Zooplankton in ballast of ships entering Puget Sound, Washington

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Abstract

Due to its physical oceanography and presence of international shipping, Puget Sound, Washington, USA may be at high risk for introductions of aquatic invasive non-indigenous species (NIS). In order to evaluate the potential risk posed by NIS in arriving ballast water, we sampled zooplankton in 246 ships of various types after they entered Puget Sound. We classified taxa into two major groups: a potentially high risk group consisting of known NIS plus coastal organisms in ballast obtained in regions other than the northeast Pacific, and a low risk group consisting of oceanic species and those of undetermined origin. Most of the ships sampled had reported conducting mid-ocean ballast water exchange (MOE). Our data indicate that, despite Washington State regulations requiring MOE, and apparent compliance by ship operators, sampled ships had relatively high densities and/or percentages of NIS and/or non-native coastal species. Percent composition of risky organisms decreased three percent per year during the five year study period, possibly due to increased regulatory scrutiny during that time. While exchange efficiency models and controlled experiments with before-and-after exchange sampling have indicated that MOE is consistently effective for most zooplankton, our study found that MOE as practiced probably has little effect in reducing the introduction of planktonic NIS to Puget Sound. Possible reasons for this are that MOE was reported by ship operators, but not conducted, or conducted incompletely, or that MOE is not effective for some ship types or ballast tank configurations. Our study found that densities of both NIS and coastal taxa and percentages of NIS were consistently and significantly higher from domestic trips dominated by articulated tug/barges and tankers carrying ballast water from California, and lower in samples from transpacific trips dominated by container ships and bulk carriers with ballast from Japan, China, and South Korea. We also found relative high NIS abundance in a few samples from other sources on the west coast of North America. These findings are probably a result of the dense and diverse NIS assemblages present in California and other west coast estuaries and comparatively short ship transit times between them and Puget Sound.

Introduction

Along with worldwide growth in shipping commerce, increasing ship speeds and ballast capacities have probably increased the risk of introductions of non-indigenous aquatic species. In the Great Lakes there were 90 known introductions during the 150 years between 1810 and 1959, in the 40 years between 1960 and 1990 there were 43 known introductions, and since 1990 the discovery rate of introduced species has increased even more (Mills et al. 1993, Holeck et al. 2004). This pattern is similar but even more pronounced in the San Francisco estuary on the west coast of North America, where the rate of new species introductions increased from about one every 55 weeks before 1960 to one every 14 weeks after 1960 for a total of over 200 species (Cohen and Carlton 1998). A recent analysis has shown that the Puget Sound region has experienced a similar cumulative increase in successful marine/estuarine non-indigenous species invasions, with a total of 76 introduced species (Wonham & Carlton 2005). The same analysis showed that compared with other locations in the region, Puget Sound and the adjacent Straits of Juan de Fuca have fewer ballast introductions and more introductions associated with aquaculture imports of the Pacific oyster *Crassostrea gigas*. However, oyster imports to the Puget Sound region peaked in the 1950s and are now rare, and almost all of the introductions in the region after the 1980s are thought to be from ballast water (Wonham & Carlton 2005).

Puget Sound, located in Washington State, is a fjord-like estuary comprising four major sub-basins and connected to the Pacific Ocean by the Straits of Juan de Fuca (Fig. 1). The main sub-basin covers about 45% of the area, contains about 60% of the water, and is the location of the major shipping ports of Seattle and Tacoma. In data from 1991, these two ports taken together ranked 6th among 17 United States ports in the volume of acknowledged ballast water discharged, receiving an estimated 2.69×10^6 metric tons (Carlton et al. 1995, Gramling 2000). Ballast water arriving at other locations on the Pacific coast of North America has been shown to contain dense and diverse assemblages of organisms (Carlton & Geller 1993, Levings et al. 2004, Choi et al. 2005) and is currently the most frequently cited method for transfer of nonnative organisms worldwide. Puget Sound is characterized by having several relatively deep basins separated from each other and from the Straits of Juan de Fuca by shallow sills (Fig. 1). These and other factors (e.g., drought, Newton et al 2003) may limit exchange both among basins and bays in Puget Sound and between Puget Sound and the ocean. Residence times of water can be quite long in some parts of Puget Sound (e.g., up to two months in the southern sub-basin, Albertson et al. 2002). These oceanographic factors and the large ballast volumes and frequency of possible non-indigenous species inoculations may enhance the risk of further invasions in Puget Sound.

Because there is a very poor record of eliminating introduced species once they are established, the best method is to interdict new introductions is by addressing major pathways, such as ballast discharge. Ballast water mid-ocean exchange (MOE) is currently the only widely approved method for reducing the risk of transferring invasive species. Ballast taken on in coastal ports and containing risky coastal species is exchanged with oceanic water that contains low risk species. Ships primarily conduct two types of MOE: flow-through exchange, in which ocean water is pumped continuously through a ballast tank to flush out coastal water from the ballast source port,

and empty-refill exchange, in which a ballast tank is first emptied of ballast water and then refilled with ocean water. Flow-through exchange usually involves exchange of at least three times the volume of the ballast tank, which is predicted to eliminate 95% of ballasted water (Hay and Tanis 1998). When properly practiced, MOE can replace between approximately 95% and 100% of the original water, and in theory, the discharge of exchanged oceanic water should significantly reduce the risk of new non-indigenous species introductions (Rigby et al. 1999). However, in practice, organism removal is a complex issue that can vary among particular ships, voyages, seasons, type of MOE options, and types of organisms (Dickman & Zhang 1999, Rigby et al. 1999, Zhang & Dickman 1999, Wohham et al. 2005).

In the United States, federal law requires ballast exchange to be conducted a minimum of 200 nautical miles from shore for international voyages. Coastal voyages are exempt from the current federal law. However, the states of Washington, Oregon, and California have enacted laws requiring ships on coastal voyages to exchange their ballast a minimum of 50 nautical miles from shore. The federal and state laws require international and coastal voyages to conduct a one time empty-refill exchange or a three times flow through exchange. No regulatory structure currently exists at federal or state levels to verify ballast exchange, other than asking the ship operators if they complied with the law. In addition, there is not a clear understanding of how much reduction in undesirable organisms is achieved by currently practiced MOE techniques, either on individual ship or aggregated bases. Washington Department of Fish and Wildlife (WDFW) was given authority to implement the Washington State ballast management law, and in 2004 began a systematic collection of ballast exchange data and zooplankton samples from ships arriving in Puget Sound. In this study we examine exchange data and zooplankton from the WDFW collections and from previous samples and data that were collected by the University of Washington (UW) beginning in 2001. The goals of this study are to (1) document the sources and exchange locations of ballast water entering Puget Sound, as reported on ship's ballast water reporting forms; (2) compare zooplankton faunas of those ships that recorded MOE vs. those that did not; (3) explore relationships between zooplankton assemblage structure and density and factors such as reported source, ship route, ship type, and temperature; and (4) examine the zooplankton and ship data for changes that have occurred during a period of increased regulatory scrutiny of ships entering Washington State waters. Our intent is to better understand the effectiveness of reported MOE in reducing the risk of new invasions in Puget Sound and the Pacific Northwest.

Methods

Ship Sampling

The UW began ship sampling on 2 February 2001. Sampling was conducted on vessels entering the ports of Seattle and Tacoma, Washington, associated with two shipping agencies worked with the UW. The Puget Sound Marine Exchange Report was consulted daily, and when a ship from one of the agencies was inbound, they were contacted and arranged for sampling so as to not interrupt ship operations. On 22 June 2004, WDFW began sampling ships. As part of its authority to implement ballast water regulations, WDFW was not required to consult with shipping companies before

boarding vessels. On a given day, if a vessel from a potentially high-risk domestic port was available (e.g., San Francisco Bay), it was sampled. Also, if a ship that had shown previously high proportions of coastal or non-indigenous ballast organisms was in port, it was sampled. Otherwise, ships were sampled randomly as they arrived. Most of the samples taken by WDFW were in the ports of Seattle and Tacoma, but several other smaller ports in the Puget Sound region were occasionally sampled, and several samples were also taken from ships in Gray's Harbor on the Washington coast and from several ports on the Columbia River.

One tank per ship was sampled, and tanks were chosen in the following order of priority: (1) tanks that had undergone a reported MOE (i.e., those that would presumably be discharged into Puget Sound); (2) fullest tanks; (3) easiest tanks to access via hatches (wing tanks were preferred, as they generally had easiest access and most unobstructed water columns; and (4) arbitrary choice by master or chief of the vessel.

We obtained the following information from each ship's Ballast Water Reporting Form (BWRF): vessel name, IMO number, owner, vessel type, last port, total ballast capacity, total discharge, exchange type, yes/no exchange, date source water was ballasted, exchange date, and exchange location.

Sampling Methods

Zooplankton were sampled with a conical 33 cm diameter 73 µm mesh plankton net. Three replicate plankton samples were taken in each ship. Depth was measured with a 30 m weighted measuring tape, from tank bottom to the top of the water column. The net was then lowered to the bottom, and after approximately 15 s, it was pulled to the surface at a rate of approximately 30 cm s^{-1}. Zooplankton was washed from the cod-end of the net into pre-labeled plastic sample jars and fixed in 10% formalin. In each tank near-surface salinity and temperature were measured with a YSI model 33 salinity-temperature meter or with a handheld refractometer and thermometer.

In the laboratory, each zooplankton sampled was filtered through a 30 µm mesh screen and placed into a plankton counting tray. Zooplankton taxa were counted under a dissecting microscope at 25X, except for some taxa, which were removed and identified using a compound microscope. Larval forms of invertebrates were generally identified to higher taxonomic levels such as order (e.g., Calanoida) suborder (e.g., Balanomorpha) or class (e.g., Bivalvia). Adults were identified to species in most cases. Based on published taxonomic and distributional literature (list of sources available from corresponding author), we assigned each species or group to one of the following categories: (1) coastal/non-indigenous taxa, which included meroplankton such as larvae of shallow water invertebrates, plus holoplankton species such as copepods which are not known to occur in the northeast Pacific; and (2) taxa of oceanic, cosmopolitan, or uncertain origin. We assumed that organisms in the first category represented source port or nearshore ballast water, and those in the second category mostly represented oceanic (exchanged) ballast water.

Data Analyses

Exploratory methods

Exploratory data analyses were conducted using graphical methods that would allow visual assessment of the distribution of both the density and the percent composition of total zooplankton, non-indigenous taxa, coastal taxa, and combined coastal and non-indigenous taxa.

Graphical methods were used to assess how the distribution of these dependent variables related to categorical and continuous independent variables believed to affect them. Categorical factors in this study included exchange method, season, ballast source, vessel category, and before and after increased scrutiny by WDFW inspectors (Table 1). Continuous covariates included time since exchange (age of ballast water), temperature, and salinity (Table 2).

Table 1. Categorical independent factors and levels within factors used. Numbers in parentheses indicated number of ship samples in each level.

Categorical Factor	Number of levels /categories	Levels (n)	
Ballast source	16 (9 levels with n>3)	Alaska (1) British Columbia (3) California (62) Washington (2) Oregon (4) China (30) Japan (88) S. Korea (18)	Taiwan (4) Pacific Ocean (21) Italy (1) Ecuador (1) Guatemala (1) Saudi Arabia (1) Atlantic Ocean (1) Unknown (8)
Trip type	4 (only west coast and transpacific included in statistical tests)	west coast: includes AK, BC, CA, WA, OR (72) transpacific: includes China, Japan, S. Korea, Taiwan, Pacific Ocean (161) Atlantic/S. America: includes Italy, Ecuador, Guatemala, Saudi Arabia, Atlantic Ocean (5) Unknown (8)	
Exchange method	3	Empty/refill (114) Flow-through (82)	None (46)
Exchange Conducted	2	Yes (198) No (45)	
Vessel Category	5	Articulated Tug/barge (20) Bulk Carrier (123) Container (57)	General Cargo (7) Tanker (39)
Season	4	Winter (11/16 – 2/29) (49) Spring (3/1 – 5/30) (63)	Summer (6/1 – 9/15) (81) Fall (9/16-11/15) (53)
Regulatory Scrutiny	2	Before 6/1/02 (n = 17) After 6/1/02 (n = 229) 6/1/02 = date that increased WDFW scrutiny began	

Table 2. Continuous independent variables and levels within factors used.

Continuous covariable	Range of Values
Salinity (PSU)	0 - 40
Temperature (degrees C)	4.7 - 23.2
Age of Ballast water (days)	2 – 114 (ballast age > 90 not used)

Boxplots are a simple tool for assessing the range of values in a data set including the maximum and minimum values, the 25th, 50th, and 75th percentiles of the data, and any values that lie beyond critical distances from the 25th and 75th percentiles. They provided insight about whether data were symmetrical or skewed and what portion, if any, of the ranges of different subsets of the data (levels within factors) of data overlapped. Bivariate scatterplots were used to assess the shape and variance of relationships between continuous variables; e.g., whether the relationships were linear and whether scatter (variance) in the data increased with the value of the data. Both of these tools provided rationale for determining whether data needed to be transformed before statistical tests of selected hypotheses were tested and to what extent smaller subsets of the data appeared to lie within larger subsets.

