of samples in each of those sites. Hybrids were found at all sites in Monterey Bay, with the highest frequencies found at Santa Cruz Harbor. Across all four Monterey Bay sampling sites, *M. galloprovincialis* accounted for 87% of all 198 samples.

Samples from Morro Bay and all sampling sites further south were found to be comprised of 100% *M. galloprovincialis.*

## Comparison to Previous Surveys

Chi-squared analysis across all sites (Table 3), using data reported in Braby and Somero (2006) for samples collected in 2003 as the expected frequency for each bay region, shows that the species composition of the populations observed in this study are significantly different than those found by Braby and Somero (2006) [$\chi^2$ (d.f. = 18) = 597.35, $p << 0.001$].

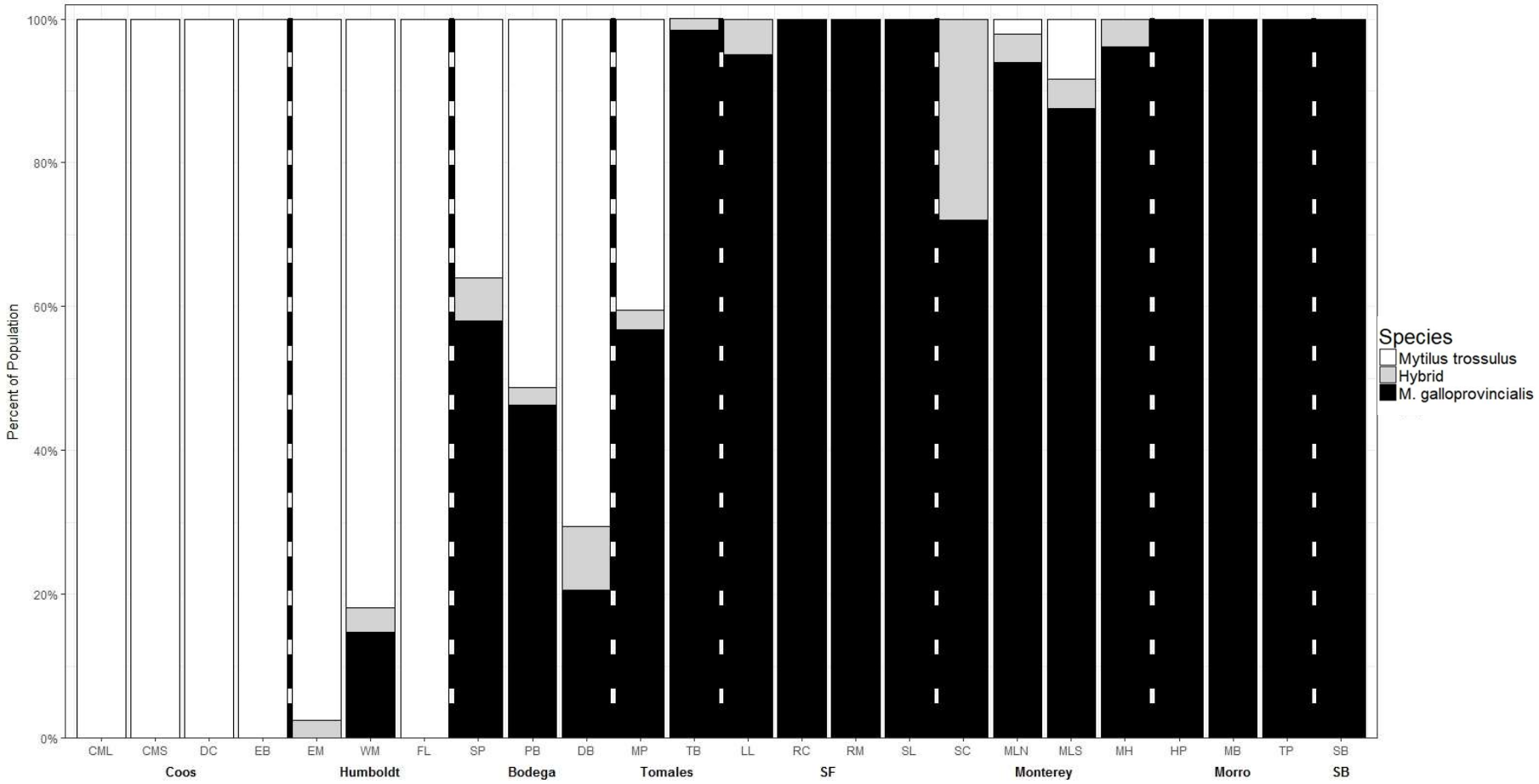


**Figure 5.** ***Mytilus*** **spp. genotype distribution by site within the hybrid zone.**

| Bay | N | *Mytilus galloprovincialis* | | | | Hybrids | | | | *Mytilus trossulus* | | | | Σ ($X^2$) by Bay |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Exp Freq | Exp Num | Obs | $X^2$ | Exp Freq | Exp Num | Obs | $X^2$ | Exp Freq | Exp Num | Obs | $X^2$ | |
| Coos | 277 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 257 | 257 | 0 | 0 |
| Humboldt* | 245 | 0.32 | 78.4 | 13 | 54.55 | 0.15 | 36.75 | 5 | 27.43 | 0.53 | 129.85 | 227 | 72.68 | 154.66* |
| Bodega | 125 | 0.58 | 72.5 | 55 | 4.22 | 0.115 | 14.375 | 7 | 3.78 | 0.305 | 38.125 | 63 | 16.23 | 24.23 |
| Tomales | 102 | 0.61 | 62.22 | 85 | 8.34 | 0.03 | 3.06 | 2 | 0.37 | 0.36 | 36.72 | 15 | 12.85 | 21.56 |
| San Francisco* | 312 | 0.507 | 158.08 | 322 | 140.00 | 0.173 | 53.976 | 5 | 44.44 | 0.32 | 99.84 | 0 | 99.84 | 284.28* |
| Monterey* | 197 | 0.51 | 100.47 | 173 | 50.93 | 0.254 | 50.038 | 20 | 18.03 | 0.236 | 46.492 | 5 | 37.03 | 105.99* |
| Morro | 159 | 0.96 | 152.64 | 159 | 0.27 | 0.04 | 6.36 | 0 | 6.36 | 0 | 0 | 0 | 0 | 6.63 |
| | | | | | | | | | | | | | **Total Σ ($X^2$)** | 597.35 |

N is number of samples collected from each bay, ExpFreq is the expected frequency of a genotype (calculated from Braby and Somero 2006a), ExpNum is expected number of each species based on N, and Obs is observed number of each species. $X^2$ is the Chi-squared value for each species in each bay, where higher values indicate greater differences between expected and observed populations, and smaller (close to 0) values indicate highly similar expected and observed populations. Total Σ ($X^2$) is the overall sum of Chi-squared values (d.f. = 18, $p < 0.001$), and indicates that in the hybrid zone, expected and observed populations are highly dissimilar. * indicates $X^2$ values for bays that are driving these differences.

**Table 3. Hybrid zone Chi-squared results.**

Further examination of the contingency table (Table 3) suggests that the observed frequency values for Humboldt Bay, San Francisco Bay, and Monterey Bay were likely behind the differences between these two data sets. This indicates that significant increases in the proportions of *Mytilus galloprovincialis* and decreases in the proportions of *M. trossulus*, in these mussel populations have occurred in these specific regions over the past decade.

