

Report of the

Puget Sound Expedition

September 8-16, 1998

A Rapid Assessment Survey of Non-indigenous Species in the Shallow Waters of Puget Sound

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Summary

A Rapid Assessment survey for non-indigenous marine organisms, based on techniques pioneered in San Francisco Bay, was conducted in Puget Sound¹ on September 8-16, 1998. Twenty-three primary stations and nine secondary stations were surveyed, mainly consisting of dock-fouling stations and adjacent shallow water benthic habitats. Material was sampled by a variety of techniques and examined in the field and laboratory by a multi-institutional team covering a broad range of taxonomic expertise. The Expedition team included core researchers from the four San Francisco Bay Expeditions conducted in 1993-1997 and marine scientists from the Puget Sound region. The Expedition was jointly organized by team members from the Washington State Department of Natural Resources, the University of Washington (UW) Friday Harbor Laboratories and the San Francisco Estuary Institute. Laboratory work was conducted at the King County Environmental Laboratory and the UW Friday Harbor Laboratories. Direct and in-kind contributions in support of the Expedition are listed in the Acknowledgments.

The Expedition collected and identified 39 non-indigenous species in six days of sampling. The number of non-indigenous species collected per site showed no obvious pattern with regard to salinity, temperature or oceanographic basin. Eleven of the non-indigenous species collected by the Expedition are new records for Puget Sound, and at least another five are previously unpublished. Several prior lists of non-indigenous marine species in Puget Sound and adjoining waters have been produced:

- Carlton (1979) listed 18 species of non-indigenous invertebrates in Puget Sound. The Puget Sound Expedition collected a total of 36 non-indigenous invertebrates, 24 of which were not listed by Carlton.
- Elston (1997) listed 31 non-indigenous marine species in the shared inland waters of British Columbia and Washington, 14 of which apparently represent valid established species in Puget Sound. The Expedition collected 30 non-indigenous species that are not on Elston's list.
- Ruiz and Hines (1997) list 67 non-indigenous species in the marine and estuarine waters of Washington and British Columbia. Based in large part on the Ruiz and Hines list, the Washington Department of Fish and Wildlife posted on the Internet² a list of 78 "non-indigenous marine species of Washington State and adjacent waters" (adjacent waters apparently referring to the coastal waters of British Columbia). Fifty-one of the species on these lists appear to reflect potentially valid records of established non-indigenous species in this region³, 27 of which appear to reflect valid records from Puget Sound. The Expedition collected 14 non-indigenous species that are not on either of these lists.

¹ For the purpose of this study, the Puget Sound Ambient Monitoring Program's definition of Puget Sound was used, it includes Washington State's inland marine waters east of Cape Flattery.

² June 17, 1998 update posted at www.wa.gov/wdfw/fish/nuisance/ans4.htm

³ Species were deleted from the lists that are not established in these waters, are purely freshwater species, are included elsewhere on the list under another name, that we judge to be better categorized as cryptogenic, or that were apparently listed as present in these waters based solely on their inclusion in Kozloff (1987).

Overall, fewer non-indigenous species were collected in Puget Sound than were collected by similar expeditions in San Francisco Bay. We developed an updated and corrected list of 52 non-indigenous salt and brackish water species that appear to be present and established in Puget Sound. This and other species lists in this report should be considered provisional, pending additional taxonomic work and review.

Methods

The Rapid Assessment survey focused primarily on non-quantitative or semi-quantitative sampling of dock fouling (organisms growing on the sides and undersides of floating docks and associated floats, bumpers, tires, ropes, etc.). Field identification of specimens was followed by examination of sampled material in the laboratory by a team of taxonomic experts. Sampling dock fouling has the following advantages:

- The habitat is easily sampled at low cost and with simple equipment.
- It can be sampled without regard to the tide level.
- There is easy and quick access to a large selection of suitable sites throughout Puget Sound.
- Most sites provide an adequate working area for a sizeable team of experts to sample simultaneously, while remaining in verbal contact.
- In many coastal regions, the dock fouling fauna has been found to include a significant non-indigenous or cryptogenic⁴ component.

Dock sampling sites were selected to obtain broad coverage in terms of spatial distribution, land use, salinity and temperature (Figure 1. Map of Study Sites). Site selection was made by Claudia Mills, assisted by Helen Berry and Betty Bookheim, and involved preliminary examination of about 60 potential sites from the South Sound to the Canadian border.

Dock-fouling organisms were sampled by a variety of simple manual techniques. Tools included hand scrapers, sieves, a long-handled scraper with a fine steel mesh net, and a long-handled (2.4 meter pole) net with 1 mm mesh. A sample of live bay mussels (*Mytilus* sp.) was obtained and frozen from each site where they were present. At most dock sites, benthic (bottom) and plankton samples were also taken.⁵ An Ekman grab was used to obtain non-quantitative bottom samples that were sieve-washed and sorted on site; unsorted bottom samples were retained for later examination for foraminifera and other microfauna. A custom-made cylindrical benthic sampler fitted with 1 mm stainless steel mesh walls was thrown out on a line and retrieved by dragging along the bottom, working like a small benthic sled to collect larger infauna. Vertical plankton hauls were taken with a 0.5 m, 102 μ m mesh net with a 211 μ m mesh cod end. Horizontal plankton tows were taken by pulling a plankton net fitted with 125 μ m mesh alongside each dock, close to the dock fouling, in an effort to obtain demersal organisms such as

⁴ "Cryptogenic" refers to species whose status as non-indigenous or native organisms is unknown (Carlton 1996).

⁵ Jason Toft and Marjorie Wonham were unable to participate during the first two days of the sampling. They returned to the missed sites and collected plankton and mussel samples within two weeks.

harpacticoid copepods.

In addition, pilings were separately sampled at several sites, and nearby intertidal sites were opportunistically sampled. In order to compare similar environments, docks were classified as primary sites, other sites such as beaches were classified as secondary sites.

Temperature and salinity were measured at each dock site. We attempted to use two electronic (YSI) meters to obtain depth profiles of temperature and salinity, but these devices produced varying and unreliable readings (as was the experience of the San Francisco Bay Expeditions). The measurements we report are near-surface measurements obtained with 2 thermometers and 2 refractometers that agreed within 0.5 parts per thousand (ppt).

From each dock site we obtained a one-liter representative voucher collection, plus additional samples of material of interest. The samples were kept on ice on days with laboratory time scheduled soon after the field work, and preserved on-site in formalin or alcohol on other days. Laboratory work was conducted at the King County Environmental Laboratory (on the evening of Sept. 8) and the University of Washington's Friday Harbor Laboratories (Sept. 11-16). The voucher collections were all examined in the laboratory and organisms identified by team members, with some material retained by individual team members for further study. The voucher collections are currently held by the Washington Department of Natural Resources, and are ultimately to be deposited in an appropriate curated facility.

Only a small portion of the data analysis was completed during the Expedition. Work completed after the Expedition and anticipated in the future is discussed on page 17. Additional information on participants, schedule, collecting sites and equipment is provided in Appendices 1-4.

Non-indigenous Species Collected in Puget Sound

The information provided in this section is our best assessment of the data available at the conclusion of the Expedition. However, a great deal of work remains in order to complete analysis of the samples. Information given here should be considered provisional and subject to refinement as analysis progresses.

The Expedition collected at least 39 non-indigenous salt and brackish water species in Puget Sound (Table 1). At least 16 appear to represent new or unpublished records for Puget Sound, however none of the species collected appears to be a new record for the Pacific Coast of North America. Twenty-two additional species we consider to be cryptogenic. Two polychaete worms may represent new species, previously unreported introductions, or substantial range extensions. The status of many of these species may be clarified by further taxonomic analysis.

All of the non-indigenous species collected by the Expedition whose native range is known are native to either the North Atlantic or the Western Pacific (Table 2), with about half from each region. However, the importance of the two source regions has shifted over time. Sixty-one percent of the species first recorded on the Pacific Coast before 1950 originated from the North Atlantic, while 78% of the species first recorded after 1950 originated from the Western Pacific.

The first record of collection on the Pacific Coast for any of the non-indigenous species collected by the Expedition is in 1871. For the species for which we have data, half were first recorded on the Pacific Coast in the latter half of the 1871-1998 period, while 70% have first records for Puget Sound in the latter half of the period. These data suggest no obvious trend over time with regard to initial appearance on the Pacific Coast, but suggest an increase in the rate at which they are appearing or being discovered in Puget Sound.

Possible mechanisms of introduction were assigned in Table 2 based on historic records, known associations of organisms with transport mechanisms, the organisms' biological characteristics, etc. The assigned mechanisms show trends over time. Ship fouling appears to have declined in importance between the earlier and later halves of the period from 1871 to 1998. It is listed as a possible mechanism for 71% of species with first records in the earlier period, and 53% of species in the later period (note that in many cases more than one mechanism is listed for each species). Introductions with Atlantic or Japanese oysters declined slightly between the two periods, while ballast water increased in importance (ballast water listed for 18% of species in the earlier period, 53% of species in the later period).

Table 1. Non-indigenous and Cryptogenic Species and other New Records Collected by the Puget Sound Expedition

This list of species is provisional pending further taxonomic work and review by expedition members and associates.

