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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

1. PROJECT INFORMATION

Title	**Marine Algae arriving on Japanese Tsunami Marine Debris (JTMD) and their invasion threat to the coasts of Oregon and Washington, USA – Year 3**
Award period	Year 3 -- April 1, 2016 – March 31, 2017 Year 2 -- April 1, 2015 – March 31, 2016 Year 1 -- August 1, 2014 – March 31, 2015
Amount of funding	Year 3 – US $37,331.08 Year 2 – US $48,092.77 Year 1 – US $39,921.00 + $10,000 (for Kawai and Hanyuda)
Report submission date	06-January-2017 -- update submitted 21-January-2017
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2. YEAR 3 PROGRESS SUMMARY

a) Describe progress.

During year 3 of the ADRIFT project (April 1, 2016-March 31, 2017), the algae on 5 boats, 1 carboy, and numerous small plastic debris items (without BF numbers) were collected, processed and identified. During the last days in March while we were finishing our annual ADRIFT reports, 4 of the major items came ashore. We were able to sample the items but could not begin processing the material until April, the beginning of our 3rd year projects. From April through August, I carried out the sorting and morphological identification of the algal species on these items and compared them with samples from the debris collections of previous years. This helped to ensure that my identifications were uniform. Since many of the species were very small and required both a dissecting and compound microscope for identification, I prepared semi-permanent (Karo) microscope slides of the samples to be used as vouchers. I then photographed these slides and saved them for later study. When they were large enough, the individual species were separated, cleaned, put into silica gel, and mailed to Japan for sequencing.

Below are the summary results of the major debris items processed during the year 3, our last sampling period. The number of species on these each item was surprisingly high. The 2012 Agate beach dock (the largest debris item sampled) contained only 29 species of algae, and 2 of the boats sampled this year contained 24 species. Moreover, 6 of the species were new to debris: *Mutimo cylindricus*, *Petroderma maculiforme, Pseudolithoderma subextensum, Chroococcus submarinus, Neosiphonia yendoi*, and *Meiodicus spetsbergensis*. *Petroderma*, *Pseudolithoderma* and *Meiodiscus* are crusts that might have been overlooked in earlier collections, but each was quite apparent on the debris item where they were found this year. Bluegreen algae/bacteria like *Chroococcus* were much more abundant on debris than earlier, possibly due to the longer time the debris items were at sea. *Mutimo cylindricus* and *Neosiphonia yendoi* are larger species that would have undoubtedly been noticed and collected earlier if they had been present.

Just as surprising was the re-occurrence of species that had been rare earlier. *Cladophora albida* and *Cladophora vagabunda* were extremely abundant on 2 of the recent boats. On the Horsfall Beach 2 boat, *Cladophora albida* coated the top and sides of the boat mimicking *Ulva prolifera* in overall appearance. On the Roads End boat, an extensive nearly black turf coated the inner sides of the boat. This turf turned out to be the deep-green colored basal system of *Codium fragile fragile*, an extremely invasive species that propagates by fragmentation. Erect thalli of this species also occurred sporadically on the interior of the boat. Another highly invasive species, *Grateloupia turuturu*, very rare earlier, was found sporadically on both the Horsfall Beach 2 boat and on the Sixes River boat.

Table 1. BF-numbered debris items processed during year 3 of the PICES-ADRIFT project

BF#	State	Site	Collector	Item	Date	Year	Total Species
BF-526	OR	Horsfall Beach 2	Hansen	boat	22-Mar	2016	24
BF-656	OR	Quail Street (plastic)	Hansen	carboy	26-Mar	2016	4
BF-545	OR	Umqua River mouth	Chapman	boat	26-Mar	2016	6
BF-533	OR	Roads End	Hansen	boat	28-Mar	2016	24
BF-538	OR	Sixes River mouth	Chapman	boat	16-Apr	2016	17
BF-652	OR	Falcon Cove beach	Chapman	boat	26-Jul	2016	6

Even taking into account the size of the debris items, the number of algal species on debris has not been predictable or particularly consistent. Larger items were likely to have more species, but this was not always true. Since the debris items that ended up on NE Pacific beaches were not necessarily from the same parts of the Tōhoku coast and they did not contain the same species originally, the final species counts were unpredictable. Numerous factors would have impacted the survival of each species at sea -- including floatation level and inundation, water temperature, competition for space, herbivory by animals, nutrient requirements, and the basic life history features. The total checklist of the algal species, their invasive and life history features (reproduction, longevity, and successional stage), and their worldwide distributions are discussed more thoroughly in the 3 year summary results to follow and in my manuscript for Marine Pollution Bulletin.

One other species should be mentioned, even though it is only loosely associated with our project. An unusual gelatinous pink crust first appeared on plastic debris along the Washington coast in March of 2015. However, the debris could not be shown to be JTMD so the species was ignored. Then from November 2015 through March 2016, the same crust was found on more than 30 small plastic debris items. None of these could be shown to be from the tsunami, and hence we could not justify working on it as a part of our ADRIFT project. So, I began culturing the material in my non-salaried time and sent both living material and DNA samples to colleagues in Australia and New Zealand who did a gratis molecular and culture study of the species for me. They discovered it was new-to-science. We decided to call it *Tsunamia transpacifica* since it was transported on currents "with tsunami debris" if not "on tsunami debris". I spent part of this past summer working on the manuscript for this study with John West. Since I did eventually find it on 3 BF numbered debris items, I am including it as a peripheral product of my JTMD project.

The latter part of this year was spent re-checking and analyzing the data from our project and preparing posters for the annual meetings of PICES and WSN. It was the 25th year anniversary meeting of PICES and the 100th year anniversary meeting of the Western Society of Naturalists. More than 600 people attended each meeting. At both, I presented my summary poster with the same title as this grant and also 2 smaller posters: "*Tsunamia transpacifica*, a new-to-science pink crust on hard plastic debris" and "Determining the source of the Seal Rock debris boat". For the PICES meeting, Kawai presented a talk on "Genetic diversity and biogeography of the macroalgal species associated with Japanese tsunami marine debris" where he discussed not only the molecular data from our project (~60% of our identified species have been sequenced) but also the genetics of our cross-Pacific species comparisons. A number of the 61% of the JTMD species that occur on both coasts are actually slightly different haplotypes. Therefore, in addition to our concerns about introduced species from JTMD, we now must also consider the threat of genetic contamination to those species that we already share.

By our project conclusion, we plan to finish our 2 major papers and an internet or hard-copy illustrated guide to the most prominent and potentially invasive JTMD algal species.

b) Describe any concerns, challenges and suggestions to PICES that you may have about your project's progress.

1. PICES-ADRIFT Reports – time requirement. PICES required each PI to prepare 2 reports/year for this project. I think that 1 annual report should be enough. These are extensive reports and with the limited time available for the project, the time required for the 2nd report would have been better spent working on research, publications and presentations.
2. The PICES-ADRIFT Annual Reports to MoE. It would be helpful if these could be made available to the PIs when they are submitted to the MoE. As Principal Investigators (PIs) we contribute to these reports, so it is important for us to see how our data is being used and also what the other scientists are doing on the project. Seeing the overall report should motivate us to adjust our projects to better fit the ADRIFT questions and perhaps to increase our output. However, It has been very useful to have the central ADRIFT committee advise us each year on how to modify our proposals so that they fit within the vision for the final MoE project report and also within the funding available.
3. PICES-ADRIFT Annual Reports and the security of sensitive pre-published data. I appreciate the promise of the ADRIFT committee not to release our data outside the ADRIFT group before we PIs have the opportunity to publish. I was very concerned about the checklists that we were required to provide at the end of our 1st year of PICES funding, particularly since I completed the identifications and analysis of many of the algal species on my checklist (50 of the final 80) before the PICES funding even began. However, with the continuing arrival of debris and with sampling and identifications still necessary, there was no way for us to summarize and publish our work until now. I am very grateful to Cathryn for setting up an issue of Marine Pollution Bulletin where we can now submit our manuscripts on 31 January 2017. I hope to finish mine by then. It is my understanding that although our report data will be incorporated into the final PICES report to MoE, the PICES-ADRIFT staff will not skim the important parts of our data and produce interpretive publications until after our own final papers are published.

4. Distance between our 2 separate laboratories. Due to being on separate sides of the Pacific, we algal PIs rarely have the chance to talk in person to discuss our project. Instead we work through e-mail for discussion and US Mail to provide the material for sequencing. It has taken some time to get used to the limited e-mail responses I receive from Japan -- and to our different customs and work schedules, but we have resolved how to work around this. Although our projects are interdependent, we now simply keep some aspects of them separate. This has not deterred our progress, and we will soon be able to produce 2-3 excellent publications and a guide to the algal species of greatest risk for invasion. I have been fortunate to work with Profs. Kawai and Hanyuda on this project. They are the best suited collaborators in Japan for this study, and the algal project would not have been possible without their extraordinary DNA sequencing contributions.
5. Although we are completing our ADRIFT study on March 31, 2017, some parts of our project will remain to be published after our PICES funding ends. I hope we will be able to complete these during the coming year – particularly the naming and description of our new species.

3. ABSTRACT

Carried across the North Pacific on currents from Japan, marine debris from the 2011 Great Tōhoku Earthquake and Tsunami has been arriving in Oregon and Washington since June 2012. Many of the debris items are laden with healthy and reproductive Japanese marine algae and there is considerable risk that many of these species could recruit to invade NE Pacific shores. Our project sampled and identified the algal species on debris using both morphological and molecular methods, and we also examined the invasion threat of these species to the NE Pacific. On the 42 debris items that we sampled between June 2012 and July 2016, we identified more than 80 marine algal species and obtained DNA data on 53, mainly those species large enough to isolate for sequencing. The majority (55%) of the algal species were found on only 1-3 debris items; while only 9% occurred on >12 debris items. The features of many of the JTMD species indicated a high invasion potential. More than 84% of the species were found to be fertile and actively releasing spores or gametes. A large percentage of the species were ephemeral (50%) and/or early successional (76%) forms capable of reproducing multiple times during a single year and of quickly colonizing new habitats. These life history and reproductive traits are reflected in the wide distribution of many of the species: 60% of the species are widespread, reported from multiple continents, and an additional 16% are well-known Asian global invaders. Only 15 % are limited to Asia, and 9% occur only on NE and NW Pacific shores. Based on published data alone, 49 of the 80 JTMD algal species (61%) were already present in the NE Pacific before the tsunami, but only 8 of these have been documented to be from earlier invasions. By using molecular methods, we have been able to more thoroughly characterize the species on JTMD and to compare their DNA sequences with populations of the same species in Asia, the NE Pacific, and other areas around the world. When subtle variations in the sequences occurred, we used these variations to determine the relationship of the JTMD species to other global populations, often shedding light on their possible origin. Our sequencing studies also enabled us to discover new cryptic species, genetically distinct but otherwise indistinguishable, in populations on both coasts. Our collections of material from Tōhoku coast of Japan and the shores of OR and WA greatly facilitated our comparative studies, and they also enabled us to survey for new introductions from JTMD in our NE Pacific study area. We have been particularly vigilant for 6 JTMD algal species that are on the Global and/or Mediterranean Worst Invasive Alien Species Lists (*Undaria pinnatifida*, *Codium fragile* subsp. *fragile*, *Grateloupia turuturu*, *Antithamnion nipponicum*, *Polysiphonia morrowii*, and *Desmarestia viridis*). Although our surveys continue, we have not, to date, found any new populations in the NE Pacific that are the result of invasions by the algae from JTMD.

4. PROJECT DESCRIPTION (for the entire 3-year ADRIFT project)

a) Research purpose

To complete a comprehensive taxonomic account of the marine algae arriving in Japanese Tsunami Marine Debris in Oregon and Washington and to evaluate the risk of these species invading Northeast Pacific shores.

Note: Our project on the algae of tsunami debris that I am covering in this report began on 5 June 2012, when the first JTMD item (the Misawa 1 Derelict Dock) landed on Agate Beach in Newport, Oregon. Oregon Sea Grant kindly provided me with 1 month of funding for my study identifying the algae on that dock and enabled me to subcontract with Prof. H. Kawai in Kobe for $1000 to sequence the problematic species. For the next 2 years (8/2012-8/2014), we continued to work on the algae of tsunami debris, but it was difficult because the project had to be funded through our personal savings. During this early time period, we completed 27 of the 42 debris items covered by the project. Then, on 1 August 2014, our luck changed! The MoE of Japan through PICES began funding the tsunami debris studies (PICES-ADRIFT, 2016). We received enough financial support from this program to carry us through until 31 March 2017. We are very grateful to both PICES and the MoE for this support.

b) Objectives

Carried across the North Pacific on currents from Japan, marine debris from the Great Tōhoku Tsunami of 2011 has been arriving on Oregon and Washington shores since June 5, 2012. Since the debris often arrives carrying a wide variety of healthy **Japanese marine algae**, we devised a 5-part project to monitor and evaluate the invasion threat of these species to NE Pacific shores. Our project has involved: (1) Identifying and characterizing the algal species found on JTMD (Japanese Tsunami Marine Debris), including their genetic structure, (2) Surveying sites along the shores of WA & OR for new invasions of these species, (3) Determining the percentage of JTMD species that already occur in the NE Pacific and mapping their distributions so that new invasions are not confused with earlier colonizations. (4) Using molecular sequencing to compare these pre-tsunami populations of JTMD species in the NE Pacific with the populations on debris, in Japan and elsewhere around the globe -- providing insight into the origin of earlier invasions, and lastly, (5) Preparing an illustrated identification guide to the JTMD algal species that are most likely to invade the NE Pacific. The Guide will be applicable to both professionals and the public so that they can detect new invasions and alert authorities so that control measures can be taken.

c) Methods

Part 1. Identifying and characterizing the JTMD algal species

A. Since the 2012 landing of the Agate Beach dock, a variety of state workers, volunteers, and scientists have helped us to collect algal samples for the project. Since the debris objects are not always found and collected when they first land on our shores, the biota brought to us can be either healthy or in various states of decay. If the material arrives alive (without preservative), processing must begin immediately since algae deteriorate rapidly.

B. Unique species in each collection are sorted under a dissecting microscope and prepared for study.

C. Vouchers of the species are made (via pressings and Karo-mounted microscope slides) and the material is preserved in (1) 5% formalin/seawater (for later anatomical study and photography), and (2) silica gel (for DNA analysis).

D. Using the available reference literature (Yoshida, 1998; Yoshida, Suzuki & Yoshinaga, 2015; and many more), preliminary morphological identifications are made in Oregon and the species are characterized. Since many of the species mimic one another in external appearance, the diagnostic identification features are most often anatomical and microscopic. Hence, for each sample, repetitive sectioning and microscopic observation is necessary to determine the species. During this process, I also:

1) Score the species for fertility (if they are actively reproductive and dropping spores)
2) Score for known features relevant to the potential spread of the species: longevity (ephemeral, annual, perennial) and successional stage (opportunistic, late successional)
3) When possible, score for the frequency and habitat of each species on the debris and determine any noticeable seasonality
4) Photograph unusual features under the microscope.