## Temporal Variation at One Location

Repeated annual sampling allows an examination of how mussel populations changed over time at one site. *Mytilus* spp. population data from Moss Landing, CA were compiled from Braby and Somero (collected 2002, published 2006), data collected by Dr. Simona Bartl and Henrik Kibak (collected 2005-9, unpublished), and the present survey (collected 2015) (Figure 6). *Mytilus galloprovincialis* comprised >50% of the Moss Landing population from 2005-2008, and in 2015. The proportion of the hybrid *Mytilus* population varied between 4-28% of the total sample over all years, and the frequency of *M. trossulus* peaked in 2009 (~70% of all samples). Because sample size is low and unequal throughout this sampling period, statistical analysis is not appropriate for these data, but they do reveal that the population of *Mytilus* spp. is dynamic with respect to relative proportions of *M. galloprovincialis*, *M. trossulus*, and their genetic hybrids.

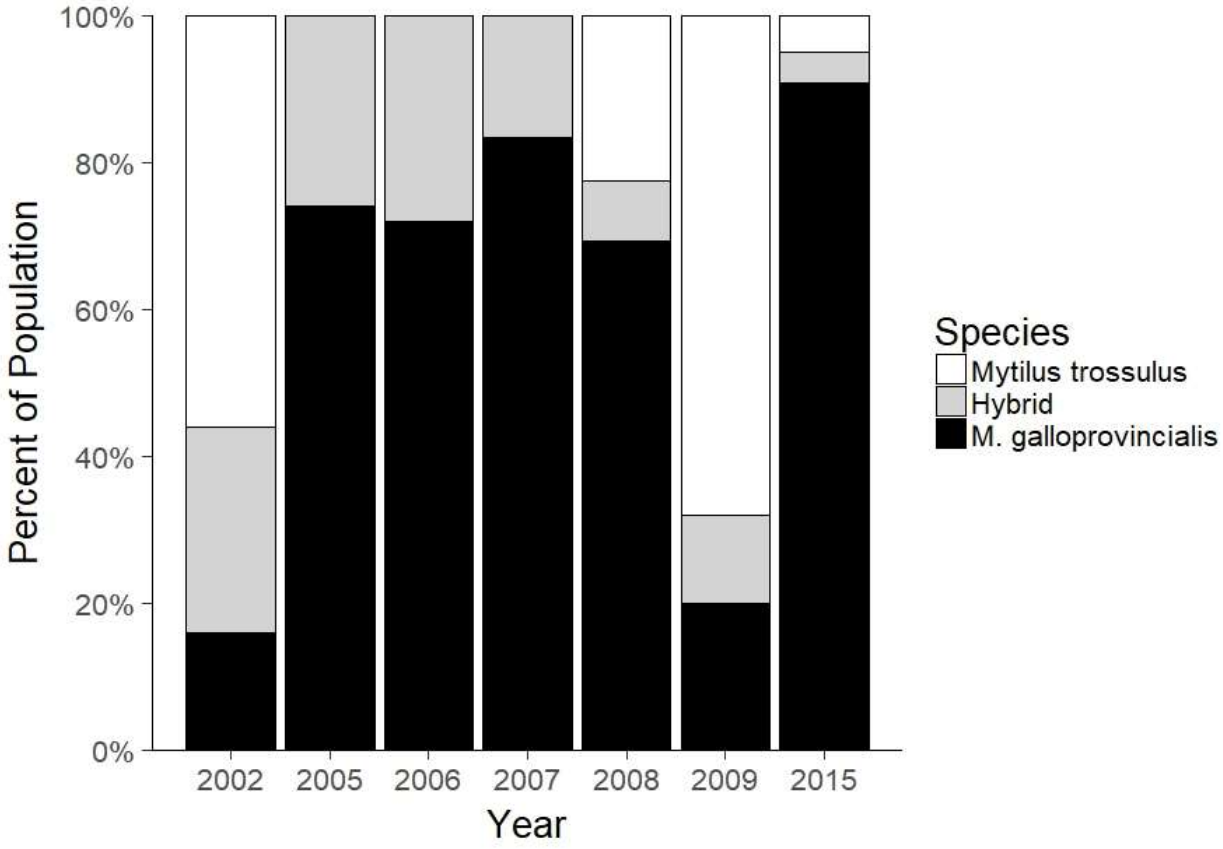


**Figure 6. *Mytilus* spp. genotype distribution in Moss Landing, CA from 2002-2015.**

## DISCUSSION

For many of the *Mytilus* spp. populations sampled in this survey, sampling effort has been significantly increased from past surveys – for example, only 13 mussels were collected from one site in Humboldt Bay by Braby and Somero (2006), compared to 245 from three sites, as presented here. These larger sampling sizes, coupled with collections from multiple sites within a bay or region allows for a finer-scale understanding of the spatial variability of mussel populations within large areas of habitat, and identifies Monterey Bay, San Francisco Bay, Tomales Bay, and Bodega Harbor as potential future study areas to determine which environmental characteristics (if any) may be driving these differences.

While past work on the distributions of *M. galloprovincialis* has established wild populations outside of aquaculture facilities in Puget Sound and British Columbia (Wonham 2004), the Oregon coastline (at least, the central and southern coast, as confirmed by the data presented here) has remained unaffected by the spread of *M. galloprovincialis* in neighboring regions. Wonham (2004) suggests that Oregon's lack of mussel aquaculture and relatively low capacity for trans-Pacific shipping (save the wood trade in Coos Bay) may be behind the absence of established *M. galloprovincialis* alleles in adult populations. On the other hand, larvae of *M. galloprovincialis* have been isolated repeatedly from the ballast water of ships in Coos Bay in the 1990s (Carlton and Geller 1993; Suchanek et al. 1997). The failure of repeated release of larvae to establish adult populations suggests environmental mismatch for early life history stages. In recent years, ballast water in U.S. ports has become more regulated, often requiring offshore ballast water exchange or treatment of ballast water before discharging in port, which may decrease further exposure of Oregon's coast to *M. galloprovincialis* larvae.

As will be discussed in Chapter 2 of this work, very large numbers (estimated at 100s of 1000s of individuals; J. T. Carlton, *pers. comm*.) of living adult *M. galloprovincialis* landed on the Oregon and Washington coasts on Japanese tsunami marine debris between 2012 and 2017 (Carlton et al. 2017, 2018; Miller et al. 2018). Ocean rafting thus provided a novel mechanism for the transfer to North America of *M. galloprovincialis*, in this case from the Western Pacific Ocean, to where it was also introduced in the 20th century. Nevertheless, our collections up to November 2015 failed to detect any colonization by *M. galloprovincialis* in Oregon.

