North Pacific Marine Science Organization (PICES)
PICES-MoE project on "*Effects of marine debris caused by the Great Tsunami of 2011*"

Year 3 Final Scientific Report

For research components conducted by Smithsonian Environmental Research Center

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Project Title:

Japanese Tsunami Marine Debris (JTMD) and Alien Species Invasions:
PICES Interception of Non-Native Species on JTMD and Genetic, Morphological, and Parasitological Analyses of JTMD North American and Japanese Vouchers

Submitted by:

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1. PROJECT INFORMATION

Title	*Japanese Tsunami Marine Debris (JTMD) and Alien Species Invasions: PICES Interception of Non-Native Species on JTMD and Genetic, Morphological, and Parasitological Analyses of JTMD North American and Japanese Vouchers*
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**Although there may be only one lead author of the report, all PIs and co-PIs of the project, as identified in the approved statement of work and listed below, are responsible for the content of the Final Report in terms of completeness and accuracy.*

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2. YEAR 3 PROGRESS SUMMARY

a. **Describe progress.**

Here, I highlight the activities and accomplishments in Year 3 of this project, representing only SERC's contributions to the overall collaborative project for this year. More specifically, I provide a summary for each of four major areas of activity in Year 3 only. These are also include in a three-year summary in Section 3 (below), including additional areas of activity from Years 1-3.

§ Detection of Invertebrates from JTMD in North American Waters

In Year 3, a primary focus of SERC research was completion of analyses for fouling community surveys along the Pacific coast of North America, to detect resident JTMD species. This aimed specifically at species-level analyses of voucher specimens collected from fouling panels in Year 2 at Yaquina Bay (OR), Prince Rupert (BC), and Ketchikan (AK). We have completed analyses for these three areas, generating species lists and also characterizing the biogeography of these species, to identify those native to North America and those shared with Japan (including native, introduced, or cryptogenic in this region).

We have also synthesized and analyzed existing data on marine nonindigenous species (NIS) for the Pacific coast of North America, based upon (a) fouling panel surveys that we have conducted and (b) records in SERC's National Exotic Marine and Estuarine Species Information System (NEMESIS; http://invasions.si.edu/nemesis/). This was done to identify possible JTMD species already present along the Pacific coast of North America that may have colonized previously, based on a mechanism other than

JTMD. In short, this serves to provide a baseline measure of exiting biota (and previous invasions), in order to identify new records that may result from JTMD.

§ **Overall data synthesis and analyses of invertebrate biota arriving on JTMD to North America and Hawaii**

SERC (Ruiz, Steves, Barnard) worked intensively in Year 3 with Williams College (Carlton) to synthesize and analyze all existing species occurrence records for JTMD objects arriving to North America and Hawaii. This includes intensive data entry and proofing, iterative updates, and analyses for the cumulative data set. The goals of the syntheses and analyses are to (a) provide a comprehensive record of JTMD species arriving to North America and Hawaii, (b) evaluate their distribution in space and time, (c) provide the core data needed for Risk Assessment, and (d) publish these results in peer-reviewed publications. We are on schedule for each of these outputs. All data are current (up-to-date), and multiple manuscripts are progressing for completion in spring 2017, as indicated in the Williams College synthesis report. In addition, data have been delivered and used for the Risk Assessment group (see next section).

§ **Contribute to Risk Assessment for invasions to these regions by JTMD species.**

SERC (Ruiz) has attended two in-person meetings in Canada and participated in multiple conference calls to advance the Risk Assessment model, being advanced by our Canadian colleagues. In addition, SERC has helped organize, deliver, and interpret data on JTMD species occurrences (i.e., data output from the syntheses described in previous section) for this specific work element. Finally, SERC has run several environmental niche models for JTMD species, to help inform discussion and interpretation of "environmental match" of JTMD species along the Pacific coast of North America.

§ **Assist in design and public access to ADRIFT species information through a web-based portal of the National Exotic Marine and Estuarine Species Information System (NEMESIS).**

SERC has worked with PICES (Clarke Murray) to create a web-based portal for JTMD / ADRIFT species information. This makes available detailed information on the taxonomy, distribution, biology, and ecology of species detected on JTMD arriving to North America and Hawaii. We have been developing a specific portal of NEMESIS to provide public access to these data, compiled by the PICES team as an element of the overall project. This portal is on schedule and will be live in spring 2017.

b. Describe any concerns or challenges you may have about your project's progress.

The only problem encountered was loss of panels in 3 Washington bays. We had fouling panels in 3 small bays x 2 sites each in Washington state in Year 2, but we were unable to retrieve these as planned (in Year 2) due to severe weather. Our intention was to retrieve these in Year 3 (15 months after initial deployment), but we had significant losses during this time. Thus, samples from these bays are unfortunately not available, but this was also a very small number of intended sites (unlike Yaquina Bay or Prince Rupert, with 10 sites each), having low impact to the overall project results. We hope to conduct a future survey of these areas, but this is beyond the scope of the current project.

3. ABSTRACT

The Great Japan Tsunami of 2011 resulted in a massive and unprecedented dispersal event of marine biota from Asian coastal waters to the shores of North America. While significant effort has focused on characterizing the spatial and temporal patterns of biota arriving to North America, the fate of these organisms and the extent of new invasions is poorly resolved. We report here the synthesis of existing

data and new surveys to evaluate (detect) the presence of non-native marine species in western North America, from California to Alaska, including free-living marine invertebrates and parasites reported on Japanese tsunami marine debris (JTMD). These data provide important baseline measures (benchmarks) for the species pool present in North America before and during the JTMD dispersal event. We explore the inferences that can be drawn from this baseline and its application to evaluate invasions associated with JTMD, while also considering the potential for both lag-times in detection and other mechanisms (vectors) of introduction of biota from the northwestern Pacific.

We used mussels (*Mytilus* spp.) as a model system to explore the opportunity (risk) of parasite transfers. Mussels, one of the most frequent organisms on JTMD arriving to North America, are known to have a large number of parasite and commensal species worldwide and in Japan, and some of these cause severe disease and mortality. We tested >1,000 mussels arriving on JTMD and detected at least 3 distinct parasite taxa. These results demonstrate that parasites (including commensals) arrived with invertebrate hosts on JTMD, increasing the total number of taxa. In the case of mussels, detected parasites increased total diversity four-fold, underscoring the potential for high levels of hidden parasite diversity among the nearly 300 invertebrate taxa detected on JTMD to date, since none of these have been evaluated (tested) for parasite species richness.