- A NON-INDIGENOUS SPECIES - New Record of an Introduction for Puget Sound (not previously published or known; Puget Sound defined as the inland marine waters of Washington State east of Cape Flattery)
- B NON-INDIGENOUS SPECIES - Introduction Known but not Published for Puget Sound
- C NON-INDIGENOUS SPECIES - Introduction Published for Puget Sound
- D CRYPTOGENIC SPECIES
- E NON-INDIGENOUS SPECIES - Total (total of A through C)

	A	B	C	D	E
Chlorophyta					0
<i>Enteromorpha</i> sp.				x	
<i>Ulva</i> sp.				x	
Rhodophyta					0
<i>Chondria dasyphylla</i>				x	
<i>Polysiphonia paniculata</i>				x	
Phaeophyceae					1
<i>Sargassum muticum</i>			x		
Anthophyta					2
<i>Spartina anglica</i>			x		
<i>Zostera japonica</i>			x		
Foraminifera					1
<i>Trochammina hadai</i>		x			
Ciliata					0
<i>Zoothamnium</i> sp.				x	
green folliculinids				x	
Porifera					0
<i>Halichondria</i> sp.				x	
Cnidaria: Hydrozoa					1
<i>Cordylophora caspia</i> (= <i>C. lacustris</i>)			x		
Cnidaria: Anthozoa					1
<i>Diadumene lineata</i> (= <i>Haliplanella luciae</i>)			x		
<i>Alcyonium</i> sp.				x	
Annelida: Polychaeta					2
Autolytinae sp. 1 (clear)				x	
Autolytinae sp. 2 (transverse bars)				x	
Autolytinae sp. 3 (transverse orange bars)				x	
Autolytinae sp. 4 (mid-dorsal orange stripe)				x	
Autolytinae sp. 5 (4 longitudinal lines)				x	
Autolytinae sp. 6 (lemon-yellow)				x	
<i>Capitella</i> sp.				x	
<i>Exogone lourei</i>				x	
<i>Harmothoe imbricata</i>				x	
<i>Hobsonia florida</i>			x		
<i>Platynereis</i> sp.				x	

Table 1 (cont'd)

	A	B	C	D	E
Annelida: Polychaeta (cont'd)					
<i>Polydora</i> sp.				x	
<i>Proceraea</i> sp. A (of Piltz, unpubl.)				x	
<i>Pseudopolydora</i> sp.			x		
<i>Typosyllis</i> sp.				x	
Mollusca: Gastropoda					
<i>Batillaria attramentaria</i> (= <i>B. zonalis</i> , = <i>B. cumingi</i>)			x		3
<i>Crepidula fornicata</i>			x		
<i>Myosotella myosotis</i> (= <i>Ovatella myosotis</i>)			x		
Mollusca: Bivalvia					
<i>Crassostrea gigas</i>			x		4
<i>Mya arenaria</i>			x		
<i>Mytilus</i> sp.				x	
<i>Nuttallia obscurata</i>			x		
<i>Venerupis philippinarum</i> (= <i>Tapes japonica</i>)			x		
Arthropoda: Crustacea: Copepoda					
Choniostomatid copepod	x				1
Arthropoda: Crustacea: Cumacea					
<i>Nippoleucon hinumensis</i>	x				1
Arthropoda: Crustacea: Isopoda					
<i>Limnoria tripunctata</i>			x		1
Arthropoda: Crustacea: Amphipoda					
<i>Ampithoe valida</i>		x			9
<i>Caprella mutica</i> (= <i>C. acanthogaster</i>)	x				
<i>Corophium acherusicum</i>			x		
<i>Corophium insidiosum</i>			x		
<i>Eochelidium</i> sp.		x			
<i>Grandidierella japonica</i>		x			
<i>Jassa marmorata</i>			x		
<i>Melita nitida</i>			x		
<i>Parapleustes derzhavini</i>	x				
Bryozoa					
<i>Alcyonidium polyoum</i>				x	6
<i>Bowerbankia</i> "gracilis"			x		
<i>Bugula stolonifera</i>	x				
<i>Bugula</i> sp. 1 (resembling <i>B. turbinata</i>)		x			
<i>Bugula</i> sp. 2 (resembling <i>B. fulva</i>)	x				
<i>Cryptosula pallasiana</i>	x				
<i>Schizoporella unicornis</i>			x		
Entoprocta					
<i>Barentsia benedeni</i>	x				1
Urochordata					
<i>Botryllus schlosseri</i>			x		5
<i>Botrylloides violaceus</i> (earlier reported as <i>Botrylloides</i> sp.)	x				
<i>Ciona savignyi</i>	x				
<i>Molgula manhattensis</i>	x				
<i>Styela clava</i>	x				
TOTAL	11	5	23	23	39

Table 2. Origins, First Records and Mechanisms of Introduction of Non-indigenous Species Collected by the Puget Sound Expedition

This list of species is provisional pending further taxonomic work and review by expedition members and associates.

Native ranges, dates of first record (planting, collection, observation or report) in Puget Sound and on the Pacific Coast of North America, and possible initial mechanisms of introduction to the Pacific Coast are given. First records consisting of written accounts that do not state the date of planting, collection or observation are preceded by the symbol "<". Mechanisms given in parentheses indicate less likely mechanisms. Mechanisms are listed as:

OA - with shipments of Atlantic oysters

OJ - with shipments of Japanese oysters

SF - in ship fouling or boring

SB - in solid ballast

BW - in ship ballast water or seawater system

MR - planted for marsh restoration or erosion control

General Taxon	Species	Native Range	First Pacific Coast Record	First Puget Sound Record	Possible Mechanism of Introduction
Seaweeds	<i>Sargassum muticum</i>	Japan	1944	?	OJ
Anthophyta	<i>Spartina anglica</i>	England	1961-62	1961-62	MR
	<i>Zostera japonica</i>	W Pacific	1957	?	OJ
Foraminifera	<i>Trochammina hadai</i>	Japan	1983	1997	BW,SF,OJ
Cnidaria	<i>Cordylophora caspia</i>	Black/Caspian Seas	ca. 1920	ca. 1920	BW,SF
	<i>Diadumene lineata</i>	Asia	1906	<1939	OA,SF
Annelida	<i>Hobsonia florida</i>	NW Atlantic	1940	1940	?
	<i>Pseudopolydora</i> sp.	?	?	?	?
Mollusca	<i>Batillaria attramentaria</i>	Japan	1924	1924	OJ
	<i>Crepidula fornicata</i>	NW Atlantic	1905	1905	OA
	<i>Myosotella myosotis</i>	Europe?	1871	1927	OA(SB,SF)
	<i>Crassostrea gigas</i>	Japan	1875	1875	OJ
	<i>Mya arenaria</i>	NW Atlantic	1874	1888-89	OA
	<i>Nuttallia obscurata</i>	Japan, Korea (China?)	1989	1991-96	BW
	<i>Venerupis philippinarum</i>	NW Pacific	1924	1924	OJ
	<i>Choniostomatid</i> copepod	?	?	1998	?
Copepoda	<i>Nippoleucon hinumensis</i>	Japan	1979	1998	BW
Cumacea	<i>Limnoria tripunctata</i>	not known	1871 or 1875	?	SF
Isopoda	<i>Ampithoe valida</i>	NW Atlantic	1941	?	BW,OA,SF
	<i>Caprella mutica</i>	Japan to Vladivostok	1973-77	1998	BW,OJ
Amphipoda	<i>Corophium acherusicum</i>	not known	1905	1974-75	OA,SF
	<i>Corophium insidiosum</i>	N Atlantic	1915	1930	OA,SF
	<i>Eochelidium</i> sp.	Japan or Korea	early 1990s?	1997	BW
	<i>Grandidierella japonica</i>	Japan	1966	?	BW,OJ,SF
	<i>Jassa marmorata</i>	NW Atlantic	1941	?	BW, SF
	<i>Melita nitida</i>	NW Atlantic	1938	1966	BW,OA,SB,SF
	<i>Parapleustes derzhavini</i>	W Pacific?	1904	1998	SF
	<i>Barentsia benedeni</i>	Europe	1929	1998	OJ,SF
Bryozoa	<i>Bowerbankia gracilis</i>	NW Atlantic?	<1923	<1953	OA,SF
	<i>Bugula</i> sp. 1	?	?	1993	?
	<i>Bugula</i> sp. 2	?	?	1998	?
	<i>Bugula stolonifera</i>	NW Atlantic	<1978	1998	SF
	<i>Cryptosula pallasiana</i>	N Atlantic	1943-44	1998	OA,SF
	<i>Schizoporella unicornis</i>	NW Pacific	1927	1927	OJ,SF
Urochordata	<i>Botrylloides violaceus</i>	Japan	1973	1977	OJ,SF
	<i>Botryllus schlosseri</i>	NE Atlantic	1944-47	?	OA,SF
	<i>Ciona savignyi</i>	Japan?	1985	1998	BW,SF
	<i>Molgula manhattensis</i>	NW Atlantic	1949	1998	BW,OA,SF
	<i>Styela clava</i>	China to Okhotsk Sea	1932-33	1998	BW,OJ,SF

The number of non-indigenous species collected and identified per site, not counting the annelids or peracarids, ranged from zero to eight (Appendix 5). Initial analysis of the distribution of the non-indigenous species collected by the Expedition reveals no obvious trends in the number of non-indigenous species collected at each dock-fouling or benthic site with regard to salinity, temperature or region (Figures 1-3). The annelid and peracarid material for dock and benthic sites has not been worked-up in the laboratory for all sampled sites; partial data are included in Appendix 5.