High freshwater input in the smaller bays/bodies of water of the Oregon and Washington coasts seems to be a barrier to the establishment of *M. galloprovincialis* in these regions. The bays and estuaries of Oregon and Washington (south of Cape Flattery) are relatively small (when compared to Puget Sound, San Francisco Bay, Monterey Bay, and San Diego Bay), and have much higher riverine input than similarly-sized bays in northern California (Bodega Harbor, Tomales Bay, Morro Bay, Humboldt Bay) due to higher average annual rainfall and smaller proximate human populations. The Puget Sound is also a complex region, comprised of differential thermohaline regimes, habitat types, and hydrology that may affect the dispersal and survival of *M. galloprovincialis* and *M. trossulus* in different ways (Moore et al. 2008).

Increased frequencies of *Mytilus galloprovincialis* and significantly decreased frequencies of the native *M. trossulus* in northern California (most notably in San Francisco Bay and Monterey Bay) indicate that the southern edge of the *M. galloprovincialis-trossulus* hybrid zone is moving northward. This may be partially due to increased drought conditions in California in recent decades.

Warmer surface water and less variable salinities (a result of infrequent rainfall) may be facilitating the spread of *M. galloprovincialis* northward in California as *M. galloprovincialis* is more tolerant of heat exposure than is *M. trossulus* (Braby and Somero 2006b; Lockwood and Somero 2011). In northern California, particularly in Monterey Bay, San Francisco Bay, and Tomales Bay, *Mytilus trossulus* larvae may be settling in fewer numbers or could have high post-settlement mortality due to thermohaline stressors or competition with similarly-aged *M. galloprovincialis.* Prolonged drought periods could open up more space in northern California harbors and

estuaries for *M. galloprovincialis* to occupy and replace the native, less tolerant *M. trossulus*.

As the global climate changes, the west coast of North America is likely to experience more frequent, prolonged periods of drought. Climate models for California predict warmer monthly air temperatures throughout the year, little change in annual precipitation rates, and stronger but more episodic winter storm events (Cayan et al. 2007). This likely translates to warmer sea surface temperatures and reductions in freshwater inputs in harbors and estuaries, which would increase the amount of suitable habitat for *M. galloprovincialis* in northern California and possibly in southern Oregon, resulting in increasing frequencies of *M. galloprovincialis* (and hybrids) in these areas. Some evolutionary models suggest that populations can adapt to physical stressors over short time scales (reviewed in Munday et al. 2013), but warming temperatures coupled with expanding populations of *M. galloprovincialis* may result in net loss of habitat and species replacement for *M. trossulus*, even if a population on its own may adapt to heat stress over several generations. The ideal environments occupied by each species likely goes further than the temperature and salinity ranges explored by many groups interested in these physiological limits of *M. trossulus* and *M. galloprovincialis*. A multitude of physical factors (e.g. currents, available habitat types, mean temperatures, etc.) and biological factors (e.g. food availability, tolerance to environmental fluctuations, competition, etc.) influence the large- and fine-scale distributions of these two species.

It is important to continue monitoring changes in species boundaries as climate and human use of marine environments change – especially since we do not yet fully understand how native and non-native *Mytilus* spp. may be interacting with other species

in estuarine communities. While some work has examined filtration rates and niche partitioning in bay mussels (particularly in *M. edulis* and *M. galloprovincialis*, in other regions), it is unknown if similarly-sized individuals of *M. trossulus* and *M. galloprovincialis* have similar energetic requirements or filtration rates, especially in areas where they coexist (Ackerman and Nishizaki 2004; Bownes and McQuaid 2010; Nielsen and Vismann 2014). Differences in mussel filtration rates might affect which organisms can settle and occupy the environment around *Mytilus* spp., and it are likely important to understand how species (particularly non-natives) associate with native and introduced mussel beds in harbors and estuaries. Additionally, *M. trossulus* and *M. galloprovincialis* may interact with each other within an aggregation, similar to the interactions between the outer coast mussel (*M. californianus*) and bay mussels (identified at the time as *M. edulis*, but probably all or largely *M. galloprovincialis*) in the rocky intertidal in southern California described by Harger (1970; 1972). Investigating these potential interactions will not only help to fully understand how the replacement of *M. trossulus* by *M. galloprovincialis* may affect estuarine communities, but will also give a broader idea of how introduced species can have small-scale impacts on their communities.

# CHAPTER 2

# *MYTILUS* SPP. ON JAPANESE TSUNAMI MARINE DEBRIS

## INTRODUCTION

Human-influenced transport and introduction of marine organisms on a trans-oceanic scale are not limited to human-aided transport mechanisms (e.g. on the bottom of ships). In March 2011, the Tohoku Earthquake (magnitude 9.0 $M_w$) and consequent tsunami generated tons of marine debris (including docks, vessels, buoys, and other material) which were moved offshore of Japan (Carlton et al. 2017). This floating debris, some of which supported existing fouling communities, has slowly been transported across the Pacific Ocean. Since the summer of 2012, when a floating dock landed on Agate Beach in Oregon covered in live organisms, debris has been reported in Hawaii and on the US west coast, from Alaska to California (Carlton et al. 2017). Phenomena of this grand scale are rare yet have the potential to act as a vector for the large-scale transport of species across an ocean basin.

*Mytilus* spp. were one of the most common organisms found on the debris in all landing sites (Miller et al. 2017). Both *Mytilus trossulus* and *M. galloprovincialis* are found in Japanese harbors (Inoue et al. 1997; Nakaoka et al. 2006). *Mytilus trossulus* is native to Japan, while *M. galloprovincialis* has been introduced (likely by shipping), and has replaced *M. trossulus* in many regions (Inoue et al. 1997). The Asian mussel, *Mytilus coruscus*, is also present on the Japanese coast, among the aforementioned *M.*

*galloprovincialis* and *M. trossulus* (Nakaoka et al. 2006). To date, *M. coruscus* has not been observed in established mussel populations in the Eastern Pacific.

The debris generated by this incident presents a novel opportunity to study both the potential for successful transoceanic dispersal of marine species, as well as the mechanisms of genetic transfer between populations of the same species that occur on opposite sides of an ocean basin (J. Geller, *pers. comm.*). The introduction of *Mytilus coruscus*, or novel genes of *M. galloprovincialis* and *M. trossulus* from Asia, could also have profound consequences for the communities in this region through competition with native populations of *Mytilus* spp., or through the introduction of associated species which are not currently found in the Eastern Pacific.

Direct observation and DNA analysis have also revealed the presence of *Eutima japonica*, a bivalve-inhabiting hydrozoan, among the mussels collected from tsunami debris (J. Carlton, J. Geller, & G. Ruiz *pers. comm.*). Bivalve-inhabiting hydrozoans in Japan (including three forms of *Eutima japonica*, and the genetically distinct related species *Eugymnanthea japonica*) live inside the mantle cavity of their host and have been observed in multiple bivalve species, such as the introduced *M. galloprovincialis* and native Japanese mussels, scallops, oysters, and clams (Kubota 1992; Piraino et al. 1994; Baba et al. 2007; Calder et al. 2014). No similar native species has been found in bivalves in the Eastern Pacific, and there have been no documented cases of *Eutima japonica* as established introduction (G. Ruiz, *pers. comm*.).