Our extensive surveys of mussels and also fouling communities along western North America, combined with comprehensive analysis of existing literature, detected no new invasions attributed clearly to JTMD-mediated transport. However, these analyses also reveal large numbers of species have colonized North America from Japan by other vectors, prior to JTMD arrival. These results indicate that many invertebrate species in Japan, including some arriving on JTMD, are able to colonize North America, suggesting a high level of environmental match.

While our morphological analyses of field-based surveys and synthesis of existing historical records have not detected JTMD-mediated invasions to date, it is still premature to draw any conclusions. The arrival of hundreds of distinct taxa and a high environmental match provide significant opportunity for invasions. Despite extensive analysis to date, the likelihood of detecting invasions during our project is low, because there are often significant lag-times in detecting new invasions, due to restricted geographic range, small population size, and still limited sampling effort. Thus, full evaluation of resulting invasions requires repeated measures over time, for selected sentinel sites, and would ideally (a) deploy molecular genetic techniques with high sensitivity and efficacy and (b) use the extensive baseline measures of historical occurrences established in our project.

4. PROJECT DESCRIPTION

a) Research Purpose

The overall purpose of the ADRIFT project was to characterize the arrival of marine biota to North America and Hawaii by JTMD and to evaluate the potential for new invasions (established populations) to result from this transfer mechanism.

SERC contributed to several components of the overall project, including:

1. Evaluation of parasites and pathogens associated with mussels on JTMD;
2. Detection of new JTMD-associated invertebrate invasions to North American waters;
3. Design and implementation of fouling panel surveys in to evaluate new invertebrate invasions in Hawaii, working with colleagues in Hawaii;
4. Design and materials for fouling panel survey collections in Japan, working with colleagues in Japan;

5. Data synthesis and analysis of all invertebrates arriving on JTMD, to evaluate spatial and temporal patterns for species occurrences;
6. Application and integration for an overall Risk Assessment; and
7. Making the ADRIFT data publically available through the NEMESIS web-based portal (NEMESIS 2003).

This report focuses on the first two of the components, where SERC assumed a lead role and devoted most of its research efforts. We will also provide a brief synopsis of fouling panel survey collections in Hawaii and Japan (3-4). The latter three components (5-7) were led by other collaborators and thus will be addressed by other individual reports.

b) Objectives

The specific objectives for SERC-led research were to:

- Test mussels arriving on JTMD objects for parasites and pathogens;
- Evaluate resident populations of mussels in North America for the presence of known JTMD parasites and pathogens;
- Evaluate resident invertebrate communities in North America for the presence of known JTMD species;
- Assist in the evaluation of JTMD-associated invertebrates in Hawaii and Japan.

c) Methods

§ Analysis of <u>mussels on JTMD</u> to detect parasites arriving to North America and Hawaii

Mussels (*Mytilus spp.*) were collected from Japanese tsunami marine debris (JTMD) objects on arrival to the coasts of California (CA), Hawaii (HI), Oregon (OR), and Washington (WA) (coll. JW Chapman, JA Miller, and others), and these were used to test for the presence of associated parasites and pathogens. Mussels were selected for this analysis, because these were frequently present on JTMD objects and also are known to have a diverse range of parasites, pathogens, and commensals (hereafter parasites) worldwide, including the hydroid *Eutima* that was detected previously on JTMD (Calder et al. 2014).

We sampled and tested 1,158 mussels from JTMD objects for the presence of parasites, combining previous work funded by the National Science Foundation with the PICES Adrift program. All mussels were visually measured for size and screened for the presence of three conspicuous metazoan parasites using a dissecting microscope: the hydroid *Eutima* sp., copepod *Mytilicola* sp., and pea crab *Pinnotheres* sp. In addition, tissue samples of mussels were collected for two different types of molecular genetic analyses. First, tissue samples were obtained and sent to MLML for genetic identification of the mussels. Second, tissue samples were obtained and processed for detection of three protistan parasites (haplosporidians, *Marteilia refringens*, and *Perkinsus* spp.) using molecular genetic techniques. For the latter, mussels collected from four JTMD objects (JTMD-BF1; JTMD-BF-6; JTMD-BF-8; and JTMD-BF-23), because these objects each had 30 or more bivalves, increasing the likelihood that parasites could be present and detected. In total, we screened n=264 mussels using molecular genetic techniques for these parasite taxa. For each molecular assay, we combined three target host tissues (gill, mantle, and digestive gland), which are known locations for the target parasites.

DNA Extraction, PCR Amplification, Sequencing: Following an overnight digestion with proteinase K, we extracted genomic DNA from all three tissues sampled, which were pooled into a single extraction, using a Qiagen Biosprint Kit (Qiagen, Valencia, CA) following the manufacturer's protocols for animal tissues. All extractions completed within the same day included a blank extraction, which served as a

negative extraction control for PCR. Aliquots of the extracted DNAs (50 μL), which were made to avoid contamination of stock DNA elutions, were stored at 4°C and stock DNA elutions were stored at -20°C.

We started with a total of 320 bivalves (JTMD and Japanese samples) and used a PCR assay to test for amplifiable DNA. The primer set (jgLCO1490/ jgHCO2198; Geller et al. 2013) amplifies the mitochondrial cytochrome oxidase I (COI) gene from a variety of mussel species. To screen for *Perkinsus* species, we used genus-specific primers **(**PerkITS85FNEW/PerkITS750R; Casas et al. 2002, Moss et al. 2007) that target the first internal transcribed spacer region (ITS1) of the ribosomal gene complex (rDNA). To screen for haplosporidian species, we used a general primer set (HAPF1/R3; Renault et al. 2000), which amplifies ~350bp of one variable region of the SSU gene and is capable of amplifying multiple genera (*Haplosporidium* sp., *Minchinia* sp., and *Bonamia* sp.) of haplosporidians. To screen for the presence of *Marteilia refringens*, we used a species-specific primer set (SS2/SAS1; Le Roux et al. 1999), which amplifies a portion of the SSU gene. To ensure that the PCR assays were amplifying the appropriate parasite DNA, positive control samples, consisting of extracted genomic DNA from infected bivalves that had successfully amplified in the past, were obtained from Drs. Ryan Carnegie for *Marteilia refrigens* (Virginia Institute of Marine Science) and our own collection for *Perkinsus* species and haplosporidians.