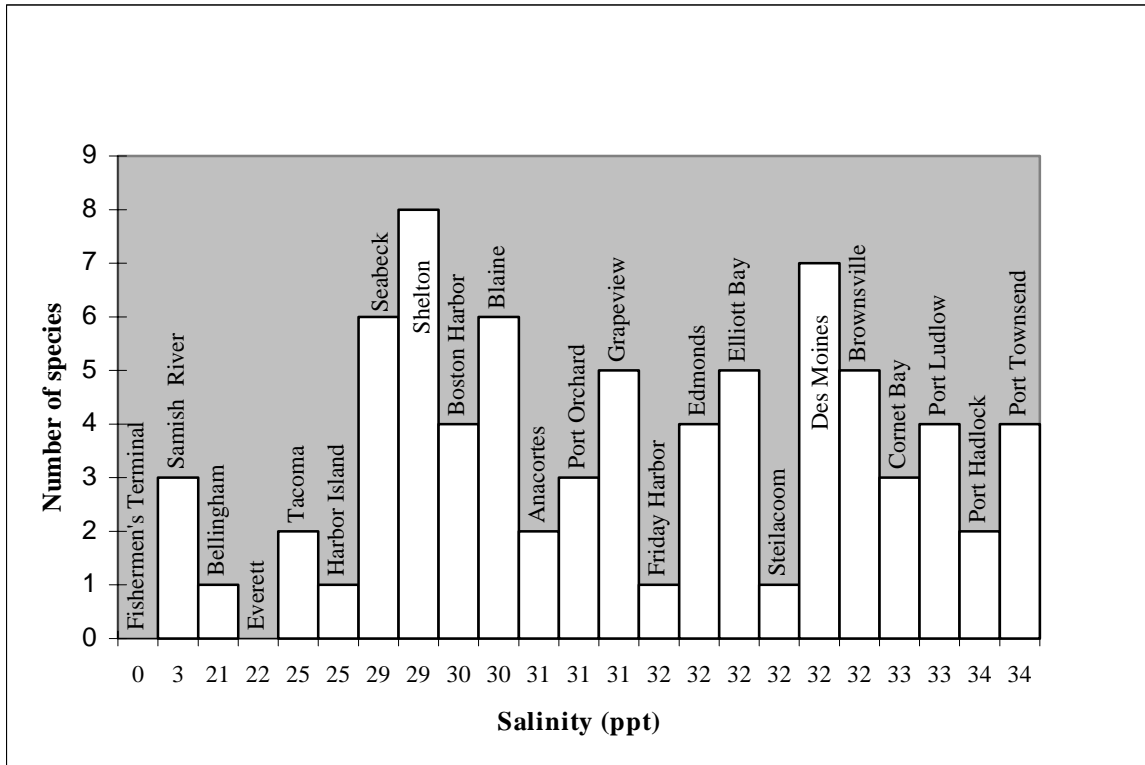


Figure 2. Non-indigenous Species Collected vs. Surface Salinity

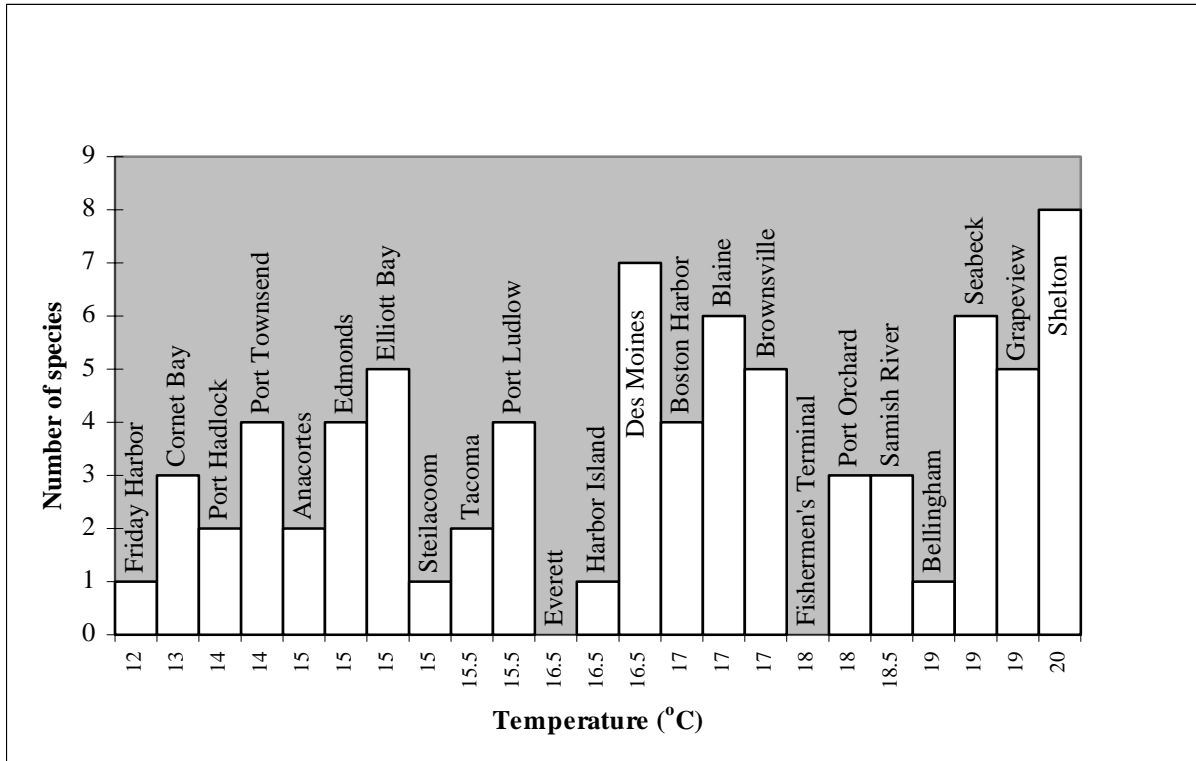


Figure 3. Non-indigenous Species Collected vs. Surface Temperature

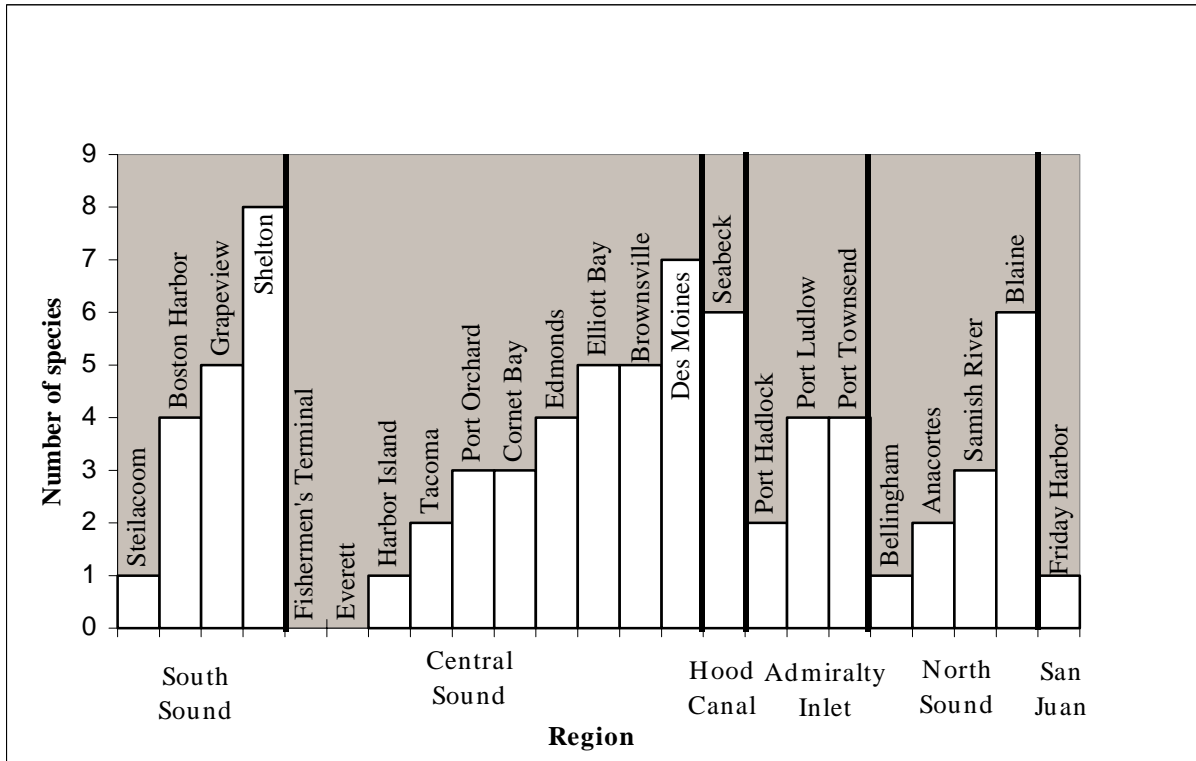


Figure 4. Non-indigenous Species By Region

The general biotic community at each sampling site is described in Table 3. Exotic species were common or dominant organisms at several sites (although generally less common than has been observed by similar expeditions in San Francisco Bay). At many sites bay mussels (*Mytilus* sp.) were the dominant organisms in dock fouling, sometimes approaching 100% cover. These mussels may consist in part of one or two exotic species from the Mediterranean and the Atlantic or their hybrids (the genetic composition of mussels collected from these sites will be analyzed by M. Wonham). At the low salinity Samish River site (3 ppt), the hydroid *Cordylophora caspia*, native to the Black and Caspian seas, covered the shaded sides and undersides of the dock and pilings, and could be collected by the tubful. The Japanese colonial tunicate *Botrylloides violaceus* was abundant in dock fouling at many sites, while the solitary tunicates *Ciona savignyi* (from Japan) and *Molgula manhattensis* (from the western Atlantic) were abundant at, respectively, the Des Moines and Shelton marinas. The small, orange-striped Asian anemone *Diadumene lineata* was also common at Shelton. *Schizoporella unicornis* (from Asia) and *Cryptosula pallasiana* (from the North Atlantic) were among the most frequently collected encrusting bryozoans. The varnish clam *Nuttallia obscurata*, a recent arrival from Asia, was common intertidally at Argyle Lagoon, Blaine and Port Ludlow. The Japanese amphipod *Grandidierella japonica* comprised nearly 100% of the organisms present at the two high intertidal mudflat areas sampled. At three other sites *G. japonica* was found in subtidal grabs or fouling samples, where it would be expected only if dense intertidal mudflat populations were nearby. The native amphipods *Corophium salmonis* and *Corophium brevis* are normally present on intertidal mudflats in the Pacific Northwest, but were not found in the intertidal mudflats where *G. japonica* was abundant.

Table 3. Characterization of the Biotic Communities at Sampling Sites

Sampling Site	Temp. Salinity	Description of Biotic Community
Anacortes (Cap Sante Boat Haven)	15° C 31 ppt	The fouling community on wood/styrofoam and concrete floats was diverse, dominated by the kelp <i>Laminaria saccharina</i> with thecate hydroids growing on its blades, <i>Metridium</i> sp. sea anemones, <i>Mytilus</i> sp. mussels, halichondria-like sponge, colonial tunicates especially <i>Botrylloides violaceus</i> and sabellid worms. Extensive sheets of <i>Perophora annectens</i> tunicates were found on submerged floats (primary site).
Argyle Lagoon (San Juan Island)		The beach and lagoon were surveyed for <i>Spartina</i> sp. and <i>Nuttallia obscurata</i> . The marsh gastropod <i>Myosotella myosotis</i> was common under pieces of wood in the <i>Salicornia virginica</i> marsh (secondary site).
Bellingham (Squalicum Harbor)	19° C 21 ppt	The fauna was depauperate, new and old floats supported sparse <i>Enteromorpha</i> sp. and filamentous diatoms, few mussels and barnacles. This site was sampled for non-indigenous species by Jim Carlton in 1976 and also found to be of very low diversity at that time. We sampled old and new floats in covered and uncovered areas (primary site).
Blaine (Blaine Marina)	17° C 30 ppt	The fouling community was dominated by <i>Mytilus</i> sp, with abundant <i>Botrylloides violaceus</i> , halichondria-like yellow sponge and <i>Metridium</i> sp.; <i>Styela clava</i> common in patches. Water silty due to harbor dredging; some resultant dieoff evident on floats. Sediments collected beneath the floats were black and sulphurous. We sampled the older wood and styrofoam floats (primary site) and the marine park north of the marina (secondary site).

