Zooplankton associated with ballast water of LNG tankers arriving to Nikiski, Alaska

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Submitted by:

Gemma Quilez-Badia, George Smith, and Gregory M. Ruiz
Smithsonian Environmental Research Center

&

Jeffery R. Cordell, University of Washington

Introduction

Relatively few non-native marine species are known from high-latitude regions around the world. This is best illustrated on the Pacific coast of North America, where intensive analyses indicate that a steep cline exists in the number of established non-native species, declining sharply from south to north (Hines and Ruiz 2000; Ruiz et al. 2000; de Rivera et al. 2005; Ruiz et al. 2006). Ruiz et al. (2006) documented only 20 non-native species in Alaska coastal waters, compared to 55–157 species from large bays and estuaries in California to Washington. Only two additional marine invaders have been reported and confirmed in Alaska in recent years, since this analysis (Ashton et al. 2008; Lambert et al. 2009).

In a recent review, Ruiz and Hewitt 2009 hypothesized this latitudinal pattern of invasion on the Pacific coast resulted primarily from historical differences in human-mediated propagule (organism) supply. Although quantitative measures of propagule supply are not available, it is clear that the historical opportunities for species transfers have been much less in Alaska than further south, due to the relative level of human activities that are responsible for species transfer (e.g., shipping, mariculture, trade of live organisms for pets and food). To date, California and especially San Francisco Bay has been the initial site of colonization for > 200 non-native species of invertebrates and algae that have colonized western North America (Cohen and Carlton 1995, Ruiz et al. unpublished data). Many of the species that invaded California are spreading northward and appear capable of tolerating environmental conditions in Alaska, especially along the southeast and south central coasts (deRivera et al. 2006 2007, Ruiz unpublished data).

In recent decades, the propagule supply to Alaska has increased, which may increase the risk of invasions. One major vector (transfer mechanism) for marine biota is the ballast water of commercial ships (Carlton 1985, Cohen and Carlton 1995). For Alaska, the export of oil and gas has resulted in a large influx of ballast water and associated biota, creating a new delivery system for non-native species that did not exist prior to the 1970s. A past study (Hines and Ruiz 2000) characterized the delivery of ballast water and plankton assemblages to Port Valdez by oil tankers, arriving primarily from the western

United States, including San Francisco Bay. In 1998, this study estimated that tankers arriving to Prince William Sound discharged an average of 32,715 m3 of segregated (non-oily) ballast water per vessel arrival from the original source ports. As the ballast water was untreated, it contained abundant and taxonically diverse plankton from coastal bays and estuaries to the south, being discharged by vessel arrivals to Port Valdez during this one-year period.

Operating in a similar fashion, the export of liquefied natural gas (LNG) has also created a transfer mechanism for plankton assemblages, in this case from overseas. In 1969, shipments of LNG began to occur from Cook Inlet to Negishi, Japan. Initially, the methane was shipped on two identical LNG carriers (gross tonnage of over 44,000 tons and deadweight carrying capacity of 36,896 tons), each of which made a roundtrip from Cook Inlet to Japan at approximately 21 day intervals (Marine_Exchange_of_Alaska 2009). In 1993, these vessels were replaced by two new larger ships with capacities of 87,500 metric tons , operating on the same trade route and approximate schedule.

To date, the ballast management practices and plankton assemblages associated with LNG tankers arriving to Nikiski have not been characterized. Federal regulations promulgated by US Coast Guard in 2004 (33 CFR 151 subparts C and D) require that vessels arriving to U.S. ports from outside the Exclusive Economic Zone treat their ballast water before discharging into coastal waters to reduce the risk of biological invasions (Minton et al. 2005). Although oil tankers arriving to Port Valdez from domestic ports were exempted from this requirement by U.S. Congress (National Invasive Species Act of 1996), the regulation applied to all LNG tankers (and oil tankers) arriving to Alaska from overseas. The current and widely used ballast management practice is ballast water exchange (BWE), whereby vessels flush their tanks in open ocean to reduce the concentrations of coastal organisms (and thereby risk of invasion) prior to discharge at ports of arrival. Although ballast treatment is required for LNG tankers, vessels are exempted if sea surface conditions (rough seas) are deemed by the ship’s master to be unsafe for this operation.

In this study, we examined the ballast management and associated biota on the LNG tankers arriving to Cook Inlet from Japan. Our overall goal was to characterize the effects of current management practices by LNG tankers on the plankton assemblages arriving to Nikiski. This was intended to provide the background information to consider potential risks for biological invasions to Alaska associated with this trade, especially if this increases in scope with the possibility of expanded exports that was under discussion at the initiation of the current project.

Project Overview

As outlined in the Scope of Work, this project examined the potential transfer of nonindigenous organisms in the ballast tanks of LNG tankers to Nikiski, Alaska. There were two specific tasks:

- **Task 1** objective was to quantify and describe the zooplankton community in ballast water upon arrival to Nikiski, AK on LNG tankers coming from Tokyo, Japan. We proposed to sample the ballast tanks of 8 different arrivals, distributed at approximately monthly intervals from March to October. On each arrival, we proposed to sample 2 tanks with replicate net tows.
- **Task 2** objective was to evaluate survivorship of zooplankton during 2 different voyages to Nikiski from Japan. We proposed to measure the effects of mid-ocean ballast water exchange (if it occurred as a routine operation on the voyages) on removal of coastal organisms entrained in ballast tanks. For this purpose, two tank pairs were to be sampled through time (3 time points and replicate net tows), to compare changes in an unexchanged and exchanged tank in each pair.

Materials and Methods

For Task 1, sampling events were conducted at the end point of nine voyages, upon arrival to Nikiski, AK (Table 1). The ballast water of nine arrivals was sampled: six from March – June 2007, and three from August – October 2008. On each sampling event, two tanks were sampled (n=3 net tows each) upon arrival to Nikiski. On one occasion (23rd April) the tanks were not totally full, therefore the samples from this date were excluded from analysis; thus, a total of eight sampling events were used for analysis. Empty/Refill (E/R) exchange was conducted on the September 2007 and August 10, 2008 voyages.

For Task 2, the first voyage from Japan to Alaska was carried out from the 10th to 18th of March 2007, and no exchange was conducted by the vessel (Table 2). The second voyage from Japan to Alaska happened from 29th August to 8th September 2007 (Table 3). During the second voyage an E/R exchange was performed between August 31st and September 1st by the North Island of Japan.

During both voyages for Task 2, two tank pairs (four tanks total) were sampled at each of three sampling time points (T0, T1 and T2). Three replicate net tows were collected at each time point. For each sampling event measurements of temperature and salinity were taken (Table 4).