E. Final DNA identifications of the species are made in Kobe, Japan, via sequencing 1-3 genes in each species and comparing the DNA with the sequences stored in the International Nucleotide Sequence Database Collaboration (NCBI - GenBank), the DNA Data Bank of Japan (DDBJ), and with personal data. The list of genes used are provided in Appendix 3, the checklist of species.

Part 2. Surveying probable sites and habitats along the coast of WA & OR for new algal invasions.

A. During Year 1, 6 floating docks, 2 jetties, and 1 bay were each surveyed 2-3 times to search for the new recruitment of JTMD species.

B. Visual searches were carried out for the larger JTMD algal species and collections of both large and small species were made for later anatomical study and sequencing for the cross-Pacific study.

C. Since no new populations of JTMD algal species were discovered – this survey was only supported for the first year of the PICES-ADRIFT project.

Part 3. Determining the pre-tsunami distributions of JTMD algal species both globally and locally

A. We used www.algae-base.org to determine the published world-wide distribution of the JTMD species, including the general extent of their ranges (widespread, North Pacific, or Asian).

B. To gather local distributions, we used public and private herbarium databases, checklists compiled by state and national surveys, and also our own collection data from PICES-ADRIFT Year 1.

C. At the end of Year 1, we produced a map of the known occurrence of pre-tsunami JTMD species in OR & WA (see our Results) and used this data to determine the probable habitats of new invasions.

Part 4*. Examining the genetic relationship between the algal species on JTMD with populations of the same species in Asia and in the NE Pacific before the tsunami.

A. Extensive genetic comparisons were made between the algae on JTMD and the native populations of these same species in Japan and in the NE Pacific – and elsewhere around the world if the sequences were available in the DNA Data Bank of Japan or NCBI-GenBank.

B. Collections for these studies were scraped from the substratum, separated into species, cleaned under a microscope, and then placed into silica gel in labeled ziplock bags for mailing to the Kawai laboratory in Kobe, Japan, where the sequencing took place.

C. Collection sites for this study included: (1) any JTMD with algae, (2) sites in my surveys along the OR and WA coast (2014-2015), (3) the WA & OR collecting sites with Kawai (2015) and Hanyuda (2016) mainly to Grays Harbor, WA, and (4) three sites along the Tōhoku coast of Japan (2016).

D. *Methodology and results for this cross-Pacific study of the JTMD algal populations will be provided in the PICES report of my colleagues, Kawai and Hanyuda.

Part. 5*. Preparing an Internet and Hard-Copy Account of the Algae on Japanese Tsunami Marine Debris.

A. Throughout the debris project, photographs of the collection material were taken by GIH.

B. Composite plates of the pictures were prepared primarily to facilitate the molecular confirmation by Kawai and Hanyuda of my morphological identifications. Unfortunately, many of the debris algal pictures were of poor quality and not suitable for publication. This was unavoidable since many of the debris samples were partially rotten – provided to our project often weeks after collection.

C. For some species, better photographs were obtained from our field collections in OR, WA & Japan.

D. By selecting our best pictures and by working together, we plan to produce a hard-copy illustrated pamphlet on the most invasive debris species, describing their appearance in the field, overall morphology, and their diagnostic anatomical features. We will include information on their occurrence and seasonality in Japan and on debris and also their invasion history around the world. A publisher has already come forward to produce this volume without cost to PICES – although we are still negotiating the details.

E. A blog-style internet account of the debris species will also be prepared – using the lower quality images and discussing the taxonomy. Since a number of the debris species are difficult to identify and have never been photographed, this will provide an important contribution that other scientists can use. Scholar's Archive at Oregon State University has offered to post this part of the project – after 31 March 2017, at the end of our PICES-MoE study.

***** Although the preparation for these accounts is well in progress, we will not report in detail on Parts 4 and 5 of our project here. For Part 4D, Kawai and Hanyuda will present the molecular methods and detailed results in their independent final report to PICES-ADRIFT. For Part 5, we will begin designing and preparing our Guide after our final reports and manuscripts have been submitted to PICES-ADRIFT.

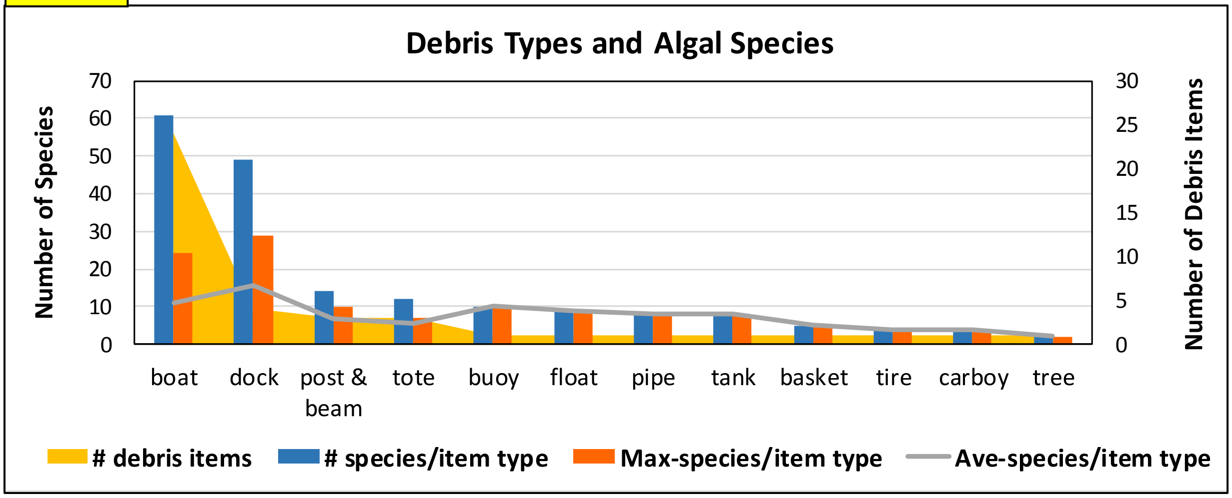
d) Results

To provide the complete story, the results for our entire study of the algae on tsunami debris are presented here, including the processing, identifying and characterizing the algae on JTMD starting with the Agate Beach Dock collected on 5 June 2012 and ending with the Falcon Cove Boat collected on 26 July 2016.

Debris landings along the OR & WA coast. From June 2012 to July 2016, many hundreds of debris items of assorted sizes and shapes came ashore on the OR and WA coast. 42 of the larger debris items carried substantial algal populations and these became available to our project for the sampling, enumerating, identifying and characterizing of the algal species. All 42 were documented by Jim Carlton as being from the Japanese tsunami and each was provided with a JTMD-BF number (see Carlton et al., 2016 personal communication). Appendix 1 provides a checklist of these items include their BF number, the debris location and type, the date of collection and the total species each item contained. In addition, 28 smaller plastic debris items, too small to be provided with BF numbers and not documentable as from the tsunami, were also collected and processed for our *Tsunamia transpacifica* sp. nov. study and publication. Appendix 2 provides a list of these items, their collection data, and also the genes used to determine the new species. The final checklist of species, provided in Appendix 3, includes algae only from the BF numbered items to be certain that all species were either from the tsunami or from items associated with the tsunami. In total, 80 marine algal species and species complexes were identified from Japanese Tsunami Marine Debris and 51 species were sequenced. These species, their taxonomic groupings and genes sequenced are all listed in this appendix.

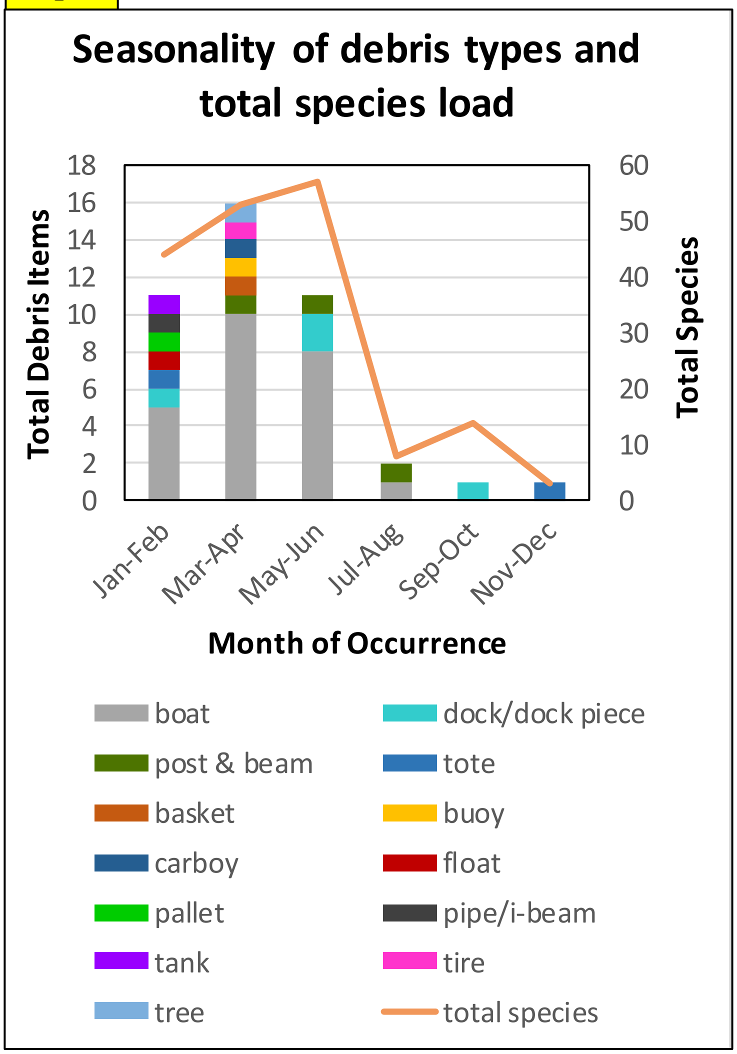
Debris types and their algal species load. The 42 BF-debris items examined for the algal project included 12 different types of debris. In order to determine the importance of each debris type in the transport of species, the algal species on each type were enumerated (Fig. 1). The greatest number of species (29) was found on the Agate Beach Dock, the first debris item to arrive in Oregon. Although there were only 4 docks or dock pieces, they averaged nearly 16 algal species each, more than on any other debris type. On all 4, a total of 49 species occurred. Derelict boats were the most common type of debris in the study and I examined 24 of these. The boats averaged only 11 species/item but in total they brought in 61 species, more than any other category of debris. The species totals on the other debris types were comparatively small. Although these counts are heavily influenced by the debris available to our

Fig. 1



project, Kawai *et al*. (PICES, 2016) correctly pointed out that the docks are one of the few types of debris that we can be certain were already in the ocean before the tsunami. It could be that many of the other debris items were colonized by algae only after the tsunami dragged them into the sea. If this were the case, they would need to be colonized by the algal spore clouds and fragments that occur in the nearshore or later by the spores and fragments that are able to jump between debris items in close proximity at sea.

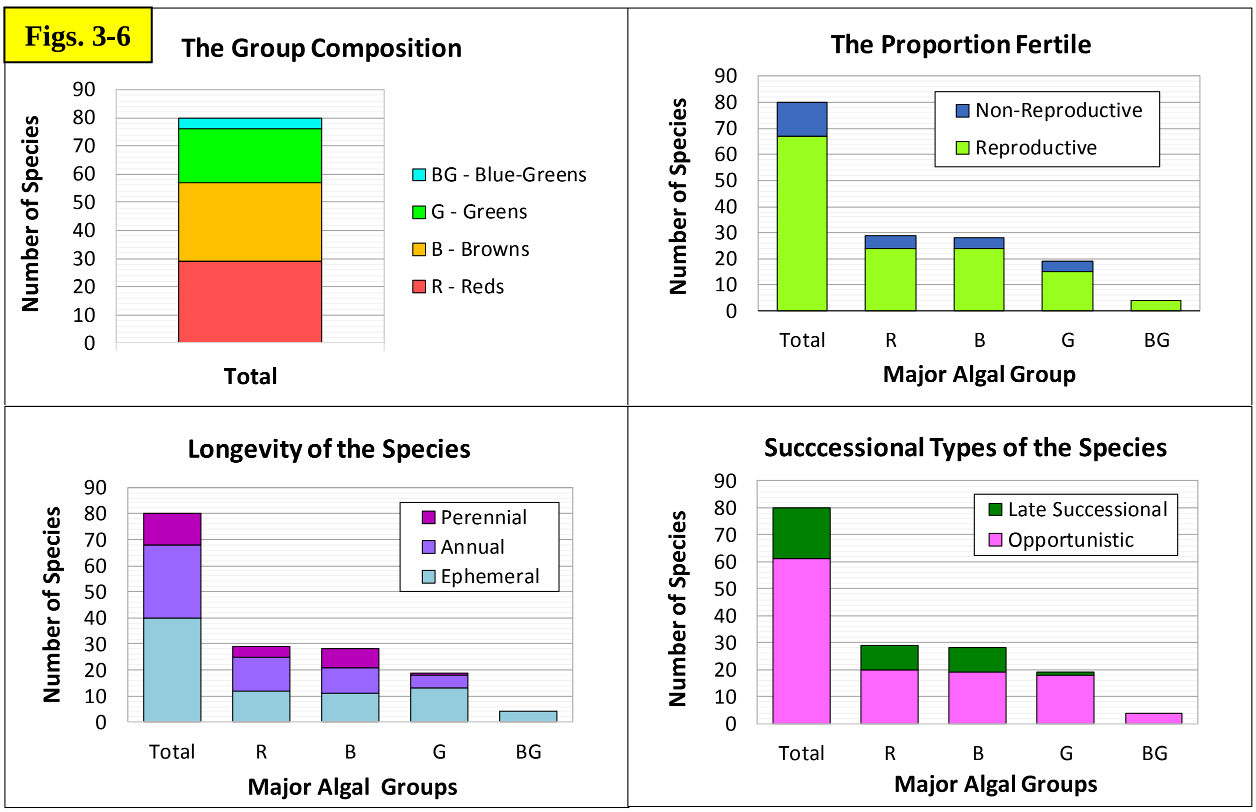
Fig. 2



The seasonality of the debris and its attached species in OR & WA. The arrival of tsunami debris along the OR & WA coast was somewhat seasonal (Fig. 2). This has been reported to be due partially to upwelling in the nearshore from April to August preventing debris from accessing the beaches. The seasonality we observed for our items was different. Most of the debris we obtained arrived between January and June and then very few items came ashore between July and December. The number of debris items peaked (at 16) in March-April when 10 of the 24 debris boats and numerous other items arrived. However, the greatest number of species/month (57) peaked later in May-June when 8 boats and the Agate Beach Dock arrived. The increase in species numbers from January through June undoubtedly relates to the number and type of debris items, but it is also influenced by the increasing daylength during this time of year. The crash in species numbers from July through December is an obvious result of the few debris items present, but there may be another cause as well. The water temperature along the Tōhoku coast of Japan ranges from is 8-22°C. On 11 March 2011 when the tsunami took place, the water temperature was about 8°C, the winter-spring low. The spring bloom of algal species would have just started and the species numbers would increase rapidly until June. Then the water temperatures climb rapidly, reaching up to 22°C in the summers. This high summer water temperature causes many of the algal populations to crash and remain in a microscopic dormant state until the water temperatures are more acceptable for growth. Some of the JTMD species could still be entrained in this seasonal cycle. Along the Oregon coast, the water temperatures range from 9-11°C and the species numbers remain high throughout the summers.