Statistical Testing Approach

Initial graphical analyses indicated that the data were positively skewed (most of the values were low with relatively few extremely large outliers) and that sample sizes and variances of the data were very heterogeneous across factor levels

Initially, we were interested in exploring and testing the main and interacting effects of all measured independent variables that could affect the density and percent composition of the zooplankton groups, particularly ballast source, vessel type, and exchange type. However, because data in this study were largely collected opportunistically, sample sizes across the levels within each factor were rarely equal and in many cases factors were partially "confounded". Variables are considered confounded when their effects on a response variable cannot be distinguished from each other. Examples of confounding in this data set include (1) most of the samples from vessel categories Articulated Tug/Barge and Tanker came from ballast source California which was the largest domestic ballast source for trip type west coast (Table 3); (2) most samples from the vessel category Bulk Carrier originated in Japan which was the largest transpacific source (Table 3); (3) trip times, which affect ballast age and the number of live organisms in a ballast sample, vary among sources and so are confounded with other (unmeasured) characteristics of a source; and (4) native zooplankton communities which differ among sources may be affected differently by treatment types. Overlapping associations such as these made it difficult to separate the effects of vessel category, ballast source, and ballast age and unequal sample sizes and/or variances reduced the power of many statistical tests. Also, confounded variables affect the assumption of tests such as analysis of variance (ANOVA) and regression that the explanatory variables be independent of one another.

To deal with the confounding and unequal sample sizes, we created a composite factor called "trip type". This factor separates sources into two major ballast source types: Pacific west coast sources and transpacific sources (we excluded a few European and South American sources). These two source types correspond to major risk factors of interest: non-indigenous species that have previously invaded the west coast of North America and are transported coastwise, and "coastal" organisms from Asian sources. In the current data set, the west coast source samples were dominated by ATBs and tanker samples from California, and the transpacific trip type is dominated by general cargo and container ships from Japan. In both our graphical analyses and in the distributions of

residuals from statistical tests, we carefully examined the distribution of other sources and vessel types within these two trip types to see if their distributions were similar to the distributions of the vessel types in each trip type.

Table 3. Vessel category * ballast source cross tabulation

		Tanker	Articulated Tug/Barge	Bulk Carrier	Container	General Cargo	Total
West Coast	Total	33	20	8	5	4	70
	Alaska	1					1
	Brit. Columbia			2	1		3
	California	*32*	*16*	*5*	*4*	*3*	*60*
	Oregon		3	1			4
	Washington		1			1	2
Trans Pacific	Total	4		112	41	2	159
	China			18	11	1	30
	Japan			*75*	*12*		*87*
	Pacific Ocean	4		5	12		21
	South Korea			13	5		18
	Taiwan			1	1	1	3

Statistical Methods

Based on the exploratory analyses, we log transformed the data to help equalize variances, reduce skewness, and enhance the power of statistical tests. Log transformation is more powerful than the square root transformation for these purposes, and was successful in reducing skewness for both the density and percent composition data, although it often did not completely homogenize variances.

To test for effects of exchange method, we considered that effects of exchange could be affected by trip type, exchange method, and season, . We conducted a three-way ANOVA to test log transformed densities of total zooplankton, coastal organisms, non-indigenous, and combined coastal and non-indigenous for an effect of trip type, exchange method, season, and all two- and three-way interactions. We were especially interested in interaction effects to determine if treatment methods performed equally across combinations of seasons and trip types. If a two- or three-way interaction was significant, we conducted *post hoc* single-factor ANOVAs to test for effects of each factor within each level the second factor. For example, if exchange method (three levels) and season (four levels) interacted, we conducted three *post hoc* single-factor ANOVAs to test for effects of season within each type of exchange method. We also conducted four, single-factor ANOVAs to test for effects of exchange method within each season. Significance results (p-values) for these tests were corrected for multiple testing using Sidak's correction factor (Sokal and Rohlf 1995) We assumed an effect of any test was significant using an *a priori* α level of 0.05.

If a main effect was significant and no interaction terms including the main effect were significant, we used the Tukey HSD *post hoc* test to determine which factor levels were different. *Post hoc* tests differ considerably in their ability to find differences

between levels – especially when sample sizes and variances are unequal as they were in this study. Tukey HSD tests maintain an experiment-wise error rate and produced similar to individual, pairwise comparisons using Sidak's correction..

After investigating the multivariate effects of trip type, exchange method, and season, we conducted several one-way ANOVAs to investigate effects of ballast source and vessel category on zooplankton densities and percent composition. These tests were conducted to investigate the effects of vessel category and ballast source within the trip-type effect. We also conducted one-way ANOVA to determine if mean ballast age differed between trip types. Log transformations and all subsequent ANOVA and *post hoc* analyses were conducted in SPSS (Version 11.5).

Results

Because of the complexity of the data set, results are presented iteratively, as follows:

1. Overall graphical presentation of combined non-indigenous and coastal taxa densities and percent composition by exchange method and major ship types to visually demonstrate range and variability of the data.
2. Effects of each factor (i.e., effects of exchange method, trip type, vessel category, ballast source) based on. three-way ANOVA and post-hoc one-way ANOVA for interaction effects
3. One way ANOVA effects of vessel category/ballast source to investigate trip type effect
4. Selected boxplots to provide examples of variability in data for total zooplankton with accompanying line graphs to display other effects, and ANOVA results to indicate significance of differences. Additional boxplots, ANOVA results, and post-hoc analyses are available in supplemental appendices.

Ballast Water Sources and Characteristics

We sampled 246 ships (this includes some ships that were sampled multiple times), beginning February 2001 and ending December 2005. Bulk carriers, container ships, tankers, and articulated tug/barge vessels made up the majority of sampled ships (Table 1). Asia was the dominant source region for ballast water: three countries—Japan, China, and South Korea—made up 55% of the pre-MOE ballast sources (Table 1, Fig. 2). The region with the second highest contribution to initial ballast source was the state of California, with 25% (Table 1, Fig. 3). Eighty two percent of the ships that discharged ballast reported conducting MOE: of these 58% used the empty-refill method, and 42% used the flow through method. Salinity was relatively high in exchanged tanks, ranging from 18.4-40 PSU with a mean of 33.5 ± 2.7 (SD) PSU (Table 2). Salinity in unexchanged tanks was lower, ranging from 0-37 PSU with a mean of 29.6 ±9.2 PSU. Water temperatures in exchanged and unexchanged vessels were similar, ranging from 4.7-22.3 °C (mean 13.6 ± 4.0 °C) in exchanged ships and from 7.5-23.2 °C (mean 14.2 ± 3.9 °C) in unexchanged ships. Age of ballast water (from time of ballasting) ranged from 2-114 days/

Zooplankton Assemblages

We identified 90 zooplankton taxa or taxa groups that were classified as coastal/non-indigenous (Table 4). Of these, the groups with the largest number of taxa were harpacticoid copepods with 27 species, and calanoid copepods with 26 species. Relatively high frequency of occurrence (>20% of samplings) was noted for several taxa, including barnacle cyprid and nauplii larvae (26.4% and 28.7%, respectively), the cyclopoid copepod *Oithona davisae* (37.5%), unidentified harpacticoid copepod juveniles (26.4%), bivalve and gastropod larvae (49.4% and 45.6%, respectively), and spionid polychaete larvae (19.9%). In terms of density, *Oithona davisae* was by far the most

abundant taxon in the sampled ballast, with a mean of 1.10 x 10^3 m^{-3} and a highest maximum density of 1.17 x 10^5 individuals m^{-3} (Table 4).

One hundred eighteen zooplankton taxa or taxa groups were classified as oceanic, cosmopolitan, or of unknown origin (Table 5). This group was dominated by calanoid copepods, with at least 45 species. High frequency of occurrences (>40% of samplings)were found for several copepod taxa/groups including copepod naupliar stages (82.8%), the cyclopoids *Oithona similis* and *Oncaea scottodicarloi* (73.6% and 51.7%, respectively), and the calanoid copepods *Paracalanus parvus/quasimodo* and *Acartia* spp. (49% and 45.6%, respectively). In addition to having a high frequency of occurrence, *Oithona similis* was the most abundant species found in this group, with average density of 1,794 individuals m^{-3} and a highest maximum density of 1.01 x 10^5 individuals m^{-3} (Table 5) (see Tables 4,5, end of draft.

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Continuous physical variables

Ballast water temperature and salinities occurred in relatively narrow ranges, and none of the zooplankton categories were significantly affected by either salinity or temperature. Of the 244 samples for which salinity was measured, only 19 had salinities less than 29 psu, and the remainder of the samples had salinities higher than 30 psu. Of those below 29 psu, only four had conducted flow-through exchange, and four empty/refill exchange; the remaining 11 had not conducted exchange. All non-exchanged ships were from US sources.

Ballast ages from transpacific vessels were significantly greater than ballast ages from west coast vessels (one-way ANOVA, $p < 0.005$, Fig. 4) although trip type explained only 16% of the variance in the data (Supplemental Appendix 1).

General Trends in combined coastal/non-indigenous Taxa

Temporal

Densities of coastal/non-indigenous taxa in sampled ships varied greatly among samples, spanning five orders of magnitude, and there was no trend across the sampling period in densities of this group (Fig. 5A). In terms of percent composition of total zooplankton, coastal/non-indigenous taxa decreased through the sampling period, about 3% per year ($p=0.023$, simple linear regression) (Fig. 5B). However, similar to the density data, the variability and range of values was high, resulting in a low R^2 value.

Mid-ocean Exchange

Percent composition of coastal/non-indigenous taxa was higher in unexchanged ships vs. those that had reported conducting MOE (Average 26.6% vs. 17.6%—Fig. 6; also see below under statistics results). Average density of coastal/non-indigenous taxa was also higher in unexchanged ships (7.2 vs. 1.3 x 10^3 organisms m^{-3}). Although densities of coastal/non-indigenous taxa decreased as percent composition decreased, there were a number of cases in both exchanged and unexchanged ships where percent composition was high but density was relatively low, and vice versa (Fig. 6). For vessels on

transpacific trips that had reported MOE, there was high variability in percent composition of coastal/non-indigenous taxa, but many cases in which this number was relatively high (Fig. 2). For trips along the Pacific coast of North America, percent composition of these taxa tended to be lower (Fig. 3).

Vessel Type

Of the three most numerous ship types sampled, container ships had the highest average percent composition of coastal/non-indigenous taxa, but the second-lowest average densities of these taxa (Fig. 7, Table 6); conversely, tankers had the lowest average percent composition of coastal/non-indigenous taxa but the highest densities. Bulk carriers, which were the most numerous ship type sampled, had the lowest average densities of coastal/non-indigenous taxa.

Table 6. Average percent composition and densities ± standard deviation, of coastal/non-indigenous taxa by ship type for ships sampled in Puget Sound, 2001-2005. Parentheses indicate number of samples in each category.

Ship Type	**Mean Percent**	**Mean Density**
Bulk Carrier (123)	17.9 ± 26.1	1034.5 ± 3525.7
Container (57)	25.6 ± 31.0	1141.0 ± 5667.2
Tanker (39)	13.0 ± 19.3	6783.3 ± 25620.4
Articulated Tug/Barge (20)	18.4 ± 18.9	4999.5 ± 11973.0
General Cargo (7)	25.6 ± 27.3	3718.8 ± 9277.9

Ballast Source Region

Of the major ballast source regions, ballast water recorded from "unknown" sources had the highest average percent composition of coastal taxa (25.5%—Table 7). Ballast recorded as obtained from Japan had the second highest percent composition of coastal organisms, followed by the Pacific Ocean, the Columbia River, South Korea, and China. Vessels arriving from the Columbia River had the highest average percent composition of organisms known to be non-indigenous to the northeast Pacific (18.9%—Table 7), followed by British Columbia and California (10.6 and 9.8%, respectively). Of the major ballast source regions, vessels from South Korea and China had very low percent compositions of non-indigenous organisms.

Table 7. Average percent numerical composition and densities (numbers m^{-3})of non-indigenous and coastal organisms in ships from various source regions (sources with n<3 not included).

		Non-indigenous		**Coastal**	
Source Region	Number Sampled	Average Percent	Average Density	Average Percent	Average Density
Columbia River	5	18.9	1916.8	12.0	905.0
British Columbia	3	10.6	1513.2	8.6	1162.5
California	58	9.8	4304.7	6.8	1992.3
Pacific Ocean	20	5.8	1323.6	14.6	435.8
Japan	85	7.5	609.9	15.0	741.5
South Korea	18	0.3	3.2	10.9	134.6
China	29	0.4	32.0	12.5	370.1
Unknown	17	0.0	0.0	25.5	158.9

Statistical Analyses

Effects of Mid-ocean Exchange

Total Zooplankton

There was a significant effect of exchange method on total zooplankton densities, with empty-refill exchange having lower densities than flow-through exchange or no exchange ($p = 0.001$, Table 8, Fig. 8A, Supplemental Appendix 2). However, exchange method interacted significantly with season, ($p = 0.027$, Table 7). Total zooplankton densities were significantly lower in unexchanged vessels in spring than in exchanged vessels (*post hoc* one-way ANOVA exchange method within season $p < 0.005$, Supplemental Appendix 3).

Table 8. P values from three-way ANOVA testing for effects of trip type, exchange method, season, and all two- and three- way interactions. Numbers in bold type indicate values ≤ 0.05.