Resulting sequences were edited using Sequencher 5.1 (Gene Codes Corporation, Ann Arbor, MI). To initially determine the organism detected, all sequences were subjected to a nucleotide search using BLAST (http://blast.ncbi.nlm.nih.gov/) in GenBank against the nr database for highly similar sequences. All duplicate sequences were concatenated prior to phylogeny constructions, which contained only unique sequences that differed by one or more base pairs. To more accurately determine the organisms detected, phylogenetic reconstructions were made comparing the sequences from this study to haplosporidian sequences obtained from GenBank (http://www.ncbi.nlm.nih.gov/).

§ Surveys of <u>resident mussel populations in North America</u> to detect established JTMD parasites

To survey for these same parasites (detected for JTMD mussels) in resident populations of mussels in western North America, mussels were collected and processed from bays in California to Alaska. We obtained mussels (50-150 per bay) through direct collections and assistance from colleagues at multiple sites, including our Canadian PICES colleagues. We obtained 4,087 mussels from western North America for multiple analyses (Table 1). Mussels were processed live in order to (a) visually survey for macroparasites, (b) preserve target tissues samples to screen genetically for protistan parasites (especially haplosporidians), and (c) preserve tissue to screen genetically for non-native mussel species. The screening for parasites focused on those taxa detected in mussels on JTMD, including the endoparasitic hydroid *Eutima* and other macroparasites, following the same protocols outlined above. The mussel tissues were sent to MLML to be screened for Japanese mussel species. Although tissue samples were also collected to test for the presence of protistan parasites (as above), PICES was not able to fund these analyses as part of the ADRIFT program.

Table 1. Mussels collected for analyses of parasites and host genetics of resident populations in western North America. Shown are year, site, total number of individuals/site, and number available for each analysis type.

Collection Year	State / Province	Bay Name	Total # Mytilus Collected	Total # DNA Samples Collected for Host ID	Total # DNA Samples Collected for Protistan Parasite ID	Total # Mytilus Samples Screened for Macroparasites
2014	OR	Yaquina Bay	247	247	134	133
2014	OR	Coos Bay	277	277	144	144
2014	CA	Humboldt Bay	252	252	168	144
2014	CA	Bodega Bay	143	143	135	135
2014	CA	Tomales Bay	119	119	107	92
2014	CA	San Francisco Bay**	202	202	202	101
2015	AK	Sitka Sound	100	100	100	100
2015	CA	Newport Bay	100	100	99	100
2015	WA	Neah Bay	50	50	50	50
2015	AK	Ketchikan	342	339	238	329
2015	AK	Kachemak Bay	50	50	50	50
2015	BC	Prince Rupert	100	100	100	100
2015	AK	Seward	150	149	149	149
2015	OR	Coast South of Yaquina Bay	50	50	50	50
2015	BC	Prince William Sound, Orca Inlet	248	248	248	248
2015	CA	San Diego Bay	150	0	150	150
2015	BC	Nanaimo	60	60	60	60
2015	CA	Mission Bay	150	0	150	150
2015	CA	Long Beach	125	125	100	100
2015	CA	Oxnard	73	73	50	50
2015	CA	Santa Barbara	84	84	50	50
2015	BC	Saanich inlet	50	50	50	50
2015	AK	Haines	47	46	44	47
2015	CA	Morro Bay	165	164	152	152
2015	CA	Elkhorn Slough	101	101	100	100
2015	CA	Monterey Bay	100	100	100	100
2015	CA	San Francisco Bay**	202	200	199	200
2016	BC	Vancouver	50	46	50	50
2016	WA	Grays Harbor	150	0	0	150
2016	WA	Willapa Bay	150	0	0	149
TOTAL (All Years, Regions, and Bays)			**4087**	**3475**	**3229**	**3483**
		** Bay sampled twice				

§ Survey of <u>resident invertebrate communities in North America</u> to detect established JTMD species.

We implemented standardized surveys of biofouling communities for bays in California, Oregon, Washington, British Columbia, and Alaska. Fouling panels were deployed in each state, and a subset of these were collected for analyses to detect free-living JTMD invertebrate species in resident coastal waters of western North America (Table 2). Over the three year PICES project, we deployed in 8 different bays. Panels consisted of bare, dark gray, lightly sanded PVC plates measuring 13.7 x 13.7 cm, attached to bricks with the collecting surface facing downward. Plates were suspended one meter below mean lower low water at randomly chosen locations on docks (Blum et al. 2007) for approximately three months, during the summer, to coincide with the period of high seasonal recruitment and provide sufficient to develop mature communities; Ruiz et al. unpublished data)

Table 2. Sites of biofouling community surveys using standardized fouling panels. Bold indicates core sites, with extensive surveys. Shown are state or province, location, total number of site and panels per location.

State/Province	Location	(# sites, # panels)
California	**San Francisco Bay**	(10, 100)*
California	**Humboldt Bay**	(10, 50) *
Oregon:	**Yaquina Bay**	(10,50)
Washington	Willapa Bay	(2, 20)
Washington	Grays Harbor	(2,20)
Washington	Neah Bay	(2,10)
British Columbia	**Prince Rupert**	(10,50)
Alaska	**Ketchikan**	(3,135)*

(* Work was funded in part or entirely by different sponsors but contributes to our PICES baseline measures)

In Year 2, we collected and processed all panels at each of these sites, except those in Washington state (see below). The retrieval and processing in Prince Rupert was a joint effort with our Canadian PICES collaborators, along with the Port of Prince Rupert and the local community college. For processing, panels were examined individually under dissecting microscopes, and invertebrates were identified initially in the field to morphospecies or lowest taxonomic level possible. Voucher specimens were collected for (a) further morphological analyses and identification and (b) genetic bar-coding for independent verification and comparison with material collected by our Japanese colleagues. All morphological analyses were done by SERC. All vouchers for molecular genetic analyses were sent to MLML.

Our highest priority for these surveys were Yaquina Bay, Oregon and Prince Rupert, British Columbia. These represented major gaps in our knowledge of non-native biota along the west coast, having only limited surveys to date for biofouling biota. Critically, Prince Rupert is a major port system but has only received limited attention to date (Gartner et al. 2016). As a major port, establishing a baseline of invasions that predate JTMD arrivals in North America was deemed a high priority, since all other commercial ports have been surveyed in the past decade for western North America. In the case of Yaquina Bay, this was a landing site and hotspot for JTMD species landings, such that measures here served both as baseline and detection effort. The dispersion of sample locations for these two sites is shown in Figure 1.