The basic composition of the debris species. A wide variety of algal species have arrived on JTMD over the 4+ years of this study. A total of 80 macroalgal species have been identified from the debris, and these consist of 36% (29) red algae, 35% (28) brown algae, 24% (19) green algae and 5% (4) bluegreen bacteria (Fig. 3). The proportion of red to brown algae (R/B), the Feldmann Index (Cormaci, 2008), is often used to evaluate the floristic affinity of samples. On the debris, this proportion is unusually low. For the Pacific coast of Japan (Tittley, 2002) and the BC-OR coast (Gabrielson, et al., 2012), the Feldmann Index is reported to be 2.7. On JTMD, it is only 1.04 – a ratio closer to that found in Arctic regions. The significance of this is not understood, but it seems likely that this ratio is not only affected by water temperature but that it can also be influenced by the unusual environmental conditions that occur around floating debris.

Figs. 3-6

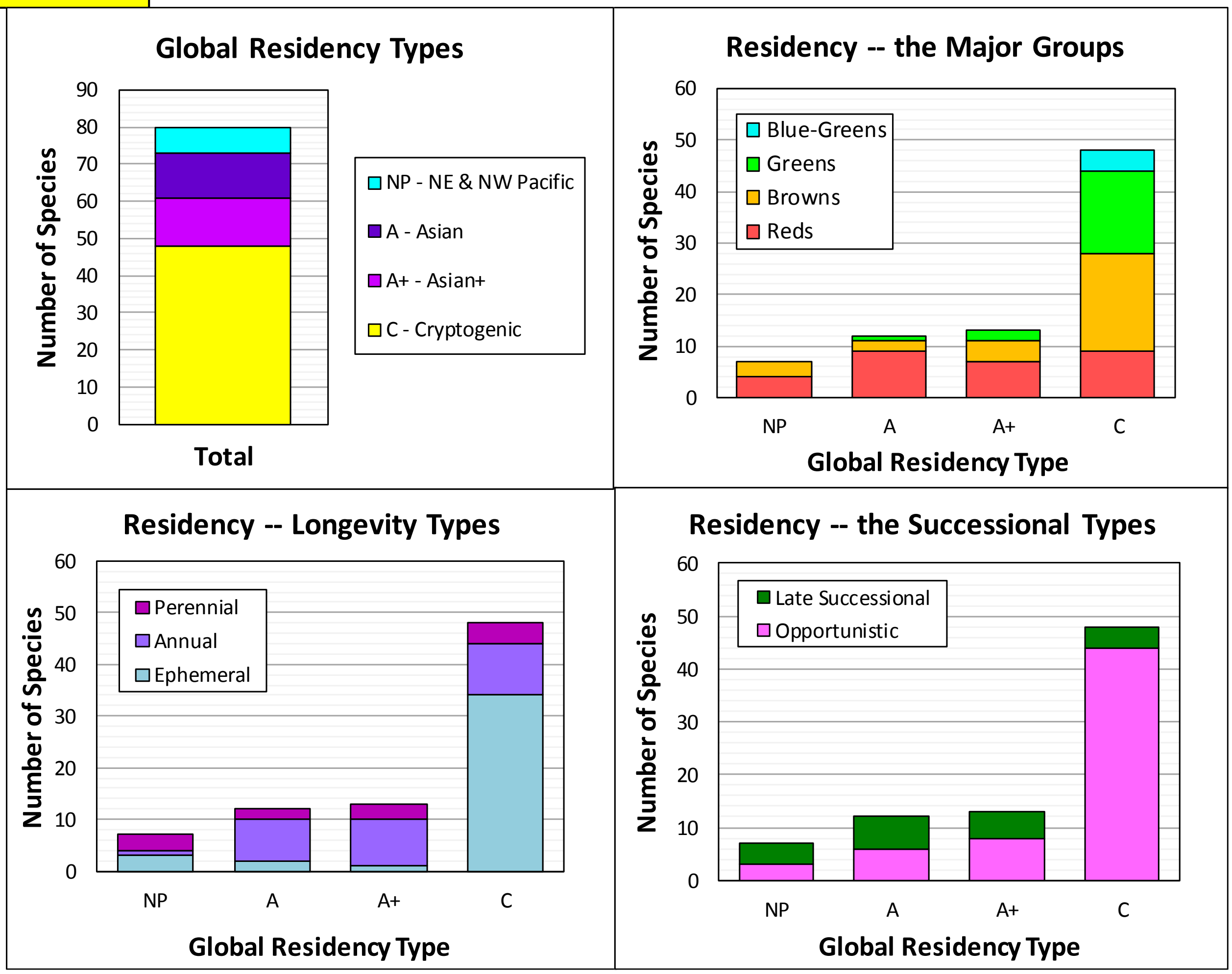


Basic life history features of the JTMD algal species. In order to evaluate the potential risk of invasion of the JTMD species in the NE Pacific, I also scored each of the species for life history features that I thought might lead to their wider dispersal and spread on arrival. These included: (a) their reproductive state, (b) their longevity type (ephemeral, annual, or perennial), and (c) their typical successional stage in the field (opportunistic early colonizers or late successional types). These features, summarized in Figs. 4-6, are quite revealing. By far the majority of the JTMD algal species (84%) were actively reproductive (Fig. 4). Most were producing gametes or spores (*Ectocarpus, Undaria, Polysiphonia, Ulva*), but some of the species were instead developing asexual propagules or fragmenting (*Sphacelaria, Codium, Scytonematopsis*). The data on the longevity types (Fig. 5) showed that a large proportion of the species were ephemerals (50%), short-lived species that are capable of reproducing repeatedly and recycling themselves throughout the year whenever the conditions are appropriate. A moderate number were annuals (35%), species that last for up to 1 year, and only a few (15%) were perennials, species that live for more than 1 year. With the perennials, I also included the pseudo-perennials, space-holding species (like true perennials) that overwinter as a small basal fraction (often a crust) of their complete thallus that regenerate in the spring. On debris, except for the crusts, none of the original thalli of any of the perennials survived beyond the first year of our study. A tabulation of the successional stage types (Fig. 6), revealed that late successional forms, like perennials, are only a minor component of the debris flora, comprising only 24% of the species. The opportunistic species, well-known for their ability to quickly colonize barren areas such as those found on debris, were far more abundant, composing 76% of the debris species.

The global occurrence of the debris species. Another feature that helps to reveal the invasive qualities of the debris species is their global distribution. Are they widespread around the world? Are they already on NE Pacific shores? Or are they normally only in Asia? To explore this feature, I grouped the species into 4 different categories of world distribution, using the published data listed in AlgaeBase (http://www.algaebase.org). My 4 categories were: C, NP, A+, and A. The C or Cryptogenic category included those widespread species with unknown origins that generally occur in different oceans and on multiple continents. The NP or North Pacific category included those species that are known only from both sides of the North Pacific -- and perhaps also Alaska. There were two A categories: the A species were those that were limited only to Asia and the A+ species were those Asian species that are also documented to be introduced and often invasive in other areas around the world.

Using these categories, the global distribution of the JTMD algal species is summarized in Figs. 7-10. It shows that that 60% (48) of the species are cryptogenic. This category contains a large proportion of ephemeral (71%) and/or opportunistic species (92%) including all of the bluegreens and most of the green algae. These highly reproductive species often foul boats and become widespread, justifying their inclusion in this category. The North Pacific group is limited to 9% (7) of the species and the Asian only group to only 15% (12) of the species. Their limited ranges possibly relate to their methods of dispersal. The Asian+ species, probably the best known of the JTMD species, include only 16% (13) of the total JTMD species (Table 2). These species have been well-studied and many are aggressive invaders around the world. Their global distributions have been documented through sequencing. Of the 13 species on debris, 8 are already known to occur on NE Pacific shores. All of the 8 are known from California, but 2 have also been found in Oregon and/or Washington. Many of the A+ species resident in the NE Pacific and elsewhere around the world are thought to have been introduced with aquaculture species (Miller, Aguilar-Rosas, and Pedroche, 2011).

Figs. 7-10



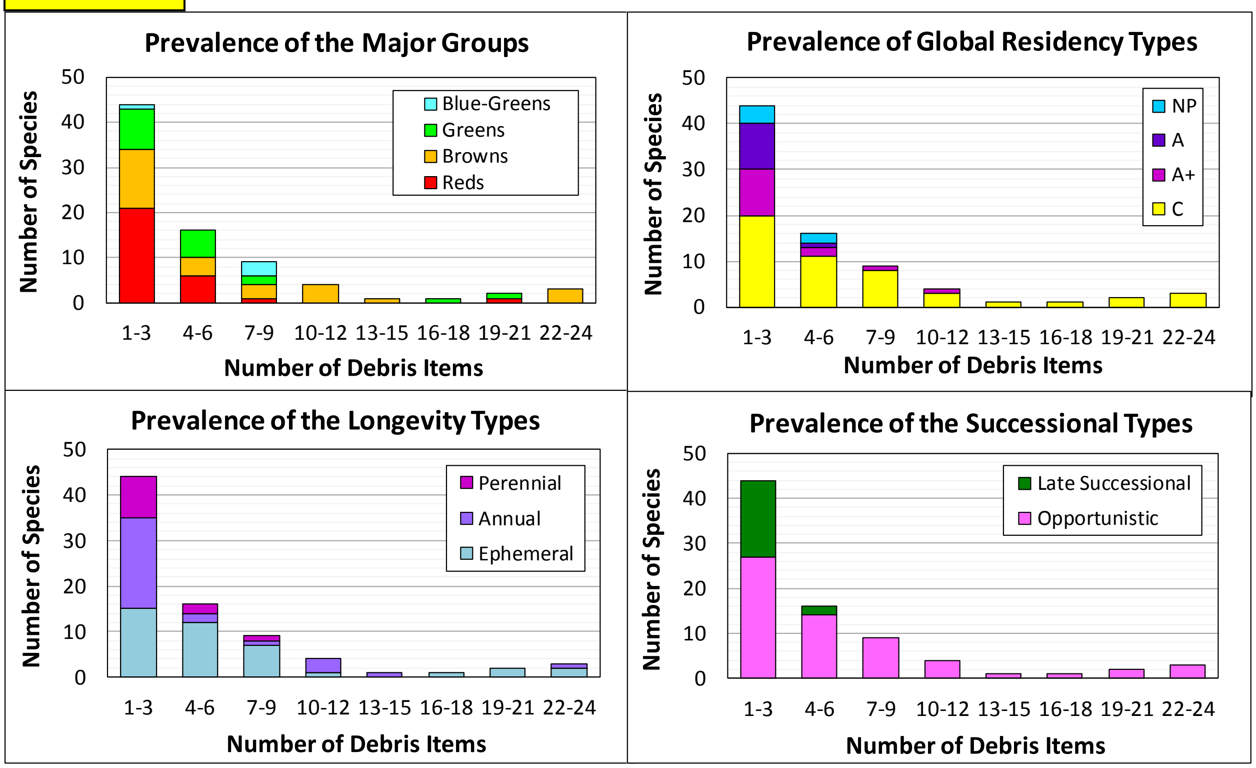
Group	Name	NE Pacific Records	Group	Name	NE Pacific Records
B	*Mutimo cylindricus*	CA	R	*Chondrus giganteus*	
B	*Saccharina japonica*		R	*Grateloupia turuturu*	CA
B	*Scytosiphon gracilis*	CA	R	*Neosiphonia japonica*	
B	*Undaria pinnatifida*	CA	R	*Polysiphonia morrowii*	
G	*Codium fragile* subsp. *fragile*	CA, WA	R	*Pyropia yezoensis*	
G	*Ulva australis*	CA, OR, WA	R	*Schizymenia dubyi*	CA
R	*Antithamnion nipponicum*	CA			

Table 2. The A+ species on Japanese Tsunami Marine Debris and their occurrence in the NE Pacific.

The basic characteristics, invasive traits, and global distribution of the JTMD species described above provide the opportunity for us to analyze more closely some of the other features of the algae on debris.

The prevalence of the algal species on debris. How widespread are the algae on debris? Are all of the species on all of the items or are they more narrowly distributed? This feature may reveal which of the debris species are common fouling species in Japan, or perhaps which species are able to jump between neighboring debris items while at sea, increasing their prevalence. The results of this study are shown in Figs. 11-14. On the 42 debris items sampled, the most widespread species were *Petalonia fascia* and *Feldmannia mitchelliae*, each found on 24 debris items. Both produce high quantities of spores and spread easily. Only 7 species in total occurred on more than 12 debris items. By far the majority (55% or 44 species) were limited to only 1-3 debris items. Of these narrow range species, nearly half (48%) were red algae. The invasive traits of these species are shown in the prevalence graphs below, and, not surprisingly, the most widespread species are primarily cryptogenic, ephemeral, and opportunistic forms.