Results

Overall a total of 120 samples were analyzed. The organisms were taxonomically identified to species or the lowest taxonomic unit (e.g. where it was not possible to identify some larval stages to species). A cumulative list of organisms identified is shown in Table 5, with 153 different taxa, providing a quick snapshot of the diversity of organisms in the ballast water of LNG tankers en route to Cook Inlet from Japan.

There was a significant decline in zooplankton abundance during both voyages (Figure 1a). A clear difference was found in the abundance of zooplankton at the different sampling times, being significantly higher at T0 (Scheirer-Ray-Hare, F = 148.11, D.F. = 2, $P < 0.001$, in March; and Scheirer-Ray-Hare, F = 297.33, D.F. = 2, $P < 0.001$, in August). No significant differences were found among the analyzed tanks in March. Differences were found among tanks in August: 2WS and P had significantly lower abundances than 3WS and P (Scheirer-Ray-Hare, F = 64.81, DF = 3, $p < 0.001$). Significant decline, however, were found for all tanks.

It is worth noting that zooplankton abundance and changes through time appeared to be different between the two voyages. For the first voyage, the initial mean density of total zooplankton (< 20 indiv/L) was much lower than that observed during the second voyage (>100 indiv/L). This may result from seasonal differences or simply short-term temporal variation at the time of ballast intake. Perhaps of greater interest is the rate of decline. Whereas total zooplankton density declined significantly with each time point for both voyages (see the percentage of remaining organisms on Figure 1a), the percent decline in density was much greater between T0 and T1 for the second voyage and then exhibited little change from T1 to T2. This more precipitous decline during the second voyage coincided with the empty/refill ballast water exchange (BWE) that occurred between T0 and T1 (only on the second voyage). A similar pattern of decline was observed, and appeared even more accentuated, when considering only coastal organisms found in our samples (Figure 1b). For the August voyage, a 93% reduction in density of coastal organisms was observed with BWE, and time did not produce any further reduction afterwards.

A similar pattern of decline was observed for the individual taxa that were most abundant on these voyages. The most abundant species from the first voyage were the three copepods *Danielssenia typica*, *Oithona davisae* and *Acartia omorii* (Figure 2a). From the second voyage, the most abundant species were the copepods *Oithona davisae*, *Paracalanus* spp., *Temora turbinate* and *Oncaea* sp1. (Figure 2b). Interestingly, after the E/R exchange (at T1) *Oithona davisae* and *Paracalanus* spp. were still both present and the two most abundant species.

To analyze the results from the End Point samples (see Table 6 for a summary of the total, coastal and most abundant species abundance for all voyages and tanks), we also included those data obtained from the T2 in the August trip (September 7, 2007). Even though it was not strictly an End Point, as it was taken the day before arrival, it can be considered as one.

Comparing the abundance found in the End Point samples, significantly higher abundances were found on June 9, 2007 (Kruskal-Wallis, H = 67.28, DF = 8, $P < 0.001$) (Table 6), perhaps due to a seasonal difference in initial plankton abundances in Japan source waters.

There was a difference in total zooplankton concentrations between voyages with exchanged versus unexchanged ballast water (Kruskal-Wallis, H = 5.24, DF = 1, $P < 0.05$). Those voyages sampled in September 7, 2007 and August 10, 2008 (with exchanged ballast tanks) had significantly lower abundance of zooplankton than the un-exchanged voyages (Table 6).

Across all voyages, the most abundant taxa found in the End Point samples were:
Nauplii
Oithona davisae
Oncaea sp1.
Acartia omorii
Paracalanus spp.
Danielssenia typica
Bivalvia larvae

Although total taxonomic diversity for all voyages was relatively high (see Table 5), it is evident that a few species dominated the observed communities, with many additional rare taxa (Table 6, % Total). Richness, Shannon's diversity index (H) and evenness (E_H) varied across all voyages (Table 7). Evenness, however, was always very low (between 0.27 and 0.65), which means that in all cases, one or few species dominated the community (Tables 6 and 7).

Differences between exchanged (September 7, 2007 and August 10, 2008 trips) and un-exchanged voyages were found (Table 7). Higher richness (Kruskal-Wallis, H = 10.51, DF = 1, $P = 0.001$) and evenness (Kruskal-Wallis, H = 5.09, DF = 1, $P < 0.05$) was found for the trips with E/R exchange.

Richness, H and E_H along the two trips from Japan to Alaska were different (Figures 3a and b). During the March trip, a decline in species richness and in diversity (although slowly) was observed from T0 to T2. Equitability however increased towards the end of the voyage. At T2, therefore, we found less species but the community was more evenly distributed.

On the other hand, during the August trip an increase in richness and diversity happened after the empty/refill exchange (Figure 3b). With time (T2), richness decreased below the initial level. Diversity also declined from T1 to T2, but the value was higher than at T0. Equitability also increased with time and at T2 the community was more evenly distributed than at the beginning of the trip.

Discussion and Conclusions

From January 1980 (no previous data are available) to July 2009, a total of 995 LNGs arrivals (i.e. between 1 and 4 (mostly 3) monthly arrivals) have occurred from Japan to Nikiski (Thompson pers. com.; NBIC unpublished data). The monthly volume of discharged ballast water since July 1999 (no previous data are available) to July 2009 varied between 12,284 to 114,778 metric tons. Since data are available (July 1999), a total of 8,410,643 metric tons have been discharged into Nikiski port originating from Japan (NBIC unpublished data), this means an average of 69,509 (± 1908) metric tons per month or 23,170 (± 636) metric tons per arrival.

Our present study has provided abundances (i.e. average of 15,475 individuals/m^3 ± 6,168) for nine vessels arriving to Nikiski from Japan. Knowing the volumes of discharged ballast water (from NBIC) for each vessel, we estimated that an average of 337×10^6 individuals (± 144×10^6) is being discharged per vessel on each arrival. When considering the average ballast discharged (above), we estimate approximately 12×10^9 zooplankton have been delivered per year by LNG tankers to Cook Inlet.

It is clear that a large proportion of ballast discharged by LNG tankers arriving to Nikiski has not been treated. Most of the vessels in our study discharged untreated ballast water, and this has also been truly historically based on information available through the National Ballast Information Clearinghouse (NBIC), as well documented in the recent report by Higman (2010). Although these LNG tankers are required to treat their ballast water before discharge under U.S. Coast Guard regulations, there is an exception when it is considered unsafe to do so (due to sea conditions), as has been reported for many of the LNG arrivals. In addition, a large part of the route for these vessels along the Aleutian Islands is close to shore, limiting the window of opportunity to conduct open ocean BWE (> 200 miles from shore).