Table 3 (Cont'd). Characterization of the Biotic Communities at Sampling Sites

Sampling Site	Temp. Salinity	Description of Biotic Community
Boston Harbor (Budd Inlet)	17° C 30 ppt	The styrofoam and tire-covered wooden docks were dominated by small (typically less than 2 cm) <i>Mytilus sp.</i> , which formed a general crust about 6 cm thick. <i>Distaplia tunicates</i> , <i>Metridium sp.</i> were common. <i>Enteromorpha sp.</i> was present at the waterline. We sampled submerged and surface floats (primary site), and the adjacent sand beach (secondary site).
Brownsville Marina	17° C 32 ppt	Floats were dominated by <i>Mytilus sp.</i> , white or brown <i>Metridium sp.</i> , <i>Botrylloides violaceus</i> , the thecate hydroid <i>Obelia dichotoma</i> , sabellid worms, barnacles and patchy strands of filamentous diatoms. Small, reproductive hydromedusae of <i>Phialidium sp.</i> were abundant near the concrete and wooden floats (primary site.)
Cornet Bay (Deception Pass Marina, Whidbey Island)	13° C 33 ppt	The fouling community was dominated by scyphozoan scyphistomae (most likely <i>Aurelia sp.</i>) in the shaded dock areas, along with <i>Laminaria saccharina</i> and other kelps, thecate hydroids, and short diatom strands. This marina was unique in not having any colonial tunicates, including the nearly ubiquitous non-indigenous species <i>Botrylloides violaceus</i> . We sampled covered and uncovered wooden docks with styrofoam floats (primary site).
Des Moines (City Marina)	16.5° C 32 ppt	In covered areas, floats were dominated by the solitary tunicate <i>Ciona savignyi</i> , with smaller numbers of other tunicates, especially the colonial <i>Botrylloides violaceus</i> . <i>Metridium sp.</i> were common at the waterline and few <i>Mytilus sp.</i> Open docks had a similar assemblage, but <i>Ciona savignyi</i> was confined to shaded float bottoms. The wood pilings were covered mostly by barnacles, with a few mussels. We sampled covered and uncovered floats on 'L' dock, made of wood with exposed styrofoam flotation (primary site).
Edmonds (Port of Edmonds Marina)	15° C 32 ppt	A relatively diverse float community, dominated by barnacles (primarily <i>Semibalanus cariosus</i>), colonial tunicates (primarily <i>Botrylloides violaceus</i> and <i>Distaplia occidentalis</i>) and mixed algae including abundant filamentous diatoms. Pilings were dominated by <i>Semibalanus cariosus</i> , with some <i>Mytilus sp.</i> We sampled the concrete floats at the Guest Dock, the adjacent covered dock (dominated by the native solitary tunicate <i>Corella inflata</i>) and a submerged, heavily-fouled power cable sheathed in black plastic (primary site).
Elliott Bay Marina (Seattle)	15° C 32 ppt	The concrete floats and pilings supported a diverse fauna and flora, but were dominated by barnacles, mussels, and large sabellid worms near the bases of the floats. Other common residents included limpets, <i>Pododesmus sp.</i> rock scallops, <i>Myxicola infundibulum</i> worms and several species of tunicates (primary site).
Everett (Port of Everett Marina)	16.5° C 22 ppt	Floats were dominated by filamentous diatoms, patchy barnacles, and mussels. Pilings were primarily covered by barnacles. We sampled covered and open areas of the concrete floats, and lines and bumpers on the 'I', 'J' and 'B' docks (primary site).
Fishermen's Terminal (Seattle)	~18° C 0 ppt	The floats, pilings and lines were characterized by small sponges, small feathery green algae, and two freshwater species of bryozoans. We sampled a work float on the outer edge of one of the central piers (primary site).
Friday Harbor (UW Lab dock)	12° C 32 ppt	The fauna on the concrete pontoons and tires was diverse and patchy. Large <i>Metridium sp.</i> were common. Mixed algae was common including large kelps (especially <i>Alaria sp.</i> , <i>Laminaria sp.</i> and <i>Costaria sp.</i>), as well as many species of red bladed algae. Other common species included <i>Balanus nubilis</i> (some covered with <i>Hydractinia</i> hydroids), the bryozoan <i>Dendrobeatia sp.</i> , thecate and atehcate hydroids, halichondria-like sponges and several species of tunicates (primary site).
Grapeview (Fairharbor Marina)	19° C 31 ppt	Dense (up to 100% cover) <i>Mytilus sp.</i> was growing on the floats, the mussels were covered by <i>Botrylloides violaceus</i> , bryozoans and <i>Metridium sp.</i> The covered and uncovered portions of the dock (primary site) and the adjacent beach (secondary site) were sampled.

Table 3 (Cont'd). Characterization of the Biotic Communities at Sampling Sites

Sampling Site	Temp. Salinity	Description of Biotic Community
Harbor Island Marina	16.5° C 25 ppt	The concrete floats were dominated by <i>Mytilus sp.</i> , barnacles, and extensive mats of filamentous diatoms. We sampled in the center of the Harbor Island Marina (primary site).
Port Hadlock Bay Marina	14° C 34 ppt	The concrete floats and a dinghy were fouled by <i>Mytilus sp.</i> , <i>Metridium sp.</i> , the branched thecate hydroid <i>Obelia longissima</i> , sabellid worms, nudibranchs, tunicates and abundant caprellids. At the waterline, <i>Ulva sp.</i> and <i>Enteromorpha sp.</i> algae were common. <i>Nereocystis leutkeana</i> and other kelps were attached to the docks (primary site).
Port Ludlow Marina	15.5° C 33 ppt	The concrete float surfaces were dominated by mussels, ascidians, <i>Metridium sp.</i> , and sponges. We sampled the floats (primary site), the adjacent man-made lagoon (secondary site) behind the marina parking lot, and the nearby debris-covered beach (secondary site). The lagoon supported masses of filamentous green algae and abundant <i>Haminoea sp.</i> . On the beach, <i>Nuttallia obscurata</i> shells were common.
Port Orchard (Kitsap Marina)	18° C 31 ppt	This marina was composed of concrete floats and wooden floats fitted with tires. The dominant organisms were <i>Mytilus sp.</i> , small <i>Metridium sp.</i> , barnacles, and clumps of the thecate hydroid <i>Obelia longissima</i> . Large and small <i>Aurelia labiata</i> jellyfish were common (approximately 3" and 12" diameter) (primary site).
Port Townsend (Boat Haven Marina)	14° C 34 ppt	The concrete floats were dominated by barnacles (mixed <i>Balanus crenatus</i> and <i>Semibalanus cariosus</i>), <i>Mytilus sp.</i> , sabellid worms, <i>Metridium sp.</i> , <i>Botrylloides violaceus</i> , and <i>Ulva sp.</i> (primary site).
Samish River (private dock, Edison)	18.5° C 3 ppt	The wooden floats and pilings were dominated by strands of diatoms, with large clumps of the hydroid <i>Cordylophora caspia</i> in shaded places, few barnacles, and a narrow band of <i>Enteromorpha sp.</i> at the waterline (primary site).
Seabeck Marina (Hood Canal)	19° C 29 ppt	The wood and styrofoam floats (some wrapped in black plastic sheeting) were dominated by <i>Mytilus sp.</i> , the colonial tunicates <i>Botryllus schlosseri</i> and <i>Botrylloides violaceus</i> , the thecate hydroid <i>Obelia longissima</i> , and thick <i>Enteromorpha sp.</i> We sampled the floats (primary site) and the adjacent gravel upper beach (secondary site).
Shelton (Shelton Yacht Club)	20° C 29 ppt	The floats were thickly encrusted with <i>Mytilus sp.</i> and dense anemones (<i>Diadumene lineata</i> at the waterline and <i>Metridium sp.</i> below), tunicates (especially <i>Botryllus schlosseri</i> and <i>Molgula manhattensis</i>) and a halichondria-like sponge. We sampled the main portion of the dock, lined and shaded by individual boathouses (primary site).
Steilacoom Marina	15° C 32 ppt	Floats and tires in the water at the Steilacoom Marina were dominated by <i>Mytilus sp.</i> , <i>Metridium sp.</i> , the thecate hydroid <i>Obelia dichotoma</i> branching out several cm, and <i>Ulva sp.</i> , with large, patchy clumps of sabellid worms. The pilings were covered with barnacles and pandalid shrimp were common several feet below the waterline (primary site). We also sampled the adjacent gravel beach (secondary site).
Swinomish Channel (South Padilla Bay)		This middle and high intertidal mud lagoon on the Swinomish Channel is adjacent to the Swinomish Casino. The mudflat is dominated by <i>Vaucheria sp.</i> We sampled only <i>Spartina anglica</i> (secondary site).
Tacoma (Ole & Charlie's Marina)	15.5° C 25 ppt	The wood and styrofoam float surfaces were dominated by <i>Mytilus sp.</i> and filamentous diatoms up to one meter long. <i>Enteromorpha sp.</i> was common at the waterline. <i>Bankia sp.</i> shipworms were found in older wood. We sampled floats and lines in open and covered areas (primary site).

Current data indicate that of the 113 non-indigenous species listed as collected or potentially

collected by either the Puget Sound Expedition of 1998 or the San Francisco Bay Expeditions of 1993, 1994, 1996 and 1997 (Table 4), the Puget Sound Expedition collected at least 39 species⁶ and may have collected another four species (pending further taxonomic analysis). John Chapman reported collecting an additional two listed species from Puget Sound immediately after the Expedition. The San Francisco Bay Expeditions collected at least 95 of the listed species, and may have collected another seven (Cohen and Carlton 1995). Including the two Chapman records, 28 of the species were collected by expeditions in both regions, 13 are listed as collected by the Puget Sound but not by the San Francisco Bay Expeditions, and 67 are listed as collected by the San Francisco Bay but not the Puget Sound Expedition. (Due to unresolved taxonomic issues, for another five listed species it remains unclear whether they were collected by any of the expeditions.) Even taking into account the fact that these data represent a greater collecting effort over a longer period of time in San Francisco Bay than in Puget Sound, they nevertheless indicate that a substantially greater number of non-indigenous species are present in San Francisco Bay.

Table 4. Non-indigenous Species from Salt or Brackish Water Collected or Observed by the Puget Sound or San Francisco Bay Expeditions

This list of species is provisional pending further taxonomic work and review by expedition members and associates.