The sites from WA were intended initially for collection in fall 2015, but strong rains and flooding occurred during the scheduled retrieval. Our intention was to retrieve these in Year 3 (15 months after initial deployment), but we had significant losses during this time. Thus, samples from these bays are unfortunately not available, but this was also a very small number of intended sites (unlike Yaquina Bay or Prince Rupert, with 10 sites each), having low impact to the overall project results. We hope to conduct a future survey of these areas, but this is beyond the scope of the current project.

In addition to the surveys conducted during the PICES ADRIFT program, we have conducted identical surveys in several other bays in California waters in the past five years. This provides further baseline data on resident species that we discuss in the Results section.

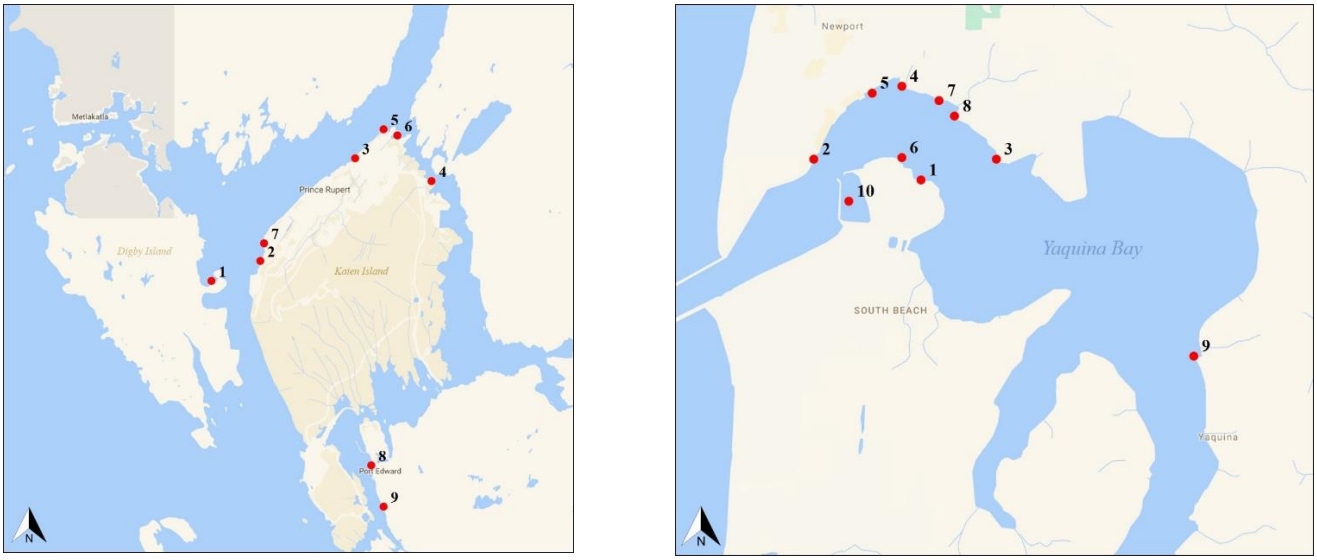

Figure 1. Sites of fouling panel surveys in Prince Rupert, BC (left) and Yaquina Bay, OR (right). Each site was surveyed with n=5 fouling panels.

Finally, our Canadian PICES colleagues collected panels using similar methods at multiple bays/sites in western British Columbia in 2015. We developed joint protocols for standard photographs and a rapid assessment for target invertebrate species, and these panels were preserved for further potential subsequent analyses, which were beyond the scope and available funding for the ADRIFT program.

§ Collect zooplankton samples in North America for future analyses, to detect JTMD species.

In Year 2, zooplankton samples were collected at several North American bays as part of the ADRIFT project (Table 3). At each bay, zooplankton samples (>1m^3; 80 micron mesh) were collected by pump sample or plankton tow. Ten sites were sampled in each Yaquina Bay OR and Ketchikan AK. Fewer sites (1-3) were sampled for Willapa Bay WA, Grays Harbor WA, and Neah Bay WA. Three replicate samples were taken per site. These were preserved in ethanol for potential molecular genetic screening of target JTMD species, including holoplankton and meroplankton (i.e., larval forms of biofouling organisms). All samples were collected by SERC and sent to MLML for potential analyses in Year 3. Thus, 90 plankton samples, 30 from Alaska and 60 from Oregon and Washington, were received at MLML for DNA extractions for potential future PCR and sequencing-based detection of JTMD-associated species. Unfortunately, the analysis of these samples was not funded by PICES in year 3.

Table 3. Sites of zooplankton community surveys using standardized pump samples. Shown are state, bay/ location, total number of sites and samples per location, and the collection dates.

State	Bay Name	Method	# Sites	Samples/Site	Total # Samples	Collection Date(s)
Oregon	Yaquina Bay	Pump	10	3	30	8/17/2015 - 8/19/2015
Oregon	Yaquina Bay	5m Vertical Tow	15	1	15	8/17/2015 - 8/19/2015
Washington	Willapa Bay	Pump	2	3	6	9/20/2015
Washington	Grays Harbor	Pump	2	3	6	9/20/2015
Washington	Neah Bay	Pump	1	3	3	9/21/2015
Alaska	Ketchikan	Pump	10	3	30	10/12/2015 - 10/14/2015

§ **Assist in advancing biofouling invertebrate surveys with colleagues in Japan, for barcoding in order to detect/confirm JTMD species in North America.**

In Years 2 and 3, we worked with our Japanese PICES colleagues to assist in their survey of biofouling invertebrates in Japan. SERC provided materials, protocols, and assisted in discussion of overall project, which was implemented by our Japanese colleagues and taxonomic experts. The primary aim of this survey was to provide identified specimens, using expertise in Japan, for DNA bar-coding. In 2015, two surveys were implemented at 3 different sites in Japan by our colleagues. In 2016, this was repeated, with some modification of site selection and seasonal timing. Specimens were sent to MLML for bar-coding. In addition, images were generously provided by our Japanese colleagues. Although outside of the scope of the current ADRIFT program, we are interested in exploring further comparisons of survey results between Japan and North America.