Our study begins to characterize the biota associated with the ballast discharged at Nikiski that includes many non-native species. In the present study, we have identified *Oithona davisae* and *Pseudodiaptomus marinus*, which have established non-native populations in San Francisco Bay. *Pseudodiaptomus marinus* was first recorded in San Francisco estuary in 1986 (Orsi and Walter 1991) and *Oithona davisae* in 1963 (Ferrari and Orsi 1984). *O. davisae* has also appeared in several other coastal estuaries in California (Cordell et al. 2008) and recently few specimens have been found from two locations in Puget Sound that are not close to shipping ports (Cordell pers. comm.). It appears therefore that *O. davisae* may have the capacity to invade outside San Francisco Bay, and possibly in colder waters.

Other non-native species found in our samples that are widely introduced and established between California and Puget Sound are *Eochelidium* sp. and *Grandidierella japonica*. *Eochelidium* sp. was first recorded on the Pacific Coast around 1993 and in Puget Sound in 1997. *Grandidierella japonica* was first recorded on the Pacific Coast in 1966 and in Puget Sound in 1977 (Cohen 2004). Thus, both species appear to have wide tolerances in temperature and in the range of other physical factors that occur within that region.

Worth noting are also the *Hemigrapsus* sp. zoea found in our samples. *H. sanguineus*, for example was first recorded in the United States at Townsend Inlet, Cape May County, New Jersey in 1988. This species is now well established and exceptionally abundant along the Atlantic intertidal coastline of the United States from Maine to North Carolina (Benson 2008). *H. takanoi*, although not present in US waters, is considered as a potential invader for Maine (SSCW 2009). This species, introduced in Europe, it was first recorded from France in 1993 (Noël et al. 1997) and now it is present in France, Spain, Belgium, the Netherlands, and Germany. Both species, therefore have been successful invaders in the Atlantic and tolerant in colder temperatures but are not currently known from the Pacific coast of North America.

We have identified to species only a subset of the zooplankton that occurs in ballast discharged at Nikiski by the LNG tankers. More species were present in the ships that we sampled but were either (a) not captured by the limited volume of ballast sampled per arrival, compared to the total amount on board, or (b) could not be identified to species, due to the life stage or quality of individual specimens. In addition, we expect a much larger species pool is available from source ports in Japan and has been entrained and delivered by ships that repeatedly fill their tanks each month over decades.

A previous study by Hines and Ruiz (2000) also characterized the large per-ship inoculation of plankton from untreated ballast water delivered to Prince William Sound, in this case by oil tankers arriving to Port Valdez primarily from the U.S. Pacific coast. Two of the dominant source ports for domestic ballast water are in California (i.e. San Francisco Bay and Long Beach) and are themselves heavily invaded. At least fourteen different nonindigenous species (13 crustaceans and 1 fish) were identified in water being discharged in Port Valdez. Oil tankers in this trade were exempted by U.S. Congress from the U.S. Coast Guard ballast management regulations.

For both Cook Inlet and Prince William Sound, we surmise that the recent ship-mediated supply of non-native species is relatively high, based on the two respective studies. To date, relatively few non-native species have been reported for these areas (see Introduction), but the capacity for colonization by these ballast-mediated transfers has not been evaluated. The recent northward spread into Alaska of several non-native marine species (Ruiz et al. 2006), combined with the apparent thermal tolerance for other species (de Rivera et al. 2006, 2007), suggest Alaska is indeed susceptible to invasion. However, the risk of invasion for this particular Alaskan sites (Cook Inlet and Prince William Sound), and these particular sources (ballast from Japan and the US Pacific coast, respectively), has not been explicitly tested. It is possible that the location of these particular arrival ports, including especially the surrounding environmental conditions and habitats, may have helped limit the current rate of colonization to date. Even if this is so, we caution that (a) some subset of species from these source regions is likely to be capable of colonization and (b) the lack of observed invasions to date may result simply from a lagtime in invasion (or detection), a well-recognized phenomenon that may apply especially to Alaska.

References

Ashton G.V., E.I Riedlecker, and G.M. Ruiz (2008) First non-native crustacean established in coastal waters of Alaska. Aquatic Biology 3:133-137.

Benson, A. (2008). Nonindigenous species information bulletin. Asian shore crab, Japanese shore crab, Pacific crab, *Hemigrapsus sanguineus* (De Haan) (Arthropoda: Grapsidae).

Carlton, J.T. (1985) Transoceanic and Interoceanic Dispersal of Coastal Marine Organisms: The Biology of Ballast Water. Oceanography and Marine Biology: An Annual Review 23:313–371.

Cohen, A.N. (2004). An Exotic Species Detection Program for the Lower Columbia River Estuary, Oakland, California, San Francisco Estuary Institute. 60p. http://www.sfei.org/bioinvasions/Reports/2004-LowerColumbiaR_ESDP381.pdf.

Cohen, A.N. and J.T. Carlton (1995) Biological Study: Non-indigenous Aquatic Species in a United States Estuary: A Case Study of the Biological Invasions of the San Francisco Bay and Delta. NTIS Report PB96-166525. U.S. Fisheries and Wildlife and National Sea Grant College Program, Springfield, Va.

Cordell, J.R., S.M. Bollens, R. Draheim and M. Sytsma (2008). "Asian copepods on the move: recent invasions in the Columbia–Snake River system, USA." ICES Journal of Marine Science: Journal du Conseil **65**(5): 753-758.

de Rivera, C.E., G.M. Ruiz, N.G. Hitchcock, S.A. Teck, B. Steves and A.H. Hines (2007). "Larval development rate predicts range expansion of an introduced crab." Marine Biology **150**: 1275-1288.

de Rivera, C.E., B. Steves, G.M. Ruiz, P.W. Fofonoff and A.H. Hines (2006). Northward Spread of Marine Nonindigenous Species along Western North America: Forecasting Risk of Colonization in Alaskan Waters Using Environmental Niche Modeling, Report Submitted to Prince William Sound Regional Citizens' Advisory Council & U.S. Fish & Wildlife Service. Available at http://www.pwsrcac.org/projects/nis/studies.html (Accessed July 2009).

Ferrari, F.D. and J. Orsi (1984). "*Oithona davisae*, new species, and *Limnoithona sinensis* (Burckhardt, 1912) (Copepoda: Oithonidae) from the Sacramento–San Joaquin estuary, California." Journal of Crustacean Biology **4**: 106–126.

Higman, B. (2010) Ballast Water Discharges into Cook Inlet, Alaska: 2002-2008. Report to Cook Inlet Regional Citizens' Advisory Council.

Hines, A.H. and G.M. Ruiz (2000). Biological invasions of cold-water ecosystems: ballast-mediated introductions in Port Valdez/Prince William Sound, Alaska, Report submitted to the Regional Citizens' Advisory Council of Prince William Sound. Available at www.pwsrcac.org/docs/d0018502.pdf (Accessed July 2009).