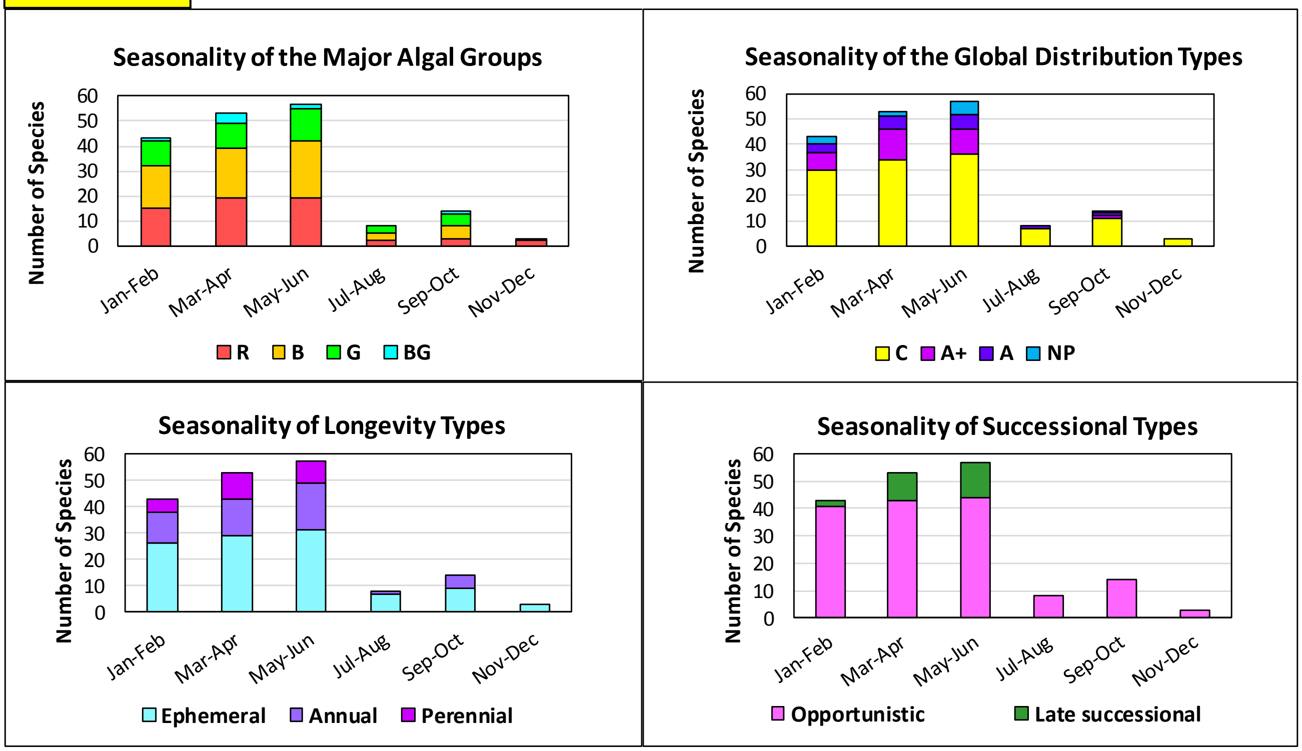
Figs. 11-14



Note: the most widespread species were *Petalonia fascia* and *Feldmannia mitchelliae* (both on 24 debris items), *Ectocarpus commensalis* (22), *Ulva compressa* and *Colaconema daviesii* (20), *Ulva linza* (17), and *Punctaria latifolia* (14).

Additional data on the seasonality of the species on debris. Although the seasonality of debris is discussed earlier, the features introduced above enable us to more finely dissect the kinds of JTMD algal species found on debris during the different months. These details, shown in Figs. 15-18, reveal that, during January to June when the debris quantities and species are abundant, the proportional composition of the species appears to be quite typical of debris. However, during July to December, when there is a dearth of debris items and species, the major survivors are primarily cryptogenic, ephemeral and opportunistic species, and the perennial and late successional forms are not present. Some annuals, such as *Ceramium cimbricum* and *Punctaria latifolia*, that commonly occur in the early months also occurred in Sep-Oct. This bi-annual occurrence of true annuals is not uncommon in temperate locations.

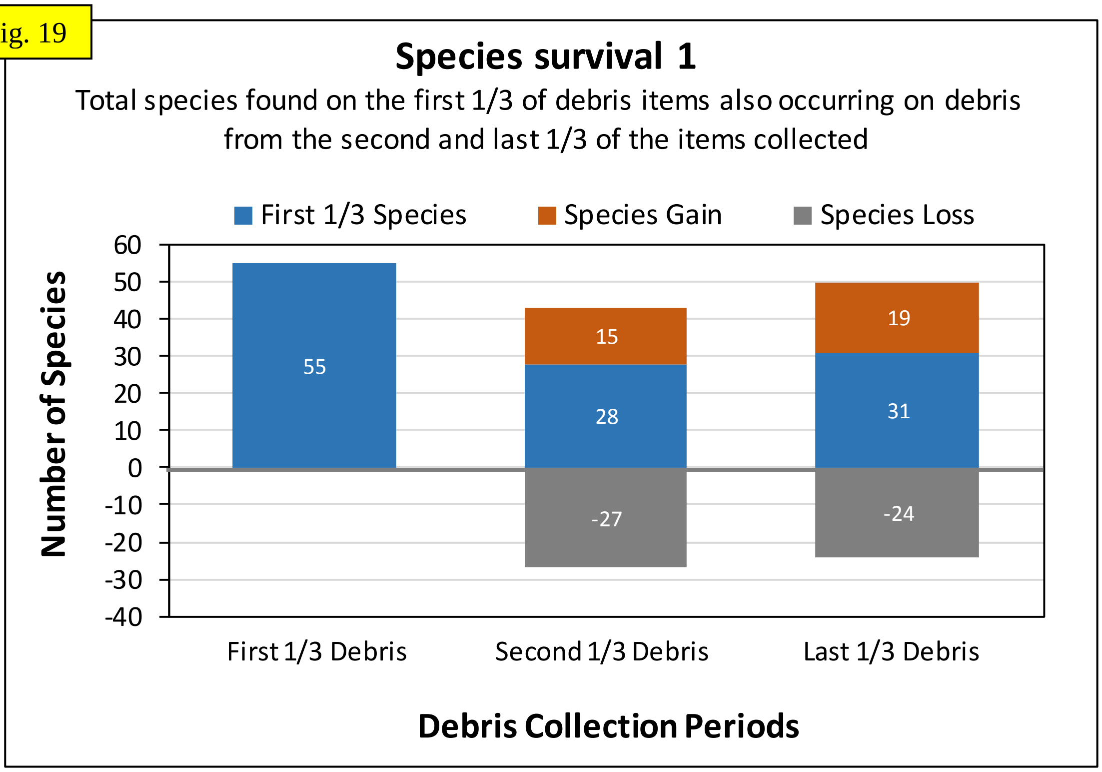
Figs. 15-18



Survival of the debris algal species over long periods of time while at sea. The risk of JTMD species colonizing our NE Pacific shores is also heavily influenced by the differential survival of the algal species at sea. Which of the Japanese species are able to survive the long journey across the North Pacific to North America? The algal species reaching our NE Pacific shores during this study survived at sea for a minimum of 15 months (>1 year, on the Agate Beach Dock of June 2012) and some survived for up to 64 months (>5 years, on the Falcon Cove Boat of July 2016). Others will perhaps last even longer on debris items that we have not yet found. For the surviving species, each must have had its basic requirements met during the journey. There would have been a suitable substrate for attachment, appropriate submergence, adequate light and temperature, sufficient nutrients, minimal herbivory & also the correct conditions for reproduction and recruitment. However, many of the species did not survive the full 5 years. This was due partly to stresses at sea, but also to the variable life history features of the species. To study this more closely, I conducted 2 survivorship investigations.

For the first, I divided the overall study into 3 parts of 14 debris items each -- all arranged by the time of collection (#1, Jun-2012–May-2013; #2, Jul-2013–Nov-2014; #3, Jan 2015-Jul-2016). Then I compared the species collected during the first 1/3 of the study with those species found during the second and third parts of the study. The results are shown in Fig. 19. It is evident that many of the original species disappeared but also that many new species were found during the second and final thirds of the debris study. Of the 55 species originally observed, only 28 occurred in the second 1/3 and 31 in the last 1/3 of the collections. Up to 19 new species were found. These differences reflect the type of study we had to conduct. This could not be a true time-series of the species on debris, because we could not enumerate the species on any of the items at the beginning of their journey. We could only tabulate those that survived. Since each debris item was independent of the rest, species were often not encountered until late in the study. In addition to their different times at sea, each item (with few exceptions) originated from a different area, undoubtedly contained a different number of species, and perhaps traveled a slightly different course across the Pacific.

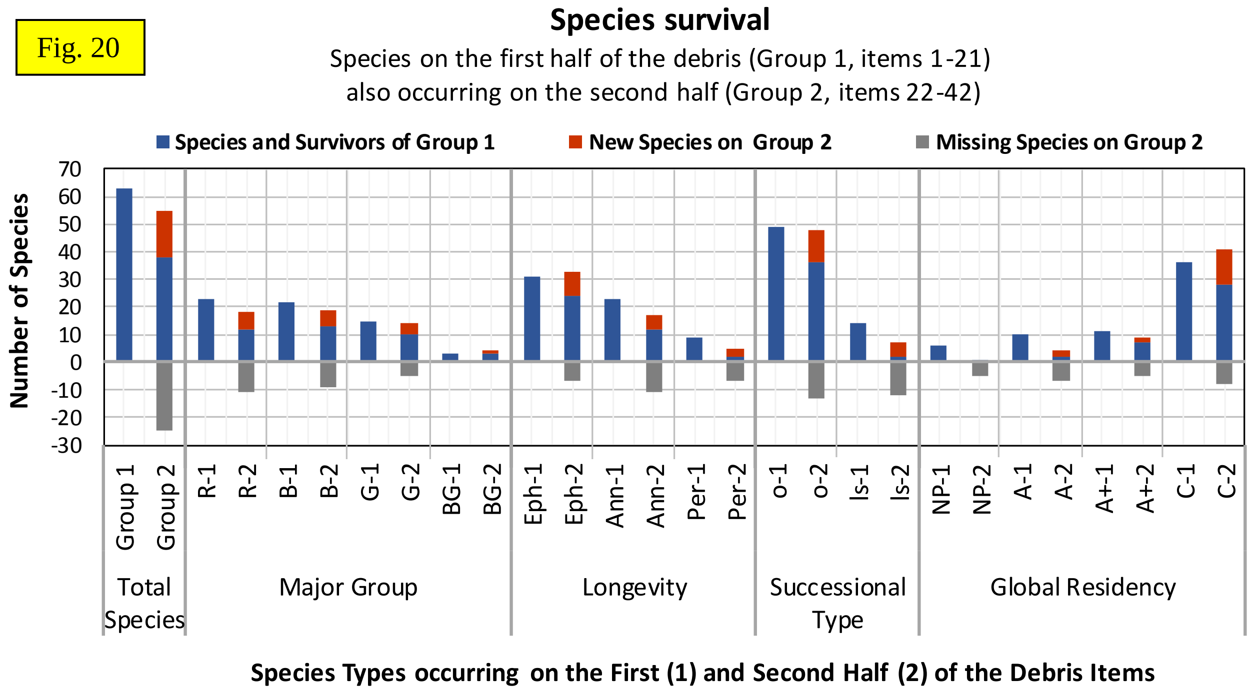
Fig. 19



For the second investigation, I divided the collections into 2 halves each containing 21 debris items. (#1, Jun-2012-Apr-2014; #2, May-2014-Jul-2016). This time I also tabulated the life history and distribution features of the species to see if there might be a difference in these features over time. The first half of the debris items were found to contain 63 species while the last half contained only 55 species. However, only 38 of the original species were present on the last half of the debris items. 17 new species were found and 25 species were lost. Fig 20 reveals the changes in species counts for the various features between the first and last halves of the study. Although this is also not a true time-series, the loss of species between the two halves does appear to reflect some valid differences in survival of the species at sea. However, the increase of species appears to be simply the new observation of survivors overlooked earlier due to the lack of appropriate debris. Within the taxonomic groups, the red algae decreased the most with a loss of 11 species; some of the missing species included the larger red algae, *Palmaria mollis*, *Neodilsea yendoana* and *Ptilota filicina*. The brown algae suffered a loss of 9 species, including all of the larger kelps and *Desmarestia* spp., and the green algae retained nearly the same count but lost the feathery *Bryopsis* species. The longevity comparison revealed that the ephemerals and annuals gained in numbers, but that many of the perennials disappeared. The perennials that did survive into the last half of the collections were mainly crusts: *Pseudolithoderma* and *Petroderma* and 3 pseudo-perennials that overwinter as a crust or turf-like stage (*Sphacelaria rigidula*, *Chondrus giganteus*, and *Codium fragile*

fragile). The crustose shape possibly facilitates their survival in adverse conditions. Opportunistic species remained dominant on debris but also lost and gained species. As might be anticipated, the

Fig. 20



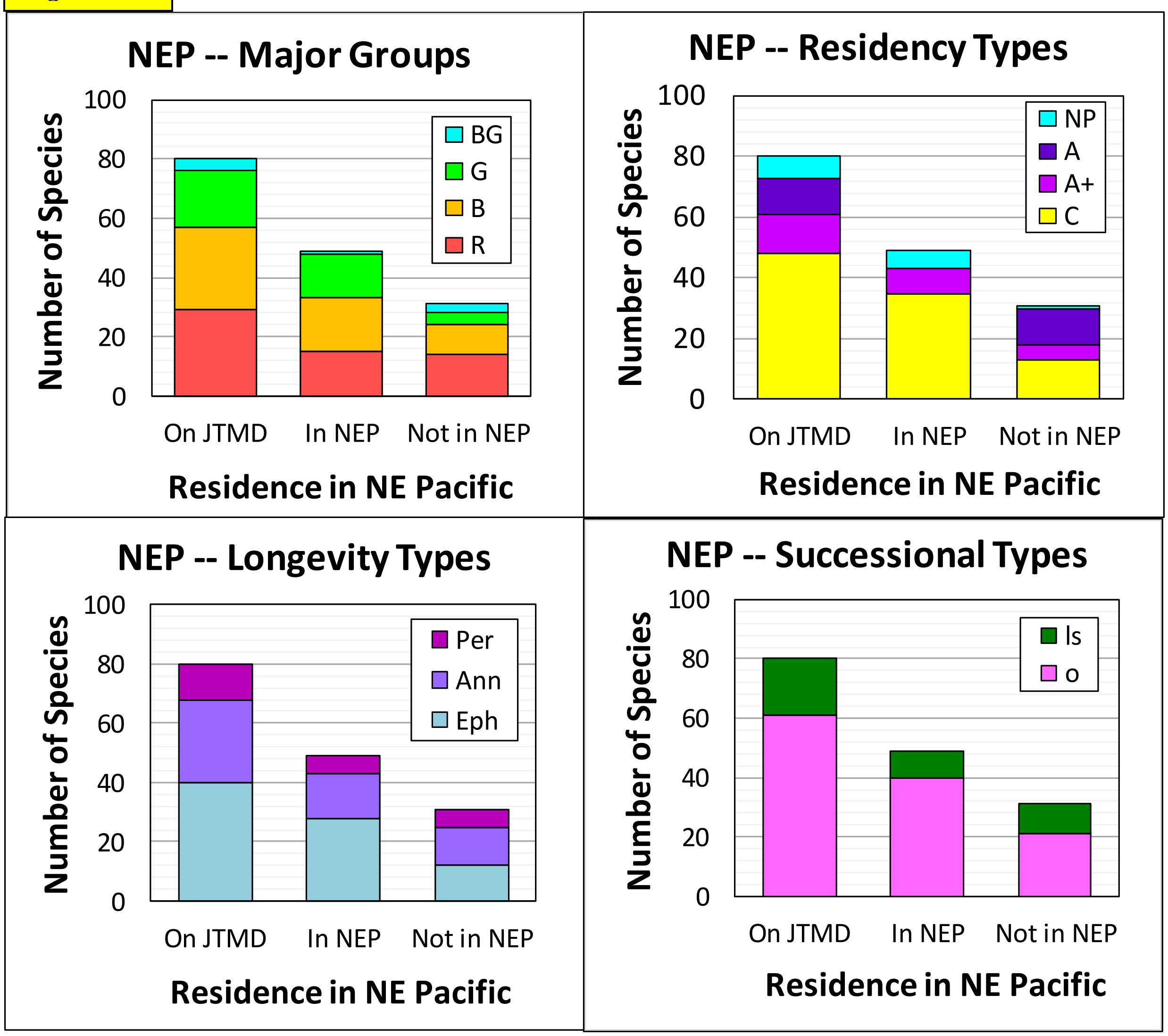
widespread cryptogenic and Asian+ species remained fairly abundant on the latter half of the debris items, but most of the more narrowly distributed North Pacific and Asian species were no longer present.