§ **Assist in advancing biofouling invertebrate surveys with colleagues in Hawaii, using similar methods as mainland US, to detect JTMD species.**

We worked intensively with colleagues in Hawaii to implement an identical biofouling survey to those along western North America (above). We provided protocols and staff time to advance this work. In Year 2, we focused on Oahu, with panels deployed at 10 sites (100 panels total). These were retrieved and processed by SERC staff from December 6-180 2015. To location of survey sites is shown in Figure 2. This survey generated a similar set of vouchers for morphological and genetic analyses to those in North America. Hawaii has funded independent taxonomists for identification of some vouchers, and SERC is continuing to provide assistance with data management and analysis.

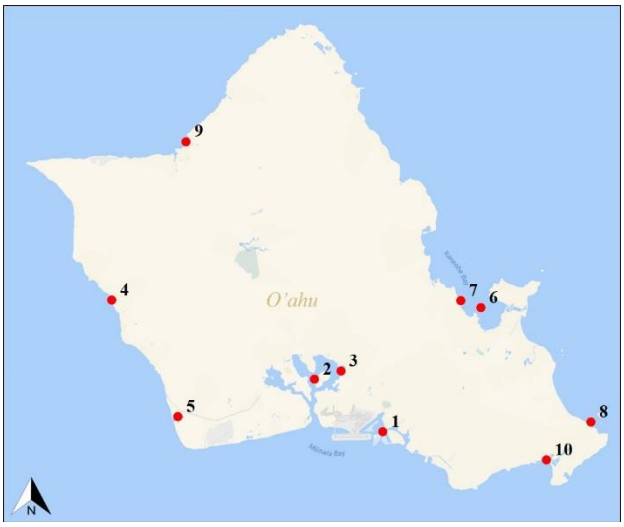

Figure 2. Sites of fouling panel surveys on Oahu, Hawaii. Each site was surveyed with n=5 fouling panels.

e) Results

§ Analysis of <u>mussels on JTMD</u> to detect parasites arriving to North America and Hawaii

We detected the hydroid *Eutima* sp. in 3.2% of the 1,158 mussels surveyed from JTMD objects. Infected mussels often exhibited high intensity of infection, with hundreds to thousands of hydorids occurring on the gills of the host organisms (Figure 3). All cases to date were detected on objects arriving to Oregon and Washington.

No positive cases of the other two macroparasties, including pinnotherid crabs and the copeopod *Mytilicola orientalis*, were detected among the 1,158 mussels screened to date.

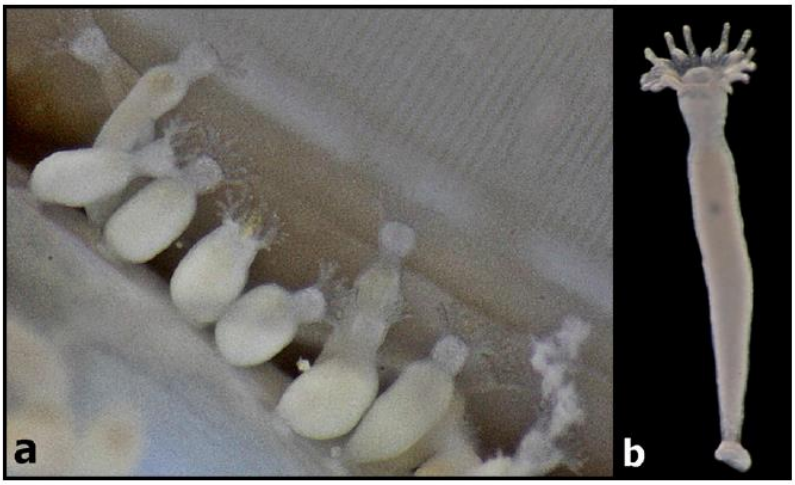

Figure 3. Pictures of the hydroid Eutima sp. from the inside of a mussel, collected from JTMD arriving to North America. Image from Calder et al. 2014.

Eight mussels (3%) tested positive for haplosporidians on JTMD, of the 264 mussels screened to date. These were on JTMD objects that arrived to Oregon and Hawaii. It appears that these are novel lineages and cluster most closely to samples from South Africa and France (Hartikainen et al. 2013). Thus, the biogeography and identity of these protists are currently unknown. None of the 264 mussels tested positive for the other two protistan parasites, *Perkinsus* sp. or *Mareilia* sp.

§ Surveys of <u>resident mussel populations in North America</u> to detect established JTMD parasites

Of the 3,483 mussels screened for *Eutima* sp. in North America during the ADRIFT project, none tested positive, from California to Alaska (Figure 4). However, 11 sites tested positive for the parasitic *Mytilicola orientalis*, including a new record for Alaska. This parasite, native to Japan, was already known to occur along western North America, and was likely introduced with the oyster *Crassostrea gigas* in the early 1900s (NEMESIS 2003).

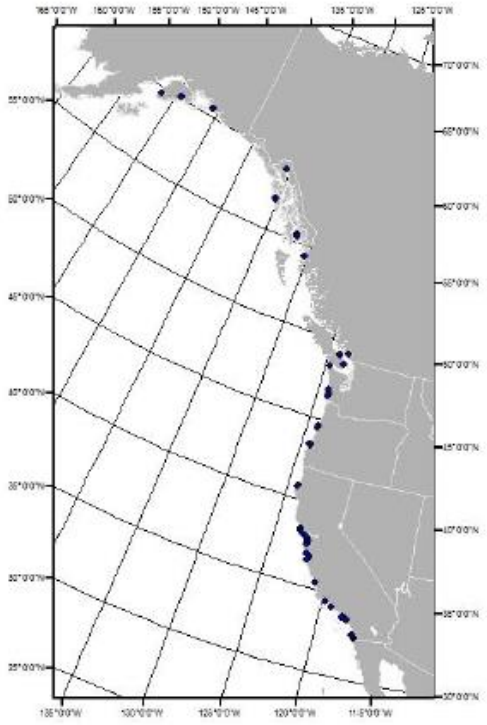

Figure 4. Mussel survey locations. Dark circles indicate location of sites sampled (n>50 mussels) and that no *Eutima* sp. were detected.

§ Survey of <u>resident invertebrate communities in North America</u> to detect established JTMD species.

Our surveys of the biofouling communities for western North America detected <u>no new records of potential JTMD species for sessile marine invertebrates</u>, based on morphological identification of specimens. While our work in the PICES ADRIFT project focused primarily on five selected bays (Table 2), we have also conducted contemporary surveys in an additional eight bays along this same coast, allowing a much broader analysis of nonindigenous species (NIS) for western North America.