In Japan, *Eutima japonica* can move from one host species to another, presumably via swimming larvae released from reproductive medusae (Baba et al. 2007). This suggests that introduced hydrozoans would spread to other nearby bivalves, once they are

established in one hose species. The nature of the relationships between hydrozoans and their bivalve hosts is largely unknown, but some authors consider them commensal (Kubota 1992; Calder et al. 2014). However, *Eutima japonica* on Japanese scallops in aquaculture has been associated with severely reduced juvenile survival (Baba et al. 2007). If bivalve-inhabiting hydrozoans become established in the Eastern Pacific, their dispersal may impact established bivalve populations (native or introduced, natural or cultured populations). In the Eastern Pacific, especially in California, the open coast mussel *M. californianus* is important in native rocky coast intertidal communities. A strong impact on this mussel (as a result of competition with congeners or the introduction of a parasite) could have profound consequences for the communities in this region.

This chapter aims to assess the potential for introduction of non-native mussels (and their associates) introduced via Japanese tsunami marine debris to existing populations, based on population frequencies of *Mytilus spp.* identified from Japanese debris. Secondarily, mussels were genetically screened for the presence of *Eutima japonica* to determine the frequency with which tsunami debris mussels were infected with the bivalve-inhabiting hydrozoan.

## METHODS

Samples were collected from 22 Japanese tsunami debris landfalls by collaborators from Oregon State University, Portland State University / Smithsonian Institution, and Williams College (Table 4). Individuals of *Mytilus* spp. from these landfalls were dissected, and tissue samples were preserved in 90% ethanol and sent to Moss Landing Marine Laboratories for molecular identification. DNA was extracted

using the MagJET Genomic kit, the nuclear adhesive protein gene was amplified using PCR, and the results were visualized and scored as described previously (see Chapter 1).

**Table 4. List of tsunami debris items sampled for *Mytilus* spp.**

| Object Number | Sampling Date | Object Description | Sample Number | Location |
|---|---|---|---|---|
| BF001 | 07-Jun-12 | Misawa dock (M1) | 101 | Agate Beach, OR |
| BF002 | 15-Jun-12 | Boat | 25 | Ilwaco, WA |
| BF006 | 01-Dec-12 | Boat | 67 | Kahana Bay, Oahu, HI |
| BF008 | 21-Dec-12 | Misawa dock (M3) | 113 | Olympic National Park, WA |
| BF011 | 24-Dec-12 | Boat | 5 | Punaluu, Oahu, HI |
| BF012 | 28-Dec-12 | Boat | 19 | Damon Point, Gray's Harbor, WA |
| BF017 | 09-Jan-13 | Float | 8 | Hanauma Bay, Oahu, HI |
| BF021 | 18-Jan-13 | Buoy | 5 | Nohili Pt, Kauai, HI |
| BF023 | 05-Feb-13 | Boat | 80 | Gleneden Beach, OR |
| BF024 | 09-Feb-13 | Wood pallet | 31 | South Beach, OR |
| BF027 | 14-Feb-13 | Pontoon | 7 | Makapuu Beach, Oahu, HI |
| BF028 | 20-Feb-13 | Boat | 33 | Horsfall Beach, OR |
| BF030 | 28-Feb-13 | Vessel fragment | 3 | Lincoln City, OR |
| BF031 | 04-Mar-13 | Rope float | 3 | Laie, Oahu, HI |
| BF040 | 22-Mar-13 | Boat | 27 | Long Beach, WA |
| BF043 | 10-Apr-13 | Boat | 27 | Lincoln City, OR |
| BF048 | 19-Apr-13 | Wood post and beam | 9 | Nye Beach, OR |
| BF049 | 04-Mar-13 | Blue bin | 28 | Lanikai Beach, Oahu, HI |
| BF135 | 18-Feb-14 | Yachats #4 | 18 | Yachats, OR |
| BF137 | 22-Feb-14 | Wood pieces | 2 | Newport, OR |
| BF170 | 23-Apr-14 | Boat | 6 | Long Beach, WA |
| BF177 | 28-Apr-14 | Boat | 1 | Ocean Shores, WA |
| **Total mussels sampled:** | | | 618 | |

Additionally, a region of the mitochondrial gene for *cytochrome c oxidase subunit III* (COIII) was amplified via PCR for these samples (Table 5) (Stewart et al. 1995). The efficacy of the adhesive protein gene marker for identifying the Asian mussel *Mytilus coruscus* is not known, but the mitochondrial genomes for *M. coruscus, M. trossulus,* and *M. galloprovincialis* have been sequenced and deposited in GenBank (NCBI), which allows for identification via BLAST comparison. PCR was conducted using a three-step program (3-minute initialization at 94°C, and 30 cycles of the following: (1) 1-minute denaturation at 94°C, (2) 1-minute annealing at 50°C, (3): 1-minute extension at 72°C). PCR products were analyzed by gel electrophoresis on a 1.5% agarose gel made with 1X Tris-Acetate-EDTA buffer and stained with ethidium bromide. COIII PCR products from mussels collected on debris were sequenced using an IonTorrent Personal Genome Machine at Moss Landing Marine Laboratories. The sequenced DNA fragments were assembled, and the sequences were analyzed using the sequence editing program Geneious version 9 (http://www.geneious.com, Kearse et al. 2012). The resulting consensus sequences were aligned to *Mytilus* spp. references from GenBank and MLML's reference sequence database using the BLAST function.

Tsunami debris mussel DNA was also used to test for the presence of *Eutima japonica*. Tissue samples were sent to MLML pre-dissected and only a subsample of gill from each mussel was received for genetic work, so we were unable to visually screen for *Eutima japonica*. Instead, a primer set was developed to amplify a region of the *E. japonica* gene for *cytochrome c oxidase subunit I* (COI) (J. Geller, *pers. comm.*) and a PCR assay on the mussel DNA samples determined what samples may include tissue or DNA from *E. japonica*. Primers were tested with DNA from a mussel that was visually

confirmed to contain *Eutima japonica*, a mussel collected outside of the known range of *Eutima japonica*, and a PCR-grade water blank. PCR was conducted using a three-step program (3-minute initialization at 94°C, and 33 cycles of the following: (1) 1-minute denaturation at 94°C, (2) 1-minute annealing at 50°C, (3): 1-minute extension at 72°C). PCR products were analyzed by gel electrophoresis on a 1.5% agarose gel made with 1X Tris-Acetate-EDTA buffer and stained with ethidium bromide - samples were scored for *Eutima japonica* presence or absence, determined by presence or absence of bands on the gel.

**Table 5. PCR primers used to identify *Mytilus* spp.**

| Gene Region | Primer Name | Primer sequence |
|---|---|---|
| COIII | MytCOIIIF | 5'-GTAACTCAAGCCCATAAGAG-3' |
| | MytCOIIIR | 5'-ATGCTCTTCTTGAATATAAGCGTACC-3' |
| Adhesive protein | Me15 | 5'-CCAGTATACAAACCTGTGAAGA-3' |
| | Me16 | 5'-TGTTGTCTTAATAGGTTTGTAAGA-3' |
| COI | Eutima_japonica-COIF | 5'-CGGAACAGCTTTAAGTATGTTAAT-3' |
| | Eutima_japonica-COIR | 5'-GCACAATGTAAACTAAATATTGCC-3' |

Primer names and sequences for PCR assays used in this study. The COIII primer pair amplifies a region of the mitochondrial gene for *cytochrome c oxidase subunit III* (Stewart et al., 1995). The adhesive protein primers amplify a nuclear gene region for the foot adhesive protein of *Mytilus* spp. which varies in length by species (Inoue et al., 1995). *Eutima japonica* assay primers were developed by J. Geller.