IHI (2002). "87,500m3 SPB LNG Carrier - POLAR EAGLE." Retrieved February 16, 2007, from http://www.ihi.co.jp/ihimu/images/seihin/pl12_1.pdf.

Lambert G., N. Shenkar, and B.J. Swalla (2009) First Pacific record of the north Atlantic ascidian *Molgula citrina*—bioinvasion or circumpolar distribution? Abstract, 6th Intl. Conference on Marine Bioinvasions. Portland, Oregon USA.

Minton, M.S., E. Verling, A.W. Miller and G.M. Ruiz (2005). "Reducing propagule supply and coastal invasions via ships: effects of emerging strategies." Frontiers in Ecology and the Environment **3**: 304-308.

NBIC (unpublished data). National Ballast Information Clearinghouse. http://invasions.si.edu/nbic/

Noël, P.Y., E. Tardy and C. d' Udekem d'Acoz (1997). "Will the crab Hemigrapsus penicillatus invade the coasts of Europe?" Comptes Rendus de l'Académie des Sciences - Series III - Sciences de la Vie **320**: 741–745.

Orsi, J.J. and T.C. Walter (1991). "*Pseudodiaptomus forbesi* and *P. marinus* (Copepoda: Calanoida) the latest copepod immigrants to California's Sacramento–San Joaquin estuary." Bulletin of the Plankton Society of Japan **Special Volume**: 553-556.

Ruiz, G.M. and C.L. Hewitt (2009) Latitudinal patterns of biological invasions in marine ecosystems: a polar perspective. In: Smithsonian at the Poles: Contributions to

International Polar Year Science, Krupnik, I, MA Lang, and SE Miller (eds.), p. 347-358. Smithsonian Institution Scholarly Press, Washington.

Ruiz, G.M., P.W. Fofonoff, J.T. Carlton, M.J. Wonham and A.H. Hines (2000). "Invasion of coastal marine communities in North America: apparent patterns, processes, and biases." Annual Review of Ecology and Systematics **31**: 481–531.

Ruiz, G.M., T. Huber, K. Larson, L. McCann, B. Steves, P.W. Fofonoff and A.H. Hines (2006). Biological invasions in Alaska's coastal marine ecosystems: establishing a baseline, Report submitted to the Regional Citizens' Advisory Council of Prince William Sound. Available at www.pwsrcac.org/docs/d0032100.pdf (Accessed July 2009).

SSCW (2009). "Guide to Marine Invaders in the Gulf of Maine. *Hemigrapsus takanoi* brush-clawed shore crab." Retrieved August 9, 2009, from http://www.salemsound.org/mis/H_takanoi.pdf.

Thompson, C. (pers. com.). Marathon Oil Corporation.

Table 1. Summary of the sampling events carried out in Nikiski.

Sampling Day	Vessel name	Tank	Replicate	Temp (^oC)	Salinity	E/R	Abundance (orgs/L)
18th March 2007	Polar Eagle	3WS	1	5	32	-	0.8
			2	5	32	-	1
			3	5	32	-	1.2
		3WP	1†	4	32	-	
			2	4	32	-	0.6
			3	4	32	-	0.4
23rd April 2007 *	Artic Sun	3WS	1	8	30	-	
			2	8	30	-	
			3	8	30	-	
		3WP	1	8	30	-	
			2	8	30	-	
			3	8	30	-	
4th May 2007	Polar Eagle	2WS	1	11	31	-	1.6
			2	11	31	-	2.3
			3	11	31	-	2.2
		2WP	1	5	31	-	2
			2	5	31	-	2.4
			3	5	31	-	1.9
29th May 2007	Polar Eagle	2WS	1	11	32	-	12.4
			2	11	32	-	16.5
			3	11	32	-	13.2
		2WP	1	11	32	-	11.4
			2	11	32	-	13.1
			3	11	32	-	16.9
9th June 2007	Arctic Sun	2WS	1	11	32	-	60.9
			2	11	32	-	56.6
			3	11	32	-	62.5
		2WP	1	11	32	-	54.9
			2	11	32	-	60.1
			3	11	32	-	66.1
16th July 2007	Polar Eagle	2WS	1	14	30	-	12.6
			2	14	30	-	14.5
			3	14	30	-	6.5
		2WP	1	14	30	-	28.6
			2	14	30	-	23.1
			3	14	30	-	19.6
10th August 2008	Artic Spirit (formerly Artic Sun)	1WS	1	14	31	Y	2.3
			2	14	31	Y	1.6
			3	14	31	Y	2.3
		1WP	1	10	31	Y	2.2
			2	10	31	Y	1.7
			3	10	31	Y	2.2

* Discarded for not having the tanks full.

† Discarded because of lost / contaminated sample.

Table 1. (continued) Summary of the sampling events carried out in Nikiski.

Sampling Day	**Vessel name**	**Tank**	**Replicates**	**Temp (^{o}C)**	**Salinity**	**E/R**	**Abundance (orgs/L)**
26th August 2008	Polar Spirit	1WS	1	16	31	-	15.5
	(formerly		2	16	31	-	14.6
	Polar Eagle)		3	16	31	-	21.5
		1WP	1	16	31	-	37.9
			2	16	31	-	24.4
			3	16	31	-	32.3
10th October 2008	Polar Spirit	2WS	1	10	30	-	9.7
	(formerly		2	10	30	-	6.3
	Polar Eagle)		3	10	30	-	12
		2WP	1	10	28	-	11.6
			2	10	28	-	8.1
			3	10	28	-	10.6

Table 2. Summary of the samples taken during the first trip from Japan to Alaska (10 March '07 – 18 March '07).

No. Tank	Sampling Time	No. replicates	Analyzed	Abundance (orgs/L)
2WS	T0 (day 2 at sea)	1	Y	17.9
		2	Y	15.6
		3	Y	22.5
	T1 (day 4 at sea)	1	Y	5.9
		2	Y	12
		3	Y	4.5
	T2 (day 8 at sea)	1	Y	1.4
		2	Y	1
		3	Y	1.1
2WP	T0 (day 2 at sea)	1	Y	13.8
		2	Y	18.9
		3	Y	13.2
	T1 (day 4 at sea)	1	Y	3.9
		2	Y	3.7
		3	Y	3.3
	T2 (day 8 at sea)	1	Y	0.6
		2	Y	1
		3	Y	1.3
3WS	T0 (day 2 at sea)	1	Y	15.1
		2	Y	20.8
		3	Y	19.1
	T1 (day 4 at sea)	1	Y	6.8
		2	Y	6.2
		3	Y	5.8
	T2 (day 8 at sea)	1	Y	1
		2	Y	0.8
		3	Y	0.9
3WP	T0 (day 2 at sea)	1	Y	20.6
		2	Y	15
		3	Y	17.6
	T1 (day 4 at sea)	1	Y	8.5
		2	Y	6.9
		3	Y	2.4
	T2 (day 8 at sea)	1	Y	0.8
		2	Y	0.9
		3	Y	0.8

Table 3. Summary of the samples taken during the second trip from Japan to Alaska (29 August '07 – 08 September '07).