Across the thirteen bays, we have detected 27-71 total sessile invertebrate species per bay, including 8-27 NIS per bay (Figure 5). NIS represented from 5-44% of all detected species per bay, with the lowest prevalence found at northern sites, including Alaska, British Columbia, and Washington sites (7, 5, and 12% NIS respectively).

Of particular relevance for the PICES ADRIFT project is the extent of shared biota with Japan, when considering only the NIS detected in each bay survey. Figure 6 shows the total number of NIS detected in each bay survey, for the sessile invertebrates, indicating many of these species also are known from Japan. The figure identifies the number of species that are not known in Japan versus those that are known to occur there, distinguishing further those that are considered native to Japan and those that are either introduced or cryptogenic there.

The majority of NIS detected in our surveys also are reported to occur in Japan, ranging from 70-100% of NIS detected per bay (Figure 6). A much smaller percentage of these (<50% --- excluding Alaska, British Columbia, and Washington sites, where the total number of NIS is low) are considered native to Japan.

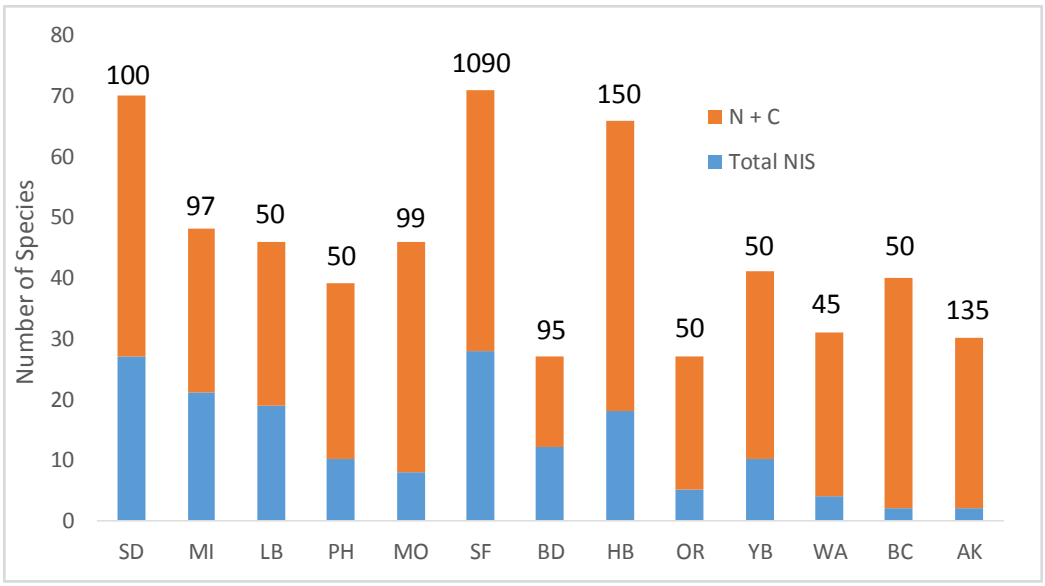

Figure 6. Total number of sessile invertebrates detected per bay survey. The figure indicates the contribution of NIS versus all other taxa. Number of panels surveyed per bay shown on top, and bay shown on x-axis includes (left to right): San Diego, CA (SD); Mission Bay, CA (MI), Long Beach, CA (LB), Port Hueneme, CA (PH), Morro Bay, CA (MO); San Francisco Bay, CA (SF); Bodega Bay, CA (BD); Humboldt Bay, CA (HB); Coos Bay, OR (OR); Yaquina Bay, Oregon (YB); Puget Sound, WA (WA); Prince Rupert, BC (BC); Ketchikan, AK (AK).

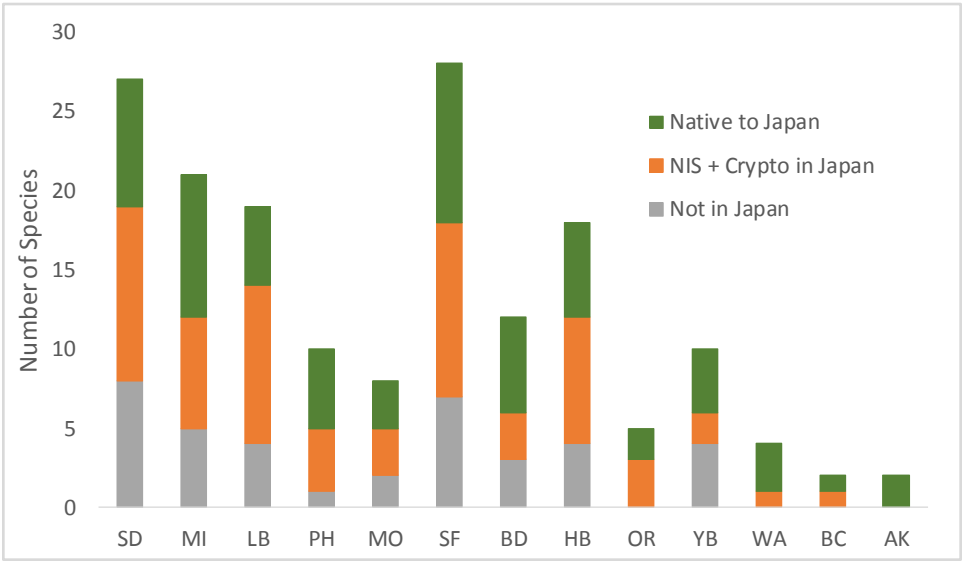

Figure 7. Total number of NIS for sessile invertebrates detected per bay survey. The figure indicates the number of species per bay that are considered (a) native to Japan, (b) occur in Japan as introduced (NIS) or cryptogenic, or (c) are not reported to occur in Japan. Bay shown on x-axis includes (left to right): San Diego, CA (SD); Mission Bay, CA (MI), Long Beach, CA (LB), Port Hueneme, CA (PH), Morro Bay, CA (MO); San Francisco Bay, CA (SF); Bodega Bay, CA (BD); Humboldt Bay, CA (HB); Coos Bay, OR (OR); Yaquina Bay, Oregon (YB); Puget Sound, WA (WA); Prince Rupert, BC (BC); Ketchikan, AK (AK). Sample size as shown in Figure 6 per bay.

Importantly, our survey results serve a dual purpose. First, we did not detect new invasions to western North America that were associated with JTMD. Second, we have established a strong baseline of field-based measures that (a) account for previously known invasions and (b) improve our capacity to potential JTMD invasions in the future (see Discussion).