## RESULTS

A total of 618 mussels across 22 tsunami debris objects were sampled for *Mytilus* spp. between 2012-2014 (Table 4). Only 255 mussels were successfully amplified using the adhesive protein primers, while 618 mussels were amplified and sequenced using the COIII primers (Table 6). For the COIII gene, across all debris items, 94% of all mussels

identified were *M. galloprovincialis*, 3% were identified as *M. trossulus*, 2% were *M. trossulus-galloprovincialis* hybrids, and 1% were identified as the Asian mussel *M. coruscus*. *Mytilus coruscus* was identified on three objects: BF001 (landed in Oregon in 2012), BF0023 (landed in Oregon in 2013), and BF040 (landed in Washington in 2013). *Mytilus trossulus* was present in debris during 2012 and 2013 sampling. Figure 7 shows the composition of *Mytilus* spp. per debris item.

**Table 6. COIII identification of *Mytilus* spp. on Japanese tsunami debris**

| Object Number | Landing State | *Mytilus coruscus* | *Mytilus galloprovincialis* | *Mytilus trossulus* | Failed PCR | Total |
|---|---|---|---|---|---|---|
| BF001 | OR | 2 | 93 | 3 | 3 | 101 |
| BF002 | WA | 0 | 24 | 1 | 0 | 25 |
| BF006 | HI | 0 | 45 | 16 | 6 | 67 |
| BF008 | WA | 0 | 113 | 0 | 0 | 113 |
| BF011 | HI | 0 | 0 | 1 | 4 | 5 |
| BF012 | WA | 0 | 18 | 0 | 1 | 19 |
| BF017 | HI | 0 | 6 | 1 | 1 | 8 |
| BF021 | HI | 0 | 5 | 0 | 0 | 5 |
| BF023 | OR | 2 | 77 | 1 | 0 | 80 |
| BF024 | OR | 0 | 28 | 0 | 3 | 31 |
| BF027 | HI | 0 | 7 | 0 | 0 | 7 |
| BF028 | OR | 0 | 33 | 0 | 0 | 33 |
| BF030 | OR | 0 | 3 | 0 | 0 | 3 |
| BF031 | HI | 0 | 2 | 1 | 0 | 3 |
| BF040 | WA | 2 | 25 | 0 | 0 | 27 |
| BF043 | OR | 0 | 27 | 0 | 0 | 27 |
| BF048 | OR | 0 | 9 | 0 | 0 | 9 |
| BF049 | HI | 0 | 28 | 0 | 0 | 28 |
| BF135 | OR | 0 | 17 | 0 | 1 | 18 |
| BF137 | OR | 0 | 2 | 0 | 0 | 2 |
| BF170 | WA | 0 | 6 | 0 | 0 | 6 |
| BF177 | WA | 0 | 1 | 0 | 0 | 1 |
| **Total mussels** | | 6 | 569 | 24 | 19 | 618 |

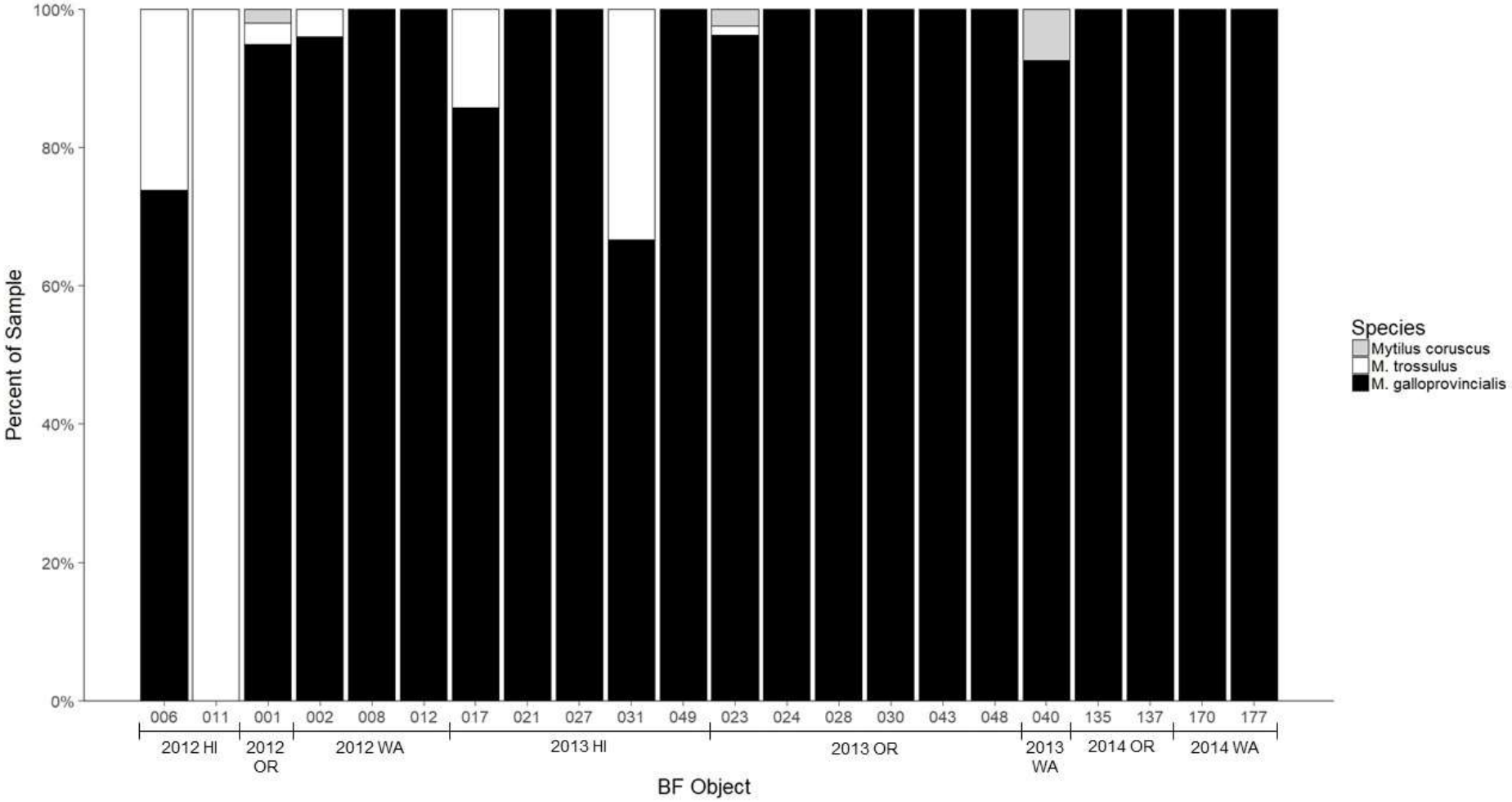


**Figure 7. *Mytilus* spp. genotype distribution on Japanese tsunami debris landings from 2012-2014.**

A total of 618 mussels were used in the genetic screening for *Eutima japonica* and 102 samples (~15%) showed amplification in the agarose gel, which indicates the presence of *Eutima japonica* in the samples (Table 7). *Eutima japonica* DNA was found in mussels from 12 debris objects (Figure 8), with the highest frequency and of *E. japonica* occurring in mussels from object BF008 (landed in Washington in 2012), which showed amplification in 67 out of 117 mussels (~60%). All mussels (n = 2) collected from object BF137 (landed in Oregon in 2014), and two-thirds of mussels (n = 6) from BF170 (landed in Washington in 2014) showed positive results for *Eutima japonica*.