No. Tank	Sampling Time	No. replicates	Analyzed	Abundance (orgs/L)
2WS	T0 (day 1 at sea)	1	Y	96.5
		2	Y	60.9
		3	Y	62.3
	T1 (day 2 at sea)	1	Y	7.6
		2	Y	7.8
		3	Y	8.2
	T2 (day 9 at sea)	1	Y	1.3
		2	Y	1.6
		3	Y	1.7
2WP	T0 (day 1 at sea)	1	Y	57.7
		2	Y	62.1
		3	Y	43.2
	T1 (day 2 at sea)	1	Y	7.7
		2	Y	9.7
		3	Y	6.7
	T2 (day 9 at sea)	1	Y	4.7
		2	Y	1.8
		3	Y	2
3WS	T0 (day 1 at sea)	1	Y	64.8
		2	Y	145.8
		3	Y	143.2
	T1 (day 2 at sea)	1	Y	16.8
		2	Y	13.4
		3	Y	13.3
	T2 (day 9 at sea)	1	Y	14.2
		2	Y	7.6
		3	Y	12.7
3WP	T0 (day 1 at sea)	1	Y	358.4
		2	Y	145.2
		3	Y	116.3
	T1 (day 2 at sea)	1	Y	21.5
		2	Y	23.5
		3	Y	26
	T2 (day 9 at sea)	1	Y	17.8
		2	Y	19.9
		3	Y	20.4

Table 4. Salinity and temperature during the two trips from Japan to Alaska.

	March trip												**August trip***											
Date	**T0 11 March ‘07**				**T1 13 March ‘07**				**T2 17 March ‘07**				**T0 31 August ‘07**				**T1 1 September ‘07**				**T2 7 September ‘07**			
Tank	**2W**		**3W**		**2W**		**3W**		**2W**		**3W**		**2W**		**3W**		**2W**		**3W**		**2W**		**3W**	
	S	P	S	P	S	P	S	P	S	P	S	P	S	P	S	P	S	P	S	P	S	P	S	P
Temp (^{o}C)	14	15	14	15	5	5	5	4	3	2	4.5	4	28	27	28	28	26	25	25	25	12	12	12	12
Sal	32	32	32	32	31	31	32	32	32	30	31	32	32	32	33	34	35	35	35	35	36	36	36	36
E/R	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	Y	Y	Y	Y	Y	Y	Y

*Salinometer was probably measuring around 5ppt higher.

Table 5. List of organisms found in the samples from the Japan-Alaska trips analyzed to date.

Phylum Arthropoda	
Class Crustacea	
Order Cladocera	
Evadne nordmannii	
Evadne spinifera	
Evadne tergestina	
Podon polyphemoides	
Penilia avirostris	
Subclass Copepoda	
Copepoda	nauplius
Order Calanoida	
Acartia omorii	
Acartia sinjiensis	
Acartia sp.	copepodid
Acrocalanus gracilis	
Calanopia minor	
Calanus pacificus	
Calanus sinicus	
Calanus sp.	copepodid
Calanidae	copepodid
Calocalanus spp.	
Candacia bipinnata	
Candacia catula	
Candacia curta	
Candacia discaudata	
Candacia sp.	copepodid
Canthocalanus pauper	
Centropages abdominalis	
Centropages elongatus	
Centropages furcatus	
Centropages orsinii	
Clausocalanus furcatus	
Clausocalanus lividus	
Clausocalanus sp.	
Cosmocalanus darwini	
Delius nudus	
Disseta sp.	
Labidocera acuta	

Pseudocyclopia	
Hemicyclops sp.	
Leptinogaster sp.	
Sapphirina opalina	
Order Harpacticoida	
Ameira sp.	
Amphiascoides sp.	
Amphiascus sp.	
Clytemnestra scutellata	
Danielssenia typica	
Ectinosomatidae	
Euterpina acutrigrons	
Goniopsyllus rostratus	
Laophontidae	
Macrosetella gracilis	
Microsetella norvegica	
Nitroka sp.	
Peltidiidae	
Tisbe sp.	
Harpacticoida	copepodid
Subclass Malacostraca	
Order Amphipoda	
Eochelidium sp.	juvenile
Gammaridae	juvenile
Grandidierella sp.	juvenile
Hyperidae	juvenile
Ischyroceridae	juvenile
Stenothoidae	juvenile
Order Euphausiacea	
Euphausiacea	calyptopis
Order Decapoda	
Brachyura	megalopa
Brachyura	zoea
Callianassidae	zoea
Cancer spp.	megalopa
Cancer spp.	zoea
Caridea	zoea
Crangonidae	zoea
Diogenidae	megalopa

Labidocera euchaeta	
Labidocera japonica	
Labidocera rotunda	
Epilabidocera longipedata	
Lucicutia flavicornis	
Monacilla sp.	
Nanocalanus minor	
Paracalanus aculeatus	
Paracalanus denudatus	
Paracalanus spp.	
Pleuromamma borealis	
Pleuromamma gracilis	copepodid
Pontellidae	
Pontellina morii	
Pseudodiaptomus marinus	
Pseudodiaptomus nihonkaiensis	
Temora discaudata	
Temora turbinata	copepodid
Temora spp.	
Undinula vulgaris	
Order Cyclopoida	
Corycaeus affinis	
Corycaeus crassiusculus	
Corycaeus erythaeus	
Corycaeus longistylis	
Corycaeus pacificus	
Corycaeus pumilus	
Corycaeus spp.	copepodid
Farranula gibbula	
Farranula sp. cf. *curta*	
Oncaea conifera	
Oncaea media	
Oncaea sp. cf. *zernovi*	
Oncaea sp. 1	
Oncaea sp. 2	
Oncaea spp.	
Oncaea ventusa	
Fam. Cyclopinidae	
Cyclopina brevifurea	
Cyclopina sp.	copepodid
Cyclopoida	copepodid
Hemicyclops sp.	copepodid