Dependent Variable	Source						
	Trip type	Exchange Method	Season	Trip type * Exchange Method	Trip type * season	Exchange Method * season	Trip type * exchange method * season
Log(Non Indigenous Density)	**0.000**	0.063	**0.003**	0.261	**0.008**	0.070	0.136
Log(coastal Density)	**0.003**	0.468	0.513	0.901	**0.027**	0.056	0.468
Log(coastal and Non Indigenous density)	**0.000**	0.375	0.125	0.769	**0.016**	0.059	0.429
Log(Non Indigenous %)	**0.000**	**0.033**	**0.001**	0.052	0.108	**0.031**	0.142
Log (coastal %)	0.355	0.091	0.957	0.874	0.178	**0.045**	0.814
Log(coastal and Non Indigenous %)	0.576	**0.037**	0.686	0.542	0.184	**0.027**	0.813
Log(Total Zooplankton Density)	**0.000**	**0.001**	**0.010**	0.811	0.500	**0.027**	0.467

Coastal and Non-indigenous Zooplankton

Average density of non-indigenous and coastal taxa tended to be higher in unexchanged ships than in exchanged ships (Fig. 9). However, in a three-way ANOVA using trip type, season, and exchange method, the effect of exchange method was only marginally significant for non-indigenous taxa (p=0.063, Table 8, Supplemental Appendix 2) and was not significant for coastal taxa (p = 0.468, Table 8, Supplemental Appendix 2). Exchange method and season had a marginally significant interaction for all density variables (p <0.07, Table 8, Supplemental Appendix 2): non exchanged densities tended to be lower in spring and higher in fall than exchanged densities (e.g., Fig. 8 B,C). One-way *post hoc* ANOVAs (exchange method within season) indicated that in spring (1) densities of both non-indigenous and coastal organisms were lower in vessels that had conducted flow-through exchange versus non-exchanged vessels (p = 0.018, p = 0.042, Supplemental Appendix 3), and (2) densities of non-indigenous organisms were lower in vessels that had conducted empty-refill versus those that had conducted flow-through exchange (p = 0.042, Supplemental Appendix 3).

Percent composition of non-indigenous and coastal taxa was higher in un-exchanged ships than in those that had reported conducting MOE: this difference was significant for non-indigenous percentages (p=0.033, 3-way ANOVA, Table 8, Supplemental Appendix 2) but only marginally significant for coastal percentages (p=0.091, Table 8, Supplemental Appendix 2). As with the density data there was a significant interaction between exchange method and season for percentages of both non-indigenous and coastal

organisms ($p = 0.031$, $p = 0.045$, Table 8, Supplemental Appendix 2): percentages of both of these categories in unexchanged ships were lower in spring and higher in fall than in exchanged ships. *Post hoc* ANOVA tests (exchange method within season) revealed that the differences between exchanged and non-exchanged vessels were significant only in autumn for non-indigenous percentages, and in autumn ($p < 0.04$) and winter ($p < 0.06$) for coastal percentages (Supplemental Appendix 3).

Effects of Trip Type

Total Zooplankton

Densities of total zooplankton were greater in vessels from west coast trip types than from transpacific trip types ($p < 0.005$, Table 8, Supplemental Appendix 2). There were no interactions between trip type and other factors, i.e. effects of season and exchange methods were similar across both trip types (e.g., Fig. 10A, Table 8, Supplemental Appendix 2).

Coastal and Non-indigenous Zooplankton

Average densities of non-indigenous, coastal, and combined coastal and non-indigenous taxa were all higher in west coast vessels than in transpacific vessels (Table 8, trip type effect, all $p < 0.003$). The difference between trip types was consistent across all exchange methods (exchange method*trip type interactions were all non-significant; Table 8), but not across seasons (trip type*season interactions were significant; Table 8). Mean densities varied much more from season to season in west coast vessels than in transpacific vessels (Fig. 10B). Although densities of coastal, non-indigenous, and combined coastal and non-indigenous taxa were similar in west coast and transpacific trip types in spring, west coast trip type densities rose sharply in summer and stayed significantly higher than those from transpacific trip types, through fall (and through winter for non-indigenous densities) (*post-hoc* one-way ANOVA tests for trip type within season, Supplemental Appendix 4).

Average percentages of non-indigenous taxa were higher in vessels from west coast trips than in those from transpacific trips (Table 8, trip type effect, $p < 0.005$, Fig.10C). The interaction with exchange method was marginally significant ($p = 0.052$): the difference between west coast and transpacific trip types was greater in non-exchanged vessels than in exchanged vessels. Although the difference between west coast and transpacific vessels was much greater in summer and fall than in spring, the interaction between trip type and season was not significant (Table 8, $p = 0.108$, Fig. 10D). For percentages of coastal taxa, there was no significant effect of trip type, and no interactions between trip type and season or exchange method (Table 8).

Effects of Vessel Category

Total Zooplankton

Sample sizes were not equal in each vessel category between trip types, with most articulated tug/barges and tankers in the west coast trip-type and most bulk carriers in the transpacific trip type (Fig. 11). However, a two-way ANOVA using vessel category and trip type found a significant effect of vessel category ($p = 0.003$, Supplemental Appendix 5), and no interaction with trip type. For both trip types, articulated tug/barges and

tankers had highest total zooplankton densities, followed by bulk carriers and container vessels, and general cargo ships had the lowest densities (Fig. 14), and there was no significant difference between trip types. A post hoc Tukey HSD test indicated that articulated tug/barges and tankers had significantly higher total zooplankton densities than general cargo, container, and bulk carrier vessels, but were not significantly different from each other (Supplemental Appendix 6).

Coastal and Non-indigenous zooplankton

In a two-way ANOVA with trip type, vessel category, and interaction factors, results were similar to the trip type analysis, with non-indigenous densities and percents higher in west coast trip types than in transpacific trip types ($p = 0.002$, Supplemental Appendix 5, Fig. 12). There was no significant effect of vessel category on the densities or percentages of nonindigenous and coastal taxa in the two-way ANOVA. For vessel types that occurred in both west coast and transpacific trips, densities of both non-indigenous and coastal taxa were highest in tankers and lowest in general cargo vessels (Fig. 13), but these differences were not significant in the two-way ANOVA (Supplemental Appendix 5). Similarly, there were differences among the vessel types in percent composition of non-indigenous and coastal taxa (Fig. 13), but these differences were not significant. There were no significant effects for vessel category* trip type interactions (Supplemental Appendix 5). However, for pooled trip types, a one-way ANOVA for effects of vessel category found a significant effect of vessel category ($p < 0.005$ for all the density variables and for non-indigenous percent (Supplemental Appendix 7). Non-indigenous and coastal taxa densities and percent composition of non-indigenous taxa were highest in articulated tug/barges and tankers; percent composition of coastal organisms was highest in container and general cargo vessels (Fig. 12, Supplemental Appendix 6).

In the three vessel categories (tankers, container ships, bulk carriers) that were represented in both trip types and had a sample size greater than three, west coast densities and percentages of non-indigenous taxa were greater than transpacific densities and percentages (post hoc one-way ANOVAs for differences between trip types within vessel categories, Supplemental Appendix 9).

Effects of Ballast Source

Total Zooplankton

Densities of total zooplankton differed among ballast sources ($p < 0.005$, Table 9, Supplemental Appendix 9). Post hoc Tukey HSD tests found that total zooplankton densities in vessels from California were significantly greater than those from vessels from China ($p = 0.003$), Japan ($p < 0.005$), the Pacific Ocean ($p = 0.041$), and South Korea ($p = 0.031$) (Supplemental Appendix 10). Differences between California and Taiwan were not significant, likely due to small sample sizes.

Table 9. Results of one-way ANOVA for effect of ballast source region. Partial Eta Squared = percent variance explained by ballast source region. Numbers in bold type indicate values ≤ 0.05.

Dependent Variable	Sig.	Partial Eta Squared
Log(Non Indigenous Density)	**0.000**	0.222
Log(Coastal Density)	**0.003**	0.096
Log(Coastal and Non Indigenous density)	**0.000**	0.135
Log(Nonindigenous %)	**0.000**	0.143
Log (Coastal %)	0.255	0.041
Log(Coastal and Non Indigenous %)	0.059	0.062
Log(Zooplankton Density)	**0.000**	0.141

Coastal and Non-indigenous Zooplankton

Densities of both non-indigenous and coastal organisms were significantly different based on ballast sources (one-way ANOVA, $p < 0.005$ for non-indigenous taxa, $p = 0.003$ for coastal taxa, Table 9, Supplemental Appendix 9). Similar to total zooplankton densities, densities of non-indigenous and coastal taxa in North American, west-coast ballast sources were greater than those from transpacific sources (Fig. 14). For ships with California as the ballast source, these differences were significant for non-indigenous taxa (except for Taiwan); for coastal taxa densities in ballast from California, the difference was not significant except for that with China. (Supplemental Appendix 10). One-way ANOVA indicated that differences among sources in percent composition were significant for nonindigenous taxa ($p < 0.005$) but not coastal taxa ($p = 0.255$) (Table 9, Supplemental Appendix 9). Post hoc tests indicated that percentages of non-indigenous taxa were significantly greater in vessels arriving from the Columbia River than from those arriving from China ($p = 0.001$) but not from other transpacific ports (Supplemental Appendix 11). The lack of significance is likely due to the small sample size from Columbia River ports since the differences between non-indigenous taxa percent compositions from California, China, and South Korea ballast sources were significant ($p = 0.001$, $p = 0.025$, Supplemental Appendix 10)

Discussion

Our data indicate that, despite Washington State regulations requiring MOE, and apparent compliance by ship operators (i.e., indicating MOE on ballast water reporting forms), ships entering Puget Sound continue to pose a large risk of introducing non-indigenous organisms. We found many cases in which ships reported MOE, but still had relatively high densities and/or percentages of organisms that were presumably not native to the northeast Pacific, e.g., known non-indigenous taxa and coastal taxa in ballast obtained elsewhere. Also, although densities and percentages of these organisms were lower in ships that had conducted MOE, the differences were not statistically significant for densities. The differences for percentage composition of these “risky” organisms was marginally significant, but we note that the mean percent composition for ships that had conducted MOE was still relatively high (17.6%). This study took place during a period of increased regulatory scrutiny (see methods), and there did appear to be a small decrease (~3%) in risky organisms in terms of percent composition in each year of our

study. However, aggregate comparison of densities of coastal/non-indigenous taxa before-and-after increased regulatory scrutiny showed no significant difference. We recommend that if current levels of regulatory activity and scrutiny are maintained or increased, ship ballast sampling in Puget Sound be continued to help understand the effects of this activity. **Scott, add some text about where this is presently headed and what we might do about this problem.**

Ballast exchange efficiency models have suggested that exchange can be quite effective at eliminating organism-bearing water (Armstrong et al. 1999). Furthermore, when conducted under controlled experimental conditions, with before-and-after exchange sampling, MOE has been shown to be consistently effective for most zooplankton. For example, Wonham et al. (2001) found in an experimental test of mid-ocean exchange, that 93 to 100% of the coastal water and 80 to 100% of the coastal organisms were removed, and Ruiz et al. (2005) found that MOE conducted on tankers resulted in reduction of non-indigenous organisms of 70 to 90% for the flow-through and empty-refill exchange methods, respectively. However, the basic finding of this study is that *as currently practiced,* MOE probably has little effect in reducing risk of introduction of planktonic NIS to Puget Sound. Why did MOE appear to be largely ineffective at reducing potentially risky zooplankton taxa in ships sampled in this study? One possibility is that in some cases MOE was reported by ship operators, but not conducted, or conducted incompletely. **Reference from Scott's intern's publication?** If this was the case, it points to the need for enforcement and verification of existing ballast exchange regulations. At present, verification consists of **Scott?** Another possibility is that MOE is less efficient in certain types of ships. While we did find that some ship types were riskier (e.g., articulated tug/barges, tankers), confounding of these results with trip type and ballast age (e.g., most tankers were from domestic routes which had lower ballast water ages) makes it impossible to pinpoint riskier ship types with the present data. Controlled before-and-after MOE experiments on several ship types/ballast tank configurations would help to answer this question, and provide information to the ship industry on ship design components that can help decrease NIS introductions. Ship owners and operators would like to transport as little ballast as possible because it does not directly contribute to revenue. Recently proposed alternative ship designs could decrease the risk of introductions by limiting amount of ballast needed, retaining more ballast on board, or changing ballast tank internal structure to facilitate drainage (National Research Council 1996). Also, ballast water treatment systems (e.g., chemical biocides, physical separation, deoxygenation, ultraviolet light) are currently being proposed and developed (summarized in Matheickal and Raaymakers 2004). Some of these systems have been tested and show promise in reducing NIS in ship's ballast (Herwig et al. in press; Sutherland et al. 2003, Waite et al. 2003,; Tamburri et al. 2002, Rigby et al. 1999).