Some survival observations could be explained through a basic knowledge of the species. *Saccharina japonica*, the largest kelp on debris is a biennial, surviving only 2 years. On the Agate Beach Dock (1 year after the tsunami), it was very abundant, the blades were 4-5 feet long, and it appeared to be 1-2 years old. By our year 2 of collecting (2 years after the tsunami), no large thalli were present, and I could find only a few small blades, apparently the first-year progeny that had seeded at sea. After that, no further plants were observed. Many of the other kelps and large algae were annuals, surviving only until our first year of observation -- they apparently did not reseed as they were not seen again during the following years. The most successful survivors were the ephemerals and opportunistic species. The lifespan of most of these species is relatively short, and to survive on debris, the species recycle themselves through sexual or asexual reproduction many times during each year and then recolonize the debris, often in greater abundance than before. The ephemerals and opportunistic species that I observed on debris were nearly always fertile and very widespread on debris. These species groups, indeed the best suited for a long survival on debris, are also well equipped for quickly colonizing and invading any new habitats that they encounter.

The NE Pacific occurrence of native "conspecific" JTMD algae. In order to understand the risk of invasion of the JTMD species in the NE Pacific, another feature is extremely important. It is critical to know the distribution of any JTMD species that have occurred in the NE Pacific before the tsunami took place. We often refer to these populations of the same species in different areas as "conspecific" populations. Although they are part of the same species (hence, "con" specific), they may not be precisely identical genetically. We will examine the genetics of the species further in the next section, but the distributions discussed here are based on the known records of conspecific populations of these species in the NE Pacific (WA, OR, & CA). It is particularly essential to document and map these conspecific JTMD species is so that we don't confuse the native populations with new invasions from actual debris. For this part of the study, I obtained my distribution information from a variety of sources: Algaebase (http://www.algaebase.org) , the Macroalgal Herbarium Portal (http://macroalgae.org/portal), herbarium databases and specimens at UW, UBC, UW, OSU, and UCB) (http://www.pnwherbaria.org; http://www.biodiversity.ubc.ca/museum/herbarium/database.htm; and https://webapps.cspace.berkeley.edu/ucjeps/publicsearch/publicsearch/) and also from my private herbarium of the algae of the outer coast of WA and OR. I also incorporated into the study the recent collections made with Kawai and Hanyuda during their visits to OR & WA that were supported by this project. Our PICES-funded collections from OR & WA and the Tōhoku coast of Japan have also been used for the cross-Pacific sequencing study of Kawai and Hanyuda, described in Part 4 of this project and summarized in the next section.

The results from the NE Pacific study are shown in Figs. 21-24 and also in Fig. 25. Of the 80 algal species found on JTMD during this study, 61% (49) had already been reported to occur in the NE Pacific before the tsunami, and only 39% (31) were not yet present. The species that were present in the NE Pacific included a high proportion of the JTMD green algae (79% of the total greens on JTMD) including many of the fouling Ulva spp. As might be expected, the JTMD species already resident in the NE

Figs. 21-24



Pacific composed a prominent part of the most invasive species on JTMD: 73% of the cryptogenic species, 70% of the ephemeral species, and 66% of the opportunistic species were already present in the NE Pacific. Many of the species with a high tendency to spread were already here. Although differences in the proportions of species already resident and not resident in the NE Pacific occurred, a surprisingly high number of species in all of the categories still remain to be introduced and are a substantial invasion threat to the NE Pacific (Appendix 4).

As a part of this study on conspecific populations of the JTMD species already occurring in the NE Pacific, I prepared the chart and map in Fig. 25 A & B in 2015. It was important to know where these populations occurred so that we could collect the species for our cross-Pacific comparative sequencing study. Although the chart and map include only a limited amount of data and do not include our more recent collections, they do reveal that the highest counts of "conspecific" JTMD species occur in sheltered bays and not on open headlands. This indicated to us that many JTMD species are likely to be quiet water forms and that sheltered areas such as bays are the most likely places to find the new introductions. The oyster cultivation bays at Grays Harbor and Willapa Bay had by far the greatest number of JTMD conspecific species – so we targeted these for our later collections of comparative DNA material.

Fig. 25A

The occurrence of JTMD algal species along the outer coast of OR and WA in 2015

Fig. 25B **The Known 2015 Occurrence of JTMD Algal Species on the Outer Coast of WA & OR before the tsunami**

Site	Pressings	Species	JTMD	% JTMD	Notes
Olympic National Park – Pacific coast	list	200	13	6.5	exposed
Grays Harbor & Jetty	278	106	18	17	oyster
Willapa Bay	589	84	26	31	oyster
Netarts Bay	93	55	9	16	oyster
Boiler Bay	916	232	15	6.5	exposed
Yaquina Bay	401	98	16	16	oyster
Coos Bay	484	102	14	14	logs
Cape Blanco	222	62	2	3.2	exposed

Interestingly, after our first year of collecting the algae on JTMD, most of the debris species were also found to be quiet water forms. For example, many of the brown algae were the small filamentous forms typical of those found on protected docks while the larger brown kelps, more typical of exposed coasts, disappeared after the first year of sampling. Perhaps the chemistry and currents of water in the boundary layer surrounding floating debris mimics in some ways the water in more sheltered locations.

Genetic studies of the Cross-Pacific shared "species". Populations of 61% (49) of the algal species found on JTMD are known to have been present in the NE Pacific before the tsunami took place. Where did these shared species come from? With the long history of drifting materials being carried from Japan by the North Pacific Current to America, it seems logical to assume that these species all originated from populations in Japan. For some species, this is true, but recent molecular investigations by my collaborators in Japan have shown that this is not always the case. By comparing the DNA sequences of populations of those species shared between debris, Japan, and the NE Pacific, they have been able to use the data from a number of species to determine if the source of our NE Pacific populations is debris, Japan, or elsewhere around the world. The requirement for this determination is that the sequences or haplotypes within an individual species vary slightly – a feature not uncommon in many species. By mapping the distribution of the different haplotypes, the historical exchanges of the species and its haplotypes between different geographic areas can be illustrated. For nearly 10 of the JTMD shared species, they have discovered that the NE Pacific populations originated from areas other than Japan. How these exchanges took place is often not well understood and we are just beginning to speculate on the vectors involved. Kawai and Hanyuda in their final report to PICES-ADRIFT will provide the details of this very important and insightful study.

e) Summary and Discussion

Using both morphological and molecular methods, our study identified and characterized 80 marine algal species that were found on 42 Japanese Tsunami Debris items that landed on OR & WA shores from 5 June 2012 (the Agate Beach Dock) to 26 July 2016 (the Falcon Cove boat).

Seasonality of the debris items and the species load. The debris landings occurred mainly from January through June of each year, and almost no debris could be found between July and December. Although a wide variety of debris items washed in, the greatest diversity of algal species occurred on the larger and more abundant items: 4 floating docks and their fragments supported a total of 49 species and 24 derelict boats carried in 61 algal species. Each item contained a diverse and often unique array of species. Only a few species were widespread*:* *Petalonia fascia* and *Feldmannia mitchelliae* each occurred on 24 of the 42 debris items. Most of the algal species (55%) were limited to only 1-3 debris items.

Features influencing the risk of invasion. In order to characterize the species for features that might be important to invasion risk, I scored each of the 80 species for their: (1) fertility, (2) longevity (ephemeral, annual, or perennial), (3) successional stage (opportunistic or late successional), and (4) published global distribution (cryptogenic, North Pacific, Asian (limited to Asia), or Asian+ (known global invaders)). These features were particularly informative.

(1) Fertility. The species arriving on NE Pacific shores were amazingly fertile – 84% of the species were actively producing spores, gametes, or asexual propagules. One environmental feature causing this might be the increase in nutrients as they arrived in the nearshore compared to the oligotrophic conditions at sea, but, since we did not sample the items midway, we could not be certain. Based on their survival success alone, many of the debris species did remain fertile throughout the trip, enabling them to recycle their populations and apparently (for the species widespread on debris) to also use their propagules to jump between any debris items in close proximity. The high proportion of species that were fertile on arrival does indicate a high invasion risk. Recruitment of these species is possible if the right conditions for growth of the spores or propagules are present (primarily temperature, nutrients, light, and available substratum). Sea water temperatures along the WA and OR coast (9-12° C) seem adequate as they are well within the range found along the Tōhoku coast of Japan (7-23° C). Since temperature and light are sufficient for the growth of Oregon species, it is likely that they are also sufficient for the growth of many of the debris species. However, one main obstacle may have impeded recruitment. Debris items along the OR & WA coast nearly always land on sandy beaches where hard substratum necessary for the recruitment of algal spores is not available. Since most algal spores and propagules are short-lived and cannot travel more than a few meters, this would prevent most recruitment. This precise feature (hard substratum vs. sand) has now been used by the State of Oregon (Office of Emergency Planning, 2012) to rank the invasion risk of various areas along our coast and use this feature to justify the costly removal of large debris items from our beaches.

(2 & 3) Longevity and successional stage. The longevity and successional stage features were also very revealing. The debris species were found to include 50% ephemerals, 32.5% annuals and 17.5% perennials (& pseudo-perennials) and also 76% opportunistic and 24% late successional types. Since ephemerals and opportunistic species can repeatedly reproduce and colonize new areas, their high incidence helped to explain the abundance of these species on debris. To determine how these features might affect their actual long-term survival on debris, I divided the debris collections into 2 halves and compared the occurrence of the species on the last half with those on the first, anticipating that this would be a good proxy for a survival study. As might be expected, the ephemeral and opportunistic species survived well, sometimes actually increasing in numbers. However, only a few of the perennials and pseudo-perennials (mostly crustose forms) were present in the latter half of the samples, undoubtedly attesting to the impact of the harsh conditions at sea on these normally-space-holding species.

(4) Global occurrence. Another important feature that influences the invasion risk of the debris species is their global occurrence. This feature is affected heavily by both the longevity and successional stage characteristics of the species. The 80 debris species were found to include mainly the widespread cryptogenic species (60%); these (cryptogenic species) consisted primarily of ephemeral (71%) and opportunistic (92%) species. The high level of reproduction and recruitment in these species apparently also leads to their widespread global distribution. The NP species (9%) and the Asian-only species (15%) were the least common on debris; their limited distributions both on debris and worldwide may reflect a lower fecundity of these species. The Asian+ species included 13 species or 16% of the total: most were red algae (7 species), a surprisingly high percentage were annuals (9 species), a few (3) were perennials, and only 1 was ephemeral. Although some (8) are opportunistic, a substantial number (5) are late successional forms. These well-studied A+ species, shown through sequencing to originate in Asia and be widespread around the world, are not dominated by the invasive life history features common to the widespread cryptogenic species.

Why then are the A+ species so widespread? Many factors undoubtedly influence this, but two should be mentioned here. (1) Many of the A+ species have been transported around the world with oysters for cultivation and do not necessarily need the high dispersal capabilities of the common fouling species.

Some A+ species have ranges in their non-native habitats limited only to their introductory bays (*Saccharina japonica*), but others (*Undaria* and *Codium*) become highly invasive after they arrive and have spread widely. (2) Most of the A+ species are relatively large. Except for *Antithamnion nipponicum*, all of the JTMD A+ species are >2-3 cm in size and several can reach up to several meters in length. Of the 8 A+ JTMD species already present in California, 7 fall into this category. The 2 cm and greater sized species can be easily detected in the field so their occurrence and spread can be documented. This is not the case for the smaller species. When populations explode, small species can be just as disruptive to an ecosystem as larger forms, but often they are they not even detected. Approximately half (52%) of the JTMD algal species are <2 cm in size and half of these are microscopic. In order to even begin to recognize the introduction and spread of these species, careful collecting in the field and both microscopic and molecular screening of the material would have to be carried out.

The JTMD algal species occurring in the NE Pacific before the tsunami. Perhaps the most intriguing discovery of this study was the fact that 61% (49) of the JTMD species were reported to already be present in the NE Pacific before the tsunami. Due to the eastward flow of the North Pacific current from Asia to the NE Pacific, we assumed that many of these species historically colonized our NE Pacific shores from Asia, but we weren't completely sure. Of the 49 species residing in the NE Pacific, 71% (35) were cryptogenic, widely dispersed and likely to be from Asia, 16% (8) were Asian+ and likely to be aquaculture transplants, and 12% (6) were limited to the North Pacific, possibly species with naturally wide NP ranges or possibly also introduced from Asia. By mapping the distributions of these species in OR and WA, we also discovered that most of the pre-tsunami JTMD species inhabited sheltered water areas such as bays and not the more exposed rocky headlands. Armed with this information, we targeted quiet water areas to search for new invasions of JTMD algae in the NE Pacific and also to locate pre-tsunami populations of these species for genetic studies.

Using material from (1) our collections from JTMD, (2) from the JTMD species residing in the NE Pacific and (3) from our collections along the Tōhoku region of Japan, my collaborators, Hanyuda and Kawai, conducted a detailed comparison of the haplotype sequences of the shared JTMD species to see if they were actually identical. If there were slight differences in the haplotypes, they could use this information, along with sequences in GenBank, to map the world distribution of the haplotypes and determine the probable source of the NE Pacific populations. Amazingly, they found nearly 10 species with variable haplotypes that could be used for this purpose. Even more surprising was their results. Most of these NE Pacific populations had originated from sources other than Japan.

