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Arrival and Expansion of the Invasive Foraminifera Trochammina hadai Uchio in Padilla Bay, Washington

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Arrival and Expansion of the Invasive Foraminifera *Trochammina hadai* Uchio in Padilla Bay, Washington

Abstract

Trochammina hadai Uchio, a benthic foraminifera native to Japanese estuaries, was first identified as an invasive in 1995 in San Francisco Bay and later in 16 other west coast estuaries. To investigate the timing of the arrival and expansion of this invasive species in Padilla Bay, Washington, we analyzed the distribution of foraminifera in two surface samples collected in 1971, in nine surface samples collected by Scott in 1972-1973, as well as in two cores (Padilla Flats 3 and Padilla V1/V2) obtained in 2004. *Trochammina hadai*, originally identified as the native *Trochammina pacifica* Cushman in several early foraminiferal studies, dominates the assemblage of most of the surface samples. In the Padilla V1/V2 and Padilla Flats 3 cores, the species' abundance follows a pattern of absence, first appearance, rapid expansion commonly seen shortly after the arrival of a successful biological invasion, setback, and second expansion. Using Q-mode cluster analysis, pre-expansion and expansion assemblages were identified. Pb-210 dating of these cores proved unsuccessful. However, based on *T. hadai's* first appearance occurring stratigraphically well above sedimentological changes in the cores that reflect deposition of sediments in the bay due to previous diversions of the Skagit River, and its dominance in the early 1970s surface samples, we conclude that the species arrived in Padilla Bay somewhere between the late 1800s and 1971. *Trochammina hadai* may have been introduced into the bay in the 1930s when oyster culturing began there or, at a minimum, ten years prior to its appearance in San Francisco Bay.

Introduction

The common Japanese estuarine foraminifera *Trochammina hadai* Uchio was first identified as an invasive along the western coast of the United States in 1995, when it was discovered in sediments of San Francisco Bay Estuary (McGann and Sloan 1996, 1999). Later it was determined that the species first appeared in that estuary in 1983, comprising a mere 1.5% of the fauna (McGann, unpublished data). By 2000, the taxon had been found in 96% of the samples collected from brackish and marine waters of the estuary, accounting for >50% of the foraminiferal fauna at most sites (McGann et al. 2000). The proliferation of *T. hadai* in San Francisco Bay Estuary is associated with a decline in relative abundance of one of the most common native foraminifera,

Elphidium excavatum. A 3900-year record of deposition in the southern part of the estuary (McGann 1995, McGann et al. 2000) reveals that *E. excavatum* declined from at least 55% of the foraminiferal fauna in all samples prior to *T. hadai's* first appearance in the core, to 19% at 2.5-1 cm depth (i.e., after its initial appearance in the core), to a present average of 5% in the south bay area (McGann and Sloan, unpublished data). Because no sedimentological or environmental changes have occurred since *T. hadai's* arrival in the early 1980s, the sharp decline in abundance of *E. excavatum* suggests the arrival and proliferation of the invasive species in San Francisco Bay Estuary has profoundly impacted the native foraminiferal fauna.

The dramatic impact *T. hadai* has had on the microfauna of San Francisco Bay Estuary prompted us to investigate other West Coast embayments. Samples taken from Puget Sound by the U.S.

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Geological Survey in 1997, the Washington State Department of Ecology in 1998, and the Puget Sound Expedition also in 1998, yielded nearly 50 sites where *T. hadai* occurred (McGann et al. 1998). Since then, the species has been identified in modern sediments in 16 ports and estuaries along the west coast of America from San Diego Bay, California to Prince William Sound, Alaska (McGann et al. 2000; McGann, unpublished data).

After these broad initial surveys, we began re-examining detailed foraminiferal studies and archived sediment samples in Puget Sound. Surface samples collected 1) in 1971 at Bayview State Park in Padilla Bay and off March Point on Fidalgo Island, 2) by Scott (1974) in 1972-1973 in Padilla Bay, and 3) two cores collected in Padilla Bay in 2004, provided us the opportunity to document the timing of the arrival of *T. hadai* in Padilla Bay and the record of its abundance and expansion afterwards.

Setting

Padilla Bay is a flat, shallow estuary about 13 km long and 5 km wide located in northeastern Puget Sound (Padilla Bay National Estuarine Research Reserve 2010; Figure 1). Most of the bay is subject to mixed semi-diurnal tides with a tidal range averaging 2.6 m (U.S. Army Engineer District 1976) and a maximum range of about 4 m (Bulthuis 1995). Salinity ranges from about 28-30 ppt (Cassidy and McKeen 1986) with a mean of about 28 ppt. Freshwater enters the bay from several small agricultural sloughs that drain the 93.1 km² watershed (National Estuarine Research Reserve System 2011) as well as a small amount from the south through the Swinomish Channel (Bulthuis and Conrad

1995) which was completed in 1937. The bay is filled with sediment from the Skagit River that no longer enters the bay directly and lies at the saltwater edge of the large Skagit River Delta. Comprised mostly of intertidal mudflats that are exposed for kilometers during low tide and flooded at high tide, this is an especially valuable habitat ecologically because of the extensive (~32.4 km²) eel grass meadows developed here which are used as a nursery by numerous invertebrates and fish
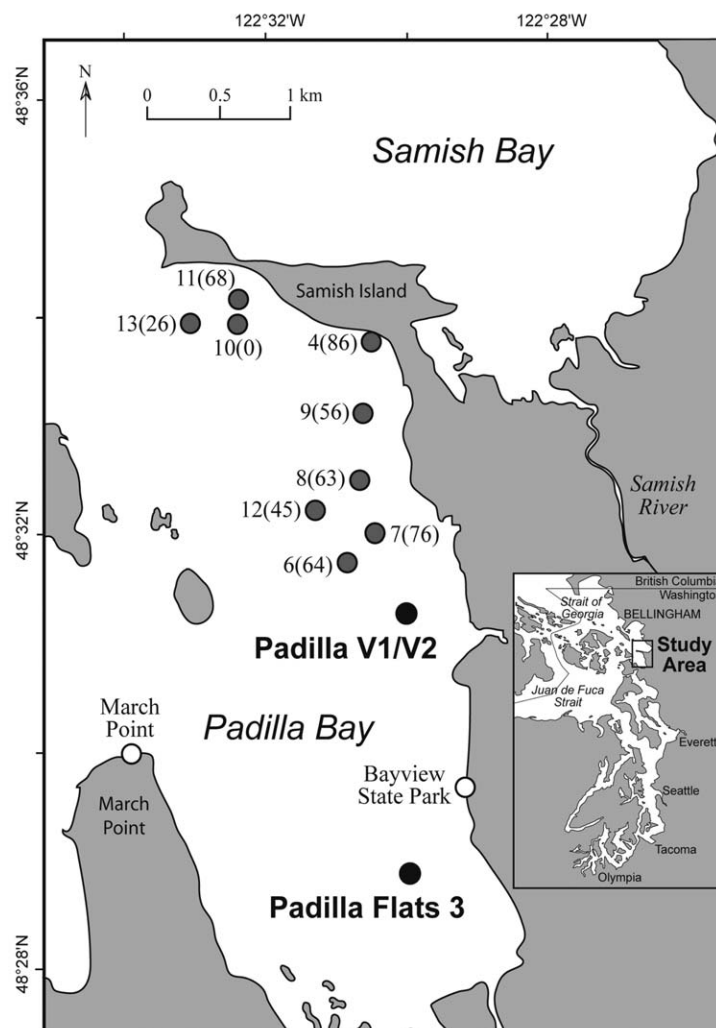
Figure 1. Location of the core sites (black circles) of Padilla V1/V2 and Padilla Flats 3 in Padilla Bay as well as the location of surface samples collected in 1971 (open circles) and in 1972-1973 (gray circles) by Scott (1974) in Padilla Bay. The numbers next to Scott's core sites indicate his sample numbers followed (in parentheses) by the percentage abundance of the invasive species *Trochammina hadai* recovered at those sites in 1972-1973.