Our graphical and statistical analyses indicate that 1) densities of both non-indigenous and coastal taxa and 2) percentages of non-indigenous taxa were significantly higher from west coast trips, which were dominated by articulated tug/barges and tankers that obtained ballast water in California. These values were lower in samples from transpacific voyages which were dominated by container ships and bulk carriers with ballast sources in Japan, China, and South Korea. While percentages of coastal organisms were higher from transpacific sources, the differences between west coast and

transpacific sources were not significant. These basic results appeared consistently in our analyses; i.e., in the significant trip type effect for non-indigenous and coastal densities in the three-way ANOVA (trip type, exchange method, and season) and in the significant one-way ANOVAs of vessel category and ballast source effects. Based on our analyses, it is not possible to determine whether the high values for coastal and non-indigenous taxa from west coast vessels are due to differences in ballast source, transit times, or the vessel types on these routes, compared to transpacific vessels. However, we think that the most likely explanation is a combination of the dense and diverse NIS assemblages present in California estuaries and comparatively short ship transit times between California and Puget Sound. San Francisco Bay appears to be a particularly risky source of NIS, with at least 212 introduced taxa, many of which dominate their invaded habitats (Cohen and Carlton 1995, 1998). It is notable that for planktonic copepods alone, much of San Francisco Bay is now dominated by an East Asian fauna (Orsi and Ohtsuka 1999). Three of these copepod species have recently been found in the Columbia River estuary, but do not occur in other west coast US estuaries (Sytsma et al. 2004, J. Cordell unpublished data). While they may have been introduced to the Columbia River via shipping from Asia, the data from the present study suggests that San Francisco Bay was a more likely source. Higher densities of both total zooplankton and of non-indigenous and coastal taxa in west coast trips is likely to be related to transit time. It has been demonstrated that most zooplankton taxa decline in ballast tanks over time, sometimes greatly (Lavoie et al. 1999, Gollasch et al. 2000, Wonham et al. 2001), and transpacific trips are much longer than west coast trips. The fact that zooplankton densities from transpacific samples were higher in exchanged samples than in unexchanged samples suggests that after decline in ballasted zooplankton densities, MOE recharges transpacific vessels with oceanic organisms.

The fact that longer transpacific trips had lower ballast plankton densities does not mean that there is no risk of non-indigenous species introductions from these sources. Percent composition of coastal and non-indigenous taxa were high in these sources, and organisms in these categories often numbered in the thousands per cubic meter. Many invasive taxa in US west coast estuaries are thought to have been originally introduced via this pathway (Wonham and Carlton 2005). Thus, transpacific vessel trips pose the risk of introduction of new non-indigenous taxa while US west coast trips pose a risk of spreading already introduced invasive taxa from highly invaded estuaries (e.g., San Francisco Bay) to new coastal locations.

In our study, season had different effects on biota in exchanged vs. un-exchanged vessels. As with the west coast-transpacific comparison, lower total and coastal/non-indigenous zooplankton densities and percents in unexchanged ballast in spring were probably a result of high mortality in unexchanged ships, combined with high densities of plankton taken on with MOE during spring bloom periods. Densities and percentages of coastal and non-indigenous organisms were higher in unexchanged ships in non-bloom periods (fall). Season also significantly different effects on the two trip types for coastal and non-indigenous taxa densities: for transpacific trips, seasonal effects were muted, whereas for west-coast trips densities were much higher in summer and fall. This again may be due to mortality patterns in transpacific trips, in which seasonal variations in plankton abundance are dampened by relatively large decreases in abundance on longer

voyages. These findings also suggest that for west-coast trips, fall and summer voyages may pose more risk for introductions than do those in other seasons.

We note with interest the relatively high densities and percent compositions of non-indigenous taxa found in vessels that obtained ballast in Vancouver, Canada, and the Columbia River. We qualitatively examined the samples obtained from these sources and found that the non-indigenous taxa found in the ballast from Vancouver was made up mostly of the Asian cyclopoid copepod *Oithona davisae*, which has not been recorded as being introduced in the northeast Pacific north of San Francisco Bay. On the other hand, the ballast samples from the Columbia River were dominated by taxa that have recently been introduced there (e.g., the calanoid copepod *Pseudodiaptomus forbesi*, Sytsma et. al 2004). While the sample sizes for these two ballast sources were quite small, we believe that they may pose a risk of non-indigenous species introduction into Puget Sound. Present Washington State law does not require MOE for ballast from these two sources, as they are considered contiguous with those of the state. But it appears that ballast from these sources may contain significant numbers of non-indigenous taxa either from residual remaining from previous ballasting events (Vancouver) or already established invasive species (Columbia River). We recommend increasing sampling and scrutiny of these two sources to further define the magnitude of the potential risk.

In a recent comparison of the Puget Sound region (including northern Washington State and southern British Columbia province) with three other coastal embayments (not including San Francisco Bay) in the northeastern Pacific, Wonham and Carlton (2005) found that the Puget Sound region had the highest number of known non-indigenous introductions. In addition, these authors found that most of the introductions in this region were invertebrates probably introduced via oyster culture (transport of oyster seed from the Atlantic and northwest Pacific). However, they suggest that this pathway has probably peaked and that ballast water discharge may be an increasing source of introductions to the region. Similar to our findings for Puget Sound, Levings et al. (2004) documented a number of potential planktonic colonizers arriving to Vancouver, British Columbia in ship's ballast. In particular, several planktonic copepod species were found by both Levings et al. (2004) and in this study that are (1) non-indigenous to the region, (2) invasive in San Francisco Bay but not in the Puget Sound region, and (3) hypothesized (based on physiological tolerances and native distributions) to be successful colonizers in the region (Levings et al. 2004). Two of these species, the calanoid *Pseudodiaptomus marinus* and the cyclopoid *Oithona davisae* were among the most common in terms of frequency of occurrence and/or abundance in our samples (see Table 4). In addition, a number of other potentially invasive taxa may be represented by meroplanktonic taxa that were not identifiable to species level (e.g., larvae of barnacles, bivalves, polychaetes) and were abundant in our ballast samples. Although relatively little studied, the ecological effects of both planktonic and benthic invaders can be large in the northeastern Pacific (summarized by Wonham and Carlton 2005). Puget Sound may be at particular risk of continued ballast-mediated invasions because of its physical geography and continued risky ballast discharges.

Ultimately, for MOE or any other ballast water treatment method to significantly reduce risk of new invasions, viable propagules of non-indigenous taxa must be reduced below a critical threshold (Choi et al. 2005, Wonham et al. 2005). In the few controlled experiments conducted, it has been shown that MOE can be very effective in replacing

potential invaders with less risky oceanic species (Wonham et al. 2001, Ruiz et al. 2005), and in theory, MOE can be conducted in ways that significantly reduce propagule pressure (Wonham et al. 2005). Although it has been suggested that ballast water treatment designed to inactivate organisms should eventually replace MOE as a ballast water management practice, at this time technologies are in the development stage, and efficacy, reliability, and costs are still being evaluated. Thus, we recommend that MOE be retained for the time being in the ballast water management "toolbox". However, a caveat to this recommendation is that in order to be effective, MOE will require more regulatory oversight and verification than is currently practiced. Currently practiced MOE verification methods such as ballast exchange reporting by ship operators and measuring ballast salinity are problematical: in the first case, ballast exchange records can be manipulated or poorly kept, and in the second case, salinity is a poor predictor of MOE because some ballast source ports are located in high salinity waters (Murphy et al. 2004). One verification method that shows potential is the use of chemical tracers indicative of coastal vs. oceanic waters (Murphy et al. 2004). We suggest that another possible verification method could be the use of taxonomic indicators, using a set of taxa that are known to occur only in coastal habitats. As Murphy et al. (2004) point out, this method could be problematical because of the taxonomic expertise required and extremely high temporal variation in abundances at a given source. However, we believe that this method bears more consideration, and recommend that studies be conducted to (1) determine whether or not there are taxa that are easily identified that can be reliable indicators of coastal waters, and (2) how variability affects the ability of the method to determine whether or not MOE has been conducted.

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Figures

Figure 1. Location of Puget Sound, Washington and its shallow sills (dark bars), and ports where ballast water sampling was conducted. Numbers in parentheses indicate total number of ships sampled at each location.

Figure 2. Location of reported mid-ocean ballast water exchange for transpacific trips. Open symbols indicate percent composition of combined non-indigenous and coastal zooplankton taxa, filled circles indicated last port visited before exchange.

Figure 3. Location of reported mid-ocean ballast water exchange for American west coast trips. Symbols indicate percent composition of combined non-indigenous and coastal zooplankton taxa; asterisks indicate last port visited before exchange.

Figure 4. Boxplot showing percentiles and outliers of ballast age and effects of trip type.

Figure 5. Densities (A) and percent composition (B) of combined coastal and non-indigenous organisms in ships sampled in Puget Sound, Washington, 2001-2005.

Figure 6. Percent numerical composition and densities of coastal and non-indigenous taxa in unexchanged and exchanged vessels sampled in Puget Sound, Washington, 2001-2005. Values are plotted in order of decreasing percent composition

Figure 7. Percent numerical composition and densities of coastal and non-indigenous taxa in four main vessel types sampled in Puget Sound, Washington, 2001-2005. Values are plotted in order of decreasing percent composition.

Figure 8. Interaction graph showing effects of exchange method and season on total zooplankton

Figure 9. Figure 9. Box plots showing percentiles of non-indigenous and coastal taxa by trip type

Figure 10. Interaction graphs showing effects of season and exchange methods on average densities of non-indigenous and coastal taxa.

Figure. 11. Interaction graphs showing effects of exchange method and trip type on average total zooplankton densities.

Figure 12. Interaction graphs showing effects of season and trip type on average non-indigenous taxa densities.

Figure 13. Interaction graphs showing effects of exchange method and trip type and season and trip type on percent composition of non-indigenous taxa.

Figure 14. Numbers of each vessel type sampled by trip type (left) and interaction graph showing effects of trip type and vessel category on average total zooplankton densities (right).

Figure 15. Effects of vessel category on non-indigenous and coastal taxa average densities (top) and percent compositions (bottom).

Fig. 16. Interaction graphs showing effects of vessel category and trip type on average densities (top) and percent composition of non-indigenous and coastal zooplankton taxa (bottom).

Figure 17. Densities and percentages of nonindigenous and coastal taxa from different ballast sources. Error bars are 95% confidence intervals.

Table 4. Taxa from sampled ships that were considered to be of coastal origin. *Denotes species that have been introduced to the Pacific coast of North America; †denotes fresh water taxa. Blank density values indicate that volume was not measured for the sample in which that taxon occurred.

Taxon	Frequency of Occurrence (%)	Mean Density (m^{-3})	Maximum Density (m^{-3})
Class Branchiopoda, Suborder Cladocera			
Bosmina longirostris O.F. Müller, 1776†	1.9	8.9	2047.2
Cladocera†	0.8	0.1	18.6
Daphnia sp. †	1.5	0.2	14.2
Leptodora kindti Focke, 1844†	0.4	0.0	0.8
Penilia avirostris Dana, 1849	1.1	0.1	11.8
Sida crystallina O.F. Müller,1776†	0.4	0.2	4.8
Class Maxillopoda, Infraclass Cirripedia			
Balanomorpha, cypris	26.4	30.6	2708.2
Balanomorpha, nauplii	28.7	83.0	4937.0
Subclass Copepoda, Order Calanoida			
Acartia (Acanthacartia) steueri Smirnov, 1936	0.8	0.1	14.0
Acartia (Acartiura) hudsonica Pinhey, 1926	13.4	110.7	17805.7
Acartia (Acartiura) omorii Bradford, 1976	4.6	97.2	20042.5
Acartia (Acartiura) spp.	11.9	10.7	1730.0
Acartia (Acartiura) omorii/hudsonica	0.8	18.1	4267.5
Acartiella sinensis Shen & Lee, 1963	1.5	0.1	19.6
Calanus jashnovi Hulsemann, 1994	0.4	0.0	2.8
Calanus sinicus Brodsky, 1965	4.6	1.4	152.9
Diaptomidae†	6.5	2.1	227.1
Euchaeta concinna Dana, 1849	0.4	0.0	7.5
Eurytemora affinis Poppe, 1880*	0.4	0.0	0.7
Eurytemora pacifica Sato, 1913	5.4	17.3	3085.6
Eurytemora sp.	1.5	0.6	71.4
Labidocera euchaeta Giesbrecht, 1889	1.1	2.6	611.5
Labidocera jollae Esterly, 1906	0.4	0.0	1.8
Labidocera kröyeri Brady, 1883	0.4	0.0	1.9
Labidocera trispinosa Esterly, 1905	5.4	1.0	178.5
Parvocalanus crassirostris F .Dahl, 1894	8.4	40.0	3604.0
Pseudodiaptomus marinus Sato, 1913*	14.9	39.4	4327.9
Pseudodiaptomus forbesi Poppe & Richard, 1890*	1.5	19.8	4367.8
Pseudodiaptomus inopinus Burckhardt, 1913*	1.1	0.2	17.7
Pseudodiaptomus sp.	6.5	1.3	63.9
Sinocalanus sinensis Poppe, 1889*	0.4	0.3	67.9
Temora turbinata Dana, 1849	0.4	0.0	3.8
Tortanus (Eutortanus) derjugini Smirnov, 1935	0.4	0.1	28.0