Grapsidae	megalopa
Graspidae	zoea
Hippolytidae	zoea
Hoplocarida (mantis shrimp)	
Majidae	zoea
Paguridae	megalopa
Pandalidae	zoea
Pinnotheridae	megalopa
Pinnotheridae	zoea
Porcellanidae	zoea
Portunidae	megalopa
Portunidae	zoea
Upogebiidae	zoea
Xanthidae	megalopa
Xanthidae	zoea
Lucifer sp.	juvenile
Order Isopoda	
Bopyridae	juvenile
Epicaridea	juvenile
Order Euphausiacea	Calyptopis and furcilia
Phylum Chaetognatha	
Class Sagittoidea	
Suborder Ctenodontia	
Sagitta sp.	
Phylum Phoronida	
Family Phoronidae	
Phylum Nematoda	
Phylum Cnidaria	
Class Hydrozoa	juvenile
Phylum Ostracoda	
Phylum Mollusca	
Class Gastropoda	juvenile
Class Bivalvia	juvenile
Phylum Annelida	
Class Polychaeta	trochophora, juvenile
Phylum Platyhelminthes	
Class Turbellaria	
Muller’s larva	
Phylum Arthropoda	
Subclass Cirripedia	nauplius, cyprid
Subclass Malacostraca	

Oithona attenuata	
Oithona davisae	
Oithona nana	
Oithona oculata	
Oithona plumifera	
Oithona similis	
Oithona simplex	
Oithona tenuis	
Oithona sp.	copepodid
Oithona longispina	
Oithona rigida	
Oithona robusta	

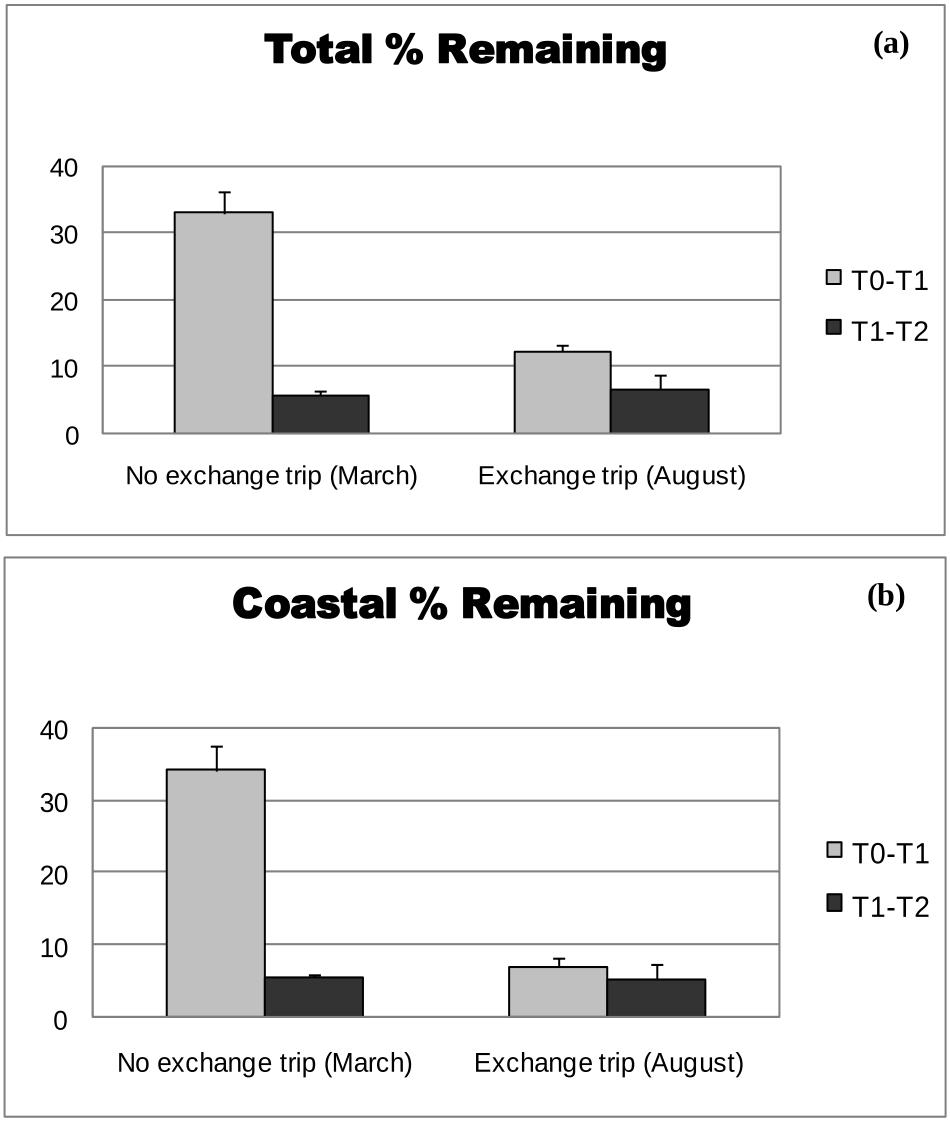
Phylum Echinodermata	
Order Echinoida	pluteus
Order Ophiuroidea	juvenile
Class Asteroidea	larva
Phylum Chordata	
Class Appendicularia	larva
Class Ascidiacea	larva
Class Pisces	larva
Unidentified soft-bodied organisms	

Table 6. Zooplankton species abundance.