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species (Padilla Bay National Estuarine Research Reserve 2010). Two species of eel grass are common: the native *Zostera marina* that inhabits most of the shallow offshore region in Padilla Bay and *Z. japonica*, an invasive from Japan that was most likely introduced into the bay when oysters were imported for culturing in the 1930s (Mach et al. 2010). In 2009, *Z. japonica* was found growing between -0.5 and +0.8 MLLW in the bay, in a thin band trending roughly north to south inshore of the *Z. marina* beds but offshore of the salt marsh bordering the land (Mach et al. 2010, Padilla Bay National Estuarine Research Reserve 2010).

The Skagit River watershed is the largest (78,000 km^2) of seven watersheds draining the western Cascade Range into Puget Sound (Collins et al. 2003) and the associated Skagit River Delta is unique for its large size, its origin from Glacier Peak volcano lahars (Beget 1982, Dragovitch et al. 2000), and the quantity, variety, and extent of its wetlands and channel habitats (Collins et al. 2003). These habitats, as well as the regional landforms, soils, and density and size of the flora they support, strongly influence riverine dynamics and, ultimately, sediment delivery to the bay and delta. Beginning in the 1860s, ditching and diking commenced in an effort to drain the extensive wetlands in order to increase farmland (U.S. War Department 1881). As a result, many distributary sloughs closed off (Collins et al. 2003), flooding and recharging of wetlands dropped, and the delivery of sediment input to Padilla Bay was dramatically reduced. Yet, anthropogenic intervention has also increased the delivery of sediment to the bay. Because the Skagit River historically drained vast areas supporting extensive forests, log jams on the river were pervasive, physically blocking sediment transport, forcing divergence of the local flow, and decreasing the natural variability in sediment transport rates (Nesbit 1885, Bilby 1981, Smith et al. 1993, Massong and Montgomery 2000, Collins and Montgomery 2002, Montgomery et al. 2003). Beginning in the 1880s, there was a concerted effort to remove snags (Collins et al. 2002) and to cut virgin low-elevation riparian forests. By 1900, the source material causing the log jams had been virtually eliminated (Plummer et al. 1902, Collins and Montgomery 2002),

thereby restoring sediment transport down the river. The historic record of sediment deposition, including the anthropogenically-induced changes, should be captured in cores obtained from Padilla Bay, thereby providing a chronology from which we can determine the timing of the arrival of *T. hadai* in the bay.

Early Foraminiferal Studies

One of the earliest studies documenting the distribution of foraminifera in the central to northern portion of Puget Sound was that of Cushman and Todd (1947) who reported on the fauna from 50 samples obtained in waters surrounding the San Juan Islands; six of 77 species recovered were dominant. In 1959 and 1960, sediment samples were taken in Bellingham and Samish bays to investigate the regional sedimentary and depositional history (Sternberg 1967). Although foraminiferal tests were recovered, no detailed taxonomic analysis was conducted. Instead, Sternberg (1967) reported that the tests comprised 0-6% of the coarse fraction and that they were distributed similarly to the shell fragments, with higher concentrations in the central portions of both bays. Phleger's (1967) study of marsh foraminiferal patterns along the Pacific coast of North America included 13 samples from the tidal marshes lining the Fraser Delta. Cockbain (1963) also identified intertidal foraminifera from Oregon to Washington, including 175 sites from Juan de Fuca and Georgia Straits as well as the Fraser River.

Foraminiferal distribution studies in and around Puget Sound continued in the 1970s. Smith (1970, 1978) reported on high latitude shallow-water foraminiferal faunas of British Columbia and southeast Alaska, and Lankford and Phleger (1973) investigated foraminifera of the nearshore turbulent zone of western North America, but only included taxonomic counts for one site near the Strait of Juan de Fuca. In 1972-1973, 23 surface samples were collected in Samish and Padilla bays to investigate the spatial distribution of foraminifera nearshore (Scott 1974). Twenty-one of these samples contained foraminifera; seventeen species were recognized. Scott (1974) identified the most abundant of these as *Trochammina pacifica*

Cushman. The species was present in 20 of 23 samples, dominant in 16 of them (26-100%), and was generally in high abundance throughout the region (mean 55%). Scott did not, however, include a figure of the taxon. This is unfortunate because we now recognize that *T. pacifica* is morphologically similar to the invasive *T. hadai* (Figure 2), although it is flatter with more elongate chambers compared to the inflated, bulbous chambers typical of *T. hadai*. Additionally, *T. pacifica* typically resides on the continental shelf (McGann 2009), whereas *T. hadai* is prevalent in very shallow, estuarine and intertidal mudflat settings (McGann and Sloan 1999, Sloan and McGann 2000). Although we were unable to examine Scott's specimens, several lines of evidence suggest that the species he recovered in Padilla Bay in 1972-1973 was actually *T. hadai*: 1) his samples were obtained from the intertidal mudflats; 2) more recent sampling in the area recovered abundant *T. hadai* but no *T. pacifica* (Sloan and McGann 2000, McGann unpublished data); and 3) molecular studies of specimens from Padilla Bay identified them as *T. hadai* (Kitazato et al. 1997, Fay et al. 2007). Because Scott's (1974) Padilla Bay samples were located close to the present study site (Figure 1), his census counts are used in this study.

Shortly after Scott's investigation, a seasonal distribution study of foraminifera in the littoral zone of Samish Bay was conducted on samples collected in 1976-1977 (Jones and Ross 1979). Ten stations were sampled nine times over a 12-month period, with Jones and Ross (1979) noting that *T. pacifica* (most likely *T. hadai*) formed the dominant part of the assemblage nearly all year long. In the same study, Jones and Ross (1979) analyzed several short cores from Samish Bay to determine the amount of seasonal variation in foraminiferal test preservation in the sediment column. They determined that the reducing substrate seemed to dissolve all calcareous specimens within five months. Also in 1979, Gallagher reported on modern foraminifera from the shelf and slope of Vancouver Island.

Several studies focusing on foraminiferal biofacies of the Fraser River Delta were published in the late 1980s and early 1990s. Williams (1989) found poor test preservation in many samples but was still able to define three elevational zonations (i.e., foraminiferal assemblages at specific elevations above and below sea level) in the mudflats (tidal flats) just west of Lulu Island (Sturgeon Bank) and two more on the foreslope, and then used these to interpret the paleoenvironment of drill-core deposits from the delta. He identified a single specimen of *Trochammina advena* Cushman in a foreslope sample. Although Williams did not illustrate his specimens, the original illustration of the species (Cushman 1922, Figure 3) is similar to that of *T. hadai*. In 1990, Patterson defined six biofacies from the surficial sediments of the marshes and mudflats based on elevation, salinity, and organic content. One of these was named the *Trochammina pacifica* Biofacies. The illustration of *T. pacifica* in this publication displays inflated chambers that look like those characteristic of *T. hadai*. Shortly thereafter, Patterson and Cameron (1991) interpreted the depositional history of the Fraser Delta based on foraminifera and ostracods.

In 1998, Patterson et al. published an atlas of common Quaternary benthic species for the western Canadian shelf; *T. hadai* was not mentioned. A brief survey of the spatial distribution of *T. hadai* in Puget Sound was presented by McGann et al. (1998) and Sloan and McGann (2000). In 2010, Martin and Nesbitt reported on a pilot study using foraminifera to assess and monitor the health of two of the most highly degraded areas of Puget Sound, Elliott and Commencement bays, based on samples collected from 1997-2010 by NOAA and the Washington State Department of Ecology that included the presence of *T. hadai*.