Tortanus (Eutortanus) dextrilobatus Chen & Zhang, 1965*	4.2	0.6	78.9
Subclass Copepoda, Order Cyclopoida			
Clausidiidae	13.8	6.2	567.52
Corycaeus (Ditrichocorycaeus) amazonicus F. Dahl, 1894	0.4	0.1	3.1
Cyclopinidae	5.0	0.5	679.41
Diacyclops thomasi S.A. Forbes, 1882.†	0.4	0.0	1.65
Hemicyclops sp.	11.9	21.1	2111.3
Limnoithona tetraspina Zhang & Li, 1976*	3.4	6.7	1528.8
Mesocyclops edax S.A. Forbes, 1890.†	0.4	0.0	1.1
Oithona brevicornis Giesbrecht, 1891	10.0	69.3	13943.3
Oithona davisae Ferrari & Orsi, 1984*	37.5	1103.3	117172.8
Oithona oculata Farran, 1913	11.1	72.8	14403.4
Subclass Copepoda, Order Harpacticoida			
Ameira sp.	0.4	0.1	2.5
Amphiascopsis cinctus Claus, 1866	0.4	0.2	3.8
Amphiascopsis sp.	0.4	0.1	11.5
Cletodidae	0.4	0.1	22.6
Coullana canadensis Willey, 1934	2.3	0.2	23.2
Dactylopusia sp.	0.4	0.0	2.7
Diathrodes sp.	0.8	0.5	9.0
Diosaccidae	0.8	0.1	22.7
Diosaccus spinatus Campbell, 1929	0.4	0.2	4.5
Diossacus sp.	0.4	0.2	34.4
Ectinosoma melaniceps Boeck, 1865	0.4	0.0	0.6
Ectinosomatidae	1.9	0.1	14.0
Euterpina acutifrons Dana, 1848	16.1	189.1	14195.3
Harpacticoida	26.4	3.7	329.2
Harpacticus sp.	1.5	0.1	14.0
Idomene purpurocincta Norman & T Scott, 1905	0.4	0.1	1.3
Leimia vaga Willey, 1923	0.4	0.0	0.6
Longipedia sp.	2.3	6.5	1025.7
Mesochra pygmaea Claus, 1863	0.8	0.5	9.0
Microarthridion sp.	0.4	0.0	0.6
Paradactylopodia sp.	0.4	0.0	0.6
Pseudobradya sp.	3.1	1.3	154.3
Rhynochothalestris sp.	0.4	0.2	34.4
Schizopera sp.	3.4	3.8	301.2
Tisbe sp .	14.2	21.5	2117.2
Tisbidae	0.8	0.5	106.6
Zaus sp.	0.4	0.0	0.7
Class Malacostraca, Order Mysida			
Acanthomysis aspera Ii, 1964*	0.8	0.1	7.8
Acanthomysis bowmani Modlin & Orsi, 1997*	0.4	0.0	0.6

Alienacanthomysis macropsis W. Tattersall, 1932	1.9	0.1	5.9
Neomysis kadiakensis Ortmann, 1908	0.8	0.0	0.7
Order Amphipoda, Suborder Gammaridea			
Eochelidium sp.*	1.1	0.1	13.4
Order Amphipoda, Suborder Caprellidea	0.8	0.0	1.3
Order Isopoda			
Epicaridea microniscid and cryptoniscid stages	9.2	0.7	52.7
Gnorimosphaeroma oregonense (Dana, 1853)	1.5	0.1	1.6
Order Cumacea			
Nippoleucon hinumensis Gamô, 1967*	1.9	0.3	5.6
Order Decapoda			
Blepharipoda sp.	0.4	0.0	0.8
Hemigrapsus sp.	0.4	0.0	1.4
Lophopanopeus bellus Stimpson, 1860	0.4	0.0	1.7
Pachygrapsus sp.	0.38		17.52
Family Callianassidae	0.4	0.1	5.1
Phylum Mollusca			
Class Bivalvia larvae	49.4	302.1	18949.7
Class Gastropoda larvae	45.6	22.7	1041.8
Phylum Annelida, Class Polychaeta			
Spionidae larvae	19.9	42.3	4032.2
Phylum Echinodermata larvae	6.1	0.9	108.3
Phylum Bryozoa larvae	8.8	3.4	297.2

Table 5. Taxa from sampled ships that were considered to be oceanic, cosmopolitan, or of unknown origin.

Taxon	Frequency of Occurrence (%)	Mean Density (m-3)	Maximum Density (m^{-3})
Subclass Copepoda			
Copepoda eggs	3.4	28.5	2006.4
Copepoda nauplii	82.8	6675.4	328832.3
Order Calanoida			
Acartia (Acanthacartia) californiensis Trinast, 1976	10.3	42.6	4430.9
Acartia (Acanthacartia) tonsa Dana, 1849	21.1	42.3	1220.0
Acartia (Acartia) danae Giesbrecht, 1889	2.7	0.2	29.2
Acartia (Acartiura) longiremis Lilljeborg, 1853	17.6	20.5	2830.9
Acartia (Odontacartia) pacifica Steur, 1915	0.4	0.0	1.4
Acartia spp.	33.0	136.8	8533.6
Aetideus sp.	0.4	0.0	2.1
Calanidae	22.2	58.2	9502.6
Calanus marshallae Frost, 1974	1.5	37.8	8900.2
Calanus pacificus Brodsky, 1948	28.4	49.7	2853.5
Calanus sp.	13.8	60.9	11889.6
Sinocalanus sp.	2.3	0.2	18.8
Sinocalanus styliremis Giesbrecht, 1888	3.8	0.5	31.6
Calocalanus tenuis Farran, 1926	2.3	0.2	18.9
Candacia bipinnata Giesbrecht, 1889	2.3	0.1	11.9
Centropages abdominalis Sato, 1913	10.3	8.3	951.2
Centropages bradyi Wheeler, 1901	0.8	0.0	2.2
Centropages sp.	2.7	0.1	2.9
Clausocalanus arcuicornis Dana, 1849	1.9	0.8	103.6
Clausocalanus furcatus Brady, 1883	6.9	6.9	462.6
Clausocalanus lividus Frost & Fleminger, 1968	7.3	1.3	100.0
Clausocalanus minor Sewell, 1929	0.4	0.0	2.0
Clausocalanus paululus Farran, 1926	0.4	0.3	61.6
Clausocalanus pergens Farran, 1926	2.7	0.4	32.2
Clausocalanus sp.	25.3	26.1	1651.3
Ctenocalanus vanus Giesbrecht, 1888	6.5	2.1	227.1
Epilabidocera longipedata Sato, 1913	3.8	0.1	8.2
Eucalanus sp.	3.4	3.4	483.4
Euchaeta spinosa Giesbrecht, 1892	0.8	0.0	1.4
Gaetanus sp.	0.4	0.0	1.8
Ischnocalanus tenuis Farran, 1926	2.7	0.3	27.4
Lucicutia gaussae Grice, 1963	0.4	0.0	5.2
Mecynocera clausi Thompson, 1888	1.9	0.3	32.2
Mesocalanus tenuicornis Dana, 1849	4.2	0.5	54.3

Metridia lucens Boeck, 1864	2.3	0.5	80.7
Metridia pacifica Brodsky, 1950	6.9	1.9	315.9
Metridia sp.	20.3	18.2	1579.6
Microcalanus pusillus Sars, 1903	3.1	4.8	895.1
Neocalanus plumchrus Marukawa, 1921	7.3	2.8	225.6
Paracalanus denudatus Sewell, 1929	0.4	0.1	25.5
Paracalanus sp. (includes *P. parvus* Claus, 1863 and *P. quasimodo* Bowman, 1971)	49.0	657.4	30437.4
Pleuromamma abdominalis (Lubbock, 1856)	1.1	0.0	2.7
Pleuromamma borealis (F. Dahl, 1893)	2.3	0.1	16.5
Pleuromamma sp.	0.4	0.1	21.3
Pontellidae	1.5	0.3	28.8
Pontellopsis villosa Brady, 1883	0.8	0.0	0.6
Pseudocalanus mimus Frost, 1989	3.1	7.0	1217.3
Pseudocalanus newmani Frost, 1989	7.7	10.2	438.6
Pseudocalanus sp.	45.6	178.3	6510.7
Rhincalanus nasutus Giesbrecht, 1888	1.5	0.2	33.2
Scolecithricella minor (Brady, 1883)	5.7	7.1	1493.5
Scolecithrix danae (Lubbock, 1856)	1.1	0.3	60.4
Subeucalanus crassus (Giesbrecht, 1888)	0.4	0.0	0.6
Tortanus (Boreotortanus) discaudatus (Thompson & Scott, 1897)	3.8	0.2	39.3
Tortanus sp.	2.7	0.5	71.4
Subclass Copepoda, Order Cyclopoida			
Caligoida	0.8	0.1	24.3
Corycaeus (Ditrichocorycaeus) anglicus Lubbock, 1857	33.0	66.0	2596.2
Corycaeus (Onychocorycaeus) catus F. Dahl, 1894	1.1	0.2	51.0
Corycaeus sp.	11.5	29.8	4755.8
Farranula curta (Farran, 1911)	0.38		1.59
Oithona atlantica Farran, 1908	0.4	0.1	14.2
Oithona nana Giesbrecht, 1892	0.4	0.0	2.7
Oithona similis Claus, 1866	73.6	1794.4	101273.9
Oithona sp.	10.0	69.4	13943.3
Oncaea clevei Früchtl, 1923	0.4	0.1	12.7
Oncaea conifera Giesbrecht, 1891	0.8	0.0	8.0
Oncaea dentipes Giesbrecht, 1891	1.1	1.2	254.8
Oncaea mediterranea (Claus, 1863)	0.8	0.4	76.4
Oncaea scottodicarloi Heron & Bradford-Grieve, 1995	51.7	152.2	5136.2
Oncaea sp.	3.4	3.9	733.0
Oncaea subtilis Giesbrecht, 1892	0.8	0.2	37.7
Oncaea venusta Philippi, 1843	1.9	3.0	495.2
Oncaea zernovi Shmeleva, 1966	0.4	0.6	133.5
Sapphirina gemma Dana, 1849	0.8	0.0	2.8
Small parasitic not Hemi or Caligoida	17.2	28.2	3527.0

Subclass Copepoda, Order Harpacticoida			
Clytemnestra scutellata Dana, 1848	4.2	0.2	12.7
Goniopsyllus rostratus Brady, 1883	1.1	0.0	2.4
Macrosetella gracilis Dana, 1848	1.5	0.1	13.2
Microsetella norvegica Boeck, 1864	26.8	29.9	1334.7
Microsetella rosea Dana, 1848	2.3	0.6	105.6
Microsetella sp.	5.0	2.4	152.9
Miraciidae	0.4	0.0	2.6
Class Ostracoda	4.2	0.2	12.7
Order Amphipoda, Suborder Gammaridea			
Corophium sp.	0.4	0.1	5.9
Gammaridea	1.5	0.3	56.6
Order Amphipoda, Suborder Hyperiidea			
Hyperiidea	9.2	0.8	59.0
Parathemisto spp.	4.2	0.7	67.9
Class Branchiopoda, Suborder Cladocera			
Evadne sp.	3.1	0.7	135.1
Podon sp.	4.6	2.2	203.8
Class Malacostraca, Order Mysida	2.68		22.65
Order Euphausiacea			
Euphausia pacifica Hansen, 1911	1.1	0.4	84.4
Euphausiacea	13.4	11.7	821.8
Order Decapoda (zoeae)			
Brachyura	2.7	0.1	7.7
Cancridae	0.4	0.9	203.8
Crangonidae	0.8	0.1	12.6
Hippolytidae	1.1	0.3	67.9
Majidae	3.8	0.1	3.1
Pinnotheridae	0.4	0.4	7.5
Sergestidae	4.6	0.7	83.2
Phylum Chaetognatha			
Sagitta spp.	29.5	27.6	4891.7
Phylum Annelida, Class Polychaeta			
Autolytus sp.	0.4	0.0	0.6
Polychaeta	26.4	79.3	3277.2
Syllidae	0.4	0.1	8.8
Tomopteris sp.	1.1	0.0	1.5
Subphylum Urochordata, Class Larvacea			
Oikopleura sp.	14.9	31.8	2169.2
Other Taxa			
Foraminiferida	4.2	0.2	8.6
Hydrozoa (medusae)	15.7	5.1	219.7
Nematoda	3.4	0.3	44.1

Oligochaeta	1.1	0.5	35.4
Phoronida	1.1	0.2	16.2
Rotifera	7.3	112.7	15237.1
Teleosti (fish) larvae	1.5	0.1	21.3
Tintinnida	4.6	10.5	2151.5
Turbellaria	15.3	2.8	277.2
Unidentified Larvae	2.7	0.2	12.8

