

Biodiversity entering US ports via ballast water discharge

An analysis using High Throughput Sequencing

*John Darling, John Martinson, Yunguo Gong, Sara
Okum, Erik Pilgrim
US Environmental Protection Agency*

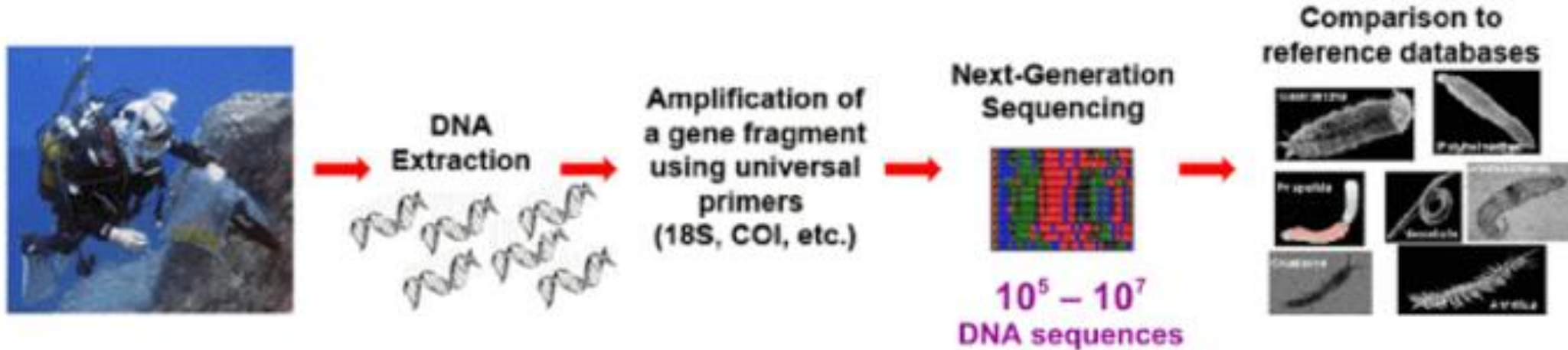
*Katrina Lohan and Greg Ruiz
Smithsonian Environmental Research Center*

*Yuping Zhang
University of Michigan*

The Questions

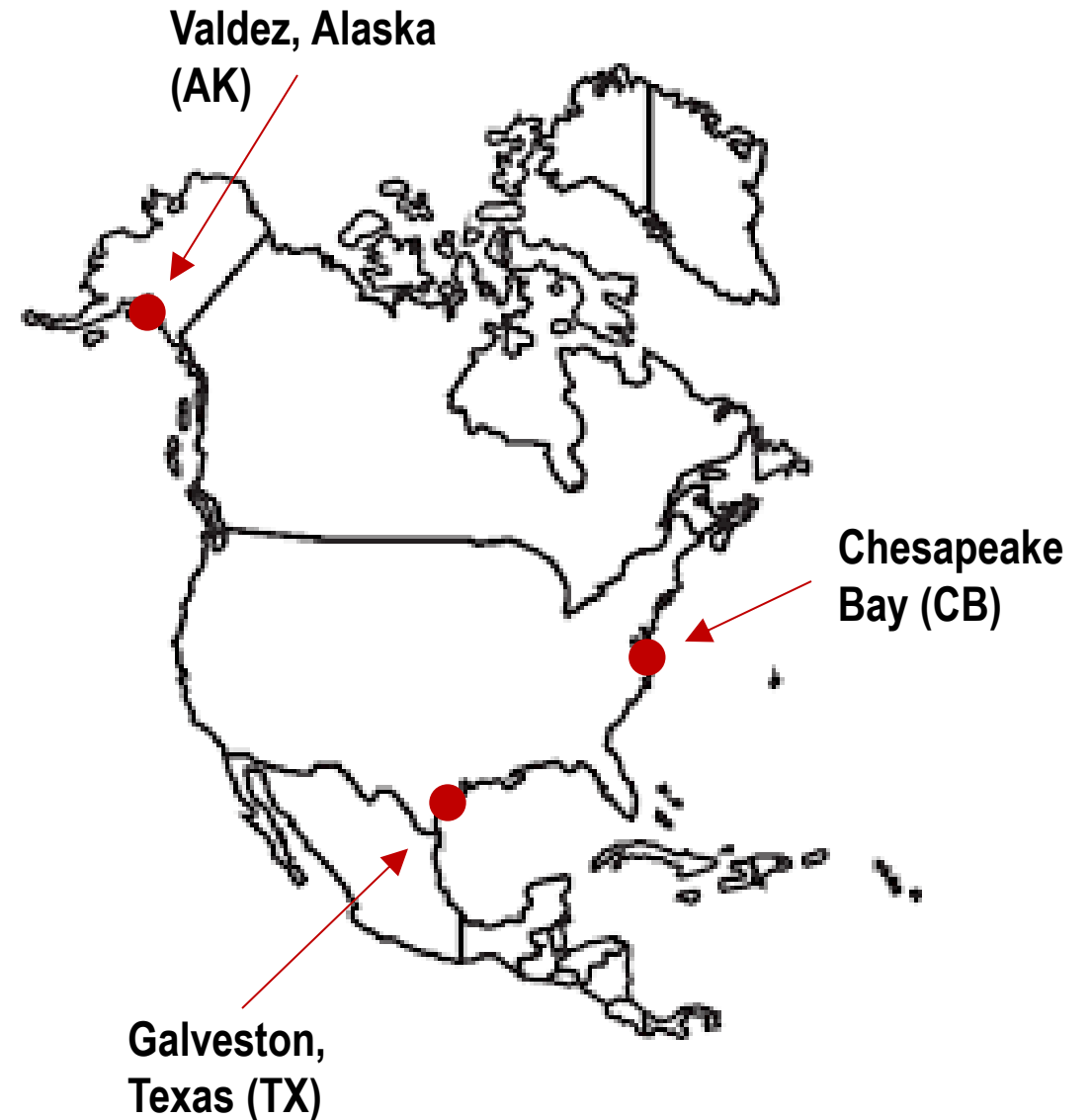
- What can DNA tell us about the biodiversity being delivered to US ports in ballast?
- Can DNA data capture biogeographic signatures (e.g. can we identify sources)?
- How much does management effect biodiversity in ballast?
- What species are being delivered, and are any of them troubling?