Because *T. hadai* has been recovered in past studies and modern surface samples from Puget Sound, and a core from San Francisco Bay Estuary was successful in documenting the arrival and expansion of *T. hadai* in that region (McGann 1995), it seemed worthwhile to attempt a similar study in Padilla Bay. We planned to investigate the abundance of foraminifera, including the invasive, in one or more cores obtained in Padilla Bay and assumed an age model could be obtained for those cores using Pb-210 dating methods.

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Figure 2. 1-3, *Trochammina hadai* Uchio. A, dorsal view; B, edge view; C, ventral view. 1, original drawing of holotype (after Uchio 1962) from off the mouth of the River Shinano, Niigata Prefecture, Sea of Japan, x58. 2-3, light micrographs of specimens from Padilla Bay, Padilla Flats 3 core, 10-12 cm, all x60. 4-6, *Trochammina pacifica* Cushman. A, dorsal view; B, edge view; C, ventral view. 4, original drawing of holotype (after Cushman 1925) from Virago Sound, off British Columbia, x66. 5-6, light micrographs of specimens from Santa Monica Bay, southern California, USGS core locator A2-98-SC, box core 306B1, 0-1 cm, all x60.
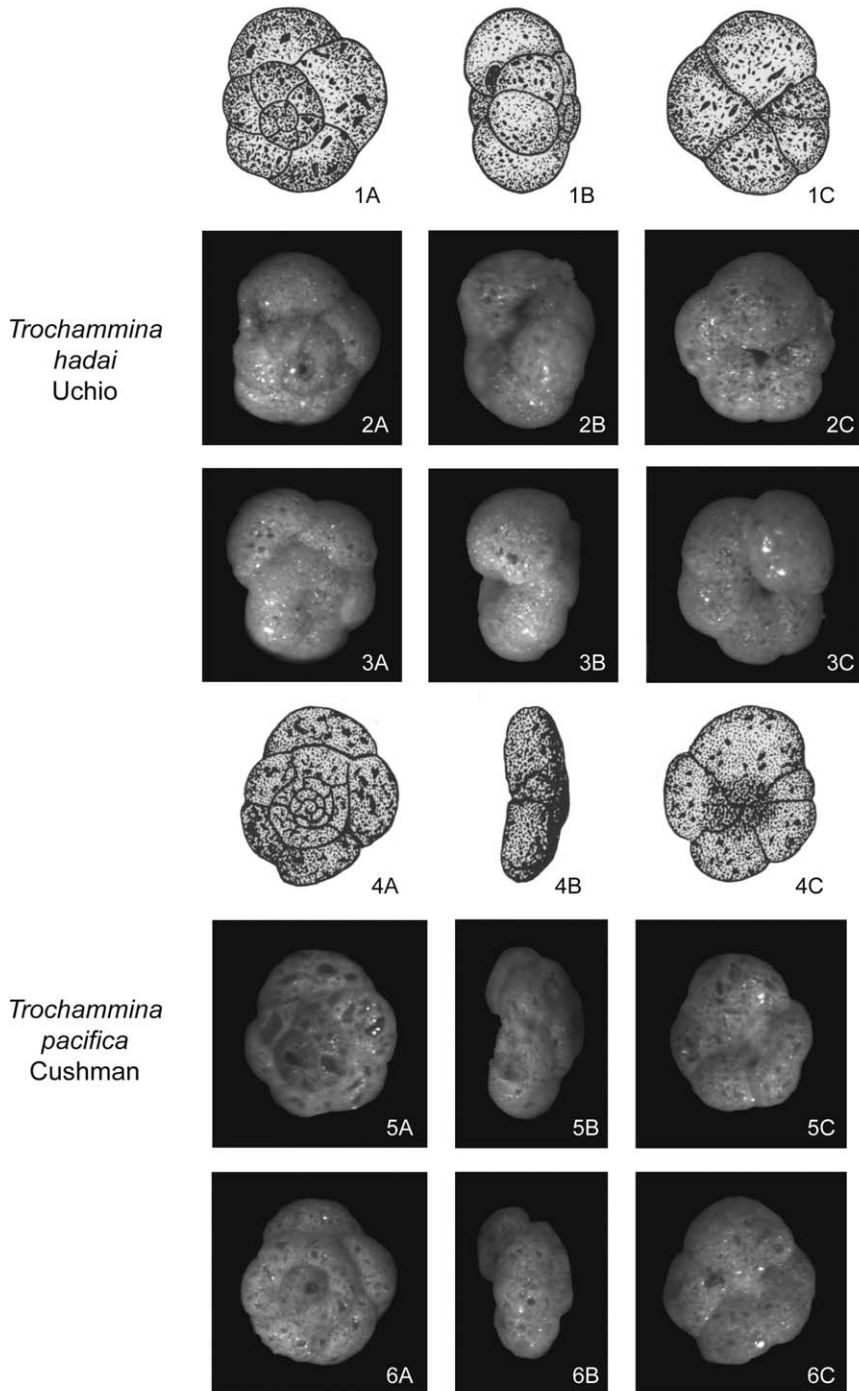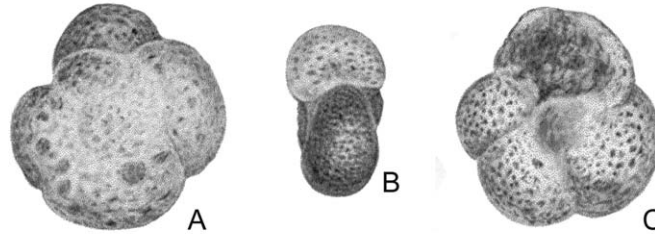
Figure 3. *Trochammina advena* Cushman. A, dorsal view; B, edge view; C, ventral view. Original drawing of holotype (after Cushman 1922) from Dry Tortugas Islands, Florida, x50.

Methods

On June 12, 1971, one of the authors (DP) collected surface samples from the shore of Bayview State Park in Padilla Bay and from March Point on Fidalgo Island (Figure 1). On September 7, 2004, a 4.03 m-long vibracore was taken on a submerged mudflat in the central-eastern portion of Padilla Bay (USGS Core Locator W-1-04-PS, Padilla V1; 48.52357°N, 122.49788°W; water depth 7.5 m relative to mean low low water [MLLW], 9.3 m relative to mean sea level [MSL]). Upon retrieval, the upper 0.6 m of the core was extruded for geochemical studies so a second short core 0.5 m in length was obtained in the same location (Padilla V2). Together, they constitute composite core Padilla V1/V2. Two days later, another vibracore, referred to as Padilla Flats 3, was taken on the submerged mudflat in the southern portion of the bay (2.06 m in length; 48.48235°N, 122.49594°W; water depth 8.0 m MLLW, 9.8 m MSL).

The cores were sampled at approximately 10 cm intervals for foraminifera. Downcore and surface sediment samples were wet-sieved through nested 0.063 mm, 0.150 mm, and 1.0 mm screens to remove clays (< 63 μm) and segregate the size fractions. After air-drying, the sediment was subjected to flotation by sodium polytungstate in order to settle out the sands and concentrate the foraminifera before picking. The foraminifera were then extracted from the > 0.63 mm fraction. Samples were split with the aid of a microsplitter into an aliquot containing at least 300 benthic foraminifers which were picked and identified. If the sample contained < 300 foraminifers, all that were present were picked. These slides and residues are on file at the U.S. Geological Survey, Menlo Park, California.