**Table 7. Occurrence of *Eutima japonica* in Japanese tsunami debris mussels.**

| Object Number | Landing Year | Landing State | Positive result | Total mussels | Proportion positive |
|---|---|---|---|---|---|
| BF006 | 2012 | HI | 0 | 67 | 0 |
| BF011 | 2012 | HI | 0 | 5 | 0 |
| BF001 | 2012 | OR | 0 | 101 | 0 |
| BF002 | 2012 | WA | 1 | 25 | 0.040 |
| BF008 | 2012 | WA | 67 | 113 | 0.593 |
| BF012 | 2012 | WA | 0 | 19 | 0 |
| BF017 | 2013 | HI | 0 | 8 | 0 |
| BF021 | 2013 | HI | 1 | 5 | 0.200 |
| BF027 | 2013 | HI | 0 | 7 | 0 |
| BF031 | 2013 | HI | 0 | 3 | 0 |
| BF049 | 2013 | HI | 0 | 28 | 0 |
| BF023 | 2013 | OR | 2 | 80 | 0.025 |
| BF024 | 2013 | OR | 0 | 31 | 0 |
| BF028 | 2013 | OR | 1 | 33 | 0.030 |
| BF030 | 2013 | OR | 0 | 3 | 0 |
| BF043 | 2013 | OR | 0 | 27 | 0 |
| BF048 | 2013 | OR | 0 | 9 | 0 |
| BF040 | 2013 | WA | 2 | 27 | 0.074 |
| BF135 | 2014 | OR | 8 | 18 | 0.444 |
| BF137 | 2014 | OR | 2 | 2 | 1 |
| BF170 | 2014 | WA | 4 | 6 | 0.667 |
| BF177 | 2014 | WA | 0 | 1 | 0 |
| **Total** | | | 88 | 618 | 0.142 |

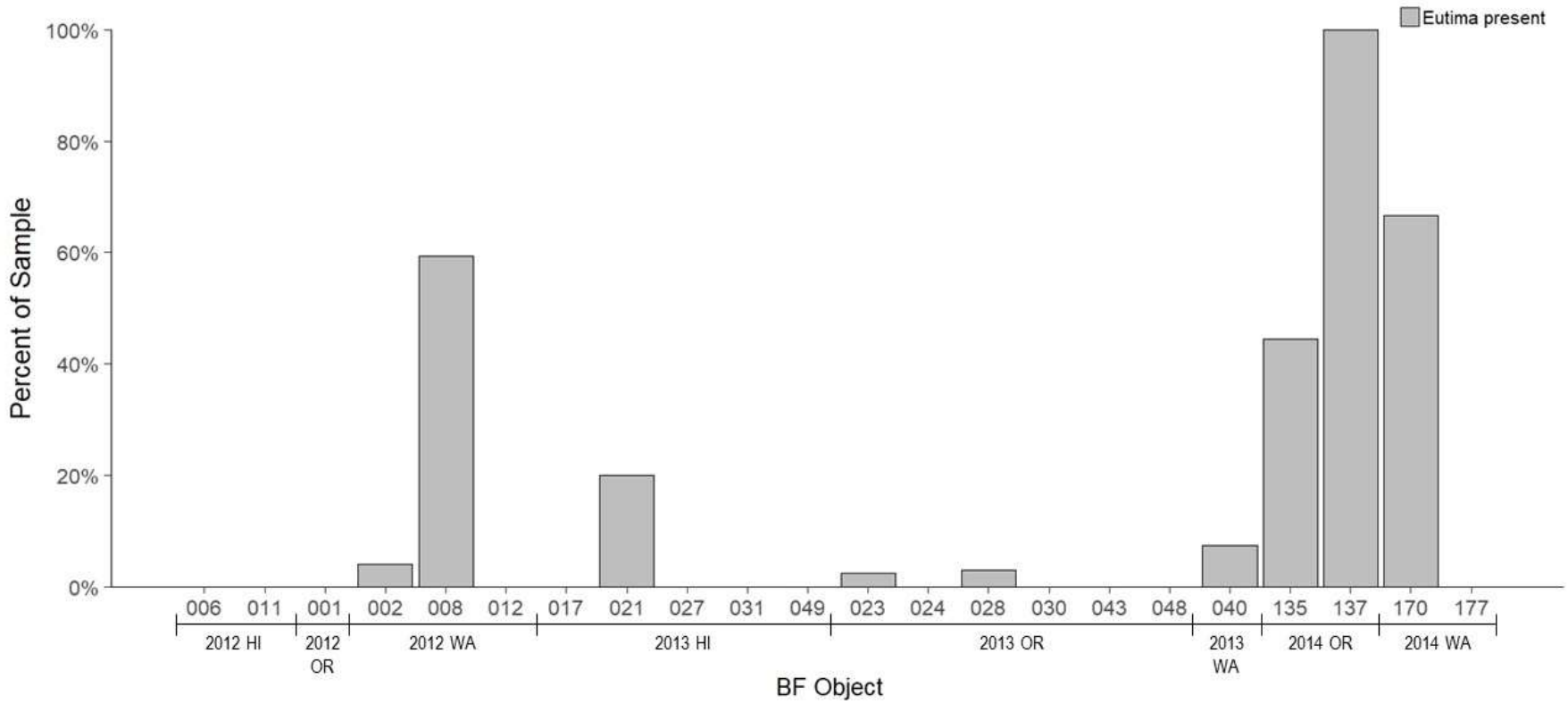


**Figure 8. Occurrence of the bivalve-inhabiting hydrozoan *Eutima japonica* in *Mytilus* spp. on Japanese tsunami debris landings from 2012-2014.**

## DISCUSSION

Japanese marine tsunami debris presented a novel opportunity to observe the transfer of whole communities of invertebrate organisms across thousands of miles of open ocean. Many organisms were found alive on debris items that washed up, even years after the tsunami had occurred (Carlton et al. 2017). Most mussels collected from the debris were *Mytilus galloprovincialis*, a non-native species to the North Pacific. Debris items sampled in this study largely landed on the outer open coasts of Oregon and Washington, which lie outside of the established introduced range of *M. galloprovincialis* on this coast, as documented in the survey of *Mytilus* spp. reported in Chapter 1. Despite the dominance of *M. galloprovincialis* on debris items landing in this region since 2012, adult populations of *M. galloprovincialis* north of California are still limited to established populations in Puget Sound and Vancouver Island (Wonham 2004).