In addition to the field-based analysis, we have also conducted a comprehensive review of NIS reported western North America and synthesized in NEMESIS (2003) through 2015. This served to evaluate the status of species detected in our surveys (above) and as well as other species known to occur in North America, expanding our baseline data to other habitats in addition to fouling panels. The results show a similar pattern to the fouling survey. Specifically, over 50% of marine and estuarine NIS reported in western North America also occur in Japan, when considering free-living invertebrates and algae, with roughly 30% native to Japan (Figure 8). Both the total number and percent overlap is smaller for known parasite species.

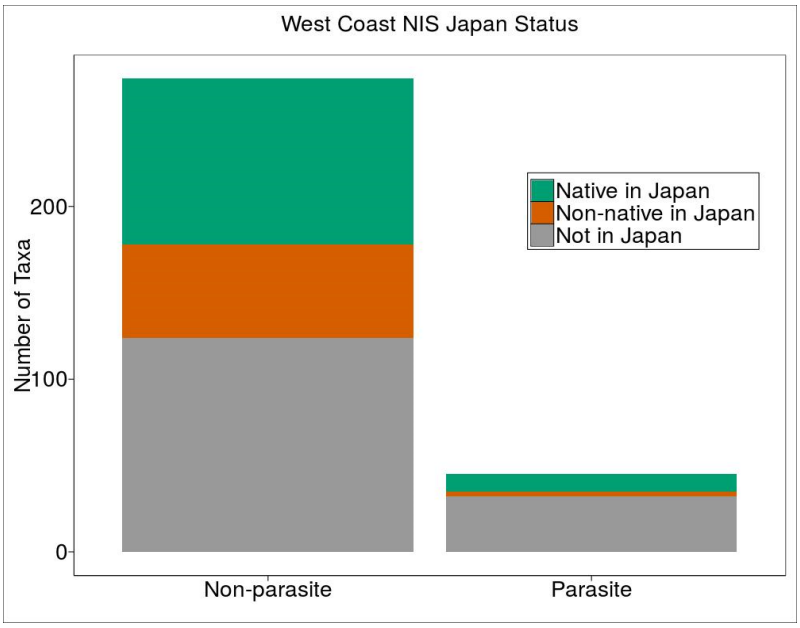

Figure 8. Total number of NIS reported for marine end estuarine habitats of western North America. The figure indicates the number of NIS for invertebrates and algae (excluding vertebrates and vascular plants), for each free-living species and parasite/commensal species. Color coding indicates the number of NIS considered (a) native to Japan, (b) occur in Japan as introduced (NIS) or cryptogenic, or (c) not reported to occur in Japan. Data synthesis from NEMESIS (2003).

f) Discussion

Our results add several dimensions to understanding of the dynamics of biota transferred by JTMD and the associated potential for new invasions in North America. First, we highlight the potential role of parasite taxa in biotic transfers with JTMD. Second, we tested for the known extent of invasions in western North America, using field-based measures and literature-based synthesis to detect new invasions. Third, we have also begun to explore the strong environmental match between Japan and western North America, evaluating further the potential for colonization. We address each of these topics below.

1. Role of parasite taxa in JTMD transfers and invasions: We identified several parasites that arrived with JTMD on multiple occasions and locations, using the mussels *Mytilus* spp. as a model system. While it is perhaps not surprising that parasites (including commensals) were associated with JTMD invertebrates, since many taxa of parasites are known and often common in subtidal communities (e.g., Lauckner 1983, Sinderman 1990, Lafferty et al. 2006), this also underscores that parasites are a potent "multiplier", serving to increase the number of taxa associated with this vector. With our current sampling effort, we added three species associated with one host (*Mytilus sp.*), quadrupling the original number of detected taxa with further analysis. Thus, not only are the total number of invertebrate and macroalgal taxa detected on JTMD an underestimate --- since many items went undetected and the biota was vastly under sampled on those detected (see report by Carlton) --- but parasite taxa are also largely overlooked in these estimates.

The parasite taxa detected are reported to have significant effects on host condition and survival. The hyroid *Eutima japonica*, which lives on the gills of mussels, scallops, and oysters and has been associated with extremely high juvenile mortality of infected bivalves (Kubota 1992, Baba et. 2007). Although the identity and biogeography of the detected haplosporidians are not known, other taxa in this group are known to cause disease and impact fishery species. Probably the best known example is *Haplospordium nelsoni*, which occurs in the native Japanese oyster *Crassostrea gigas* and was introduced to the eastern United States, causing widespread mortality (Burreson et al. 2000, Burreson & Ford 2004). Thus, while the detected parasites may cause severe pathology, and also appear to be generalists capable of infecting diverse taxa, the potential risks (effects) on North American taxa are not known. Moreover, there is currently no evidence that these species have colonized North America successfully.

For *Eutima* sp., we conducted extensive surveys across many sites in North America, from Alaska to southern California, failing to detect an individuals. This demonstrates that the species is certainly not a common resident and unlikely to have colonized historically due to another vector. We surmise that the parasite would be widespread if introduced historically, given its high prevalence in Japan (Baba et al. 2007) and also the fact that the same species of mussel host is abundant throughout North America.

While we have confidence in the historical absence of *Eutima* sp., it is more challenging to assess whether a recent invasion may have resulted from JTMD-mediated transfers. Specifically, the probability that we would detect a nascent population is low, because it would likely be very restricted geographically (to a small area) and low in prevalence. This challenge is well-recognized in invasion ecology, and can result in significant lag-times between initial colonization and detection (Crooks & Soule´1999, Ruiz et al. 2000, Solow & Costello 2004, Crooks 2005). Thus, it is premature to assess whether an invasion of Eutima has occurred, as detection may lag years to decades from any colonization event(s) and will be greatly dependent upon search effort, sensitivity of methods, and dynamics of any such population.

For haplosporidians detected on JTMD, the situation is more complicated. In addition to the challenges outlined for *Eutima* sp., it is not clear whether these particular haplosporidians are already present in western North America. While we have collected tissue samples for such an assessment, resources were not available for analysis during the project. Thus, further analysis is required to resolve both the taxonomic identity and biogeography for these parasites.