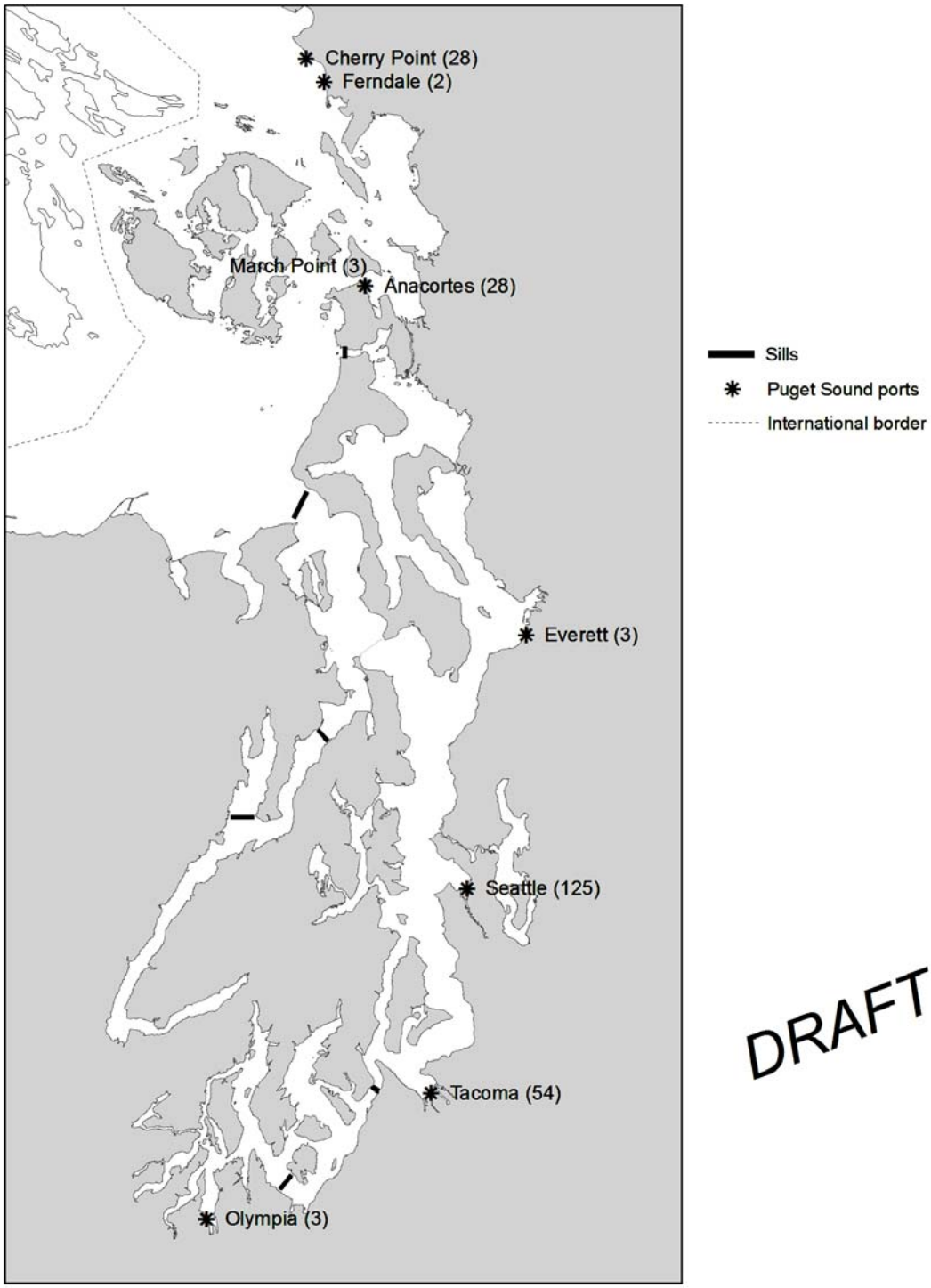

Figure 1. Location of Puget Sound, Washington and its shallow sills (filled circles), and ports where ballast water sampling was conducted. Numbers in parentheses indicate total number of ship samples taken at each location.

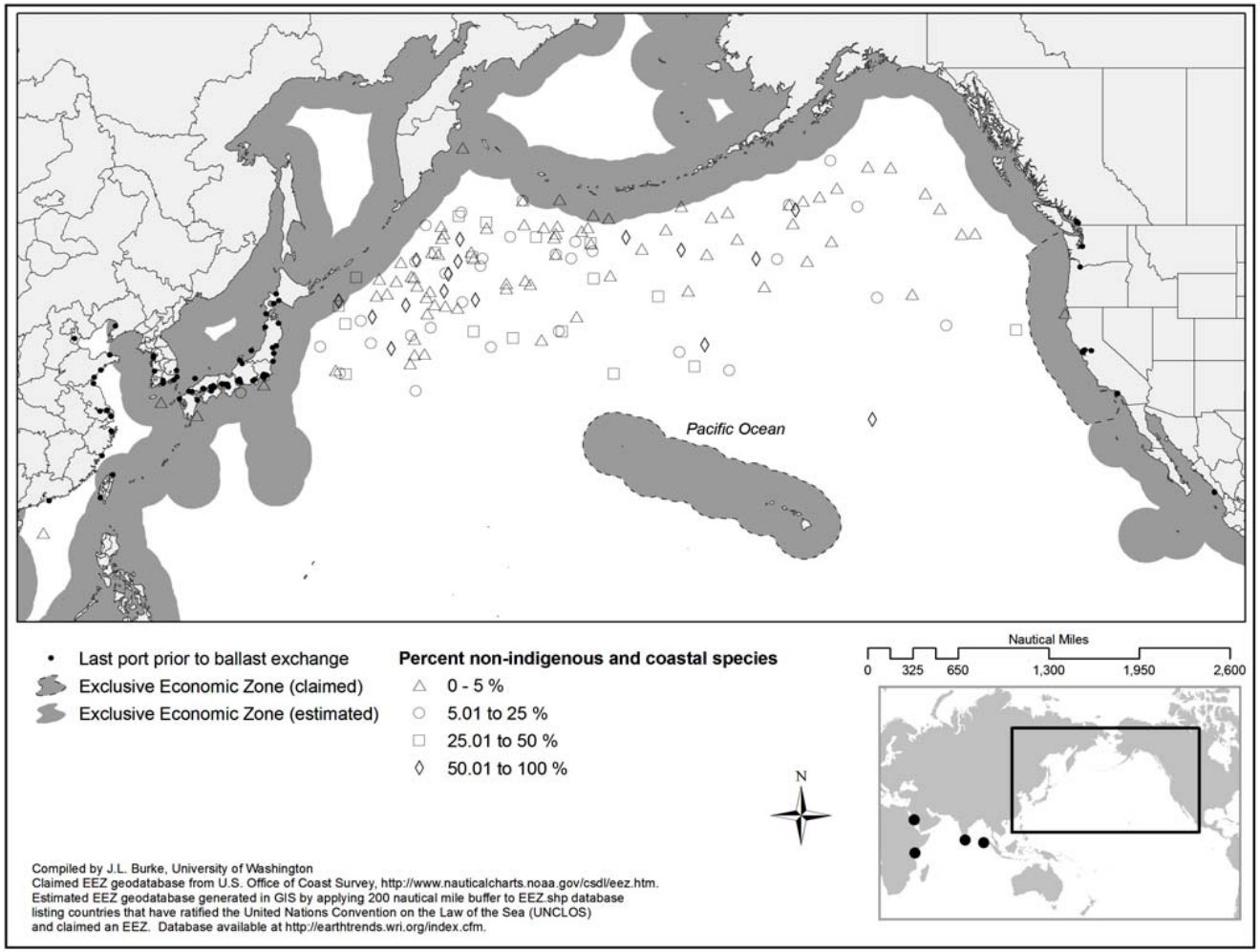

Fig. 2. Locations of reported mid-ocean ballast water exchange for transpacific trips. Open symbols indicate exchange location and percent composition of combined non-indigenous and coastal zooplankton taxa, filled circles indicated last port of call before exchange. Inset shows four additional last ports of call and region of exchange locations.

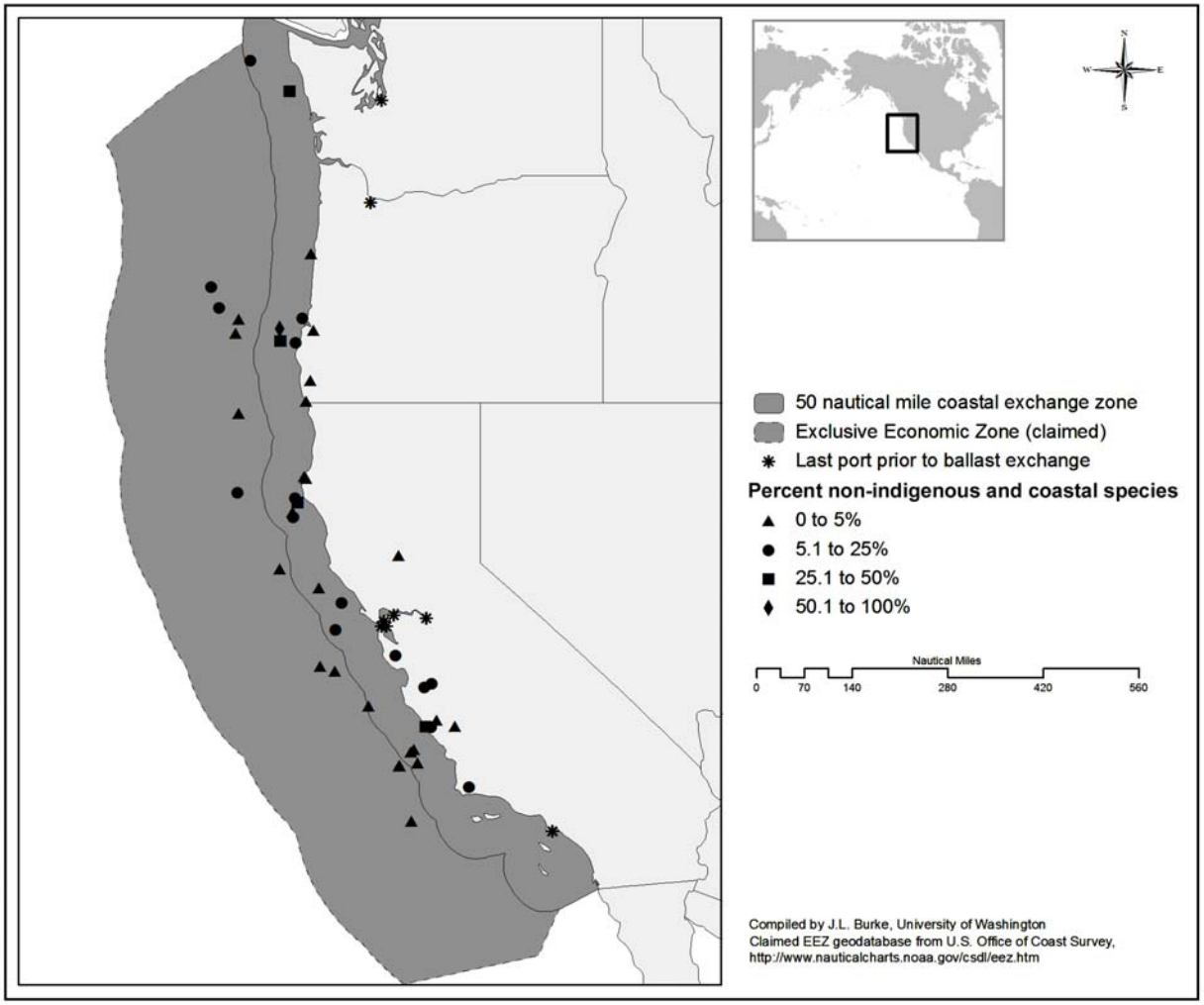

Fig. 3. Locations of reported mid-ocean ballast water exchange for American west coast trips. Symbols indicate percent composition of combined non-indigenous and coastal zooplankton taxa; asterisks indicate last port of call before exchange. Inset shows region of exchange locations.

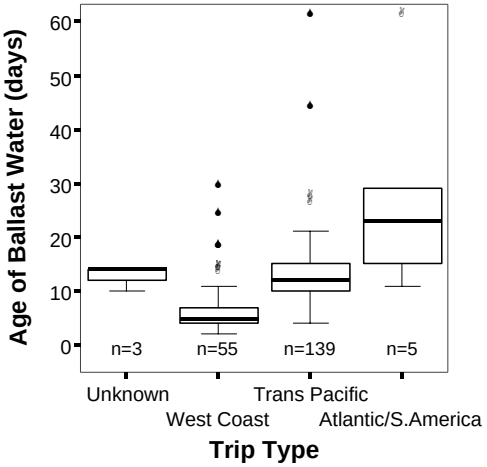

Fig. 4. Boxplot showing percentiles and outliers of ballast age and effects of trip type.

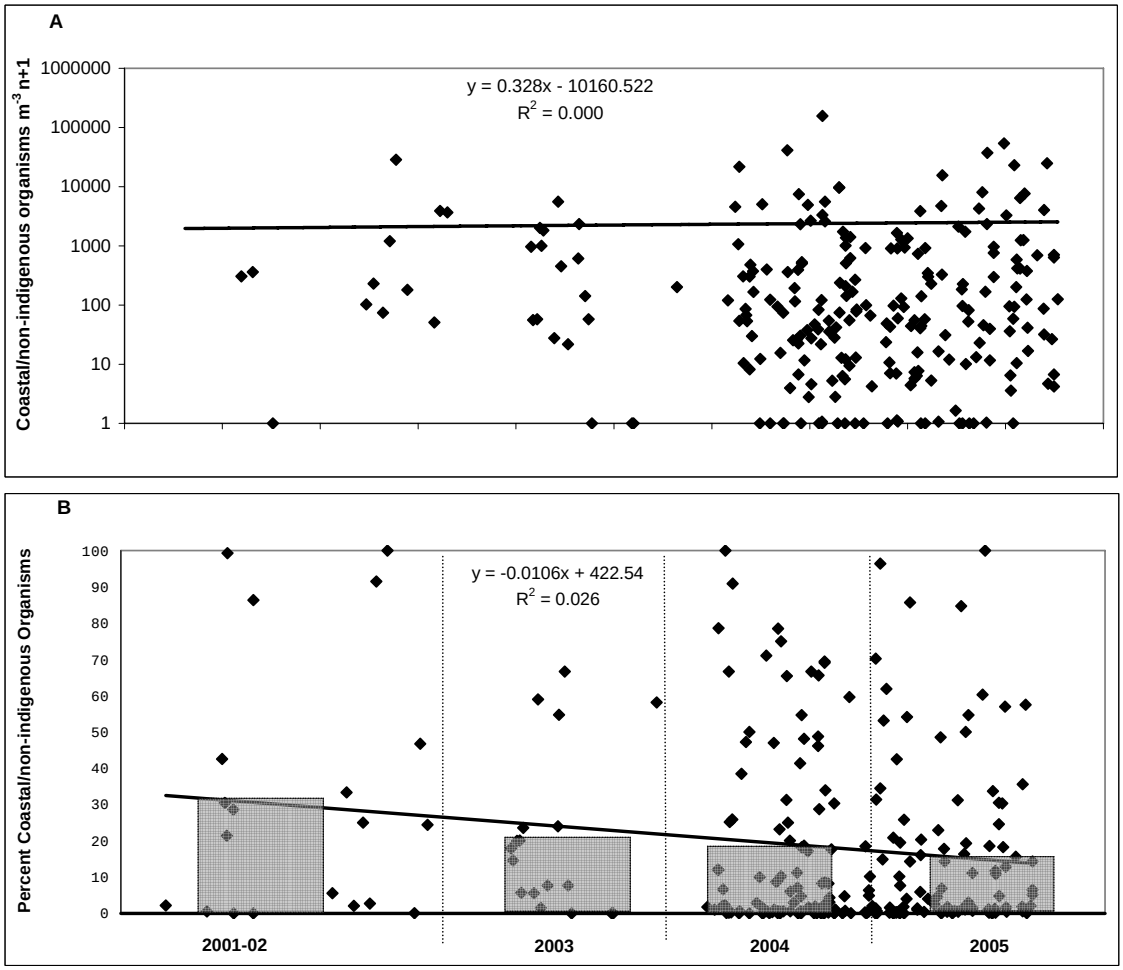

Figure 5. Densities (A) and percent composition (B) of combined coastal and non-indigenous organisms in ships sampled in Puget Sound, Washington, 2001-2005. Shaded bars indicate means for indicated periods.