The survival of the Asian mussel, *Mytilus coruscus*, on tsunami debris arriving in the East Pacific is a significant finding. This mussel has not been previously recorded in mussel populations on the North American coast (J. Geller & J. Carlton, *pers. comm.*), despite frequent co-occurrence with *M. galloprovincialis* and *M. trossulus* in Asia (Nakaoka et al. 2006). Despite its similar morphology to the bay mussels, *M. coruscus* is more phylogenetically similar to the outer coast mussel *M. californianus* (native to the East Pacific) than to *M. galloprovincialis* and *M. trossulus* (Inoue et al. 1996; Li et al. 2013; Gaitán-Espitia et al. 2016). This phylogenetic relationship suggests that *M. coruscus* may occupy an ecological niche more similar to *M. californianus*. Like *Mytilus californianus, M. coruscus* is found on rocky shores and in more wave-exposed areas

than the bay mussels, and likely plays a similarly key role in intertidal communities where it occurs (Kulikova et al. 2011).

In the East Pacific, *Mytilus californianus* is an important species in rocky intertidal communities – it provides space and food for other organisms. If *M. coruscus* is able to establish populations in the East Pacific from repeated exposure by debris, it may pose a threat to the rocky intertidal system as we know it. As mentioned in the first chapter of this work, not enough is known about the role *Mytilus* spp. play in their communities on small-scales. Successful introduction of *M. coruscus* may have little or no effect on the rocky intertidal community, or it could introduce associates (such as *Eutima japonica*), that East Pacific mussels and bivalves are unable to handle. Similarly, establishment may have a large impact on rocky intertidal communities if *M. californianus* predators (such as the ochre star *Pisaster ochraceus*) are unable to recognize *M. coruscus* as a prey item.

It is also important to monitor for the spread of *Mytilus* associates along the Pacific coast. Epibionts or parasites may not have the same temperature or salinity restrictions as *Mytilus galloprovincialis* and might move from debris mussels to native mussel populations.

This study found that approximately 14% of *Mytilus* spp. sampled from debris items were positive for the presence of a parasitic hydroid, *Eutima japonica*, which is unknown in the northeast Pacific (J. Carlton, J. Geller, & G. Ruiz, *pers. comm.*). This hydroid lives within the mantle cavity of several species of Japanese and Asian bivalves, and has been shown to limit the growth and survivorship of scallops raised for aquaculture in Japan and is therefore considered parasitic, although it is considered a non-

parasitic commensal in other species (Kubota 1992; Baba et al. 2007; Calder et al. 2014). *Eutima japonica* lives in *Mytilus trossulus* and *M. galloprovincialis* in Japan (Baba et al. 2007). *Eutima japonica* were transported in live mussels on tsunami debris from Japan and were still alive when the debris landed in the Eastern Pacific (J. Carlton, *pers. comm*.; living *E. japonica* observed in the laboratory in live *Mytilus* from JTMD-BF-008). These hydroids are likely to be able to tolerate the environmental conditions of the Northeast Pacific, though it is unknown if *E. japonica* is able to reproduce under those conditions.

*Eutima japonica* is also known to inhabit multiple bivalve species and may be capable of moving between host species (Kubota 1992, Baba et al. 2007). Life history traits such as these could contribute to rapid expansion after establishment in a new region. To assess the potential risk of *Eutima japonica* introduction in the Eastern Pacific, it is important to determine if *E. japonica* can survive and reproduce in this new environment. Because these hydroids do occur in *Mytilus* spp. in Japan, *M. galloprovincialis*, *M. trossulus*, and *M. californianus* make good candidates for controlled growth and reproductive studies on *Eutima japonica* in the cold waters of the Eastern Pacific.

As climate continues to change and humans continue to use marine and nearshore environments, the amount of debris introduced to the ocean will likely increase due to storms, natural disasters, or large-scale pollution events. While large-scale marine debris events may not be as large of a vector for marine introductions, the work presented here provides further insight into the transport of living adult organisms (as opposed to larvae transported in ballast tanks) and their associates across the Pacific Ocean to a new environment.

# REFERENCES

Ackerman JD, Nishizaki MT. 2004. The effect of velocity on the suspension feeding and growth of the marine mussels *Mytilus trossulus* and *M. californianus*: Implications for niche separation. J. Mar. Syst. 49:195–207.

Baba K, Miyazono A, Matsuyama K, Kohno S, Kubota S. 2007. Occurrence and detrimental effects of the bivalve-inhabiting hydroid *Eutima japonica* on juveniles of the Japanese scallop *Mizuhopecten yessoensis* in Funka Bay, Japan: relationship to juvenile massive mortality in 2003. Mar. Biol. 151:1977–1987.

Bax N, Williamson A, Aguero M, Gonzalez E, Geeves W. 2003. Marine invasive alien species: a threat to global biodiversity. Mar. Policy 27:313–323.

Bownes SJ, McQuaid CD. 2010. Mechanisms of habitat segregation between an invasive (*Mytilus galloprovincialis*) and an indigenous (*Perna perna*) mussel: adult growth and mortality. Mar. Biol. 157:1799–1810.

Braby CE, Somero GN. 2006a. Ecological gradients and relative abundance of native (*Mytilus trossulus*) and invasive (*Mytilus galloprovincialis*) blue mussels in the California hybrid zone. Mar. Biol. 148:1249–1262.

Braby CE, Somero GN. 2006b. Following the heart: temperature and salinity effects on heart rate in native and invasive species of blue mussels (genus *Mytilus*). J. Exp. Biol. 209:2554–66.

Branch GM, Steffani NC. 2004. Can we predict the effects of alien species? A case-history of the invasion of South Africa by *Mytilus galloprovincialis* (Lamarck). J. Exp. Mar. Bio. Ecol. 300:189–215.

Burrows MT, Schoeman DS, Buckley LB, Moore P, Poloczanska ES, Brander KM, Brown C, Bruno JF, Duarte CM, Halpern BS, et al. 2011. The pace of shifting climate in marine and terrestrial ecosystems. Science 334:652–5.

Calder DDR, Choong HHCH, Carlton JTJ, Chapman JW, Miller JA, Geller J. 2014. Hydroids (Cnidaria: Hydrozoa) from Japanese tsunami marine debris washing ashore in the northwestern United States. Aquat. Invasions 9:425–440.

Carlton JT, Chapman JW, Geller JB, Miller JA, Carlton DA, McCuller MI, Treneman NC, Steves BP, Ruiz GM. 2017. Tsunami-driven rafting: Transoceanic species dispersal and implications for marine biogeography. Science 357:1402–1406.

Carlton J, Geller J. 1993. Ecological roulette: the global transport of nonindigenous marine organisms. Science 261:78–82.

Cayan DR, Maurer EP, Dettinger MD, Tyree M, Hayhoe K. 2007. Climate change scenarios for the California region. Clim. Change 87(Suppl 1): S21-42.

Cohen AN, Carlton JT. 1998. Accelerating invasion rate in a highly invaded estuary. Science 279:555–8.

Elton CS. 1958. The ecology of invasions by animals and plants. New York: John Wiley and Sons, Inc.

Gaitán-Espitia JD, Quintero-Galvis JF, Mesas A, D'Elía G. 2016. Mitogenomics of southern hemisphere blue mussels (Bivalvia: Pteriomorphia): Insights into the evolutionary characteristics of the *Mytilus edulis* complex. Sci. Rep. 6:1–10.