Due to the small number of foraminifers recovered in some of the samples, relative foraminiferal species abundances were computed using a sum of benthic foraminifers in those samples in which greater than 35 specimens were recovered in the Padilla V1/V2 core (8 of 10 samples) and in which at least 50 specimens were recovered in the Padilla Flats 3 core (20 of 23 samples); all other samples had > 300 specimens. Also, in order to compare all of the abundance data available from Padilla Bay at one time (Padilla V1/V2, Padilla Flats 3, the two 1971 surface samples, and the 1972-1973 surface samples from Padilla Bay collected by Scott [1974]), it was first necessary to make the data sets compatible. This was accomplished by assuming what Scott identified as *T. pacifica* was actually *T. hadai*, *Ammobaculites dilatatus* Cushman and Brönniman and *Glabratella opercularis* (d'Orbigny) were equivalent to *Ammobaculites exiguus* and *Neoconorbina opercularis*, respectively, and that his *Cribroelphidium* spp. were equivalent to *Elphidium* spp. in this study. Once converted to frequency data, a Q-mode cluster analysis was utilized to describe the relationship between the benthic foraminiferal samples. The cluster analysis grouped the samples according to their degree of similarity. Clustering was based on a square root transformation of the data, a Bray-Curtis similarity coefficient, and amalgamated by a group averaged linkage strategy. Primer (v. 6, Primer-E, Ltd.), a statistical software package, was used for this analysis (Clarke and Gorley 2006).

Nine samples each in the Padilla V1/V2 and Padilla Flats 3 core were also measured for Pb-210 (alpha and gamma) and Cs-137 radionuclides to provide a chronology for the cores. Unfortunately, the data indicate that excess Pb-210 was only

present in the upper few centimeters of each core. As a result, sediment accumulation rates and a chronology for these cores could not be derived. However, the cores still looked promising because lithologic changes seen in the upper part of both is inferred to represent the removal of log jams between 1898 and 1908, allowing continuous deposition at the study site to resume at a rate of about 0.9-1.7 cm/yr.

Results

Sedimentology

The Padilla V1/V2 core is characterized by reverse bedding reflected by a slight coarsening-upward sequence (Figure 4). The bottom of the core between 403 and 180 cm is largely dark silty sand or sandy silt, the region between 180 and 50 cm

is characterized by fine to very fine dark sands, and the upper 50 cm of the core is comprised of fine to medium brown to dark sands. Within these broad units, an isolated coarse dark sand unit was observed between 102 and 95 cm and a normal bedded unit (slight fining upward sequence) occurs between 95 and 60 cm. Sparse shell fragments occur between 40 and 30 cm and wood debris was observed at 350 and 102 cm. The wood sample at 350 cm was analyzed by ^{14}C accelerator mass spectrometry and determined to have a ^{14}C age of 3945 ± 35 years.

Three distinct sedimentological units characterize the Padilla Flats 3 core: 1) medium to coarse sand from 206-171 cm; 2) silt from 171-92 cm, and 3) fine sand from 92 cm to the core top (Figure 4). These differences in core lithology most likely reflect natural and anthropogenically-induced
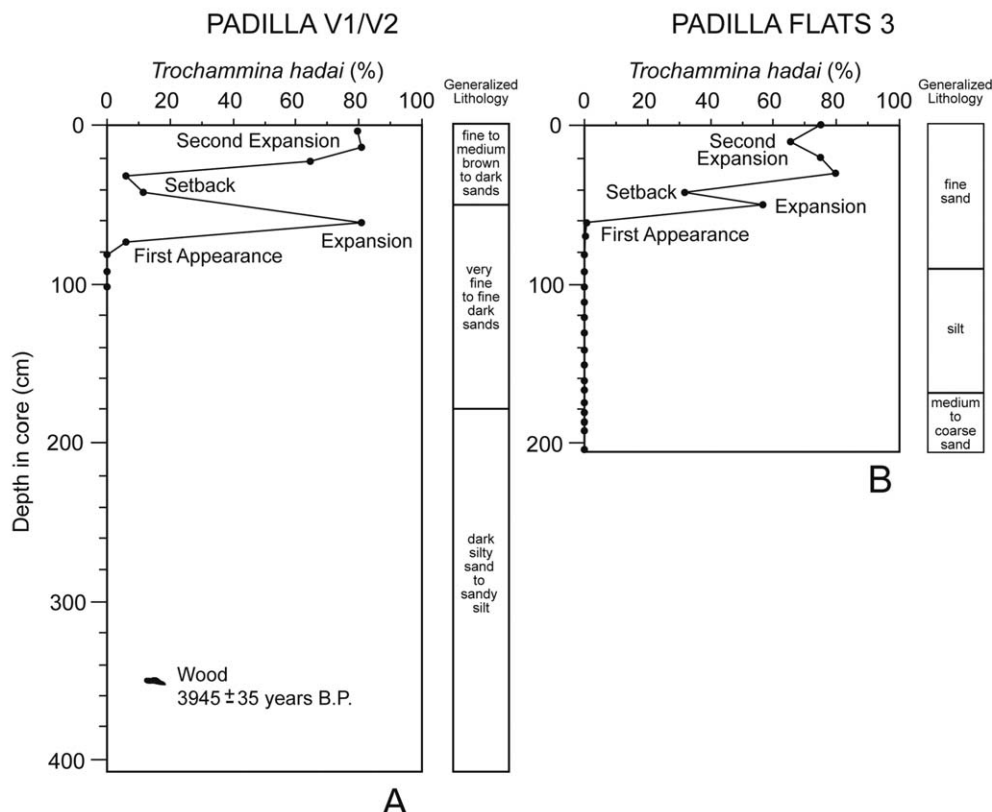
Figure 4. Percentage abundance of *Trochammina hadai* downcore and general lithology in (A) the Padilla V1/V2 core and (B) the Padilla Flats 3 core. The species' first appearance, first expansion, setback, and second expansion are noted. The location of a wood sample, taken for carbon dating from 350 cm downcore in the Padilla V1/V2 core, is shown. The age of the wood was determined to be 3945 ± 35 years B.P.

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TABLE 1. Arenaceous and calcareous benthic foraminiferal species from Padilla Bay identified in this study and their synonymies as reported in Scott (1974).

Arenaceous Taxa
Ammobaculites exiguus Cushman and Brönnimann
Scott: *Ammobaculites dilatatus* Cushman and Brönnimann
Eggerella advena (Cushman)
Haplophragmoides subinvolutum Cushman and McCulloch
Jadammina macrescens (Brady)
Miliammina fusca (Brady)
Spiroplectammina biformis (Parker and Jones)
Trochammina charlottensis Cushman
Trochammina hadai Uchio
Trochammina inflata (Montagu)
Trochammina kelletae Thalmann
Trochammina sp.
Calcareous Taxa
Ammonia beccarii (Linné)
Bolivina vaughani Natland
Buccella frigida (Cushman)
Cibicides lobatulus (Walker and Jacob)
Elphidiella hannai (Cushman and Grant)
Elphidium excavatum (Terquem)
Elphidium frigidum Cushman
Elphidium gunteri Cole
Elphidium magellanicum Heron-Allen and Earland
Elphidium spp.
Scott: *Cribroelphidium* spp.
Neoconorbina opercularis (d'Orbigny)
Scott: *Glabratella opercularis* (d'Orbigny)
Quinqueloculina sp.

changes in the Skagit River that impacted the deposition of sediments at the core site.

Foraminifera

Twenty-three species of arenaceous and calcareous foraminifera were recovered from the surface and downcore samples from Padilla Bay (Table 1). Besides the invasive species *T. hadai*, all of these species commonly reside in estuarine waters of the Pacific Northwest (Phleger 1967, 1970; Scott 1974; Jones and Ross 1979; Williams 1989; Patterson 1990; McGann et al. 1998).

Nine species were recovered from the surface sample collected in 1971 along the east shore of Padilla Bay at Bayview State Park (Table 2). *Trochammina hadai* overwhelmingly dominates (94%) this assemblage. In contrast, *Elphidium excavatum* dominates (67%) a more diverse assemblage of eleven species recovered in 1971

off March Point. In this latter sample, *T. hadai* accounts for only 17% of the assemblage and *Buccella frigida* another 8%.