2. Extent of invasions to North America from Japan: Our summary of extensive field-based measures and a comprehensive synthesis of existing historical records provides several important insights about (a) past invasions from Japan and (b) the potential for invasions associated with JTMD arriving to North America. Our analyses quantify the extent to which past invasions from Japan have occurred, due especially to live importation of oysters (and associated biota) in the 20th century (Carlton 1979, Cohen and Carlton 1995, Ruiz et al. 2011), prior to any species transport by JTMD. Critically, this synthesis of

data allows us to remove the confounding effect of historical invasions, to evaluate whether new species of putative JTMD origin have been detected to date.

From a positive perspective, we have no evidence to date of new invasions in western North America that are attributed to JTMD, based on the morphological analyses conducted (but see also report by Geller for molecular genetic analyses). We have also established a solid quantitative baseline and historical record to evaluate future invasions, to assess whether JTMD is a plausible mechanism, based on geographic distribution and other potential vectors.

However, as was the case for *Eutima* sp., it is also unlikely that we would detect new invasions within a few years of arrival, unless they underwent a population explosion and spread rapidly in an area where we sampled. It is premature to draw any conclusions about actual invasions, especially without further and repeated measures over time. Ideally, this would include molecular genetic methods, which promise high sensitivity and efficacy, drawing on the DNA bar-code library developed during this project (see report by Geller). Moreover, this could use initial zooplankton community samples already collected from several areas to advance this analytical approach, as initially proposed but unfunded in the project (see Table 3).

Environmental match between Japan and western North America: Another important outcome from our analysis is the large number of NIS in western North America that also occur in Japan, whether native or not in the latter region (Figures 7 & 8). This underscores the high potential environmental match between these two regions, demonstrating that many species have the capacity (indeed the history) of successfully colonizing both regions. Moreover, several of the species detected on JTMD are already established along western North America, indicating further that this vector is delivering species capable of colonization. While this species overlap provides a coarse measure of potential "match", a next step in analysis is to use environmental niche models to formally assess the potential climatic range for several of these species. This may further refine predictions and also be useful in identifying locations and taxa for future detection measures, to assess whether JTMD invasions have occurred.

g) Challenges

The largest challenge during the project was keeping pace with (a) the intensive field sampling schedule (effort) during Year 2 and (b) synthesis and analysis of the large amount of data on species identification from JTMD (as reported in Carlton's report). The former was challenging, because of the sheer magnitude of this effort. The latter was challenging due to the continuous and ongoing arrival of JTMD through 2017 with live biota, nearly six years after the event.

As evident in this report, SERC was funded to collect many samples in Year 2 (e.g., for plankton and parasites), for which analyses were not funded in Year 3. As a result, we have archived these samples, hoping for a future opportunity to complete the analyses initially proposed. The only other associated challenge was that some of this field sampling in Year 2 limited the time available for collection of fouling panels in Washington state, contributing to our loss of survey results from a few sites in each of three bays, as noted above.

h) Achievements

My project contributed strongly to the overall analysis of biota on JTMD, by (a) considering the and evaluating the potential importance of parasite transport and (b) playing a major role with Carlton and other collaborators in synthesis, formal analysis, and interpretation of species richness on JTMD.

My project made a major contribution in evaluating historical invasions to western North America, and the detection of new invasions, for invertebrates.

My project and participation also contributed to evaluation of environmental match and the potential risk of invasions, including active participation in the Risk Assessment component being led by our Canadian PICES colleagues.

Finally, my project contributed substantively to advance (a) standardized fouling panel surveys in Hawaii, (b) fouling panel surveys in Japan, and (c) genetic voucher specimens and plankton samples for analysis by MLML.

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5. OUTPUTS

a. Completed and planned publications

Cathryn Clarke Murray and Jim Carlton have compiled detailed and comprehensive lists of manuscripts planned to date, including those that involve me.

To date, I have published no papers on the PICES ADRIFT project.

There are however several papers planned for publication, including:

- A synthesis paper of invertebrate biota on JTMD, led by Carlton (Possible venue: Science);
- A paper on *Eutima* sp. and parasite analysis for JTMD and western North America (Possible venue: Journal of Parasitology);
- A short paper on surveys for Prince Rupert (Possible venue: BioInvasion Records);
- A paper on environmental match for JTMD species arriving to North America (Possible venue: Global Ecology and Biogeography).
- Paper on risk assessment that combines biological and oceanographic data to model risk, led by Canadian PICES colleagues (Possible venue: TBA).

b. Poster and oral presentations at scientific conferences or seminars

My lab and I have given oral presentations at three separate meetings, including:

- International Conference on Marine BioInvasions, Sydney, Australia (January 2016);
- PICES Science Meeting, San Diego, California (November 2016);
- Workshop on parasite ecology and invasions, National Zoological Park, Smithsonian Institution (Fall 2014).

I have also been coauthor on several papers presented by Carlton and other PICES collaborators.

c. Education and outreach

I have not played a lead role in this area. However, I have engaged at least five undergraduate and graduate students in the research for our project, including several undergraduate interns and graduate fellows who were funded fulltime on this project for portions of a year.

I also am now working with PICES colleagues to serve the ADRIFT database as a portal through the NEMESIS website, for public access.

6. RESEARCH STATUS AND FUTURE STEPS/PLANS

I feel my project accomplished the major objectives of characterizing (a) the flux of JTMD, (b) the biota associated with JTMD, and (c) the baseline of existing invasions in western North America shared with Japan.

It has been challenging keeping pace with the magnitude of effort required to advance all aspects of this project, but I feel we have stayed on track and adhered to timelines.

It is gratifying to see the overall syntheses being completed (e.g., biotic flux and species composition, led by Carlton) and also the increased interactions across projects, including (a) the exploration of interactions between ocean currents/dispersal and biota and (b) Risk Assessment project.

I regret that the full analysis of parasite data (for North America) and plankton samples were not funded, as I believe both of these are still important components. I would like to see these advance, especially as testing/detection the presence of JTMD-mediated invasions. As discussed above, we cannot yet assess whether such invasions have occurred, due to time lags in detection. Moreover, molecular genetic approaches (including use of bar code libraries that include results of Japan surveys) would offer the best resolution for the least effort/cost. A few sentinel sites (e.g., hotspots of JTMD biota landings, such as Yaquina Bay, Oregon) for repeated measures would be especially useful in this regard.

In addition to completing the publications outlined above, I would very much like to conduct comparative analyses of fouling communities and parasite communities between western North America and Japan. I intend to explore opportunities with Japanese colleagues to advance collaborative work in this direction.