PSX = Puget Sound Expedition (1998); SFX = San Francisco Bay Expeditions (1993, 1994, 1996, 1997)

x = collected or observed; 0 = not collected or observed; ? = unclear whether collected pending further taxonomic work or review; * = reported by J. W. Chapman from Mud Bay near Olympia after the Puget Sound Expedition

	<u>PSX</u>	<u>SFX</u>			
Chlorophyta			<i>Blackfordia virginica</i>	0	x
<i>Codium fragile tomentosoides</i>	0	x	<i>Cordylophora caspia</i>	x	x
Phaeophyceae			<i>Ectopleura crocea</i>	0	x
<i>Sargassum muticum</i>	x	x	<i>Garveia franciscana</i>	0	x
Anthophyta			<i>Gonothyraea clarki</i>	0	?
<i>Cotula coronopifolia</i>	0	x	<i>Maeotias inexpectata</i>	0	x
<i>Lepidium latifolium</i>	0	x	<i>Turritopsis nutricula</i>	0	x
<i>Salsola soda</i>	0	x	Cnidaria: Anthozoa		
<i>Spartina alterniflora</i>	0	x	<i>Diadumene "cincta"</i>	0	x
<i>Spartina anglica</i>	x	0	<i>Diadumene franciscana</i>	0	x
<i>Zostera japonica</i>	x	0	<i>Diadumene leucolena</i>	0	x
Foraminifera			<i>Diadumene lineata</i>	x	x
<i>Trochammina hadai</i>	x	x			
Porifera					
<i>Halichondria bowerbanki</i>	?	?			
<i>Haliclona loosanoffi</i>	?	?			
<i>Microciona prolifera</i>	0	x			
	<u>PSX</u>	<u>SFX</u>			
Cnidaria: Hydrozoa					

⁶ For this analysis, the species listed as *Pseudopolydora* sp. in Tables 1 and 2 is assumed to be either *P. kempii* or *P. paucibranchiata*.

	<u>PSX</u>	<u>SFX</u>		<u>PSX</u>	<u>SFX</u>
Annelida: Polychaeta			Arthropoda: Crustacea: Tanaidacea		
<i>Ficopomatus enigmaticus</i>	0	x	tanaid sp. 1	?	x
<i>Heteromastus filiformis</i>	0	x	Arthropoda: Crustacea: Amphipoda		
<i>Hobsonia florida</i>	x	0	<i>Ampelisca abdita</i>	0	x
<i>Marenzelleria viridis</i>	0	x	<i>Ampithoe valida</i>	x	x
<i>Neanthes succinea</i>	*	x	<i>Caprella mutica</i>	x	x
<i>Polydora ligni</i>	0	?	<i>Corophium acherusicum</i>	x	x
<i>Pseudopolydora kempfi</i>	?	?	<i>Corophium alienense</i>	0	x
<i>Pseudopolydora paucibranchiata</i>	?	?	<i>Corophium heteroceratum</i>	0	x
<i>Streblospio benedicti</i>	*	x	<i>Corophium insidiosum</i>	x	x
<i>Typosyllus</i> sp. 1	0	x	<i>Eochelidium</i> sp. 1	x	x
Mollusca: Gastropoda			<i>Gammarus daiberi</i>	0	x
<i>Batillaria attramentaria</i>	x	0	<i>Grandidierella japonica</i>	x	x
<i>Crepidula fornicata</i>	x	0	<i>Jassa marmorata</i>	x	0
<i>Ilyanassa obsoleta</i>	0	x	<i>Leucothoe</i> sp. 1	0	x
<i>Littorina saxatilis</i>	0	x	<i>Melita nitida</i>	x	x
<i>Myosotella myosotis</i>	x	x	<i>Melita</i> sp. 1	0	x
<i>Okenia plana</i>	0	x	<i>Paradexamine</i> sp.	0	x
<i>Philine auriformis</i>	0	x	<i>Parapleustes derzhavini</i>	x	x
<i>Tenellia adspersa</i>	0	x	<i>Stenothoe valida</i>	0	x
Mollusca: Bivalvia			<i>Transorchestia enigmatica</i>	0	x
<i>Crassostrea gigas</i>	x	0	Arthropoda: Crustacea: Decapoda		
<i>Gemma gemma</i>	0	x	<i>Carcinus maenas</i>	0	x
<i>Geukensia demissa</i>	0	x	<i>Eriocheir sinensis</i>	0	x
<i>Macoma petalum</i>	0	x	<i>Palaemon macrodactylus</i>	0	x
<i>Musculista senhousia</i>	0	x	<i>Rhithropanopeus harrisi</i>	0	x
<i>Mya arenaria</i>	x	x	Bryozoa		
<i>Nuttallia obscurata</i>	x	0	<i>Anguinella palmata</i>	0	x
<i>Potamocorbula amurensis</i>	0	x	<i>Bowerbankia "gracilis"</i>	x	x
<i>Teredo navalis</i>	0	x	<i>Bugula neritina</i>	0	x
<i>Theora fragilis</i>	0	x	<i>Bugula stolonifera</i>	x	x
<i>Venerupis philippinarum</i>	x	x	<i>Bugula</i> sp. 1	x	0
Arthropoda: Crustacea: Copepoda			<i>Bugula</i> sp. 2	x	0
Choniostomatid copepod	x	0	<i>Conopeum tenuissimum</i>	?	x
Arthropoda: Crustacea: Cirripedia			<i>Cryptosula pallasiana</i>	x	x
<i>Balanus amphitrite</i>	0	x	<i>Schizoporella unicornis</i>	x	x
<i>Balanus improvisus</i>	0	x	<i>Watersipora "subtorquata"</i>	0	x
Arthropoda: Crustacea: Nebaliacea			<i>Zoobotryon verticillatum</i>	0	x
<i>Epinebalia</i> sp. 1	0	x	Entoprocta		
Arthropoda: Crustacea: Cumacea			<i>Barentsia benedeni</i>	x	x
<i>Nippoleucon hinumensis</i>	x	x	Urochordata		
Arthropoda: Crustacea: Isopoda			<i>Ascidia zara</i>	0	x
<i>Dynoides dentisinus</i>	0	x	<i>Botrylloides</i> cf. <i>diegensis</i>	0	x
<i>Iais californica</i>	0	x	<i>Botryllus schlosseri</i>	x	x
<i>Ianiropsis serricaudis</i>	0	x	<i>Botrylloides violaceus</i>	x	x
<i>Limnoria tripunctata</i>	x	?	<i>Ciona intestinalis</i>	0	x
<i>Munna</i> sp. 1	0	x	<i>Ciona savignyi</i>	x	x
<i>Paranthura</i> sp. 1	0	x	<i>Molgula manhattensis</i>	x	x
<i>Sphaeroma quoyanum</i>	0	x	<i>Styela clava</i>	x	x
<i>Sphaeroma walkeri</i>	0	x	Chordata: Pisces		
<i>Synidotea laevidorsalis</i>	0	x	<i>Acanthogobius flavimanus</i>	0	x
			<i>Lucania parva</i>	0	x
			<i>Morone saxatilis</i>	0	x
			<i>Tridentiger trigonocephalus</i>	0	x

Based on the data developed by the Puget Sound Expedition, and a brief review of the extant lists of non-indigenous species that include the Puget Sound area (including reviews of the Cnidaria by Claudia Mills and Polychaeta by Leslie Harris), we offer a provisional list of 52 non-indigenous species that have been collected from and appear to be established in the salt or brackish waters of Puget Sound (Table 5). In addition, a single specimen of an Asian copepod, *Pseudodiaptomus inopinus*, was collected in the fall of 1991 in the Snohomish River estuary, and a few specimens of another Asian copepod, *Pseudodiaptomus marinus* were collected in the spring of 1998 in Elliott Bay, but it is unclear whether either of these specimens is established in Puget Sound (information from J.F. Cordell).

Table 5. Non-indigenous Species in Puget Sound

This list of species is provisional pending further taxonomic work and review by expedition members and associates.

For the purposes of this list, Puget Sound is defined as the inland marine waters of Washington State east of Cape Flattery. * = species reported by J. W. Chapman from Mud Bay near Olympia after the Puget Sound Expedition.

Phaeophyceae	Mollusca: Bivalvia (con't)
<i>Sargassum muticum</i>	<i>Nuttallia obscurata</i>
Anthophyta	<i>Venerupis philippinarum</i> (= <i>Tapes japonica</i>)
<i>Spartina alterniflora</i>	Arthropoda: Crustacea: Copepoda
<i>Spartina anglica</i>	Choniostomatid copepod
<i>Spartina patens</i>	<i>Mytilicola orientalis</i>
<i>Zostera japonica</i>	Arthropoda: Crustacea: Cumacea
Foraminifera	<i>Nippoleucon hinumensis</i>
<i>Trochammina hadai</i>	Arthropoda: Crustacea: Isopoda
Cnidaria: Hydrozoa	<i>Limnoria tripunctata</i>
<i>Cladonema radiatum</i>	Arthropoda: Crustacea: Amphipoda
<i>Cordylophora caspia</i> (= <i>C. lacustris</i>)	<i>Ampithoe valida</i>
Cnidaria: Anthozoa	<i>Caprella mutica</i> (= <i>C. acanthogaster</i>)
<i>Diadumene lineata</i> (= <i>Haliplanella luciae</i>)	<i>Corophium acherusicum</i>
Platyhelminthes	<i>Corophium insidiosum</i>
<i>Pseudostylochus ostreophagus</i>	<i>Eochelidium</i> sp.
Annelida: Polychaeta	<i>Grandidierella japonica</i>
<i>Hobsonia florida</i>	<i>Jassa marmorata</i>
<i>Neanthes succinea</i> *	<i>Melita nitida</i>
<i>Pseudopolydora</i> sp.	<i>Parapleustes derzhavini</i>
<i>Pygospio elegans</i>	Bryozoa
<i>Streblospio benedicti</i> *	<i>Bowerbankia "gracilis"</i>
Mollusca: Gastropoda	<i>Bugula stolonifera</i>
<i>Batillaria attramentaria</i> (= <i>B. zonalis</i> , = <i>B. cumingi</i>)	<i>Bugula</i> sp. 1
<i>Ceratostoma inornatum</i> (= <i>Ocenebra japonica</i>)	<i>Bugula</i> sp. 2
<i>Crepidula fornicata</i>	<i>Cryptosula pallasiana</i>
<i>Crepidula plana</i>	<i>Schizoporella unicornis</i>
<i>Myosotella myosotis</i> (= <i>Ovatella myosotis</i>)	Entoprocta
<i>Urosalpinx cinerea</i>	<i>Barentsia benedeni</i>
Mollusca: Bivalvia	Urochordata
<i>Crassostrea gigas</i>	<i>Botryllus schlosseri</i>
<i>Musculista senhousia</i> (= <i>Musculus senhousia</i>)	<i>Botrylloides violaceus</i>
<i>Mya arenaria</i>	<i>Ciona savignyi</i>
<i>Mytilus galloprovincialis</i>	<i>Molgula manhattensis</i>
	<i>Styela clava</i>

Future Research and Reports, and Research Needs

Follow-up work

Jeff Cordell and Jason Toft of the University of Washington's Fisheries Research Institute identified the zooplankton. Mary McGann of the U. S. Geological Survey in Menlo Park, CA has completed a preliminary examination and identification of the foraminifera. John Chapman of Oregon State University will complete identification of peracaridan crustacean species. Marjorie Wonham will examine mussels with molecular genetic techniques as part of her University of Washington dissertation research to determine if two nonindigenous mussels (*Mytilus galloprovincialis* and possibly *M. edulis*) are present in Puget Sound in addition to the native bay mussel (*M. trossulus*). Additional taxonomic work remains, which will be completed by expedition participants and associates as time allows.