This discovery had accidentally uncovered yet another invasion risk. Although the JTMD, NE Pacific and Japanese populations of these shared species are truly within the same species, the introduction of the different haplotype would genetically contaminate the NE Pacific populations. So, the invasion risk even for the shared species is still high.

The Japanese species on JTMD that are not yet resident in the NE Pacific. The JTMD algal species that are not already present in the NE Pacific undoubtedly pose the greatest invasion threat. These include 31 (39%) of the 80 species identified from JTMD. A large percentage of the 31 species are red algae (48%, 14 species) and brown algae (32%, 10 species). It seems logical to assume that the JTMD species with more invasive traits would already be present in the NE Pacific, and that those without would still be absent. In terms of overall percentages this is mostly true, but the numbers are not as high as might be expected. Within the 31 species, the proportion of the species remaining only on JTMD and not in the NE Pacific included: (1) a considerable number of the species groups with invasive traits (30% of the ephemerals and 34% of the opportunistic species), and (2) only a slightly higher number of those considered less invasive (46% annuals, 50% perennials, 53% of the late successional types). Within the distributional types, similar percentages were found: 29% of the cryptogenic species and 43% of the Asian + species have not yet been introduced into the NE Pacific.

The risk of JTMD algal species invading the NE Pacific. Based solely on the invasive features described above, there is a high risk that populations of many of the 80 JTMD marine algal species will recruit and possibly invade NE Pacific shores. The majority of the species have been reproductive (84%), ephemeral (50%), and/or opportunistic (76%) forms – all fully capable of quickly invading new habitats.

Also, sea water temperature along the Washington and Oregon coast (9-12° C) appears to be compatible with JTMD species as it is well within the range found along the Tōhoku coast of Japan (7-23° C). The risk of invasion is reduced somewhat by the fact that 49 (61%) of the JTMD algal species are already present in the NE Pacific, but if haplotypes of these resident species differ from those on debris (e.g., *Petalonia*), there is still a risk of genetic contamination. Among the species already present in the NE Pacific are 8 well-known Asian exports (A+) that have been introduced primarily into California. There is some risk that these species will be reintroduced into Oregon and Washington and become invasive in this area. However, it is the 31 species not yet resident in the NE Pacific that pose the greatest new threat. About 30% of these are large, potentially invasive species fully capable of altering habitat. To date, no new populations of JTMD algal species have been found recruiting in WA, OR, or CA. Perhaps there are features of NE Pacific shores that have prevented their survival, or perhaps the species have already become established cryptically and will expand their populations to invade at a later date. Time, careful field surveys, and molecular screening are now required if we are to fully understand the true impact of the JTMD algae in the NE Pacific.

f) Challenges – See also 2B

This has been a lengthy project. In order to finally analyze, summarize and write up our data on this study, we simply had to stop collecting and identifying material. With the end of the project in sight, many of the challenges I encountered seem irrelevant. But, perhaps by mentioning them here, you will see why some aspects of the project had to be limited.

1. Obtaining algal material from the JTMD items. Although I was able to do the collections of algal material from debris items that landed near Newport, most of the items studied in this project were collected by others. Due to the algal and invertebrate projects being separate, it was not uncommon for the collections to be sent directly to Connecticut for invertebrate processing and, for many of these, I did not receive the algae. Luckily, most of our WA and OR collectors did provide the material to John Chapman, and then I was able to subsample his material. Very often the collections arrived deteriorated, but, fortunately, I did obtain enough items in adequate condition to process for the project. I am grateful to my collectors for the hard work of obtaining samples that would have been otherwise impossible for me to get.

2. Disintegrated material. During this study, I have become an expert at interpreting disintegrated remains – based mainly on their reproductive structures. Our trip to Japan and local collections in OR & WA helped to compensate for this as they enabled me to see and preserve many of the species that I had never seen in good condition. I am now hoping that I will have enough good preserved material to complete our Guide.

3. Unavailable sequences. Once the morphological determinations were made, we needed to sequence the species and match our sequences to those of known species in the International Nucleotide Sequence Database (GenBank and the DNA Bank of Japan) in order to confirm our identifications. Unfortunately, many of the species had never been sequenced. So for these, we relied completely on morphology for our identifications and deposited our sequences as the first DNA references for the species in this worldwide public repository of sequence data. Matching sequences would have been much easier.

4. Incorrect genes and gene sequences. On a number of occasions, the sequences in GenBank appeared to be wrong and I would get incorrect identifications back from Hanyuda. We handled this in several ways – the easiest was simply to use a different gene for the same species. For suspicious identifications, we perused the literature and asked other experts for advice on the best genes to use for the species – and the new genes usually gave us a better identification.

5. Writing the papers. Now that our reports are complete, the biggest challenge will be writing our papers. We will write our 2 main papers separately. Although all 3 of us will "author" both, I will write

the checklist and analysis paper and Kawai will write the cross-Pacific sequencing paper. Hanyuda has generously done the sequencing for both projects. It is my hope that we will be able to submit both manuscripts to Cathryn's issue of Marine Pollution Bulletin.

g) Achievements

The main purpose of the ADRIFT project was to determine the risk of species invasions to the NE Pacific coast. Without our project, no one would be aware of the high risk of invasion by the algal species. We identified the JTMD algal species using both morphology and molecular methods. We determined their invasive features. We noted their local and global distributions before the tsunami and we searched (unsuccessfully) for new recruitment of the major species along the OR/WA coast after the tsunami. For the scientific community, we provided new sequences to the DNA Bank of Japan and pressings of the species to the OSU herbarium. In addition to our publications, we will provide a guide for the public and government agencies to use for detecting the most invasive JTMD algal species.

h) Literature Cited

Most of the references for this project will be provided in our upcoming manuscripts for publication. The references here are only the limited papers and websites cited in this report.

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5. RESEARCH OUTPUTS (2014-2017) + earlier contributions on debris algae

a) Completed and planned publications

1. West, John A., Gayle I. Hansen, Takeaki Hanyuda and Giuseppe C. Zuccarello. 2016. Flora of drift plastics: a new red algal genus, *Tsunamia transpacifica* (Stylonematophyceae) from Japanese tsunami debris in the northeast Pacific Ocean. *Algae* 31 (4): 289-301. http://www.e-algae.org/
2. Hansen, Gayle I., Takeaki Hanyuda, and Hiroshi Kawai. (To be submitted Jan. 2017). Marine algae carried across the North Pacific on Japanese Tsunami Marine Debris (JTMD) and their invasion threat to the coasts of Oregon and Washington. Marine Pollution Bulletin – special issue
3. Hanyuda, T., H. Kawai, and G. I. Hansen. (To be submitted in early 2017). A comparative molecular study of seaweed species on Japanese Tsunami Marine Debris (JTMD) that were present in both the NE and NW Pacific before the tsunami.
4. Hansen, G. I., T Hanyuda, and H. Kawai (March 2017). An illustrated guide to the most invasive marine algal species on Japanese Tsunami Marine Debris.
5. Hansen, G. I. (summer of 2017). An internet guide/blog on identifying the algae from Japanese Tsunami Marine Debris.

b) Poster and oral presentations at scientific conferences or seminars

(presenter is underlined)

- Regional Preparedness and Response Workshop to address Bio-fouling and Marine Invasive Species on Japanese Tsunami Marine Debris, Portland, Oregon. July 31-August 1, 2012. (~100 people attending). Power Point Talk by <u>G. Hansen</u>: Marine algae of the Japanese Tsunami Floating Dock -- a progress report.
- Heceta Head Coastal Conference, Florence, Oregon. October 26 & 27, 2012. (~180 people attending). Power Point Talk by <u>J. Miller</u>: Invasive Species and Tsunami Debris. (Included 3 slides by G. Hansen summarizing her algal studies)
- The 3 most invasive algal species on marine debris. 2013. Power Point slides given to <u>J. Miller, J. Chapman, S. Chan, and J. Carlton</u> for their talks to Sea Grant and NSF on bioouling on JTMD.
- Japan-US Marine Debris Public Workshop. Lincoln City and Newport, OR. February 15 & 16, 2013. Sponsored by SOLVE. Power Point Presentation and Display by <u>GI Hansen</u>, T Hanyuda, and H Kawai. Marine Algae on Tsunami Debris, a study in progress. (~100 people attending)
- Japanese Phycological Society Meeting, Yamunashi University, Japan, March 27-29, 2013. Poster presented by <u>GI Hansen</u>, T Hanyuda, and H. Kawai: Japanese marine algae on tsunami debris

reaching western North America. (~100 people attending)

- Japanese Memorial Dock Dedication, Hatfield Marine Science Center, Newport, OR. March 10, 2013. Poster and Specimen Display by G. Hansen: Some marine algae on Japanese Tsunami Debris. (~150 people attending)
- Hatfield Marine Science Center Open House, Newport, OR. April 13, 2013. Poster and Specimen Display by G. Hansen: Marine Algae of Tsunami Debris, also Manning the booth. (300+ attending)
- Oregon State University, Dept. of Botany and Plant Pathology, Corvallis. June 6, 2013. Seminar by G. Hansen. Marine algae on Japanese tsunami debris, the preliminary results of the survey. (60 attending).
- Hatfield Marine Science Center, Newport, OR. June 21,2013, Seminar by G. Hansen. Marine algae on Japanese tsunami debris and the risk of invasion. (80 attending).
- Mid-Coast Watersheds Council Meeting, Newport, OR. September 5, 2013. A seminar by G. Hansen, Marine algae on Japanese tsunami debris and the risk of invasion. (50 attending).
- 27th Northwest Algal Symposium, Camp Casey Conference Ctr, WA. October 18-20, 2013. A talk by G. Hansen, Marine Algae on Japanese Tsunami Debris 1: the Risk of Invasion. (50 people attending)
- 27th Northwest Algal Symposium, Camp Casey Conference Center, WA. October 18-20, 2013. A talk by G. Hansen, T. Hanyuda, and H. Kawai Kawai. Marine Algae on Japanese Tsunami Debris 2: a Tour of the Algae. (~50 people attending)
- 1st PICES ADRIFT Project Science Team Meeting. Seattle, WA. 30 July–1 August 2014. Talk by G. Hansen. Marine algae on tsunami debris and their invasion threat to the NE Pacific. (~25 people attending)
- 2nd PICES ADRIFT Project Science Team Meeting. Honolulu, HA. 15-20 March 2015. Talk by G. Hansen. Marine algae on tsunami debris, update on the species. (~30 people attending)
- 9th International Conference on Marine Bioinvasions. Sydney, Australia. 19-21 January 2016. A poster presented by H. Kawai: GI Hansen, T Hanyuda, and H Kawai. Marine Algae arriving on Japanese Tsunami Marine Debris (JTMD) and their invasion threat to the coast of Oregon and Washington, USA.

Carried across the North Pacific on currents from Japan, recognizable debris from the 2011 Great Tōhoku Earthquake and Tsunami has been arriving in Oregon and Washington since June 2012. The debris items are often laden with healthy Japanese marine algae that could recruit to invade NE Pacific shores. On 24 of the most heavily colonized items, we identified 62 marine macroalgal species. Of these species, 32% species were found on only 1 debris item, and only 8% occurred on >10 debris items. More than 80% of the species were fertile bearing mature reproductive structures. The majority of the species were ephemeral (53%) and/or early successional (76%) forms capable of reproducing multiple times during a single year and quickly invading new habitats. More than half of the species on JTMD have already been reported to occur in the NE Pacific. These include widespread species, native species common to both the NW and NE Pacific, and also non-indigenous species by earlier introductions. Currently, we are using multiple genetic markers to analyze the JTMD specimens and determine their relatedness to native populations in the NW and NE Pacific. Our comparative studies are also revealing new cryptic species in populations on both coasts. Well-known global invaders on JTMD include: *Undaria pinnatifida*, *Codium fragile* subsp. *fragile*, *Grateloupia turuturu*, *Antithamnion nipponicum*, *Polysiphonia morrowii*, and *Saccharina japonica*. New populations of these species have not yet been found in Oregon or Washington. However, if they do recruit and become invasive here, they could dramatically impact the marine environment.

- Marine Sciences Day, Hatfield Marine Science Center. Newport, Oregon. 11 April 2016. 3 posters were presented by GI Hansen. Updated Abstracts are given with the PICES 25 meeting below. (~300 people attending)
    a. GI Hansen, T Hanyuda and H Kawai. Marine algae carried across the North Pacific on Japanese Tsunami Marine Debris (JTMD) and their invasion threat to the coasts of Oregon and Washington, USA. (Poster)

b. GI Hansen, JA West, T Hanyuda and GC Zuccarello. The Pink Crust, a new-to-science species on hard plastic debris. (Poster)

c. T Hanyuda, H Kawai, and GI Hansen. Determining the source of the Seal Rock debris boat. (Poster)

- 11th EMECS – Sea Coasts XXVI Joint Conference. St. Petersburg, Russia. 22-27 August 2016. A Poster presented by H. Kawai. H Kawai, T Hanyuda, and GI Hansen. Species diversity and the threat of introduced macroalgal species arriving on Northwestern American shores via Japanese Tsunami Marine Debris (JTMD).

Due to the tsunami generated by the 2011 Great East Japan Earthquake, large amounts of debris from Japan have been arriving on NW American coasts since June 2012. Numerous marine organisms have arrived alive (macroalgae and benthic invertebrates including herbivores and marine pathogens) and there is a considerable threat that they may invade the coast. In order to determine the diversity of macroalgal species on debris and to evaluate the introduction risk, we have undertaken a monitoring project supported by PICES and MOE. Using both morphology and molecular study, we have identified 66 macroalgal species (19 green, 24 brown and 23 red algae) on 36 debris items collected from Oregon and Washington. On these items, more than 75% of the species were fertile, indicating a high risk for recruitment occurring along the coast. More than 33% of the species, including several large taxa over 50 cm in length (e.g. *Alaria crassifolia*, *Saccharina japonica*, *Undaria pinnatifida*, *Neodilsea yendoana*), are not known in the NE Pacific, and their introductions would substantially impact the ecosystem. Even among the debris species that are common to both the NW and NE Pacific (e.g. *Petalonia fascia*, *Palmaria mollis*), there are genetic differences that could pollute the populations. The search for new recruitment of the JTMD algal species along the NW American coast has just begun. With our JTMD checklist established, we must now carefully monitor and molecularly screen the NE Pacific populations to detect the new introductions and finally reveal the actual impact of the JTMD on the native algal populations.