Thirteen species of foraminifera were recovered in the Padilla V1/V2 core (Table 3). *Trochammina hadai* is absent from 104-74 cm in the core (Figure 4) then first appears at 74-72 cm (5%). A significant change occurs at 64-62 cm where *T. hadai* dominates the assemblage (81%), followed by a decrease up core in abundance to 33% and 6% at 44-42 and 34-32 cm, respectively. From 24 cm to the top of the core, *T. hadai* once again dominates the assemblage (64-81%).

A far more diverse assemblage of 21 foraminiferal species, all common in Pacific Northwest estuaries, characterizes the Padilla Flats 3 core (Table 4). *Trochammina hadai* is absent from the lower two-thirds of the core (206-71 cm; Figure 4) then first appears as a single speciment at 71-69 cm. A few centimeters higher (62-60 cm), the species comprises 1% of the assemblage. By 52-50 cm, its abundance increases to 56%, then drops to 31% of the assemblage at 42-40 cm. Faunal dominance by the invasive returns at 32-30 cm and continues to the top of the core, accounting for 65-80% of the assemblage.

Q-mode Cluster Analysis

The Q-mode cluster analyses performed on various combinations of the data sets from the 1971 surface samples (Bayview State Park and March Point; Table 2), Scott's (1974) 1972-1973 Padilla Bay samples (Table 2), and the Padilla V1/V2 and Padilla Flats 3 cores (Tables 3 and 4, respectively), clearly define two foraminiferal assemblages (Figures 5-7). The first groups together samples prior to and shortly after the first appearance of *T. hadai* in Padilla Bay, and the second, after the species' expansion in the region. The results, therefore, are referred to in terms of *T. hadai* invasion phases (i.e., pre-expansion and expansion). The Padilla V1/V2 data grouped into separate pre-expansion and expansion clusters as well as a pre-expansion outlier. The Padilla Flats 3 foraminiferal counts separated into two pre-expansion and one expansion clusters and all of the abundance data from Padilla Bay (Padilla V1/V2, Padilla Flats

TABLE 2. Species abundances of the benthic foraminifera in the Bayview State Park and March Point surface samples collected in 1971, and Scott's (1974) surface samples collected in Padilla Bay in 1972-1973, given as a percentage of total foraminifera/sample. Abundances reported in Scott (1974) given only as whole numbers and those originally designated as < 1% changed to 0.5% for statistical purposes in the present study.

Foraminifera	Bayview State Park (1971)	March Point (1971)	Scott 4	Scott 6	Scott 7	Scott 8	Scott 9	Scott 10	Scott 11	Scott 12	Scott 13
						(1974)				
Alveophragmium? sp.			1			0.5					
Ammobaculites exiguus	1.0	0.2									
Ammonia beccarii		0.2									
Bolivina vaughani	0.2										
Buccella frigida		7.7		3		1	4		2	19	36
Eggerella advena	0.2	0.5		6	1	1	1		1	7	2
Elphidiella hannai		0.2		10		0.5			1	18	18
Elphidium spp.	0.2	66.7		1	1	6	1			2	8
Haplophragmoides subinvolutum	0.2										
Miliammina fusca	2.9	1.7	12	12	19	20	36		27	6	5
Neoconorbina opercularis	0.2						1		1		
Quinqueloculina sp.	0.2	4.3	2	1	3	9			1	2	3
Spirillina vivipara					1						
Trochammina charlottensis		0.7									
Trochammina hadai	94.3	17.2	86	64	76	63	56		68	45	26
Trochammina inflata		0.5	0.5	1		0.5					2
Other	0.5										
Total Foraminifera counted	419	418	9650	606	2260	3360	1567	0	2491	298	160

TABLE 3. Species abundances of the benthic foraminifera in the Padilla V1/V2 core samples collected in 2004, given as a percentage of total foraminifera/sample. PV1 = Padilla V1 core; PV2 = Padilla V2 core.

Foraminifera	PV2 3-5	PV2 13-15	PV2 22-24	PV2 32-34	PV2 42-44	PV1 62-64	PV1 72-74	PV1 82-84	PV1 92-94	PV1 102-104
Ammobaculites exiguus	0.5	2.7	13.3	31.4	50.4	11.3	81.6	47.2	100.0	8.3
Buccella frigida	0.3		0.5	5.7						
Eggerella advena	0.3		0.5							
Elphidium excavatum		0.3	0.9	11.4						
Elphidium frigidum	0.5									
Elphidium sp.	5.4									
Haplophragmoides subinvolutum				5.7						2.1
Jadammina macrescens	0.3	1.2	0.9	11.4	3.7		1.3	19.4		33.3
Miliammina fusca	12.1	14.0	17.0	5.7	32.6	1.9	9.2	27.8		2.1
Trochammina charlottensis	0.5									
Trochammina hadai	79.9	81.1	64.2	5.7	11.9	81.1	5.3			
Trochammina inflata	0.3		2.8	20.0	1.5	5.7	2.6	5.6		52.1
Trochammina kelletae		0.6		2.9						
Other										2.1
Total Foraminifera counted	373	328	218	35	135	53	76	36	4	48

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TABLE 4. Species abundances of the benthic foraminifera in the Padilla Flats 3 core samples collected in 2004, given as a percentage of total foraminifera/sample. PF3 = Padilla Flats 3 core.

A.

Foraminifera	Core/Sample Depth (cm)											
	PF3 0-2	PF3 10-12	PF3 20-22	PF3 30-32	PF3 40-42	PF3 50-52	PF3 60-62	PF3 69-71	PF3 80-82	PF3 92-94	PF3 100-102	PF3 110-112
Ammobaculites exiguus	1.7	1.7	7.8	3.0	4.5	2.5	11.3	10.1	7.8	2.6	5.2	3.2
Ammonia beccarii										0.4	0.7	0.8
Buccella frigida							15.5	1.7	1.6	8.6	9.7	
Cibicides lobatulus										0.4	0.4	
Eggerella advena	2.6	1.1	5.8	3.4	3.0	2.5	4.2	4.2	4.7	2.6	4.1	0.8
Elphidiella hannai						0.8	5.6		1.6	0.4		
Elphidium excavatum	1.3				3.3	4.2	6.7	13.3	27.8	34.9		0.8
Elphidium frigidum										0.4	0.4	
Elphidium magellanicum										0.4		
Haplophragmoides subinvolutum	0.4	0.3	0.3	0.7	3.0			2.5	3.9	3.0	1.5	8.1
Jadammina macrescens	0.9	2.8	0.3	1.7	3.0		11.3	14.3	8.6	15.0	9.3	18.5
Miliammina fusca	8.7	6.2	2.8	1.7		1.7	2.8	6.7	13.3	10.5	4.8	2.4
Neoconorbina opercularis										1.1	0.4	
Spiroplectammina biformis			0.8									
Trochammina charlottensis	1.3	2.3	3.0	1.7	4.5	5.8	4.2	4.2	3.1	1.5	1.5	
Trochammina hadai	73.9	64.8	73.8	79.5	31.3	56.2	1.4	0.8				
Trochammina inflata	9.1	20.3	4.0	8.4	49.3	27.3	39.4	48.7	40.6	24.8	27.1	65.3
Trochammina kelletae		0.6	1.5							0.8	0.4	
Other				1.5				0.8				
Total Foraminifera counted	230	355	397	298	67	121	71	119	128	266	269	124

B.