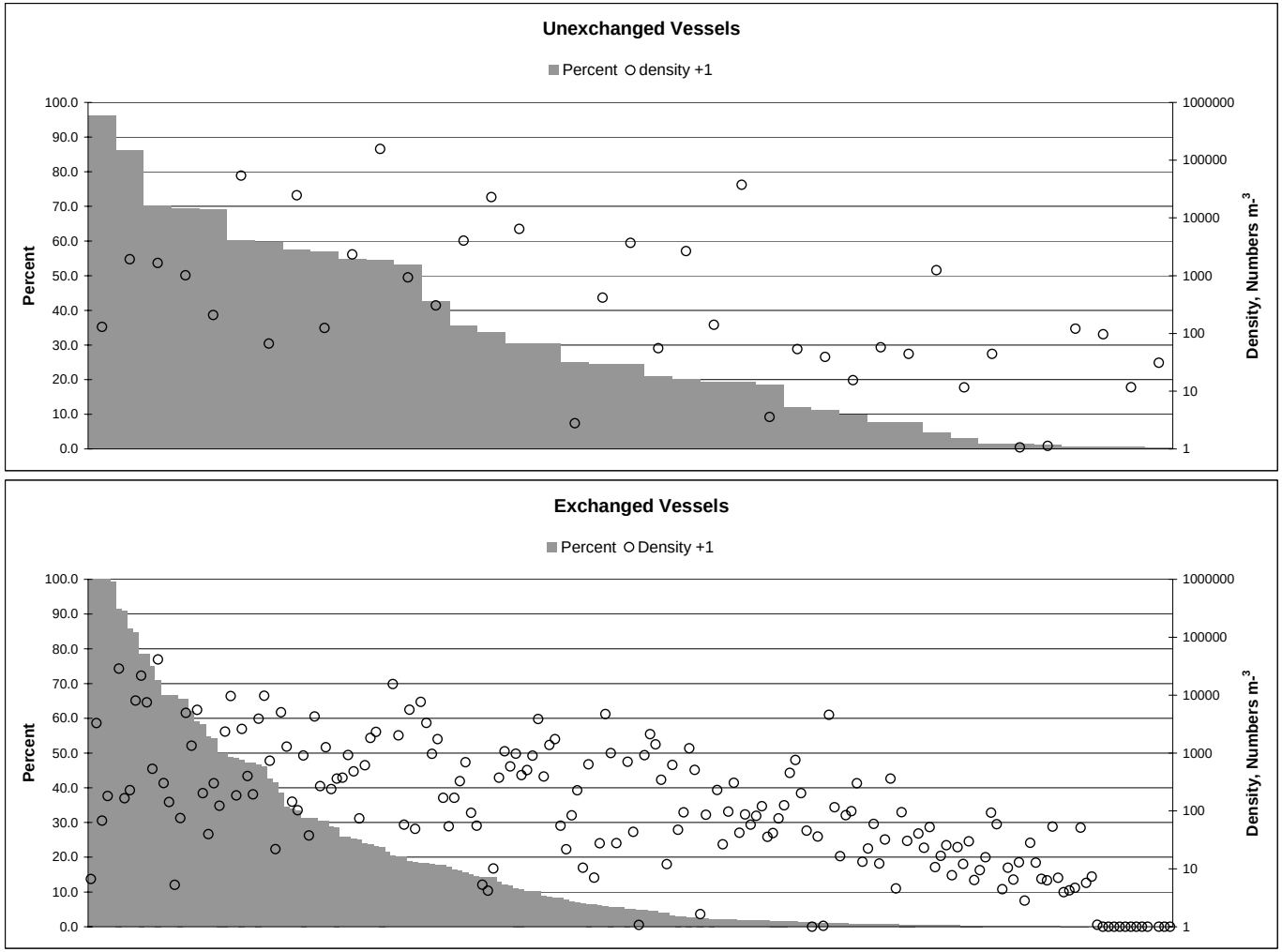

Figure 6. Percent numerical composition and densities of coastal and non-indigenous taxa in unexchanged and exchanged vessels sampled in Puget Sound, Washington, 2001-2005. Values are plotted in order of decreasing percent composition. Note densities are plotted on a log scale.

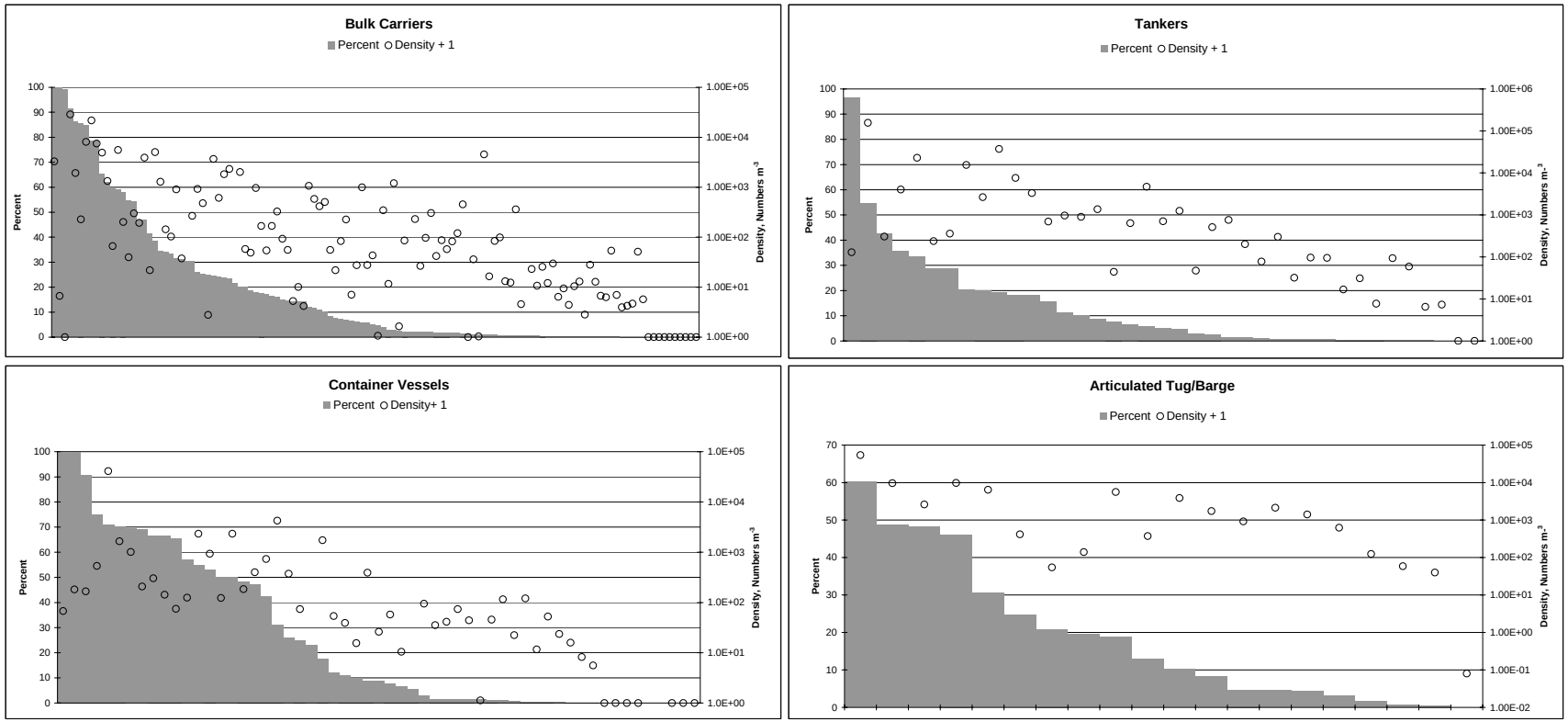

Figure 7. Percent numerical composition and densities of coastal and non-indigenous taxa in four main vessel types sampled in Puget Sound, Washington, 2001-2005. Values are plotted in order of decreasing percent composition.

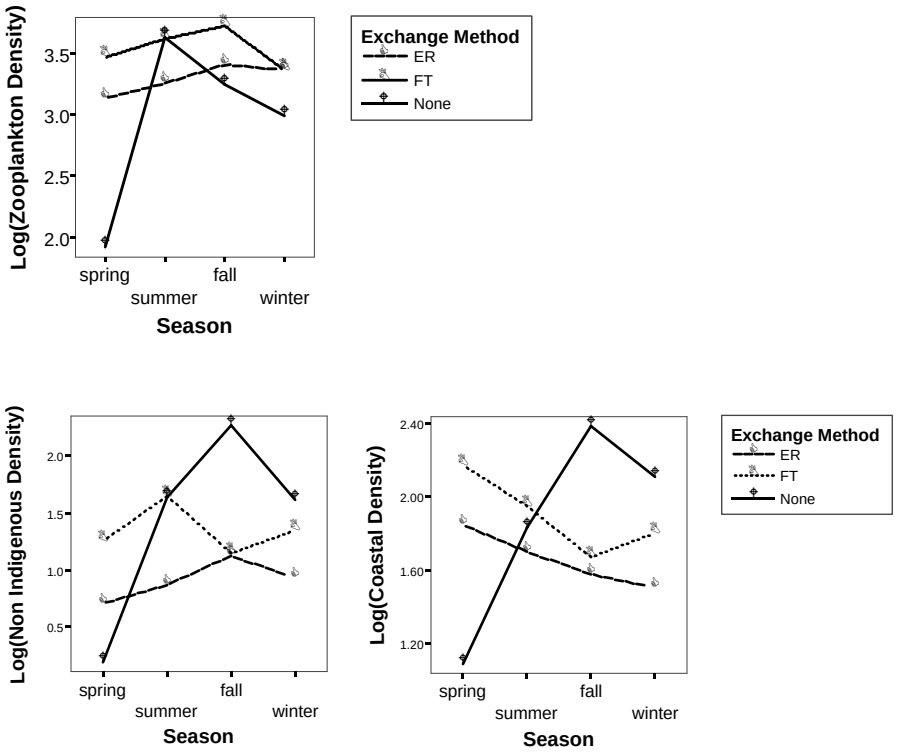

Figure 8. Interaction graphs showing effects of season and exchange methods on average densities of (A) total zooplankton, (B) non-indigenous taxa, and (C) coastal taxa.

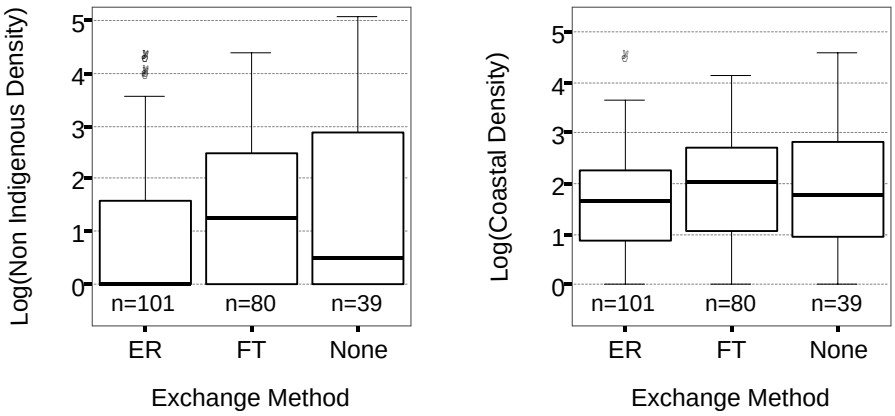

Figure 9. Box plots showing percentiles of non-indigenous and coastal taxa by exchange method.