- PICES 25 -- North Pacific Marine Science Organization -- 2016 Annual Meeting. San Diego, California. 2-13 November 2016. 3 posters were presented by GI Hansen + 1 talk by H Kawai. (~650 people attending)

a) GI Hansen, T Hanyuda and H Kawai. Marine algae carried across the North Pacific on Japanese Tsunami Marine Debris (JTMD) and their invasion threat to the coasts of Oregon and Washington, USA. (Poster)

Carried across the North Pacific on currents from Japan, debris from the 2011 Great Tōhoku Earthquake and Tsunami has been arriving in Oregon and Washington since June 2012. The debris items are often laden with healthy Japanese marine algae that could recruit to invade NE Pacific shores. On 40 of the most heavily colonized items, we have identified 75 marine macroalgal species and species complexes. Of these species, 55% were found on only 1 or 2 debris items, and only 8% occurred on >15 debris items. 77% were fertile bearing mature reproductive structures, and many were ephemeral (48%) and/or opportunistic (77%) forms capable of reproducing multiple times during a single year and quickly invading new habitats. Two-thirds (66%) of the species on JTMD have been reported to already occur in the NE Pacific before the tsunami. These include widespread species, native species common to both the NW and NE Pacific, and also non-indigenous species by earlier introductions. Currently, we are using multiple genetic markers to analyze the JTMD specimens and determine their relatedness to native populations in the NE and NW Pacific. Our comparative studies are also revealing new cryptic species in populations on both coasts. Well-known global invaders on JTMD include: *Undaria pinnatifida*, *Codium fragile* subsp. *fragile*, *Grateloupia turuturu*, *Antithamnion nipponicum*, *Polysiphonia morrowii*, and *Saccharina japonica*. New populations of these species have not yet been found in Oregon or Washington. However, if they do recruit and become invasive in this area, they could dramatically impact the marine environment.

b) GI Hansen, JA West, T Hanyuda and GC Zuccarello. *Tsunamia transpacifica*, a new-to-science pink crust on hard plastic debris. Poster. (Poster). A pdf. was provided to PICES.

From 2015-2016, quantities of hard plastic debris items have been washing ashore on the beaches of Oregon and Washington. Since these items float, many appear to have been carried with currents across the North Pacific before arriving on our shores, and many appear to be from the Great Tōhoku Tsunami of 2011. Some of these items arrive on our beaches partially covered by a thin growth of a gelatinous pink crust. The crust is most noticeable on white plastic but also has been found on a variety of light-colored plastic items. It appears to be a primary colonizer of plastic and may settle at sea before the recruitment of pelagic fouling invertebrates. Microscopic examination of the crust reveals that it is a primitive red alga consisting of single or small clusters of cells in a thick gelatinous sheath. Each cell contains a single highly lobed chloroplast without a pyrenoid. In culture, the alga produces spores and tiny branched filaments, and, morphologically, it does not resemble any known alga. To further explore its identity, we examined the species using molecular methods. By sequencing 3 different genes in the alga (*psb*A, *rbc*L, and SSU), we have discovered that it is an undescribed member of the red algal class Stylonematophyceae. Commemorating its discovery on plastic arriving with Japanese tsunami debris, we are naming the new alga ***Tsunamia transpacifica***.

c) T Hanyuda, H Kawai, and GI Hansen. Determining the source of the Seal Rock debris boat. (A Poster composed by GI Hansen). A pdf. was provided to PICES.

On April 9, 2015, a 26-foot derelict boat arrived off shore at Seal Rock, Oregon (USA), bearing none of the invertebrate species typically used to identify Japanese Tsunami Marine Debris (JTMD). Where was it from? Could it have been from elsewhere in Asia and not related to the Great Tōhoku Tsunami of 2011? The boat did bear a wide variety of algal species, particularly green algae in the "sea lettuce" family, the Ulvaceae. One particular species in this family, *Ulva pertusa/australis*, is a well-known invader of many places around the world, including NE Pacific shores. Its DNA had been studied earlier by my two colleagues, Takeaki Hanyuda and Hiroshi Kawai, and their associates. They had mapped the distribution of its haplotype (DNA) strains in Japan and in other areas in the Pacific. By carefully comparing their data with 2 haplotype sequences from our sample, they determined that the Seal Rock boat debris sample of *Ulva pertusa/australis* and also the Seal Rock boat itself were not only from Japan, but also from Kamaishi in the Iwate Prefecture -- an area particularly hard hit by the Tsunami. Some of the data from their study will be illustrated.

d) T Hanyuda, GI Hansen, and H Kawai. Genetic diversity and biogeography of the macroalgal species associated with Japanese tsunami marine debris. (Talk)

Recognizable debris from the 2011 Great Tōhoku Earthquake and Tsunami (JTMD) was carried across the North Pacific on currents from Japan, and has been arriving on Northeastern Pacific shores since 2012. Often healthy marine macroalgae are attached on JTMD, and there is a threat that they may become introduced to the coasts and disturb their ecosystems. In the PICES ADRIFT project sponsored by the Ministry of Environment, Japan, we have monitored the macroalgae on JTMD, and identified them by morphology and by using genetic markers. We have identified ca. 70 macroalgal species on JTMD, genetically analyzed the specimens using multiple genetic markers, and compared them with those from natural habits in Tōhoku and NW America. We have genetically examined following taxa: *Ulva* spp. (*U. compressa*, *U. lactuca*, *U. linza*, etc.), *Blidingia* spp. [Ulvophyceae]; *Feldmannia mitchelliae*, *Ectocarpus* spp., *Kuckuckia* sp., *Desmarestia* spp. *(D. japonica*, *D. viridis*, etc.), *Petalonia fascia*, *P. zosterifolia*, *Saccharina japonica*, *Scytosiphon lomentaria, S. gracilis* [Phaeophyceae]; *Chondrus giganteus, C. yendoi*, *Grateloupia turuturu*, *Palmaria palmata* and *P. mollis* [Rhodophyceae]. We have further analyzed the geographical distributions of representative haplotypes (genetic types) of the following taxa: *Ulva pertusa, Blidingia* spp., *Petalonia fascia*, *Desmarestia* spp. *Ectocarpus* spp., *Palmaria palmata*/*P. mollis*. These analyses suggest that although half of the species on JTMD have already been reported to occur in the NE Pacific, many of the debris specimens are genetically distinct from NW and NE Pacific populations, and their introductions to NE Pacific coasts may cause genetic disturbance to the local populations.

- Western Society of Naturalists – 100th Anniversary. Monterey, California. 10-13 November 2016. Three posters were presented by GI Hansen. Abstracts are provided with the PICES 25 meeting. (~950 people attending)

a) GI Hansen, T Hanyuda and H Kawai. Marine algae arriving on Japanese Tsunami Marine Debris (JTMD) and their invasion threat to the shores of Oregon and Washington, USA. (Poster)
b) GI Hansen, JA West, T Hanyuda, and GC Zuccarello. *Tsunamia transpacifica*, a new-to-science crust on hard plastic debris. (Poster).
a) T Hanyuda, H Kawai and GI Hansen. Determining the source of the Seal Rock debris boat. (Poster).

- Oregon Department of Fish and Wildlife. Marine Science Poster Review. Newport, Oregon. 15 November 2016. 3 posters
- Cape Perpetua Land/Sea Symposium IV. Yachats, Oregon. 17 November 2016. 3 posters

Versions of the three posters presented at WSN were also presented by GI Hansen at these 2 meetings.

- The Green Planet – Past, Present, and Future. Calcutta, India. 21-23 December 2016. A talk was presented by JA West: JA West, GI Hansen, T Hanyuda and GC Zuccarello. Flora of Drift Plastics: a new red algal genus, *Tsunamia transpacifica* (Stylonematophyceae) from Japanese tsunami debris in the northeast Pacific. (Talk)

Floating debris provides substrates for dispersal of organisms by ocean currents, including algae that thrive on plastics. The 2011 earthquake and tsunami in Tōhuku, Japan resulted in large amounts of debris carried by the North Pacific Current to North America from 2012 to 2016. In 2015-2016, the plastics in the debris bore a complex biota including pink algal crusts. One sample (JAW4874) was isolated into culture and a three-gene phylogeny (*psb*A, *rbc*L, and SSU) indicated it was an unknown member of the red algal class Stylonematophyceae. It is a small pulvinate crust of radiating, branched, uniseriate filaments with cells containing a single centrally suspended nucleus and a single purple to pink, multi-lobed, parietal plastid lacking a pyrenoid. Cells can be released as spores that attach and germinate to form straight filaments by transverse apical cell divisions, and subsequent longitudinal and oblique intercalary divisions produce masses of lateral branches. This alga is named *Tsunamia transpacifica* gen. nov. et sp. nov. Sequencing of additional samples of red algal crusts on plastics revealed another undescribed Stylonematophycean species, suggesting that these algae may be frequent on drift oceanic plastics.

c) Education and outreach

- *Watch for Invasive Wakame on Tsunami Debris!* June 15, 2012. A Flyer by GI Hansen on *Undaria pinnatifida* that has been widely distributed, posted on the Coast Watch website, and incorporated into the training program for Oregon Parks and Recreation volunteers.
- *Collecting Marine Algae from Tsunami Debris for Identification.* June 19, 2012. An OPRD educational handout by G. Hansen.
- *Coping with Marine Debris. July 2012.* A library exhibit prepared by S. Gilmont that included my Flyer and my specimens of *Undaria pinnatifida.*
- *Ideal collections of biota from marine debris*. March 16, 2013. A Handout for Oregon State Agencies and Volunteers by.J. Miller, G. Hansen, and J. Chapman.
- *Some Marine Algae from Japanese Tsunami Debris*. April 13, 2013. A general public poster by G. Hansen, updated in 2016 and widely distributed.
- *Japanese Tsunami Marine Debris, Key Aquatic Invasive Species Watch*. 2015. Oregon Sea Grant, 12 pp. Authors include: Jennifer Lam and Sam Chan (OSU/Oregon Sea Grant); Gayle Hansen, John Chapman, and Jessica Miller (OSU); Jim Carlton (Williams College); Rick Boatner (ODFW); Rick Cooper, Pat Kight, Tania Siemens, and Kayla Martin (Oregon Sea Grant); Jared Corcoran (designer).
- *Marine Algae of Washington Debris: the Cape Disappointment Tsunami Boat. 2014.* An Instructional Power Point Presentation for use in Washington -- provided to Allen Pleus, Aquatic Nuisance Species Coordinator of Washington Dept. of Fish and Game and to J. Carlton for an NSF Biofouling Report.

d) Other grant reports & products

- Hansen, G. I. 4/15/2013. Identification and Biology of Seaweeds of the Japanese Tsunami Floating Dock. Oregon Sea Grant Project Completion Report for Grant # NA10OAR4170059, NA223C R693
- Carlton, J. T, J. R. Cordell, G. I. Hansen, and A. Pleus. Feb. 2013. Biofouling Community on Japanese Vessel 20-210-42909 Washed Ashore June 15, 2012 on Benson Beach at Cape Disappointment State Park, Ilwaco, Washington. NSF RAPID Japanese Tsunami Marine Debris Biofouling Register # JTMD-BF-2
- *2012 Algal Herbarium collections from the Agate Beach Dock.* For deposit at the OSU Hebarium

e) Newspaper, radio and television interviews

- *Coos County Public Radio*-- Phone interview on potential introductions from the Agate Beach Dock. Jessica Miller & Gayle Hansen. 9/2012.

- *Japan Broadcasting Corporation* -- TV Interview on the introductions -- Gayle Hansen and John Chapman 10/27/12
- *BBC on-line*. Video-Interview on the beach on the risks of invasive seaweeds 8/2/12. (just before the Agate Beach Dock removal)
- *News-Times*, *Newport* – Interview & photos by Larry Coonrod on preserving & cataloging the dock algal species for future study - 6/6/12 (article on 6/8/12)
- Numerous others in 2012 – not documented, including several in Japan
- *KEZI television*, Eugene, Oregon. 4/10/2015. Marine algae on the Seal Rock off-shore derelict boat
- *News-Times, Newport*. Interview & photos by Dennis Anstine. 4/10/2015 (article on 5/15/2015). Arrival of a slow boat from Japan.
- Undocumented radio interviews via phone. 4/10–4/16/2015. On the new derelict boat & its algae.

f) JTMD-related awards

- *Ten Fingers in the Dike Award* -- presented to several of us for our work on the Agate Beach Floating Dock -- by the Oregon Invasive Species Council, February 12, 2013, in Salem.

6. RESEARCH STATUS AND FUTURE STEPS/PLANS

After submitting my report and checklist/risk manuscript, I will devote my time to working with Kawai and Hanyuda on a hard-copy illustrated pamphlet/guide to the ~20 most invasive species found on Japanese Tsunami Marine Debris. In this booklet we will discuss our own knowledge of the species as well as the published accounts on the species and their distributions. We have already had one publisher come forth offering to produce our guide, but we are still discussing the possibilities. In addition to the guide, I will begin posting my internet-based taxonomic "blog" on all of the JTMD algal species. Since the information on many of these species is only available in Japanese publications and photographs often do not exist, this illustrated discussion of the species will help other scientists in looking for new recruitment and spread of these species along the NE Pacific coast. As a part of my normal algal studies, I will also continue checking the Oregon and Washington coast for new algal invaders, including those identified on JTMD.