Anticipated additional reports/presentations

Claudia Mills plans to present a summary of the Expedition at the National Conference on Marine Bioinvasions at the Massachusetts Institute of Technology in January 1999. John Chapman has submitted a paper to the same conference titled "Climate and Non-indigenous Species Introductions in Northern Hemisphere Estuaries." Charles and Gretchen Lambert will include tunicate data from the Expedition in their final report to California Sea Grant on the tunicates of San Diego Bay, as all of the non-indigenous tunicates collected by the Puget Sound Expedition are also known from southern California.

Research needs

As noted in this report, the results reported for the Expedition are provisional and incomplete. This is primarily due to the lack of adequate funding for the Expedition as a whole. Most of the Expedition members participated in the project without benefit of funding for their time, out of an interest in the marine ecology of Puget Sound and the phenomenon of biological invasions. However, judging from past experience with the San Francisco Bay Expeditions, much of the desired follow-up work -- including the time-consuming task of identifying the organisms that could not be identified to species in the immediate round of laboratory work, and compiling, analyzing and reporting on the data -- will likely not take place without additional funding. Much of the potential value of the Expedition could therefore be lost. Funding would make needed research possible, including: taxonomic analysis, publication of results, surveys to monitor invasions over time, further sampling in Puget Sound, and expansion of sampling to British Columbia and other areas.

The substantial ecological and economic impacts of biological invasions in aquatic ecosystems have been well documented, and are increasingly referenced by Washington State agencies and discussed by the public and the press. However, the overall level of funding provided for research into both the nature of these invasions and potential solutions remains a small fraction of the cost of the impacts. Unless this situation changes, efforts to reduce the rate of biological invasions or mitigate their impacts are likely to be hampered.

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APPENDIX 2

Puget Sound Expedition Schedule

Day 1 . Sept. 8 (Tuesday) EAST-CENTRAL PUGET SOUND	TIME SAMPLED
Low tide +0.9 ' at 1151 (Seattle prediction).	
1. Port of Everett Marina	9:30-10:20 am
2. Port of Edmonds Marina	11:15-12:05 pm
3. City of Des Moines Marina	2:30-3:25 pm
4. Harbor Island Marina, near mouth of Duwamish River	4:25-5:10 pm
5. Elliott Bay Marina, Seattle	5:40-6:20 pm
6. Fishermen's Terminal, Seattle	6:45-7:10 pm
Evening work in laboratory at the King County Environmental Lab.	
Day 2. Sept. 9 (Wednesday) SOUTH PUGET SOUND	
Low tide +1.9 ' at 1237 (Seattle prediction).	
7. Ole & Charlie's Marina, Tacoma	9:50-10:40 am
8. Steilacoom Marina	12:10-12:55 pm
9. Boston Harbour Marina, near Olympia	3:05-4:10 pm
10. Port of Shelton Marina	5:20-6:05 pm
11. Fairharbor Marina, Grapeview	6:45-7:20 pm
Day 3. Sept. 10 (Thursday) KITSAP PENINSULA AND OLYMPIC PENINSULA	
Low tide +3.1 ' at 1326 (Seattle prediction).	
12. Kitsap Marina, Port Orchard	8:00-8:45 am
13. Brownsville Marina	9:30-10:30 am
14. Seabeck Marina, Hood Canal	11:50-12:50 pm
15. Port Ludlow Marina	3:15-4:00 pm
16. Port Hadlock Bay Marina	5:00-5:30 pm
17. Boat Haven Marina, Port Townsend	6:10-6:45 pm
Day 4. Sept. 11 (Friday) NORTH PUGET SOUND	
Low tide +4.0 ' at 1328 (Pt. Townsend prediction).	
18. Deception Pass Marina, Cornet Bay	8:50-9:40 am
19. Blaine Marina	11:15-12:10 pm
20. Squalicum Harbor, Port of Bellingham	2:25-2:45 pm
21. Samish Bay - small float in Samish River near Edison	3:35-4:05 pm
22. Padilla Bay - <i>Vaucheria</i> flats east of the Swinomish Channel	4:30-4:45 pm
23. Cap Sante Boat Haven, Anacortes	4:50-5:40 pm
Day 5. Sept. 12 (Saturday) Friday Harbor Laboratories, San Juan Island.	
Work in laboratory.	
C. & G. Lambert sampled 2 marinas on San Juan Island for tunicates	
Day 6. Sept. 13 (Sunday) Friday Harbor Laboratories, SAN JUAN ISLAND.	
Low tides +0.2 ' at 0322 and +5.5 ' at 1610 (Pt. Townsend prediction).	
Work in laboratory.	
24. UW Friday Harbor Laboratories dock, San Juan Island	3:30-4:45 pm
C. and G. Lambert sampled 5 additional marinas on San Juan Island for tunicates.	
Day 7. Sept. 14 (Monday) Friday Harbor Laboratories, SAN JUAN ISLAND.	

Low tides +0.2 ' at 0430 and +5.6 ' at 1740 (Pt. Townsend prediction).

Work in laboratory.

25. Argyle Lagoon and adjacent beach on North, San Juan Island

7:15-8:15 am

C. and G. Lambert sampled 6 marinas on Orcas Island for tunicates.

Sept. 15, 1998 (Tuesday) At the Friday Harbor Laboratories, SAN JUAN ISLAND.

Work in laboratory and begin report.

26. Mud Bay, at base of Eld Inlet, near Olympia sampled by J. Chapman noon for 45 minutes at around noon.

C. and G. Lambert sampled 2 additional marinas on San Juan Island for tunicates.

Sept. 16, 1998 (Wednesday) At the Friday Harbor Laboratories, SAN JUAN ISLAND.

Complete work in laboratory and continue work on report.

C. & G. Lambert sampled 1 marina on Lopez Island and 3 additional marinas in Anacortes for tunicates.

Sept. 17-20 1998 (Thursday-Sunday)

Complete work on report.

APPENDIX 3
Description of Sampling Sites
(listed in the order they were sampled)

Site 1. Port of Everett Marina

Everett is in Possession Sound, in the central Puget Sound basin. The marina is located near the mouth of the Snohomish River in an industrial area north of the Kimberley-Clark facility and the U.S. Naval Base. Salinity in the area is lowered by fresh water input from the Snohomish River, making this site potentially susceptible to non-indigenous brackish water species.

Site 2. Port of Edmonds Marina

Edmonds is located north of Seattle in the central basin. The marina is south of the Edmonds-Kingston ferry terminal. Many of the concrete floats at the marina were replaced after suffering heavy snow damage in December, 1996.

Site 3. City of Des Moines Marina

Des Moines was settled in 1870. The large marina is located in the central basin, approximately mid-way between Elliott Bay, Seattle, and Commencement Bay, Tacoma. The area is heavily used by recreational boats.

Site 4. Harbor Island Marina, Seattle

This small facility with commercial and recreational boats is located in central Seattle, near the mouth of the Duwamish River. The primary land use in southern Elliott Bay is water-based commerce and industry. Reduced salinity makes it a potential introduction site for brackish water species.

Site 5. Elliott Bay Marina, Seattle

Built 6 years ago, this large 1,200 slip marina is situated at the north end of Elliott Bay, near Seattle's commercial / industrial area. Elliott Bay has been a major shipping center in Puget Sound since the mid-1800s.

Site 6. Fishermen's Terminal, Seattle

This large marina for commercial fishing boats lies just inside the Hiram M. Chittenden Locks that connect the freshwater lakes (Lake Washington and Lake Union) with Puget Sound. The site was examined primarily for potential freshwater species such as the hydroid *Cordylophora*.

Site 7. Ole & Charlie's Marina, Tacoma

The marina is located near the mouth of the Hylebos Waterway in Commencement Bay, Tacoma. Tacoma was first settled by Euro-Americans in 1852. Commencement Bay is one of the earliest and most heavily used areas for water-based commerce and industry. Natural mudflat and marsh habitat were dredged and filled in order to create the current waterway system. Significant contamination exists as a result of historical uses. Near the marina, the area is currently dominated by commercial shipping, including log yards and scrap steel.

Site 8. Steilacoom Marina

This small private marina is the northernmost site in the south sound basin. It lies south of the McNeil and Anderson Island ferry terminal in Steilacoom. Steilacoom was established in 1851, and was an early center for West Coast shipping in Puget Sound. The marina is closed to business, yet some boats remain in the area.

Site 9. Boston Harbor Marina, near Olympia

This small, neighborhood marina is located at the northeast end of Budd Inlet, near Olympia. Olympia was an early population center, with a population larger than Seattle during early settlement years. Boston Harbor was recommended to us by Erik Thuesen who teaches marine science at the Evergreen State College, as more diverse than marinas deeper inside Budd Inlet. The site was selected to represent a south sound site that maintained higher salinity and lower temperature than the heads of the bays in Eld Inlet, Budd Inlet and Totten Inlet.

Site 10. Port of Shelton Marina

The Shelton Marina in Oakland Bay at the end of Hammersley Inlet was selected to represent a geographic endpoint of habitats farthest from the entrance to Puget Sound. Activities in the area that might lead to species introduction include aquaculture, log shipping, and recreational boating. Currently, logs are stored in much of the nearshore area in the Shelton embayment. Oyster growing areas were severely impacted by sulfite from pulp mills in the 1920s and 1930s.