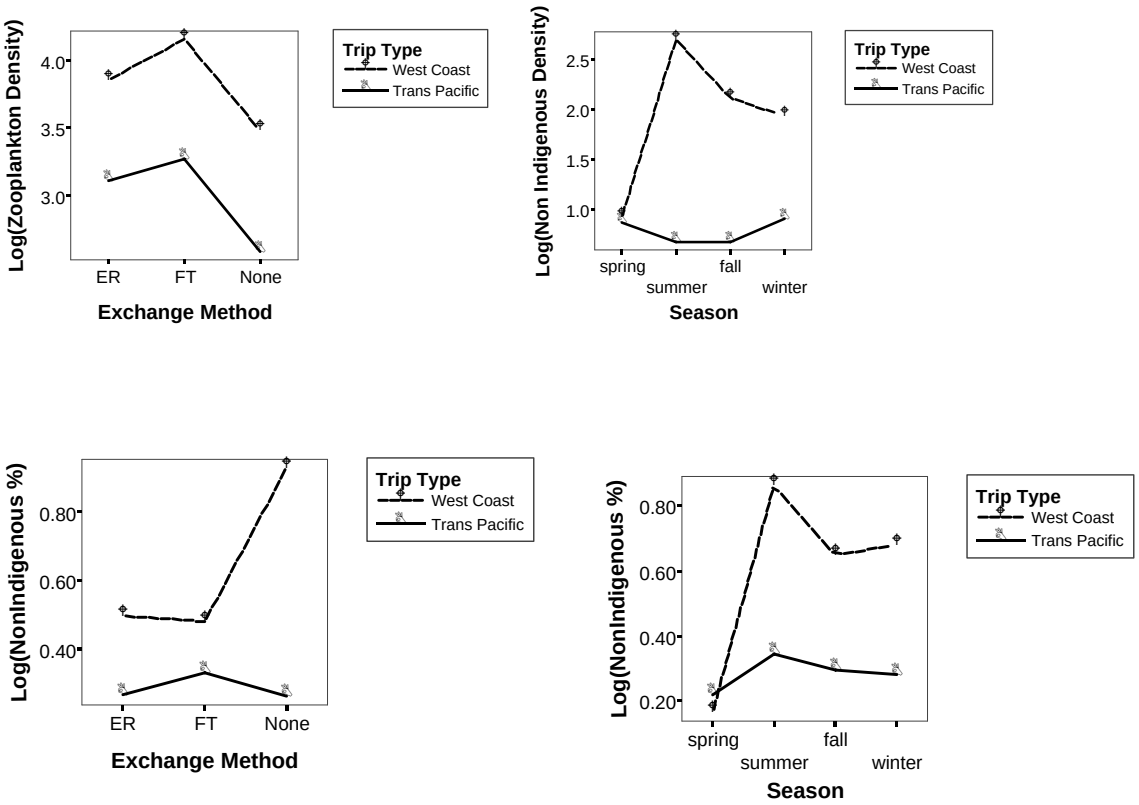

Figure. 10. Interaction graphs showing effects of (A) exchange method and trip type on average total zooplankton densities, (B) season and trip type on average non-indigenous taxa densities, and (C) exchange method and (D) trip type on percent composition of non-indigenous taxa

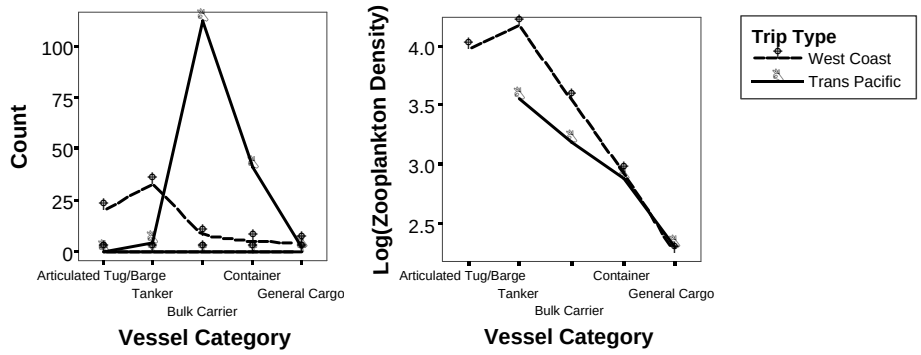

Figure 11. Numbers of each vessel type sampled by trip type (left) and interaction graph showing effects of trip type and vessel category on average total zooplankton densities (right).

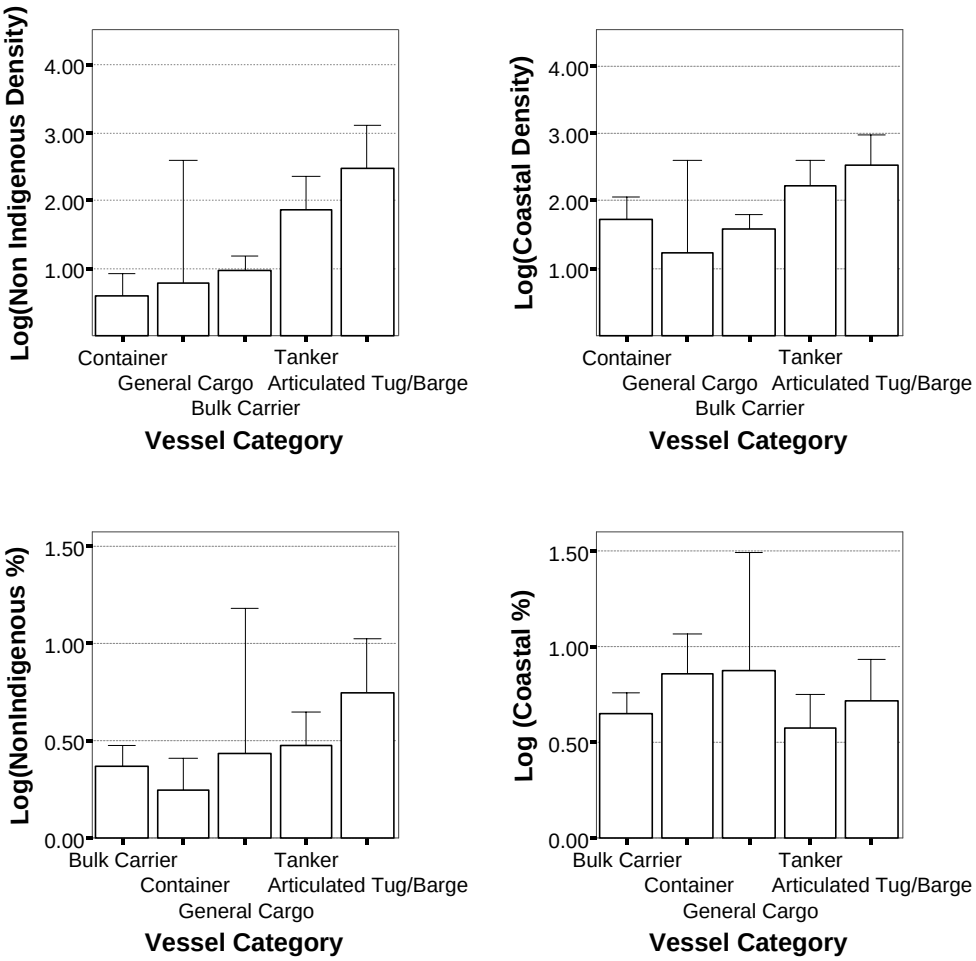

Figure 12. Effects of vessel category on non-indigenous and coastal taxa average densities (top) and percent compositions (bottom).

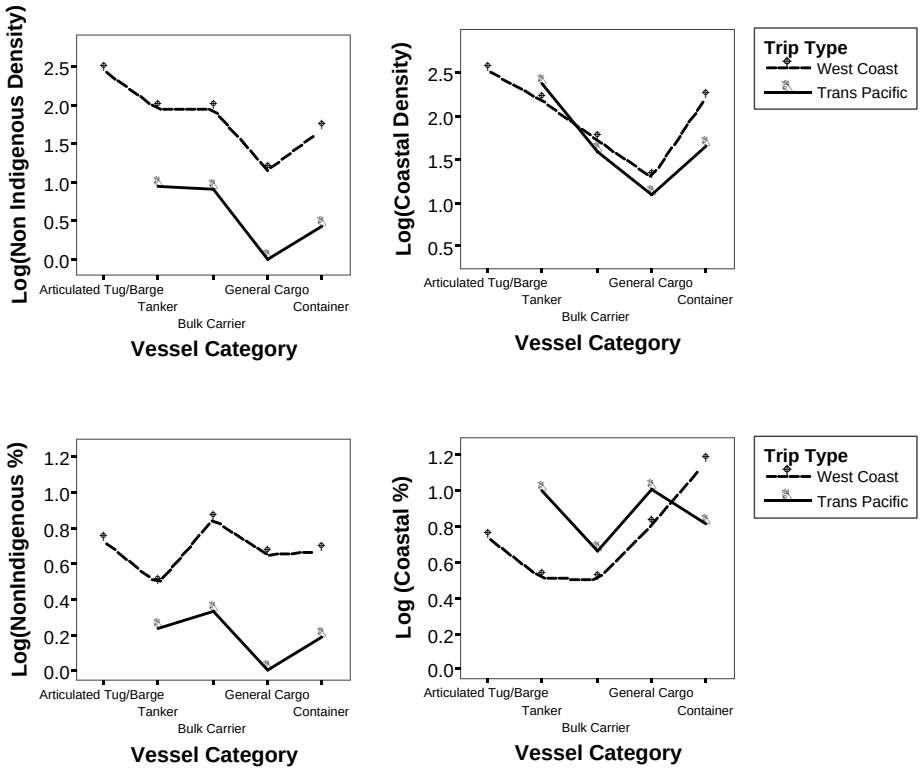

Fig. 13. Interaction graphs showing effects of vessel category and trip type on average densities (top) and percent composition of non-indigenous and coastal zooplankton taxa (bottom).

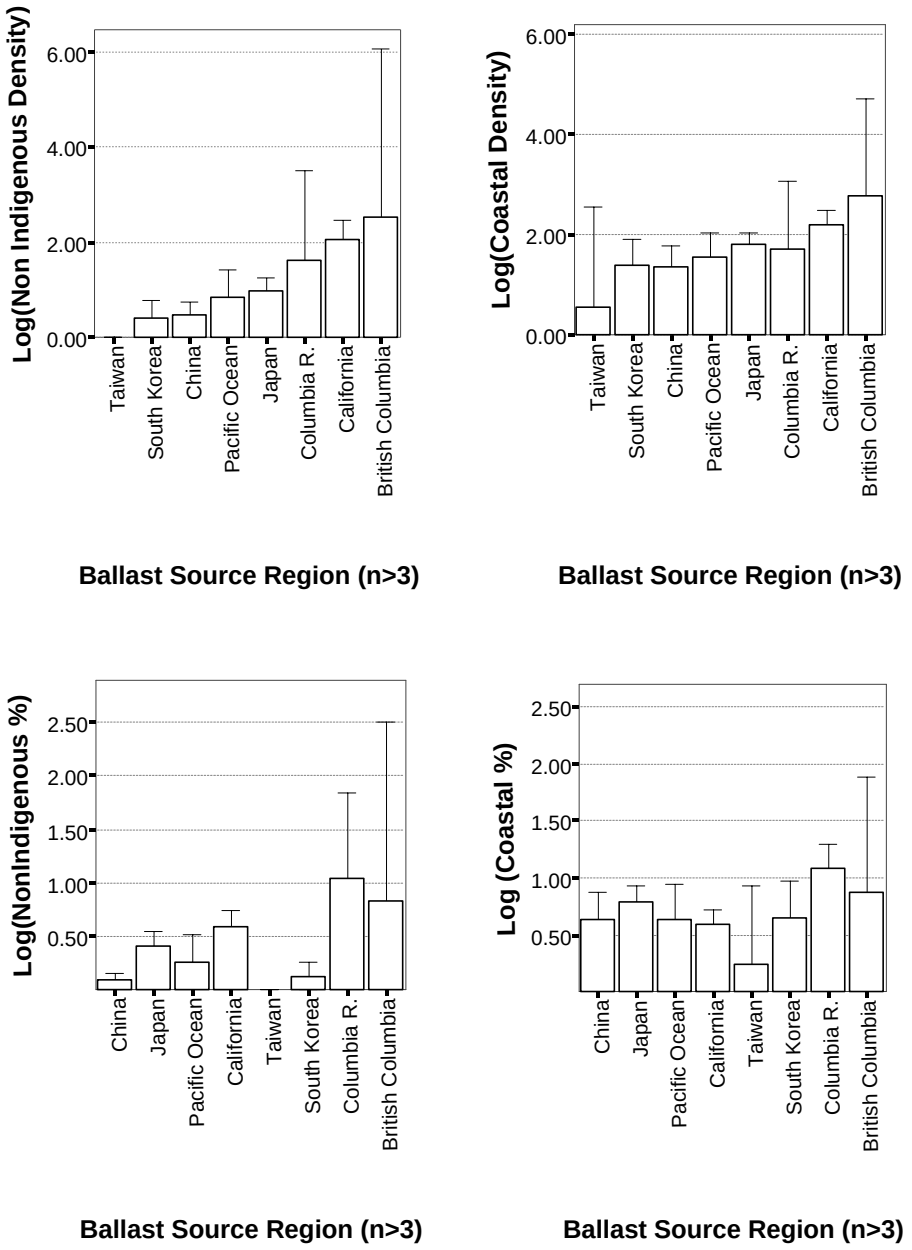

Figure 14. Densities and percentages of nonindigenous and coastal taxa from different ballast sources. Error bars are 95% confidence intervals.