DNA Meta-barcoding



The Samples

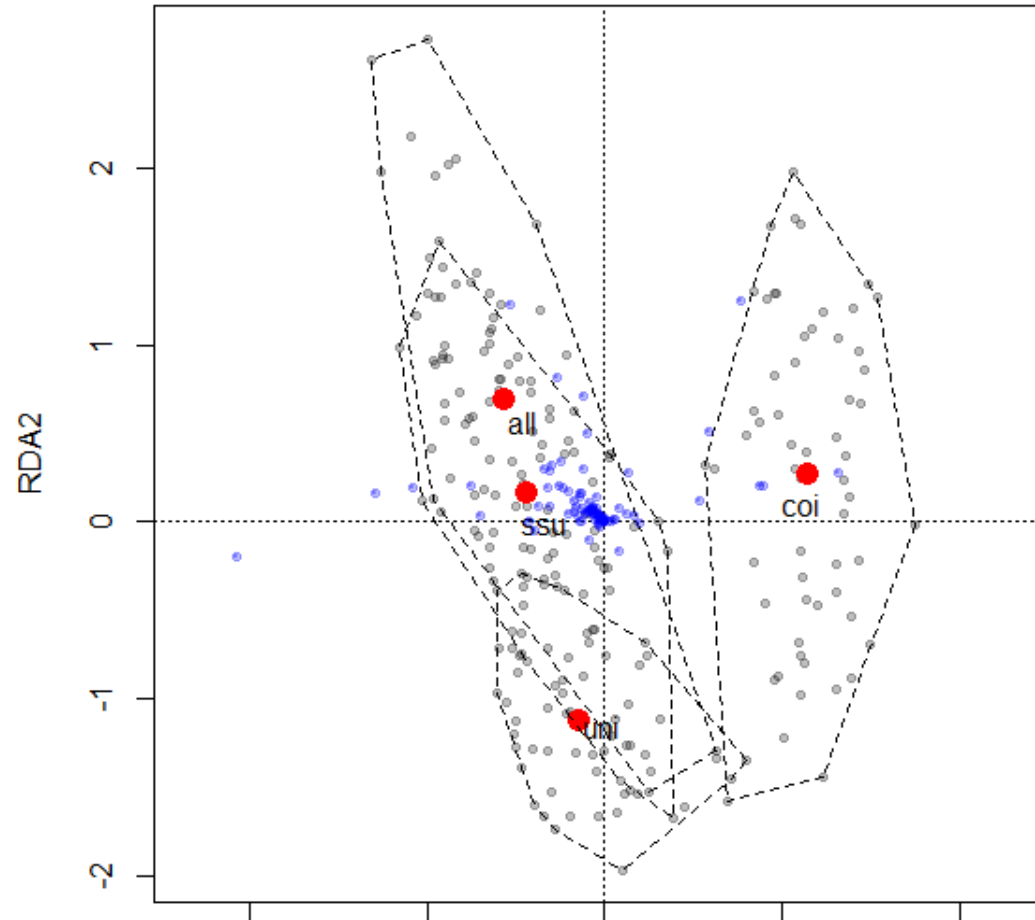
- Chesapeake: 51 samples sourced from 32 ports in 12 countries, mostly Europe and North America
- Texas: 45 samples from 32 ports in 16 countries, mostly North and South America
- Alaska: 47 samples from 14 ports, all in the US
- Sequence generated from 3 18S loci and COI