Site 11. Fairharbor Marina, Grapeview

Grapeview is located in the northern portion of Case Inlet, in the south sound. Fairharbor Marina is a relatively small neighborhood marina for local and visiting recreational boats. Aquaculture in the area is common. Grapeview was originally platted in 1891.

Site 12. Kitsap Marina, Port Orchard

The Kitsap Marina is a small facility near the Bremerton Naval Shipyard in Sinclair Inlet. Sites in Port Orchard were deemed more accessible than sites in Bremerton. A lumber mill in Port Orchard was functioning for shipping as early as 1855. Heavy historic use of the water for commerce and defense makes the area a potential site of species introductions.

Site 13. Brownsville Marina

Brownsville is located on the Kitsap Peninsula facing western Bainbridge Island. The marina is a small, residential Yacht Club. It was decided to sample at Brownsville, rather than Poulsbo, which is a little further north and at the end of Liberty Bay, based on preliminary investigations by Claudia Mills, who found that the Poulsbo fauna was more depauperate than that on the floats at Brownsville.

Site 14. Seabeck Marina, Hood Canal

Seabeck is on the Kitsap Peninsula on Hood Canal. The Seabeck Marina is the only marina in this primarily residential area. The site represents an approximate mid-point in energy, temperature and salinity gradients found in Hood Canal. Euro-American use of Seabeck began in 1857 with the establishment of a company-owned mill town. Seabeck was the site of a major port facility, shipyards, two sawmills producing 80,000 board feet per day, and a logging camp with 600-1,000 people. The mill was destroyed by fire in 1886.

Site 15. Port Ludlow Marina

Port Ludlow is the name of both a small inlet and the community occupying its shores on the east side of the Olympic Peninsula. Between the 1850s and 1938, Port Ludlow had a mill and shipyard and was one of the most active shipping ports. Closure of the mill changed the nature of this town, which is now predominantly a residential and resort development with little commercial traffic. In addition to visiting the marina area, we walked an adjacent beach strewn with what is said to be old solid ballast.

Site 16. Port Hadlock Bay Marina

Port Hadlock is approximately mid-way between Port Ludlow and Port Townsend. It is a small historic working port on the eastern shore of the Olympic Peninsula, which was the site of an alcohol plant. The Old Alcohol Plant is currently a lodge with a fairly small, private marina.

Site 17. Boat Haven Marina, Port Townsend

Port Townsend, located on the northeast tip of the Olympic Peninsula, was a thriving port in the early decades of settlement of Puget Sound. The port's regional importance decreased when the cross-continental railroad chose Tacoma as its terminus. The city is a popular area for recreational boating and tourism.

Site 18. Deception Pass Marina, Cornet Bay

This small, private marina is situated on Cornet Bay, just east of Deception Pass. The small marina serves private pleasure boats in a small residential community that is situated near the smaller of the two passes through which marine water enters Puget Sound. *Spartina* infestations are known west of the marina.

Site 19. Blaine Marina

The Blaine Marina in Drayton Harbor is run by the Port of Bellingham and is adjacent to the Canada-U.S. border. It was the northernmost point in our expedition and might more accurately be described as Strait of Georgia than Puget Sound. The town of Blaine was platted in 1884. The Blaine marina serves both a small fishing fleet and recreational boaters; there are several fish processing plants and a shipyard at the port facility. The marina is currently being expanded; the water was silty due to dredging and some dieoff on the floats was evident.

Site 20. Squalicum Harbor, Port of Bellingham

This is a very large marina facility run by the Port of Bellingham primarily for pleasure boats. The downtown area is a center for water-dependent commerce. Industrial shipping as well as a large ferry terminal for the Alaska State ferry are located further south in Fairhaven. A number of rivers drain into Bellingham Bay. Bellingham Bay was an early settlement site in Puget Sound. Contamination is known in the bay, stemming from historic activities. Shellfish growing areas were severely impacted by sulfite from pulpmills in the 1930's and 1940's.

Site 21. Samish Bay - small float in Samish River near Edison

This site consisted of a small series of wooden floats in line along the bank of the Samish River near its mouth into Samish Bay. It is used by recreational boaters and fishermen.

Site 22. Swinomish Channel, South Padilla Bay

Spartina populations are known to inhabit the mudflat adjacent to the Swinomish Casino on the Swinomish Reservation. We stopped to survey the status of control efforts there. We collected *Spartina* shoots, no other sampling took place.

Site 23. Cap Sante Boat Haven, Anacortes

The city of Anacortes contains a number of marinas serving recreational boaters, many of which are located on the west side of Fidalgo Bay. Commercial shipping facilities are primarily located either in Guemes Channel to the north, or at the oil refineries on March Point across Fidalgo Bay, with some commercial activity also utilizing the west side of Fidalgo Bay. In addition to oil, Anacortes is the site of log and petroleum coke shipping. The Cap Sante Boat Haven on Fidalgo Bay was selected for its diverse fouling community and its proximity to commercial activities.

Site 24. UW Friday Harbor Laboratories dock, San Juan Island

The University of Washington dock facility is composed of 3 large concrete pontoons, whose sides are ringed with old tires. For nearly one hundred years, many animals and plants that have been studied at the Friday Harbor Laboratories have been returned to the sea near the lab dock. Nearby in Friday Harbor, the Washington State ferry terminal transports cars and passengers north to Sidney, British Columbia, and south to Anacortes. The harbor also receives substantial private passenger ferry traffic from Seattle, recreational boaters from both Puget Sound to the south and the Strait of Georgia to the north, and tour boats. The San Juan Archipelago lies along the route of commercial vessels from the Seattle/Tacoma and Bellingham/Vancouver areas. Ballast water exchange has been observed by residents living on the west side of San Juan Island during 1998.

Site 25. Argyle Lagoon and adjacent beach on San Juan Island

This site was chosen based on known non-indigenous populations. It was invaded by the cordgrass *Spartina alterniflora* in the early 1990s. The cordgrass has been controlled by hand-pulling by classes from the UW Friday Harbor Laboratories, and application of Roundup herbicide -- no shoots were spotted during our rapid survey. Earlier this summer, Claudia Mills discovered that Argyle Lagoon and the adjacent beach on North Bay were host to a several-year old population of the purple varnish clam, *Nuttallia obscurata*, including the largest known specimen, measuring 62 mm in length. The site was surveyed only for *Spartina alterniflora*, *Myosotella myosotis* and *Nuttallia obscurata*.

APPENDIX 4
Equipment list for the Puget Sound Expedition, Sept. 8-16, 1998

2 vans	DNR
2 radios and cell phone	DNR
vouchers for WA State ferries	DNR
48 liter-jars for voucher samples	DNR
300 scintillation vials	DNR
24, 125ml. jars	DNR
18, 250 ml jars	DNR
24, 500 ml jars	DNR
Ziploc bags in various sizes	DNR
duct tape for labels	DNR
pencils, Sharpies for labels	DNR
YSI salinity/temperature meter	DNR
cameras and film	DNR
Rubbermaid tubs - 2 about 15 gal	DNR
2 buckets	DNR
2 clipboards and large rubber bands	DNR
3-ring notebook for field notes	DNR
200 sheets punched lined paper for 3-ring	DNR
books: Kozloff's Keys to NW Invertebrates and Light's Manual	DNR
3 coolers	DNR
boat ramp/ marina guides	DNR
salinity/temperature meter	Lamberts
refractometer	Lamberts
lab thermometer	Lamberts
vehicle	Lamberts
evening lab facility with microscopes	King County Environmental Lab
dip net sampler on 8 foot (2.4 meter) pole	King County Environmental Lab
benthic sampler with 1 mm steel screen	King County Environmental Lab
additional bottles	King County Environmental Lab
vehicle	King County Environmental Lab
Ricoh digital camer	King County Environmental Lab
coolers	King County Environmental Lab
cellular phone	King County Environmental Lab
ID info for barnacles, littorines	Jim Carlton
original field notes from 1970s	Jim Carlton
label paper	Leslie Harris
3 buckets	Claudia Mills
3 dishpans	Claudia Mills
3 dipnets	Claudia Mills
10% formalin, about 5 gallons	Claudia Mills
6 gallons 70% ETOH	Claudia Mills
squeeze bottle for formalin	Claudia Mills
squeeze bottle for ethanol	Claudia Mills
squeeze bottle for water	Claudia Mills
1 lab thermometer	Claudia Mills
camera and film	Claudia Mills

40 pre-printed data cover sheets	Claudia Mills
15 copies of the agenda	Claudia Mills
15 maps for participants notes	Claudia Mills
maps for drivers and participants	Claudia Mills
bowls and petri dishes for IDs	Claudia Mills
vials for <i>Aurelia</i> samples	Claudia Mills
jellyfish scoop	Claudia Mills
1 gallon container for seawater for evening labwork	Claudia Mills
custom-built scraper for pilings	John Chapman
steel sieves	John Chapman
5 gallon plastic rectangular tub	John Chapman
field scope and light	John Chapman
field thermometer	John Chapman
Ekman benthic grab sampler	John Chapman
small sieve for formalin samples	Andy Cohen
refractometer	Andy Cohen
102-125 μm mesh plankton nets and associated gear	Jeff Cordell/UW
YSI salinity/temperature meter	Jeff Cordell/UW
gloves, scrapers	everyone
personal dissecting tools	everyone
additional vials or bags for individual use	everyone
sleeping bags	everyone
specialized literature for identifications	everyone
access to FHL library provided on last 3 days	UW Friday Harbor Labs

APPENDIX 5
Collections by Station of Non-indigenous Species

Insert spreadsheet here

Insert spreadsheet here

APPENDIX 6
Ascidians in Puget Sound and the San Juan Islands
by Gretchen Lambert, Charles Lambert, and Claudia Mills
September 1998

Ascidians are prominent members of the fouling community and are often present in very high densities on marina floats and boat hulls. We found that, after mussels and barnacles, they comprised perhaps the most important group of dock inhabitants in Puget Sound. Boat harbors were surveyed from a northern extreme of Blaine to a southernmost point near Olympia on the Washington mainland, as well as sites on Fidalgo and Whidbey Islands within the context of the Puget Sound Expedition. Sampling was accomplished by reaching under floating docks to remove adherent ascidians and by raising ropes, hoses and wires to examine attached organisms. Salinity and temperature were measured at each collection site.