| Foraminifera	Core/Sample Depth (cm)										
	PF3 120-122	PF3 130-132	PF3 140-142	PF3 150-152	PF3 160-162	PF3 166-168	PF3 174-176	PF3 181-183	PF3 187-189	PF3 190.5-192.5	PF3 204-206
Ammobaculites exiguus	4.4	3.7	5.4	4.8	4.1	4.4	3.7			8.6	1.2
Ammonia beccarii				1.6		0.9					
Buccella frigida				6.5	13.3	14.9	18.5	7.7	12.3	20.0	8.4
Eggerella advena	0.5	1.9	1.5	8.1	8.2	3.5				2.9	
Elphidiella hannai					1.0	1.8		7.7			0.6
Elphidium gunteri				4.8		0.9	3.7				
Elphidium excavatum	3.9		0.3	38.7	45.9	41.2	37.0		34.2	25.7	47.6
Elphidium frigidum				3.2		1.8					
Haplophragmoides subinvolutum	9.6	11.1	10.0	1.6	3.1						2.1
Jadammina macrescens	14.6	29.6	42.6	9.7	5.1	6.1	11.1	15.4	9.6	5.7	16.5
Miliammina fusca	19.1	1.9	12.8		2.0	0.9		30.8	4.1		2.4
Neoconorbina opercularis									2.7		1.5
Quinqueloculina sp.									1.4		
Trochammina charlottensis	0.2		0.8	3.2	1.0	1.8	3.7		1.4		1.2
Trochammina hadai											
Trochammina inflata	47.7	51.9	26.4	16.1	14.3	17.5	11.1		34.2	22.9	14.1
Trochammina kelletae			0.3	1.6	1.0	4.4	11.1	23.1		14.3	4.5
Trochammina sp.					1.0			15.4			
Total Foraminifera counted	952	54	390	62	98	114	27	13	73	35	334

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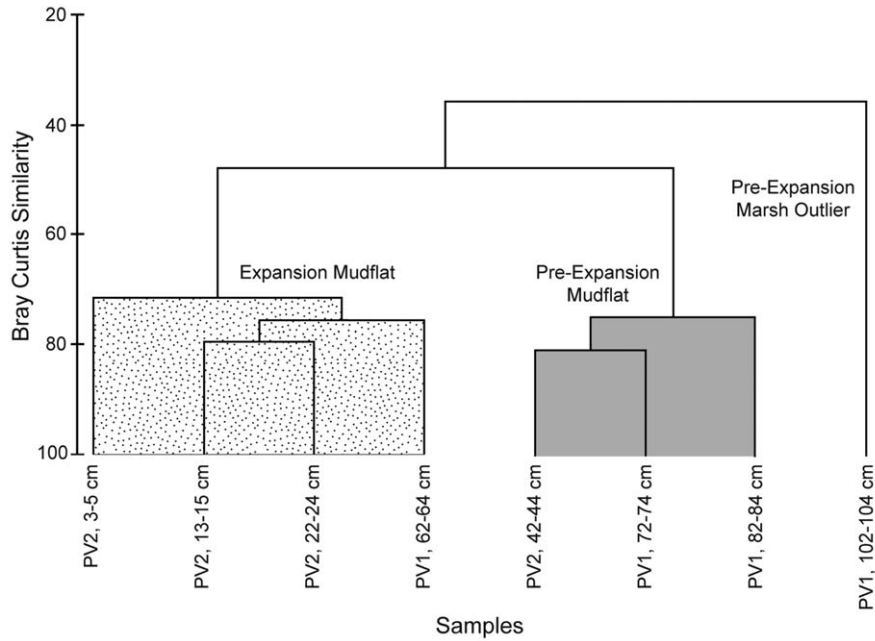
Figure 5. Q-mode cluster diagram of the Padilla V1/V2 core samples. PV1 = Padilla V1 core sample; PV2 = Padilla V2 core sample.

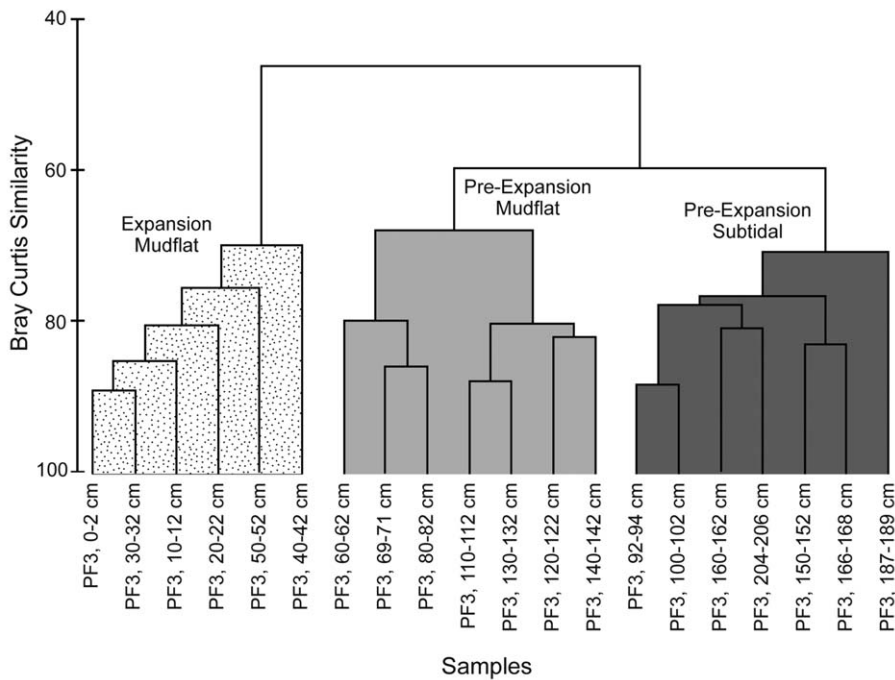Figure 6. Q-mode cluster diagram of the Padilla Flats 3 core samples. PF3 = Padilla Flats 3 core sample.
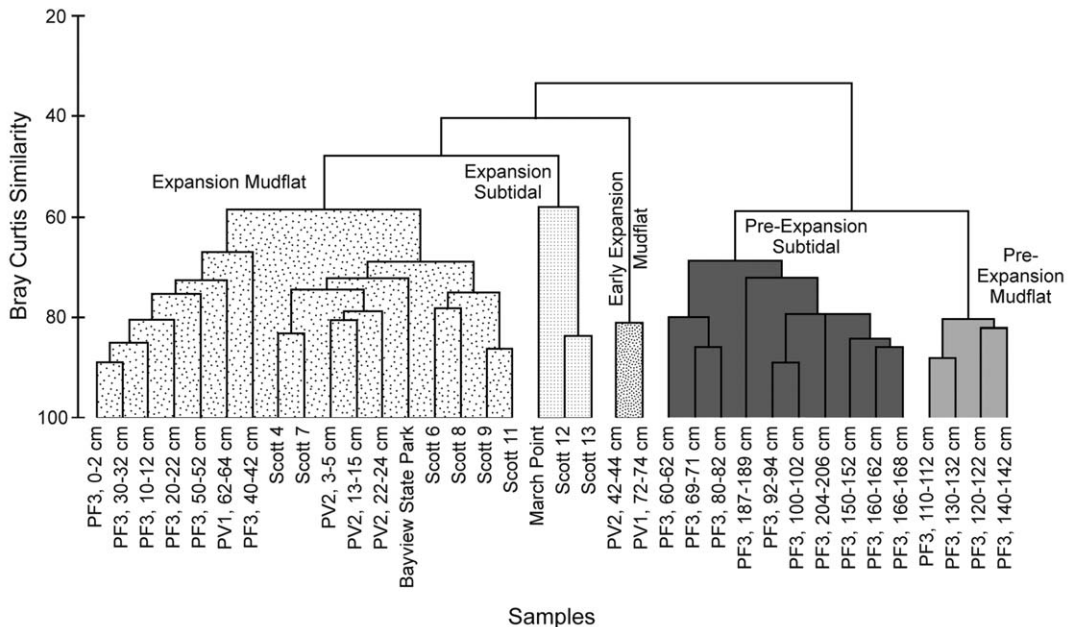
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Figure 7. Q-mode cluster diagram of the Bayview State Park and March Point surface samples collected in 1971, Scott's (1974) surface samples collected in Padilla Bay in 1972-1973, and the Padilla V1/V2 and Padilla Flats 3 core samples collected in 2004. PV1 = Padilla V1 core sample; PV2 = Padilla V2 core sample; PF3 = Padilla Flats 3 core sample.