Geller JB. 1999. Decline of a native mussel masked by sibling species invasion. Conserv. Biol. 13:661–664.

Grant WS, Cherry MI. 1985. *Mytilus galloprovincialis* Lmk. in Southern Africa. J. Exp. Mar. Bio. Ecol. 90:179–191.

Harger J. 1970. The effect of species composition on the survival of mixed populations of the sea mussels *Mytilus californianus* and *Mytilus edulis*. Veliger 13:147–52.

Harger J. 1972. Competitive co-existence: Maintenance of interacting associations of the sea mussels *Mytilus edulis* and *Mytilus californianus*. Veliger 13:387–410.

HilleRisLambers J, Ettinger AK, Ford KR, Haak DC, Horwith M, Miner BE, Rogers HS, Sheldon KS, Tewksbury JJ, Waters SM, et al. 2013. Accidental experiments: ecological and evolutionary insights and opportunities derived from global change. Oikos 122:1649–1661.

Inoue K, Odo S, Noda T, Nakao S. 1997. A possible hybrid zone in the *Mytilus edulis* complex in Japan revealed by PCR markers. Mar. Biol. 128:91–95.

Inoue K, Takeuchi Y, Takeyama S, Yamaha E, Yamazaki F, Odo S, Harayama S. 1996. Adhesive protein cDNA sequence of the mussel *Mytilus coruscus* and its evolutionary implications. J. Mol. Evol. 43:348–356.

Inoue K, Waite JH, Matsuoka M, Odo S, Harayama S. 1995. Interspecific variations in adhesive protein sequences of Mytilus edulis, *M. galloprovincialis*, and *M. trossulus*. Biol Bull.:370–375.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649.

Koehn RK, Hall JG, Innes DJ, Zera AJ. 1984. Genetic differentiation of *Mytilus edulis* in eastern North America. Mar. Biol. 79:117–126.

Kubota S. 1992. Four bivalve-inhabiting hydrozoans in Japan differing in range and host preference. Sci. Mar 56:149–159.

Kulikova VA, Lyashenko SA, Kolotukhina NK. 2011. Seasonal and interannual dynamics of larval abundance of *Mytilus coruscus* Gould, 1861 (Bivalvia: Mytilidae) in Amursky Bay (Peter the Great Bay, Sea of Japan). Russ. J. Mar. Biol. 37:342–347.

Li J, Ye Y, Wu C, Qi P, Guo B, Chen Y. 2013. Genetic variation of *Mytilus coruscus* Gould (Bivalvia: Mytilidae) populations in the East China Sea inferred from mtDNA COI gene Sequence. Biochem. Syst. Ecol. 50:30–38.

Lockwood BL, Somero GN. 2011. Transcriptomic responses to salinity stress in invasive and native blue mussels (genus *Mytilus*). Mol. Ecol. 20:517–29.

McDonald JH, Koehn RK. 1988. The mussels *Mytilus galloprovincialis* and *M. trossulus* on the Pacific coast of North America. Mar. Biol. 99:111–118.

McDonald JH, Seed R, Koehn RK. 1991. Allozymes and morphometric characters of three species of *Mytilus* in the Northern and Southern Hemispheres. Mar. Biol. 111:323–333.

Miller JA, Carlton JT, Chapman JW, Geller JB, Ruiz GM. 2017. Transoceanic dispersal of the mussel *Mytilus galloprovincialis* on Japanese tsunami marine debris: An approach for evaluating rafting of a coastal species at sea. Mar. Pollut. Bull.:1–10.

Moore SK, Mantua NJ, Newton JA, Kawase M, Warner MJ, Kellogg JP. 2008. A descriptive analysis of temporal and spatial patterns of variability in Puget Sound oceanographic properties. Estuar. Coast. Shelf Sci. 80:545–554.

Munday PL, Warner RR, Monro K, Pandolfi JM, Marshall DJ. 2013. Predicting evolutionary responses to climate change in the sea. Ecol. Lett. 16:1488–1500.

Nakaoka M, Ito N, Yamamoto T, Okuda T, Noda T. 2006. Similarity of rocky intertidal assemblages along the Pacific coast of Japan: effects of spatial scales and geographic distance. Ecol. Res. 21:425–435.

Nielsen P, Vismann B. 2014. Clearance rate of *Mytilus edulis* (L.) as a function of current velocity and mussel aggregation. J. Shellfish Res. 33:457–463.

Parmesan C. 2006. Ecological and evolutionary responses to recent climate change. Annu. Rev. Ecol. Evol. Syst. 37:637–669.

Piraino S, Todaro C, Geraci S, Boero F. 1994. Ecology of the bivalve-inhabiting hydroid *Eugymnanthea inquilina* in the coastal sounds of Taranto (Ionian Sea, SE Italy). Mar. Biol. 703:695–703.

Rawson P, Agrawal V, Hilbish T. 1999. Hybridization between the blue mussels *Mytilus galloprovincialis* and *M. trossulus* along the Pacific coast of North America: evidence for limited introgression. Mar. Biol. 134:201–211.

Ruiz GM, Fofonoff PW, Steves B, Foss SF, Shiba SN. 2011. Marine invasion history and vector analysis of California: a hotspot for western North America. Divers. Distrib. 17:362–373.

Sagarin RD, Barry JP, Gilman SE, Baxter CH. 1999. Climate-related change in an intertidal community over short and long time scales. Ecol. Monogr. 69:465–490.

Sarver S, Foltz D. 1993. Genetic population structure of a species' complex of blue mussels (*Mytilus* spp.). Mar. Biol. 117:105–112.

Shinen JS, Morgan SG. 2009. Mechanisms of invasion resistance: competition among intertidal mussels promotes establishment of invasive species and displacement of native species. Mar. Ecol. Prog. Ser. 383:187–197.

Sorte CJB, Williams SL, Carlton JT. 2010a. Marine range shifts and species introductions: comparative spread rates and community impacts. Glob. Ecol. Biogeogr. 19:303–316.

Sorte CJB, Williams SL, Zerebecki RA. 2010b. Ocean warming increases threat of invasive species in a marine fouling community. Ecology 91:2198–204.

Suchanek T, Geller J, Kreiser B, Mitton J. 1997. Zoogeographic distributions of the sibling species *Mytilus galloprovincialis* and *M. trossulus* (Bivalvia: Mytilidae) and their hybrids in the North Pacific. Biol. Bull. 193:187–194.

Stewart DT, Saavedra C, Stanwood RR, Ball AO, Zouros E. 1995. Male and female mitochondrial DNA lineages in the blue mussel (*Mytilus edulis*) species group. Mol. Biol. Evol. 12:735–47.

Varvio SL, Koehn R, Vainola R. 1988. Evolutionary genetics of the *Mytilus edulis* complex in the North Atlantic region. Mar. Biol. 98:51–60.

Wilkins NP, Fujinof K, Gosling EM. 1983. The Mediterranean mussel *Mytilus galloprovincialis* Lmk. in Japan. Biol. J. Linn. Soc. 20:365–374.

Wonham MJ. 2004. Mini-Review: Distribution of the Mediterranean mussel *Mytilus galloprovincialis* (Bivalvia: Mytilidae) and hybrids in the Northeast Pacific. J. Shellfish Res. 23:535–543.