		Abundance (indiv/L) (mean, SE (% Total))								
Voyage	**Tank**	**Nauplii**	***Oithona davisae***	***Oncaea* sp1.**	***Acartia omorii***	***Paracalanus* spp,**	***Danielssenia typica***	**Bivalvia larvae**	**COASTAL**	**TOTAL indiv/L (mean, SE)**
18 March 2007	3WS	0, 0.1 (0.0)	0, 0.0 (17.6)	0, 0.0 (0.0)	0, 0.0 (14.7)	0, 0.0 (0.0)	0, 0.1 (46.3)	0, 0.0 (0.0)	1, 0.1 (79.2)	1, 0.1
	3WP	0, 0.0 (0.0)	0, 0.0 (13.7)	0, 0.0 (0.0)	0, 0.0 (12.7)	0, 0.0 (0.0)	0, 0.1 (59.5)	0, 0.0 (0.0)	0, 0.1 (87.2)	0, 0.1
4 May 2007	2WS	0, 0.0 (13.0)	0, 0.0 (8.7)	0, 0.0 (0.0)	1, 0.2 (58.8)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	2, 0.2 (78.3)	2, 0.2
	2WP	0, 0.0 (7.5)	0, 0.0 (4.5)	0, 0.0 (0.0)	1, 0.2 (70.4)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	2, 0.2 (83.0)	2, 0.2
29 May 2007	2WS	1, 0.0 (0.0)	8, 0.5 (53.9)	0, 0.0 (0.0)	2, 0.3 (13.6)	0, 0.0 (0.0)	0, 0.0 (0.0)	2, 0.3 (14.8)	12, 1.1 (83.9)	14, 1.3
	2WP	1, 0.1 (0.0)	8, 0.8 (55.9)	0, 0.0 (0.0)	2, 0.4 (16.5)	0, 0.0 (0.0)	0, 0.0 (0.0)	2, 0.2 (13.5)	12, 1.5 (87.7)	14, 1.6
9 June 2007	2WS	26, 2.9 (43.9)	24, 2.7 (40.4)	0, 0.0 (0.0)	8, 0.6 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	32, 3.3 (53.3)	60, 1.7
	2WP	22, 1.3 (37.2)	29, 2.8 (47.5)	0, 0.0 (0.0)	6, 0.4 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	35, 3.2 (57.4)	60, 3.2
16 July 2007	1WS	4, 1.0 (39.6)	6, 1.7 (56.4)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	6, 1.2 (57.9)	11, 2.6
	1WP	8, 1.3 (32.5)	15, 1.2 (63.7)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	16, 1.7 (65.8)	24, 2.4
7 September 2007	2WS	0, 0.0 (8.6)	0, 0.0 (24.0)	1, 0.1 (17.2)	0, 0.0 (0.0)	0, 0.0 (17.2)	0, 0.0 (0.0)	0, 0.0 (0.0)	1, 0.0 (32.8)	2, 0.1
	2WP	0, 0.0 (12.6)	1, 0.0 (35.6)	0, 0.0 (0.0)	0, 0.0 (0.0)	1, 0.8 (33.1)	0, 0.0 (0.0)	0, 0.0 (0.0)	1, 0.0 (37.0)	3, 0.9
	3WS	1, 0.3 (10.3)	8, 1.3 (66.3)	0, 0.0 (0.0)	0, 0.0 (0.0)	1, 0.1 (3.9)	0, 0.0 (0.0)	1, 0.4 (0.0)	9, 1.6 (76.9)	11, 2.0
	3WP	2, 0.3 (0.0)	9, 0.5 (46.7)	1, 0.1 (0.0)	0, 0.0 (0.0)	0, 0.0 (2.3)	0, 0.0 (0.0)	6, 0.1 (0.0)	15, 0.7 (79.7)	19, 0.8
10 August 2008	1WS	2, 0.2 (80.1)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.0 (7.5)	2, 0.2
	1WP	1, 0.2 (73.6)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.0 (11.1)	2, 0.2
26 August 2008	1WS	1, 0.2 (0.0)	16, 3.7 (91.9)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.1 (0.0)	16, 2.1 (94.1)	17, 3.9
	1WP	1, 0.1 (0.0)	27, 2.1 (85.1)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	1, 0.1 (0.0)	28, 3.6 (88.4)	32, 2.2
10 October 2008	1WS	0, 0.0 (0.0)	8, 1.0 (83.2)	0, 0.0 (0.0)	0, 0.0 (0.0)	1, 0.1 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	8, 1.6 (86.9)	9, 1.0
	1WP	0, 0.1 (0.0)	9, 1.6 (85.7)	0, 0.0 (0.0)	0, 0.0 (0.0)	1, 0.1 (0.0)	0, 0.0 (0.0)	0, 0.0 (0.0)	9, 1.0 (88.1)	10, 1.7
OVERALL MEANS		**4, 1.6 (17.9)**	**8, 2.1 (44.0)**	**0, 0.1 (0.9)**	**1, 0.5 (9.3)**	**0, 0.1 (5.3)**	**0, 0.0 (2.8)**	**1, 0.3 (1.4)**	**10, 4.0 (66.8)**	**15, 4.0**

Table 7. Species richness, Shannon's diversity (H) and Eveness E_H for the End Point samples.

Voyage	Tank	H (mean, SE)	E_H (mean, SE)	Richness (mean, SE)
18th March 2007	3WS	2.2, 0.0	0.7, 0.0	26.7, 2.3
	3WP	1.9, 0.0	0.6, 0.0	22.0, 1.0
4th May 2007	2WS	1.7, 0.0	0.5, 0.0	29.3, 1.5
	2WP	1.3, 0.0	0.4, 0.0	26.3, 1.3
29th May 2007	2WS	1.9, 0.0	0.6, 0.0	27.0, 0.6
	2WP	1.8, 0.0	0.6, 0.0	25.3, 0.9
9th June 2007	2WS	1.5, 0.0	0.5, 0.0	26.7, 1.7
	2WP	1.6, 0.0	0.5, 0.0	29.0, 1.2
16th July 2007	2WS	1.4, 0.1	0.4, 0.0	27.3, 1.3
	2WP	1.4, 0.0	0.4, 0.0	26.3, 2.0
7th September 2007	2WS	2.6, 0.0	0.7, 0.0	39.7, 1.3
	2WP	2.3, 0.1	0.6, 0.0	45.7, 6.3
	3WS	1.8, 0.0	0.5, 0.0	56.7, 3.0
	3WP	1.7, 0.0	0.4, 0.0	49.0, 0.6
10th August 2008	1WS	0.9, 0.0	0.3, 0.0	22.3, 2.4
	1WP	0.8, 0.4	0.3, 0.1	25.3, 0.7
26th August 2008	1WS	1.1, 0.0	0.3, 0.0	32.0, 0.0
	1WP	1.3, 0.0	0.4, 0.0	30.3, 1.5
10th October 2008	1WS	1.3, 0.1	0.4, 0.0	21.0, 0.6
	1WP	1.2, 0.0	0.4, 0.0	25.7, 2.2
OVERALL MEAN		**1.6, 0.1**	**0.5, 0.0**	**30.7, 2.1**


Total % Remaining (a)

Coastal % Remaining (b)

Figure 1. Percentage of organisms remaining between the three sampling times (T0-T1-T2) on the two trips from Japan to Alaska, for (a) total number of organisms and (b) coastal organisms.