How much diversity can we see? Which loci work best?

EXAMINING THE METHOD

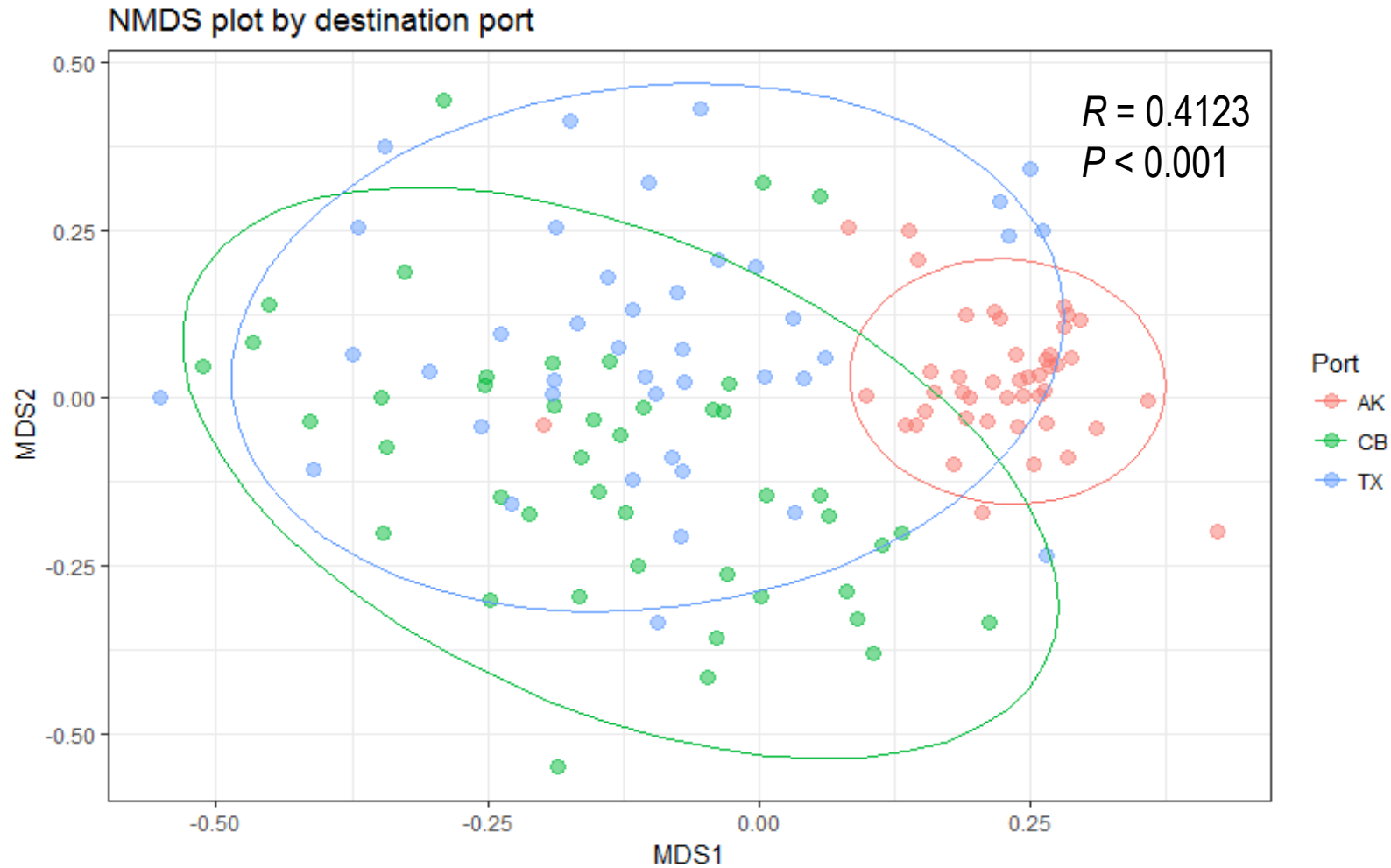