3, and the surface samples collected in 1971, and in 1972-1973 [Scott, 1974]) clustered into two pre-expansion and three expansion clusters. These assemblages are further described below by the environment (marsh, mudflat, or subtidal) they represent.

Discussion

Foraminiferal Distribution

Mudflat and marsh foraminiferal assemblages typically are characterized by low species diversity because they reside in stressful environments resulting from wide fluctuations in water temperature and salinity (Phleger 1970, Murray 1973, Patterson 1990, Jennings and Nelson 1992, de Rijk 1995). The faunas are also often dominated by arenaceous taxa because high organic input is common, which lowers sediment pH (especially < 7.0), dissolving calcareous tests (Parker and Athern 1959, Arnal 1961, Phleger 1967, Scott and Medioli 1980, Scott and Leckie 1990, Jennings and Nelson 1992). The 1971 shoreline sample from Bayview State Park does, in fact, have a low diversity (9 species)

fauna dominated by arenaceous specimens (98%). The same is true for the Padilla V1/V2 core (11 species and 83-100% arenaceous specimens) and the upper 150 cm of the Padilla Flats 3 core (61-100% arenaceous specimens, except at 100-102 cm [54%]). The 1972-1973 surface samples collected in Padilla Bay (Scott 1974) also follow this trend with 11 species and 84-99% arenaceous specimens, except for two samples (Scott-12 and Scott-13) lying furthest away from the mainland that have only 35-58% arenaceous specimens. In contrast, the 1971 March Point locality, with its more open-ocean exposure, has a fauna of only 21% arenaceous specimens. Similarly, the percentage of arenaceous specimens drops to 39-54% in the lower 50 cm of Padilla Flats 3 (206-150 cm), suggesting the presence of slightly deeper water (subtidal) and an enhanced marine influence on the locality.

In the Padilla V1/V2 core, the sudden and dramatic increase in abundance of *T. hadai* from its first appearance at a depth of 74-72 cm where it accounts for 5% of the foraminiferal assemblage to 81% of the assemblage only ten centimeters

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up core (64-62 cm) (Figure 4), follows a pattern of abrupt dominance commonly seen among invasive species that is best illustrated by recent macrofaunal introductions such as the European zebra mussel (*Dreissena polymorpha*) in the Great Lakes (Mills et al. 1993, Nalepa and Schloesser 1993) and the Asian clam (*Potamocorbula amurensis*) in San Francisco Bay (Carlton et al. 1990, Nichols et al. 1990). The dominance by *T. hadai* continues to the top of the core except for a brief decline from 44-32 cm that perhaps may be attributed to a short-term environmental change (e.g., a temporary increase in input of freshwater) that favors other species. The overall pattern of abundance of *T. hadai* in the Padilla V1/V2 core then is one of first appearance, expansion, setback, and second expansion (Figure 4). In the Padilla Flats 3 core, *T. hadai* also displays the typical explosive growth of an invasive organism and a similar overall pattern of first appearance, expansion, setback, and second expansion (Figure 4), suggesting comparable environmental conditions existed at both core locations.

Q-mode Foraminiferal Assemblages

The Q-mode cluster analysis of the Padilla V1/V2 core census data grouped the two samples from 84-72 cm (Figure 5) because they are both characterized by a low abundance of *T. hadai* (0-5%), high abundance of *Ammobaculites exiguus* (47-82%), and moderate to high abundance of *Miliammina fusca* (9-28%). Dominance by *A. exiguus* and *M. fusca* is common in mudflat environments from Oregon to British Columbia (Hunger 1966, Phleger 1967, Jennings and Nelson 1972) so this assemblage is referred to as the Pre-Expansion Mudflat Assemblage. The samples from 64-3 cm (exclusive of 44-42 cm) are all dominated by *T. hadai* (64-81%) as well as moderate abundances of *M. fusca* (2-17%) and *A. exiguus* (3-13%) and form the Expansion Mudflat Assemblage. The sample from 44-42 cm is an anomaly because it occurs well after the expansion of *T. hadai* but also clusters with the Pre-Expansion Mudflat Assemblage due to its relatively low abundance of *T. hadai* (12%; i.e., a setback in abundance), as well as high abundances of *A. exiguus* (50%), and *M. fusca* (33%). The lowermost sample in the

core, 104-102 cm, is added on last in the Q-mode cluster analysis. No *T. hadai* were recovered in this sample. Instead, *Trochammina inflata* (52%) and *Jadammina macrescens* (33%) occur in very high abundance and *A. exiguus* (8%) is common. Dominance by *T. inflata* and *J. macrescens* is representative of the marsh environment in the Pacific Northwest (Hunger 1966, Phleger 1967, Patterson 1990, Jennings and Nelson 1992). Therefore, this is referred to as the Pre-Expansion Marsh Outlier.

The Padilla Flats 3 core Q-mode cluster analysis separated the foraminiferal samples into three groups: two before the proliferation of *T. hadai* (pre-expansion) and one in which the species occurs in high abundance (expansion) (Figure 6). The first, the Pre-Expansion Subtidal Assemblage, includes samples from 206-150 cm and 102-92 cm. Calcareous taxa that commonly reside in the subtidal zone (*Elphidium excavatum* [28-48%] and *Buccella frigida* [7-15%]) dominate the assemblage, yet arenaceous taxa are still abundant, including *T. inflata* (14-34%) and *J. macrescens* (5-17%). Samples from 142-60 cm (exclusive of 102-92 cm) grouped into the second assemblage, referred to as the Pre-Expansion Mudflat Assemblage. Arenaceous taxa dominate this assemblage (*T. inflata* [26-66%], *J. macrescens* [9-43%], *M. fusca* [2-19%], *A. exiguus* [3-11%], and *Haplophragmoides subinvolutum* [3-11%]), but subtidal calcareous species are also present (*E. excavatum* [<1-13%], *B. frigida* [2-16%], and *Elphidiella hannai* [2-6%]). The third combined the samples from 52 cm to the core top forming the Expansion Mudflat Assemblage. *Trochammina hadai* dominates this assemblage (31-80%) but other arenaceous species are also abundant locally, including *T. inflata* (4-49%), *M. fusca* (2-9%), and *A. exiguus* (2-8%). The abundance of these arenaceous species and low abundance of calcareous taxa suggests this has remained a mudflat environment to the present.

A Q-mode cluster analysis was performed using all of the foraminiferal samples available for Padilla Bay, including the Padilla V1/V2 and Padilla Flats 3 cores, the 1971 samples from Bayview State Park and March Point, and the surface samples collected by Scott (1974) in 1972-1973

in order to reveal any relationship between the downcore and surface samples and possibly suggest the timing of the *T. hadai* invasion (Figure 7). Because only those samples with > 50 specimens were used (eliminating two samples from the Padilla V1 core: 82-84 cm and 102-104 cm) and all species of *Elphidium* were combined into *Elphidium* spp. to make the data sets compatible with Scott's (1974) (i.e., =*Cribroelphidium* spp.), the samples clustered slightly differently than the previous Padilla V1/V2 and Padilla Flats 3 core data sets when clustered alone. In this combined cluster analysis, two assemblages prior to the expansion of *T. hadai* and three after the species' expansion were identified.

The largest grouping of samples prior to the expansion combined Padilla Flats 3 core samples from 204-150 and 82-60 cm and is referred to as the Pre-Expansion Subtidal Assemblage; those from 142-110 cm are the Pre-Expansion Mudflat Assemblage. These assemblages are generally the same as those that resulted from the Padilla Flats 3 data analyzed alone, except that the samples from 82-60 cm clustered with the Pre-Expansion Subtidal Assemblage instead of the Pre-Expansion Mudflat Assemblage. This change in the order of clustering is most likely due to the fact that the three samples between 82 and 60 cm had faunal elements common to both subtidal and mudflat assemblages, and so could have aligned with either assemblage. The ordering may also reflect the fact that the Bray-Curtis similarity coefficient is sensitive to outlying values (Michie 1982). When a Euclidean similarity coefficient was used, the samples from 82-60 cm clustered with the Pre-Expansion Mudflat Assemblage.