7. APPENDICES

Appendix 1. Japanese Tsunami Debris Items Collected for the Algal Study

#	BF #	State	Site Name (my additions)	Item	Collection Date	Year	Species Count
1	BF-1	OR	Agate Beach	dock	5-Jun	2012	29
2	BF-2	WA	Ilwaco (Benson Beach)	boat	15-Jun	2012	10
3	BF-8	WA	Mosquito Creek	dock	5-Jan	2013	16
4	BF-293	WA	Long Beach (Seaview rusty)	pipe/I-beam	28-Jan	2013	9
5	BF-23	OR	Gleneden Beach	boat	6-Feb	2013	9
6	BF-234	OR	South Beach	tank	9-Feb	2013	7
7	BF-28	OR	Horsfall Beach	boat	21-Feb	2013	15
8	BF-235	WA	Long Beach	tire	1-Mar	2013	4
9	BF-36	OR	Florence (Muriel Ponsler)	boat	14-Mar	2013	8
10	BF-39	OR	Cannon Beach (S-Jockey Cap)	boat	22-Mar	2013	8
11	BF-40	WA	Long Beach	fish boat	22-Mar	2013	8
12	BF-50	OR	Coos Bay Spit	boat	22-Apr	2013	3
13	BF-58	OR	Clatsop Beach	boat	30-May	2013	10
14	BF-59/61	OR	Nye Beach	post & beam	30-May	2013	10
15	BF-108	OR	Cape Arago (Lighthouse Beach)	post & beam	11-Jul	2013	2
16	BF-130	OR	Clatsop Beach	dock piece	9-Oct	2013	15
17	BF-134	WA	Twin Harbors State Park	boat	17-Jan	2014	11
18	BF-135	OR	Yachats (Fiberglass fragment)	boat	18-Feb	2014	19
19	BF-331	WA	Oysterville	Boat	14-Mar	2014	9
20	**BF-160**	OR	Tillamook Bay spit	tree	26-Apr	2014	2
21	**BF-171**	OR	Tillamook Bay spit	post & beam	25-Apr	2014	7
22	BF-173	OR	South Beach (Lost Creek black)	buoy	27-Apr	2014	10
23	BF-188?	OR	Cape Lookout Beach #1	boat	3-May	2014	4
24	BF-196	OR	Waldport	boat	12-May	2014	8
25	BF-208	OR	Cape Arago (North Cove)	boat	19-May	2014	14
26	BF-223/224	WA	Long Beach (Ilwaco)	boats 2	29-May	2014	9
27	BF-227/228	WA	Long Beach	boats 2	5-Jun	2014	7
28	BF-277	OR	Seal Rock	tote	30-Nov	2014	3
29	BF-285	WA	Long Beach (Styrofoam fragment)	boat	4-Jan	2015	5
30	BF-462	WA	Long Beach (black)	float	4-Jan	2015	8
31	BF-288	OR	Beverly Beach	pallet	20-Jan	2015	7
32	BF-461	OR	Manzanita (blue)	baskets	2-Mar	2015	5
33	BF-356	OR	In ocean -Seal Rock	boat	10-Apr	2015	16
34	BF-397	WA	Long Beach	dock piece	1-May	2015	3
35	BF-402	WA	Long Beach (Seaview)	boat	12-May	2015	17
36	BF-500	WA	Long Beach (red)	tote	16-Feb	2016	6
37	BF-526	OR	Horsfall Beach 2	boat	22-Mar	2016	24
38	BF-656	OR	Quail Street (plastic)	carbuoy	26-Mar	2016	4
39	BF-545	OR	Umqua River mouth	boat	26-Mar	2016	6
40	BF-533	OR	Roads End	boat	28-Mar	2016	24
41	BF-538	OR	Sixes River mouth	boat	16-Apr	2016	17
42	BF-652	OR	Falcon Cove beach	boat	26-Jul	2016	6

Appendix 2. Samples of plastic debris collected on OR and Wa beaches in 2015 and 2016 containing Stylonematophyceae crusts

GIH #	psbA Tsunamia	rbcL Sp. 2	Plastic Debris Object	Site	State	Col-Date	Collector
130	x		white tray fragment	Long Beach	WA	3/2/2015	Lewis
416*	x		white bottle	Long Beach	WA	11/5/2015	Lewis
422			black buoy	Beverly Beach	OR	12/17/2015	Sarver
426			light blue basket	Otter Crest	OR	12/20/2015	Sarver
430	x		pink float fragment	Nye Beach	OR	12/26/2015	Sarver
431			yellow basket	Nye Beach	OR	12/26/2015	Sarver
432		x	white box fragment	Nye Beach	OR	12/26/2015	Sarver
433	x		white tray & basket fragments	Long Beach dumpster	WA	1/9/2016	Barton
434			black buoy	Long Beach dumpster	WA	1/9/2016	Barton
435			black basket fragment	Long Beach dumpster	WA	1/9/2016	Barton
478			red beer tote -Japanese writing	Leadbetter Point	WA	2/19/2016	Lewis
485			black buoy	Leadbetter Point	WA	2/9/2016	Lewis
490			white jug - Clover Chemical	Leadbetter Point	WA	2/9/2016	Lewis
494			white float	Leadbetter Point	WA	2/9/2016	Leswis
496			white jug - Clover Chemical	Grayland Beach	WA	3/5/2016	Hansen
610		x	white float fragment	Nye Beach	OR	3/18/2016	Hansen
611			white broken basket	Nye Beach	OR	3/18/2016	Hansen
612	x	x	white tray fragment	Lost Creek N.	OR	3/18/2016	Hansen
613	x	x	white tubular fragment	Lost Creek N.	OR	3/18/2016	Hansen
614	x	x	white tray fragment	Lost Creek N.	OR	3/18/2016	Hansen
615	x	x	white tray fragment	Lost Creek N.	OR	3/18/2016	Hansen
616	x	x	blue basket fragment	Yaquina Bay Lighthouse Beach	OR	3/26/2016	Hansen
617			white tray fragment	Yaquina Bay Lighthouse Beach	OR	3/26/2016	Hansen
618		x	black broken grid	Yaquina Bay Lighthouse Beach	OR	3/26/2016	Hansen
619			blue basket fragment	Otter Crest Beach	OR	3/26/2016	Hansen
620			white plastic disc	Otter Crest Beach	OR	3/26/2016	Hansen
621		x	white tray fragment	Nye Beach	OR	3/26/2016	Snell
622		x	white jug - Nissan Chemicals	Quail Street Beach	OR	3/24/2016	Custer
623			white bucket- Miyabe writing	Quail Street Beach	OR	4/26/2016	Custer
630			white tray fragment	Hubbard Creek Beach	OR	11/25/2015	Treneman

* The type culture, JAW-4874, was taken from this collection.

Appendix 3.

Marine algae identified from Japanese Tsunami Marine Debris arriving in OR & WA from June 2012 to July 2016, including additional verification methods

Group	Name	Genes Sequenced and/or Expert ID
B	*Alaria crassifolia* Kjellman *in* Kjellman *et* Petersen	*cox* 3, *rbc* L
B	*Analipus japonicus* (Harvey) M.J. Wynne	*cox* 3
B	*Costaria costata* (C.Agardh) De A. Saunders	*cox* 3
B	*Desmarestia japonica* H. Kawai *et al. in* Yang *et al.*	ITS, *cox* 1
B	*Desmarestia viridis* (O.F. Müller) J.V. Lamouroux	
B	*Ectocarpus acutus* Setchell *et* N.L. Gardner	*cox* 3 **
B	*Ectocarpus* cf. *penicillatus* (C. Agardh) Kjellman	*cox* 3 **
B	*Ectocarpus commensalis* Setchell *et* N.L. Gardner *cpx*.	*cox* 3 **
B	*Ectocarpus corticulatus* De A. Saunders	
B	*Ectocarpus crouaniorum* Thuret *in* Le Jolis	*cox* 3
B	*Feldmannia irregularis* (Kützing) Hamel	*cox* 3 **
B	*Feldmannia mitchelliae* (Harvey) H.-S. Kim *cpx*.	*cox* 3, *rbc* L
B	*Hincksia granulosa* P.C. Silva *in* Silva, Meñez *et* Moe	
B	*Hincksia sandriana* (Zanardini) P.C. Silva *in* Silva, Meñez *et* Moe	
B	*Kuckuckia spinosa* (Kützing) Kornmann *in* Kuckuck	*cox* 3 **
B	*Mutimo cylindricus* (Okamura) H. Kawai *et* T. Kitayama	*cox* 3
B	*Petalonia fascia* (O.F. Müller) Kuntze	*cox* 3, *rbc* L
B	*Petalonia zosterifolia* (Reinke) Kuntze	*cox* 3, *cox* 1, ITS
B	*Petroderma maculiforme* (Wollny) Kuckuck	*rbc* L
B	*Protectocarpus speciosus* (Børgesen) Kornmann *in* Kuckuck	#
B	*Pseudolithoderma subextensum* (Waern) S. Lund	
B	*Punctaria latifolia* Greville	*cox* 3, *rbc* L
B	*Saccharina japonica* (Areschoug) C.E. Lane, C. Mayes, L. Druehl *et* G.W. Saunders	*cox* 3, ITS
B	*Scytosiphon gracilis* Kogame	*cox* 3, *cox* 1, *rbc* L
B	*Scytosiphon lomentaria* (Lyngbye) Link	*cox* 3
B	*Sphacelaria rigidula* Kützing	*psb* C, *rbc* L, #
B	*Sphacelaria solitaria* (Pringsheim) Kylin	
B	*Undaria pinnatifida* (Harvey) Suringar	*cox* 3
G	*Blastophysa rhizopus* Reinke	
G	*Blidingia marginata* (J.Agardh) P.J.L. Dangeard *ex* Bliding	ITS, *rbc* L
G	*Blidingia minima* var. *minima* (Nägeli *ex* Kützing) Kylin	ITS, *rbc* L
G	*Bryopsis plumosa* (Hudson) C.Agardh	*rbc* L
G	*Bryopsis stolonifera* W.J. Lee, S.M. Boo *et* I.K. Lee	
G	*Cladophora albida* (Nees) Kutzing	rDNA
G	*Cladophora vagabunda* (Linnaeus) Hoek	rDNA
G	*Codium fragile* subsp. *fragile* (Suringar) Hariot	*rbc* L
G	*Epicladia* cf. *phillipsii* (Batters) R. Nielsen	
G	*Halochlorococcum moorei* (N.L. Gardner) Kornmann *et* Sahling	
G	*Ulothrix implexa* (Kützing) Kützing	
G	*Ulva australis* Areschoug	*cob* -*cox* 3
G	*Ulva compressa* Linnaeus	ITS, *rbc* L

Appendix 3 - continued

Group	Name	Genes Sequenced and/or Expert ID
G	*Ulva intestinalis* Linnaeus	
G	*Ulva lactuca* Linnaeus	ITS, *rbc* L
G	*Ulva linza* Linnaeus	ITS
G	*Ulva prolifera* O.F. Müller	ITS
G	*Ulva simplex* (K.L. Vinogradova) H.S. Hayden *et al. sensu* Ogawa	ITS **
G	*Ulvella viridis* (Reinke) R. Nielsen, C.J. O'Kelly *et* B. Wysor *in* Nielsen *et al.*	
R	*Acrochaetium microscopicum* (Nägeli *ex* Kützing) Nägeli *in* Nägeli *et* Cramer	
R	*Acrochaetium pacificum* Kylin	
R	*Antithamnion nipponicum* Yamada *et* Inagaki	#
R	*Bangia fuscopurpurea* (Dillwyn) Lyngbye	*rbc* L
R	*Ceramium cimbricum* H.E. Petersen *in* Rosenvinge	*rbc* L, *cox* 1
R	*Chondrus giganteus* Yendo	*rbc* L
R	*Chondrus yendoi* Yamada *et* Mikami *in* Mikami	*rbc* L, *cox* 1
R	*Colaconema daviesii* (Dillwyn) Stegenga	*rbc* L
R	*Colaconema thuretii* (Bornet) P.W. Gabrielson *in* Gabrielson *et al.*	*rbc* L
R	*Erythrocladia irregularis* Rosenvinge	
R	*Erythrotrichia carnea* (Dillwyn) J. Agardh	
R	*Erythrotrichia incrassata* T. Tanaka	
R	*Grateloupia livida* (Harvey) Yamada	*rbc* L
R	*Grateloupia turuturu* Yamada	*rbc* L
R	*Leptofauchea leptophylla* (Segawa) M. Suzuki *et al* .	#
R	*Meiodiscus spetsbergensis* (Kjellman) G.W. Saunders *et* J. McLachlan	
R	*Neodilsea yendoana* Tokida	ITS
R	*Neosiphonia japonica* (Harvey) M.-S. Kim *et* I.K .Lee	
R	*Neosiphonia yendoi* (Segi) M.-S. Kim *et* I.K .Lee	#
R	*Palmaria mollis* (Setchell *et* N.L.Gardner) van der Meer *et* C.J. Bird	*rbc* L, *cox* 1
R	*Polysiphonia koreana* D. Bustamante, B.Y. Won & T.O. Cho	*rbc* L **
R	*Polysiphonia morrowii* Harvey	*rbc* L
R	*Polysiphonia scopulorum* var. *villum* (J. Agardh) Hollenberg	rbcL **
R	*Porphyrostromium japonicum* (Tokida) Kikuchi *in* Kikuchi *et* Shin	
R	*Ptilota filicina* J. Agardh	*rbc* L
R	*Pyropia pseudolinearis* (Ueda) N. Kikuchi, M. Miyata, M.S. Hwang *et* H.G. Choi *cpx.*	*rbc* L
R	*Pyropia yezoensis* (Ueda) M.S. Hwang *et* H.G. Choi *in* Sutherland *et al.*	*rbc* L
R	*Schizymenia dubyi* (Chauvin *ex* Duby) J. Agardh	18S rDNA
R	*Tsunamia transpacifica* J. West, G. Hansen, T. Hanyuda *et* G. Zuccarello *cpx.*	18S rDNA
BG	*Calothrix confervicola* C.Agardh *ex* Bornet *et* Flahault	
BG	*Chroococcus submarinus* (Hansgirg) Kovácik	
BG	*Lyngbya confervoides* C. Agardh *ex* Gomont	
BG	*Scytonematopsis crustacea* (Thuret *ex* Bornet *et* Flahault) Kovález *et* Komárek	

Note: Morphological identifications were made for all species. Verifications in addition to morphology included: sequencing; ** = sequencing with additional study in progress; # = identification by monographic experts. **Group designation:** B = brown algae (Ochrophyta); C = green algae (Chlorophyta); R = red algae (Rhodophyta); BG = bluegreen bacteria (Cyanobacteria); R = red algae (Rhodophyta).

Appendix 4.

JTMD algal species not yet present in the NE Pacific (WA, OR, CA) and their global residency

Group	Name	Global
B	*Alaria crassifolia*	A
B	*Desmarestia japonica*	A
B	*Ectocarpus crouanionum*	C
B	*Ectocarpus penicillatus*	C
B	*Kuckuckia spinosa*	C
B	*Petalonia zosterifolia*	C
B	*Protectocarpus speciosus*	C
B	*Pseudolithoderma subextensum*	C
B	*Saccharina japonica*	A+
B	*Sphacelaria solitaria*	C
G	*Blastophysa rhizopus*	C
G	*Bryopsis stolonifera*	A
G	*Epicladia phillipsii*	C
G	*Ulva simplex*	C
R	*Chondrus giganteus*	A+
R	*Chondrus yendoi*	A
R	*Erythrotrichia incrassata*	A
R	*Grateloupia livida*	A
R	*Leptofauchea leptophylla*	A
R	*Neodilsea yendoana*	A
R	*Neosiphonia japonica*	A+
R	*Neosiphonia yendoi*	A
R	*Polysiphonia koreana*	A
R	*Polysiphonia morrowii*	A+
R	*Porphyrostromium japonicum*	A
R	*Pyropia pseudolinearis* cpx	A
R	*Pyropia yezoensis*	A+
R	*Tsunamia transpacifica* cpx.*	NP
BG	*Calothrix confervicola*	C
BG	*Chroococcus submarinus*	C
BG	*Lyngbya confervoides*	C

*Note that *Tsunamia transpacifica* cpx. is known on debris in the North Pacific, but it has not yet been discovered on either coast.