Effect of primer on community structure



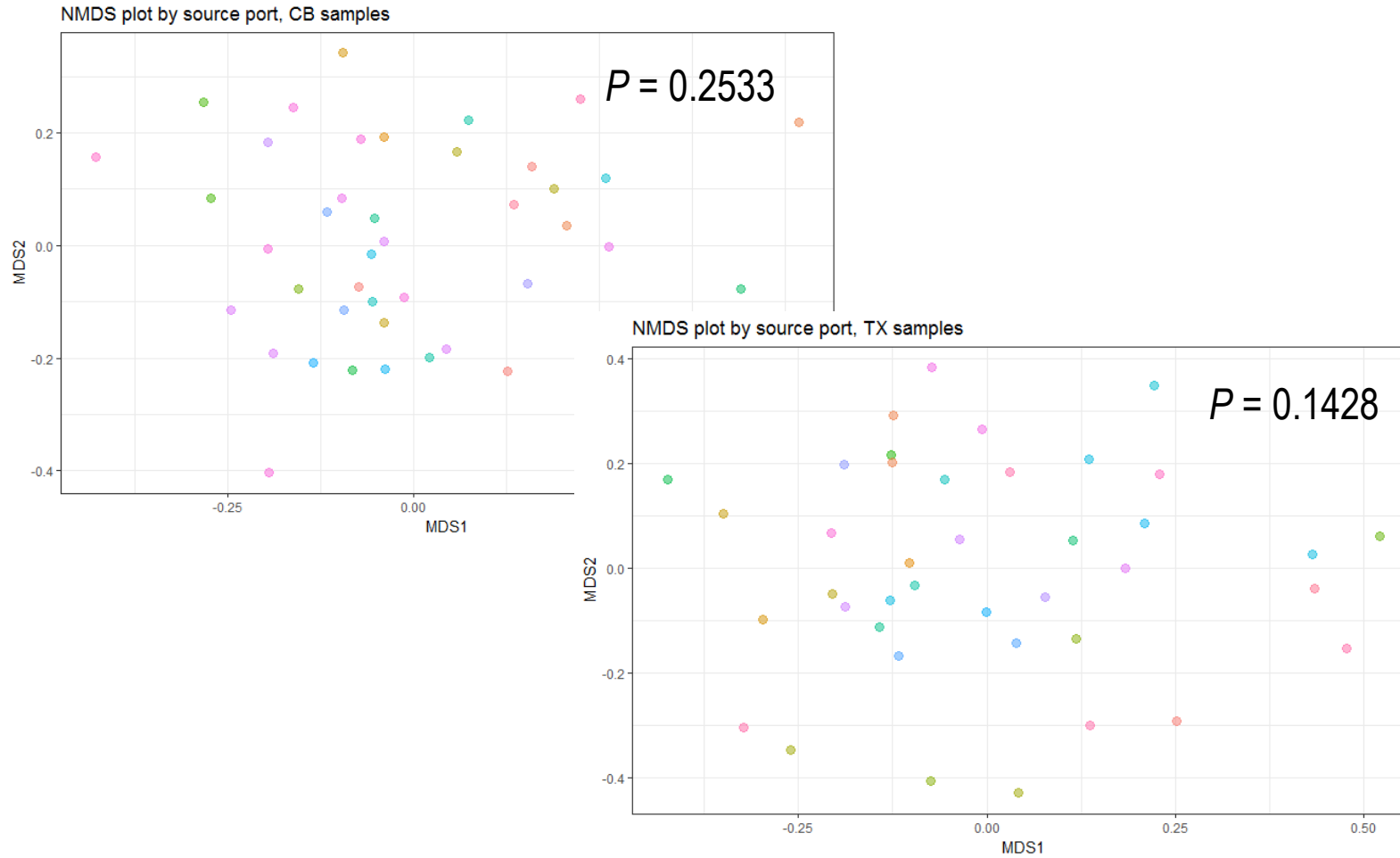
What are the drivers of differences in ballast water communities?