Summarizing our findings from north to south, Drayton Harbor at Blaine contained the native species *Distaplia occidentalis* and *Corella inflata* along with the introduced species *Botrylloides violaceus* and *Styela clava*. This is the first report of *S. clava* in American waters north of San Francisco Bay in the NE Pacific. Squalicum Harbor in Bellingham, with reduced salinity, had only a few colonies of *Botrylloides violaceus*. The Edmonds Marina had the introduced *Botrylloides violaceus* as well as the native *Distaplia occidentalis*, *Corella inflata*, *Styela gibbsii* and *Diplosoma listerianum*. Similar ascidians were encountered in the Elliott Bay Marina. At the Des Moines Marina several native species were common, but in addition there were huge numbers of *Ciona savignyi*, a species first recorded in California in 1985 but never before noted north of San Francisco Bay. The Des Moines Marina also contained *Botryllus schlosseri* and *Botrylloides violaceus*. In Tacoma at Ole and Charlie's Marina, we found *B. schlosseri* to be numerous and only found a few native *S. gibbsii* and *Distaplia occidentalis*. At the Shelton Marina Yacht Club floats, both *B. violaceus* and *B. schlosseri* were very numerous as was a third introduced ascidian *Molgula manhattensis* which was very abundant, covering all submerged objects. At the Brownsville Yacht Club we found a number of native species along with *B. violaceus* and a few *Ciona savignyi*. The Boat Haven Marina at Port Townsend had all the previously mentioned native species and also the native *Perophora annectens* and *Ascidia callosa*. In addition, the non-native *Botrylloides violaceus* was present in large numbers.

Ascidians are thus an important component of nearly every float-fouling community in Puget Sound. Non-indigenous species comprise a substantial part of this ascidian load, in places forming the dominant biota. At the Des Moines Marina, *Ciona savignyi* occupied a roughly-estimated 90% of the concrete float surface area under covered (shaded) docks, although it was much less abundant in uncovered areas and limited to float bottoms. At many sites, the non-indigenous *B. violaceus* probably accounted for 10-20% of the float cover, often overgrowing other organisms.

The Lamberts examined an additional seven boat harbors on San Juan Island, six more on Orcas Island, and one on Lopez Island in the San Juans on the days immediately following the group collections. Primarily native species were found in most harbors, but *Botrylloides violaceus* was in Roche Harbor, Mitchell Bay and Westcott Bay on San Juan Island, in most harbors on Orcas Island, and in Fishermen's Bay on Lopez. *Ciona savignyi*, *Molgula manhattensis* and *Styela clava* seem to be absent from the San Juan Archipelago at this time.

Introductions of ascidian species seem to fall on a north-south gradient. In Puget Sound we found five introduced species; in San Francisco Bay we found eight and in southern California we found 14 (Lambert & Lambert 1998). It would be very instructive to sample southern California for non-ascidian species to make this north-south comparison comprehensive.

APPENDIX 7
Commentary on species of Hydrozoa, Scyphozoa and Anthozoa (Cnidaria)
Sometimes Listed as Non-Indigenous in Puget Sound
by Claudia Mills
October 1998

A number of lists of non-indigenous marine and aquatic species for Washington State have recently been developed (see page 1 of this report) without the specific guidance of taxonomic experts for various groups. I offer here my assessment of the cnidarian species that have been included on some of those lists, ending with a list of those species that should henceforth be included as either non-indigenous or cryptogenic in Puget Sound.

HYDROZOA

Bougainvillia muscus (Allman, 1863). Sometimes listed under its junior synonym *B. ramosa*. I collected hydroids of this species on floats of the town docks of Friday Harbor in 1978. Medusae from the hydroids were raised in the laboratory in order to positively identify this species. The status of this species, which has been recorded from all over the world, is uncertain, as it may in fact represent more than one cryptic species (Calder, 1988). It should be listed as cryptogenic in Washington.

Cladonema radiatum Dujardin, 1843. This very small hydromedusa and its polyp have been very abundant amongst *Zostera marina* on the east shore of Padilla Bay during the past decade. It is considered to be a West-Atlantic boreal species, although it has also been collected at least in Bermuda, the Bahamas, Florida and perhaps Japan (Kramp, 1959; Calder, 1988). The species was first collected in Washington in 1988 by Eugene Kozloff, Judy Friesen and Francis Ambrose and identified by Claudia Mills. The population was probably established for several to many years before it was noticed. I have continued to collect it regularly in Padilla Bay since 1988 and have made some unpublished observations on its natural history.

Cordylophora caspia (Pallas, 1771). Sometimes referred to by its junior synonym *Cordylophora lacustris*, this species is reported to have been collected in Lake Union by Trevor Kincaid about 1920 (Carlton, 1979). We found luxurious growths of this species at Edison, near the mouth of the Samish River. The Puget Sound Expedition site at Fishermen's Terminal was included in order to look for this species and I further searched during October 1998 at several sites on Lake Union and in the Montlake cut, but none was found. This species is generally considered to be native to the Caspian and Black Seas, but has been found worldwide including a variety of low salinity sites from British Columbia to California (Carlton, 1979).

Ectopleura crocea (L. Agassiz, 1862). There are records of this Atlantic species in the San Juan Islands from the 1930s (see Carlton, 1979), but except for an unsuccessful intentional attempt to introduce this species using East Coast material in the early 1980s, I know of no recent collections. Confusion with several of what are thought to be native species of *Ectopleura*, *Tubularia* or *Hybocodon* is likely, and species names should only be assigned to this group with great care. This species should be removed from the list of non-indigenous species in Washington until such time as new collections show that it is really present and established.

Gonothyraea clarki (Marktanner-Turneretscher, 1895). This is one of several *Gonothyraea* species reported from the Pacific Coast by Fraser (1937), who records it from numerous locations between the Aleutian Islands and southern California including the San Juan Islands. If all are a single species, it could be either native eastern-Pacific or circumpolar. This genus can be separated from other members of the family Campanulariidae based on its reproductive structures, which are also needed to make positive identification to species. This species should be removed from lists of non-indigenous west coast species pending further study.

Obelia spp. I remain skeptical that *Obelia* is an exotic genus in the eastern Pacific; in a poll of my colleagues at the September 1998 Workshop of the Hydrozoan Society in California, most hydrozoan taxonomists present felt confident that this genus is cosmopolitan. Species identification within this genus is highly problematic and the number of valid species is hotly debated by specialists. At the Hydrozoan Workshop, Professor Wim Vervoort of the National Museum of Natural History in Leiden, Netherlands, identified both *Obelia dichotoma* (Linnaeus, 1758) and *Obelia longissima* (Pallas, 1766) from the Puget Sound Expedition samples. Until someone does an in-depth molecular and genetic study of this genus, both *O. dichotoma* and *O. longissima* might be listed as cryptogenic in Washington.

Sarsia tubulosa (M. Sars, 1835). This species should not be on any list of non-indigenous west coast species, as it almost certainly occurs natively on the west coast. All of the problems related to *Sarsia* identification, including early references to *Syncoryne mirabilis*, *Coryne rosaria* and others (see Carlton, 1979), actually reflect purely taxonomic (rather than biogeographic) problems. There are at least 4 species of *Sarsia* that seem to be native to the Puget Sound/Strait of Georgia region, including *S. tubulosa*, which appears to be a circumboreal species (see Arai and Brinckmann-Voss, 1980). Although the genus is well known and easily recognized, most species of *Sarsia* are very difficult to identify correctly, and detailed morphology of both the medusa and its polyp must be known in order to apply a species name in most cases.

SCYPHOZOA

Aurelia aurita (Linnaeus, 1758) and *Aurelia labiata* Chamisso and Eysenhardt, 1821. Recent unpublished work by Lisa Gershwin of the University of California at Berkeley has convinced me that both species are good and that both have been found in recent times on the west coast. Certainly both species can presently be found on display in public aquariums on the west coast, sometimes in the same tank. Although the name *A. labiata* has been used in various publications including Light's Manual Third Edition, it is Gershwin's new morphological observations that indicate this species to be distinctly different from *A. aurita*. New unpublished molecular data by a number of scientists appears to support that conclusion. I summarize what are believed to be valid morphological species descriptions in Wrobel and Mills (1998). Most *Aurelia* medusae on the west coast, including all specimens known from Puget Sound, seem to be *A. labiata*, which was originally described from near San Francisco. Specimens of *A. labiata* collected in Port Orchard during the Puget Sound Expedition were sent to Werner Schroth of Johann Wolfgang Goethe-University, Frankfurt am Main, Germany, for molecular analysis. *Aurelia aurita* should be removed from the list of non-indigenous species in Washington until such time as new collections show that a second species is also present here.

ANTHOZOA

Diadumene lineata Merrill, 1870. Often listed as its junior synonym *Haliplanella luciae*. This anemone with worldwide distribution was collected by the Puget Sound Expedition at the Shelton Marina where it occurred in great numbers at the waterline of the floats. It has also been collected by Jim Carlton, Art Siebert, and Claudia Mills on separate occasions in the 1970s on rocks in the high intertidal at the Friday Harbor Laboratories, below the library. Bruno Pernet collected it in Padilla Bay in May 1998 on cobbles just beyond the boulders supporting the beginning of the dyke trail, along with lots of non-indigenous *Batillaria* snails. Carlton (1979) gives a fairly cohesive argument that it is likely to be non-indigenous to Washington State, favoring the theory of an Asian Pacific origin, but since its real origin may never be understood, it might fairly be listed as either non-indigenous or cryptogenic in Washington.

Nematostella vectensis Stephenson, 1935. Known in Puget Sound from Jaekle's Lagoon on San Juan Island

and a lagoon near Camp Casey on Whidbey Island. This estuarine species has an extremely broad worldwide distribution, not so different from *Diadumene lineata*. Hand and Uhlinger (1994) have located it in all but two of the United States with coastlines. There is some question of whether or not this species with widespread global distribution is native or non-indigenous, but Hand and Uhlinger weigh in slightly favoring the spread of this low salinity, quiet water species with mariculture and shipping. Listing this species as cryptogenic in Washington seems most appropriate.

In summary, the following non-indigenous (NIS) and cryptogenic (C) species should be listed as present and established in Puget Sound:

HYDROZOA

<i>Bougainvillia muscus</i> (= <i>B. ramosa</i>)	C
<i>Cladonema radiatum</i>	NIS
<i>Cordylophora caspia</i> (= <i>Cordylophora lacustris</i>)	NIS
<i>Obelia dichotoma</i>	C
<i>Obelia longissima</i>	C

SCYPHOZOA (NONE)

ANTHOZOA

<i>Diadumene lineata</i> (= <i>Haliplanella luciae</i>)	NIS or C
<i>Nematostella vectensis</i>	C

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