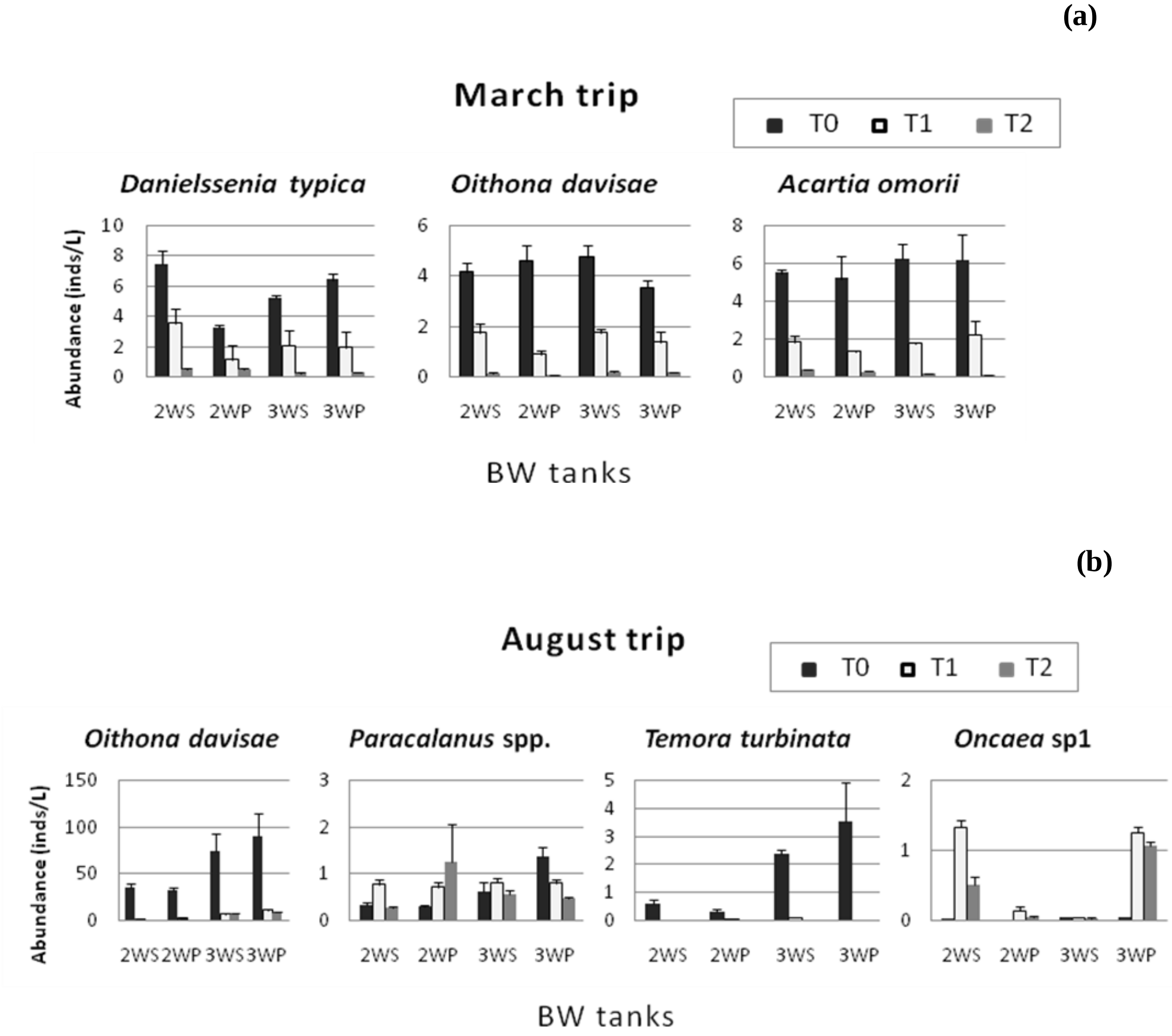


Figure 2. Abundance of the most abundant species found in the samples at different sampling points (mean ± SE) during the winter trip (**a**) and the summer trip (**b**).

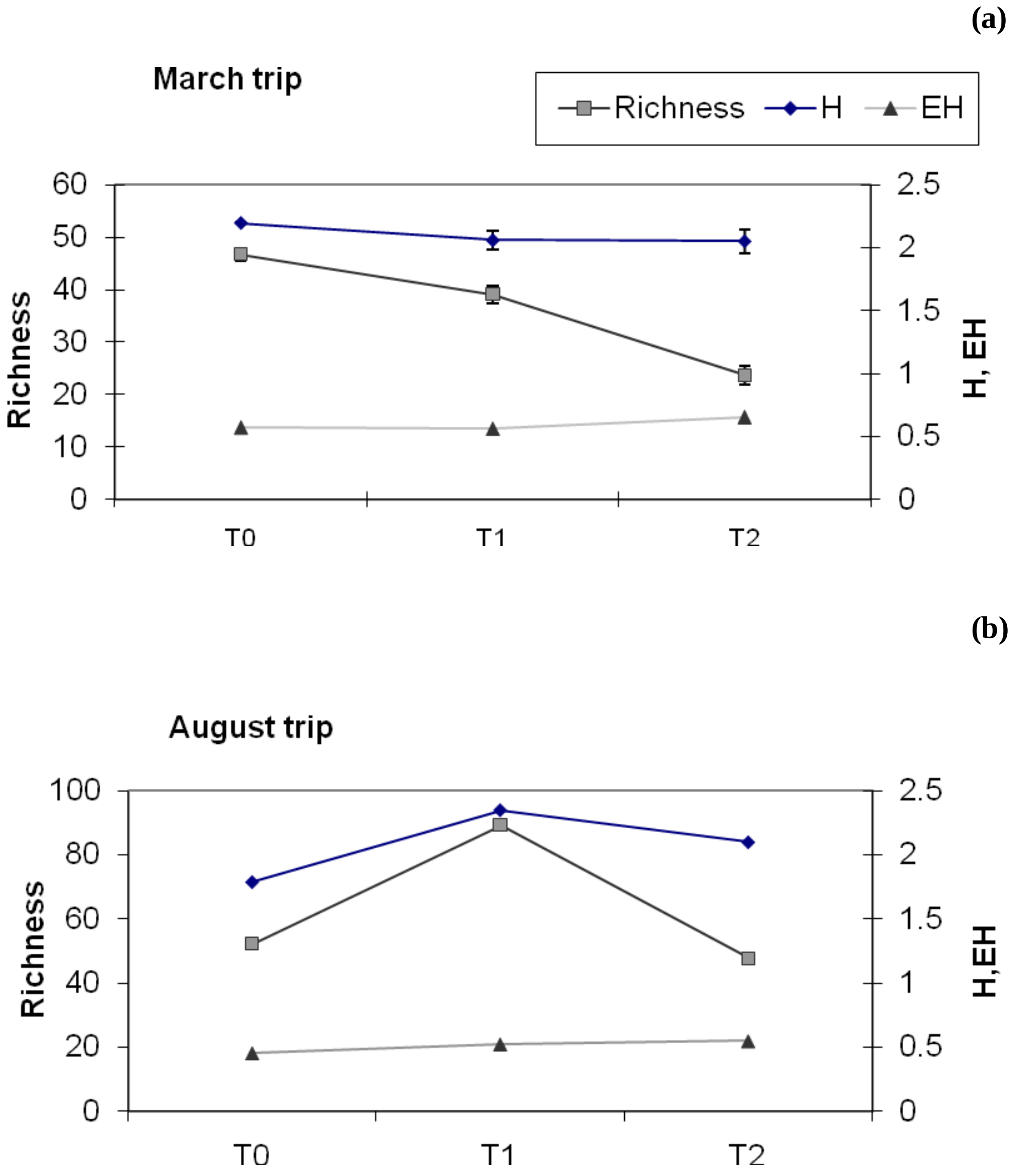


Figure 3. Total abundance of organisms found in the End Point samples (mean ± SE).