Three groupings resulted from the Q-mode cluster analysis of those samples after the *T. hadai* expansion. The largest cluster combined 17 samples with dominant *T. hadai* (31-94%) from all of the data sets: Padilla V1/V2 from 64-62 cm and 24-3 cm, Padilla Flats 3 from 52 cm to the core top, the 1971 Bayview State Park sample, and six of Scott's (1974) 1972-1973 samples to form the Expansion Mudflat Assemblage. These samples further separated into two sub-clusters based on the abundance of secondary species: *M. fusca* (12-

36%) and *A. exiguus* (2-11%) in the Padilla V2 and Scott's samples, and *T. inflata* (4-49%) in the Padilla Flats 3 and PV1 64-62 cm samples. The difference in species abundances between these two sub-clusters may be due to slight changes in environmental conditions that favor one species over another, the most likely factor being changes in porewater salinity from periodic freshwater input due to groundwater seepage or surface runoff (de Rijk 1995). *Miliammina fusca* appears to prefer regions prone to daily flooding as well as sandy substrates with low organic content whereas *T. inflata* does not appear to be sensitive to small changes in salinity, grain size, or organic content (de Rijk 1995).

Three more samples, the 1971 March Point and two of Scott's from 1972-1973, joined to form another expansion assemblage. These samples are characterized by abundant *T. hadai* (17-46%) and subtidal calcareous species (*E. excavatum* [2-67%], *B. frigida* [8-36%], *E. hannai* [<1-18%]), as well as common *M. fusca* (2-6%). The presence of subtidal species suggests these localities are more influenced by the marine environment than most of the others in the study. This cluster is referred to as the Expansion Subtidal Assemblage.

The last cluster to join the expansion samples grouped together the Padilla V1 74-72 cm and Padilla V2 44-42 cm samples. Both have common *T. hadai* (5-12%), are dominated by *A. exiguus* (50-82%), and have abundant *M. fusca* (9-33%). In the Q-mode cluster analysis of the Padilla V1/V2 core samples alone, these two samples clustered with the Pre-Expansion Mudflat Assemblage. Here, they associate with the expansion samples although *T. hadai* does not yet dominate the assemblage. For that reason, they are referred to as the Early Expansion Mudflat Assemblage.

Timing of the *Trochammina hadai* Invasion in Padilla Bay

Analyses by Pb-210 gamma spectroscopy determined that excess Pb-210 was only present in the top 10 cm of the Padilla Flats 3 core and that an accurate sediment accumulation rate could not be derived for this core based on these data. Similarly, the Padilla V1/V2 core failed to produce a

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meaningful chronology. This is in contrast to the initial Pb-210 alpha radionuclide data obtained which provided an erroneous sediment accumulation rate of 1.5 cm/year for the Padilla Flats 3 core and was used to suggest that *T. hadai* first appeared in Padilla Bay about 1958 and expanded around 1972 (McGann et al. 2009), coincident with the species' dominance in surface samples obtained by Scott (1974) in 1972-1973.

The sedimentologic record of the Padilla cores may provide some indication of the timing of the invasion. If it is assumed that the change to fine sand deposition at 92 cm of the Padilla Flats 3 core occurred at about 1900 when the log jams were removed and sedimentation could proceed naturally, and if a constant rate of sedimentation is assumed since then (92 cm/104 yr = 0.9 cm/yr), the first appearance of *T. hadai* would be estimated at about 1927 in the Padilla Flats 3 core. This estimate closely approximates the 1930s oyster culturing date in Padilla Bay. In the Padilla V1/V2 core, the change to fine sand deposition occurs at 180 cm and the sedimentation rate is considerably higher (180 cm/104 yr = 1.7 cm/yr). The first appearance of the invasive would then be estimated at about 1962. This date is much higher than expected and may reflect the fact that the sedimentation rate may not have been constant in this core as the lithology changes (i.e., becomes more coarse) at 50 cm (Figure 4). In either case, the lithologic changes seen in both cores occur prior to *T. hadai*'s arrival and suggests the species' presence is independent of past diversions of the Skagit River.

The combined surface and downcore abundance counts and the manner in which the samples grouped in the Q-mode cluster analysis also provide an approximate indication of the timing of the *T. hadai* invasion in Padilla Bay. The two samples collected in 1971 (Bayview State Park and March Point) and those collected by Scott (1974) in Padilla Bay in 1972-1973 were dominated by *T. hadai* and grouped with the expansion assemblage samples of the Padilla V1/V2 (74-3 cm) and Padilla Flats 3 (52 cm to the core top) cores. These data indicate that the expansion of *T. hadai* took place at the latest in 1971. Furthermore, the pattern of

minor abundances of the species followed by its explosive growth in the two downcore records suggests that the invasion took place just a few years prior to its expansion. In contrast, *T. hadai* did not arrive until sometime between 1981 and 1983 in San Francisco Bay (McGann et al. 2000). Within 12 years, the species spread to nearly all regions of that bay, most often dominating the assemblage (McGann and Sloan 1999). Until we have a more complete chronologic record, we can only conclude that *T. hadai* arrived in Padilla Bay sometime between the late 1800s and 1971, at a minimum 10 years before it invaded San Francisco Bay. The vectors responsible for the species' introduction were likely ballast sediment, anchor mud, or in sediments associated with oysters imported from Japan for mariculture (McGann et al. 2000, 2003; Mach et al. 2010). Perhaps *T. hadai* was introduced into Padilla Bay at the same time as the invasive Japanese eel grass *Z. japonica* that is hypothesized to have been introduced when Japanese oyster culturing began in the 1930s (Mach et al. 2010).

Trochammina hadai may have also arrived in other Pacific Northwest locations prior to San Francisco Bay. For example, Patterson (1990) identified a *Trochammina pacifica* Biofacies on the Fraser River Delta of British Columbia occurring at elevations between 1.1 and 0.5 m below mean sea level in a sheltered area on the southern portion of Robert's Bank between the Robert's Bank Port Causeway and the Tsawwassen Causeway on mudflats vegetated by eel grass (*Zostera marina*) and stated that its occurrence, "…is probably a fairly recent phenomenon, related to the construction of the flanking causeways…[and]…is due to the high level of organic mud in sediment of this area [p. 237]." The Tsawwassen Causeway was completed in 1960 and the Robert's Bank Causeway in 1969; widening and expansion efforts have taken place every decade since (Vancouver Port Authority 2004). The specimen Patterson identified in his biofacies as *T. pacifica* (plate 2, figures 5-7 of Patterson 1990) looks remarkably similar to *T. hadai* and its occurrence may, in fact, be due to an anthropogenic introduction which may or may not have had anything to do with the construction of the causeways. It remains unclear whether *T. hadai* was introduced to different locations on

the west coast through repeated innoculations directly from Japan or from a single invasive event followed by its spread among west coast ports by localized traffic.

Conclusions

Surface samples collected from 1971 to 1973 and two cores obtained in 2004 provide evidence that the invasive Japanese foraminifer *T. hadai* arrived and proliferated in Padilla Bay somewhere between the late 1800s and 1971. The pattern of the species' invasion is consistent with, but considerably earlier than, its introduction in San Francisco Bay in 1981 to 1983. *Trochammina hadai* was probably transported from Japan to western North America in ballast sediment, in anchor mud, or in sediments associated with oysters imported from Japan for mariculture in the 1930s, possibly with the invasive eel grass *Z. japonica*. It is not clear whether the species was introduced to the west coast of the United States through a single or repeated innoculations.

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