COMPARING DIVERSITY ACROSS SAMPLES

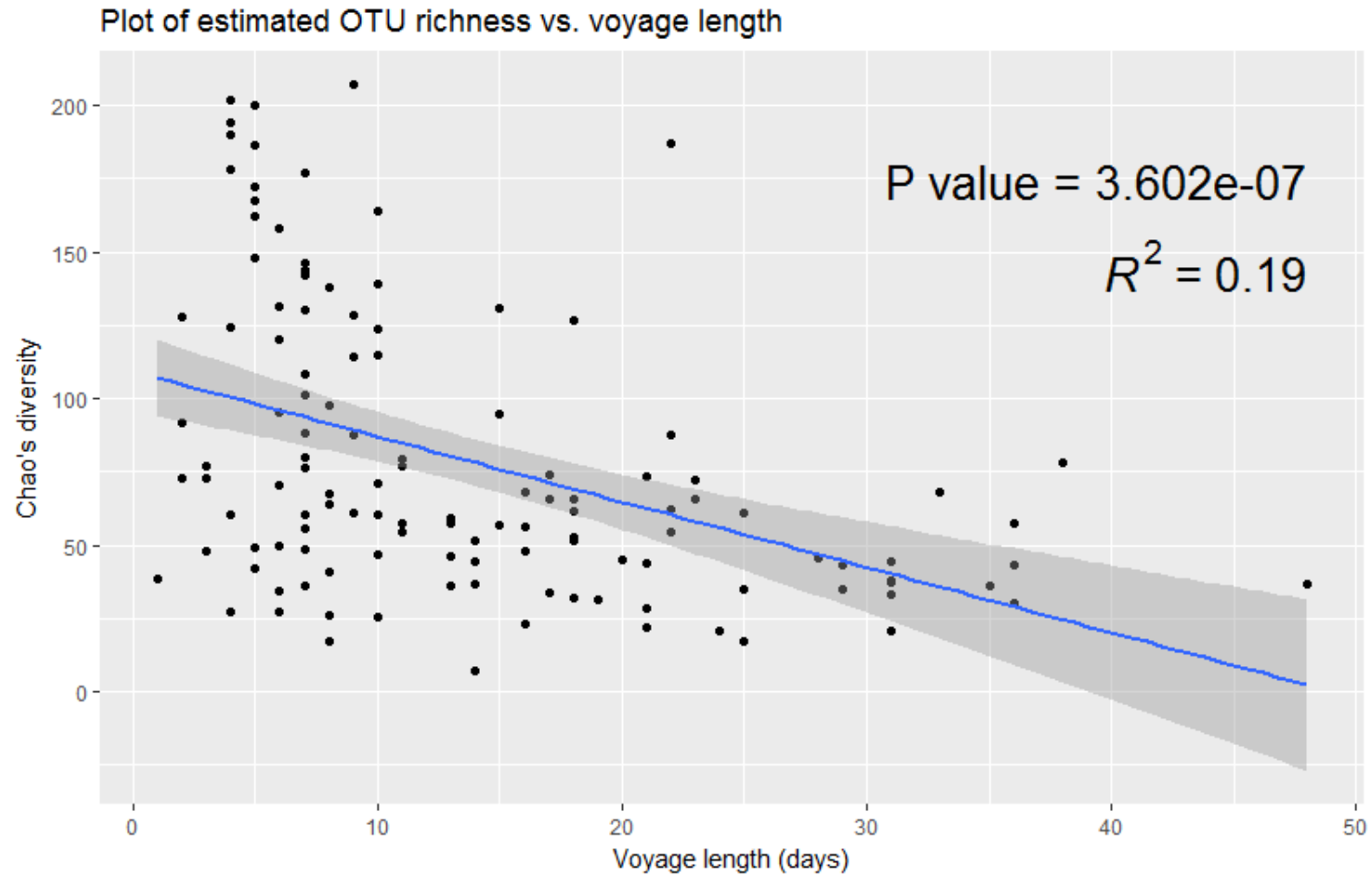
Effect of source region on community structure



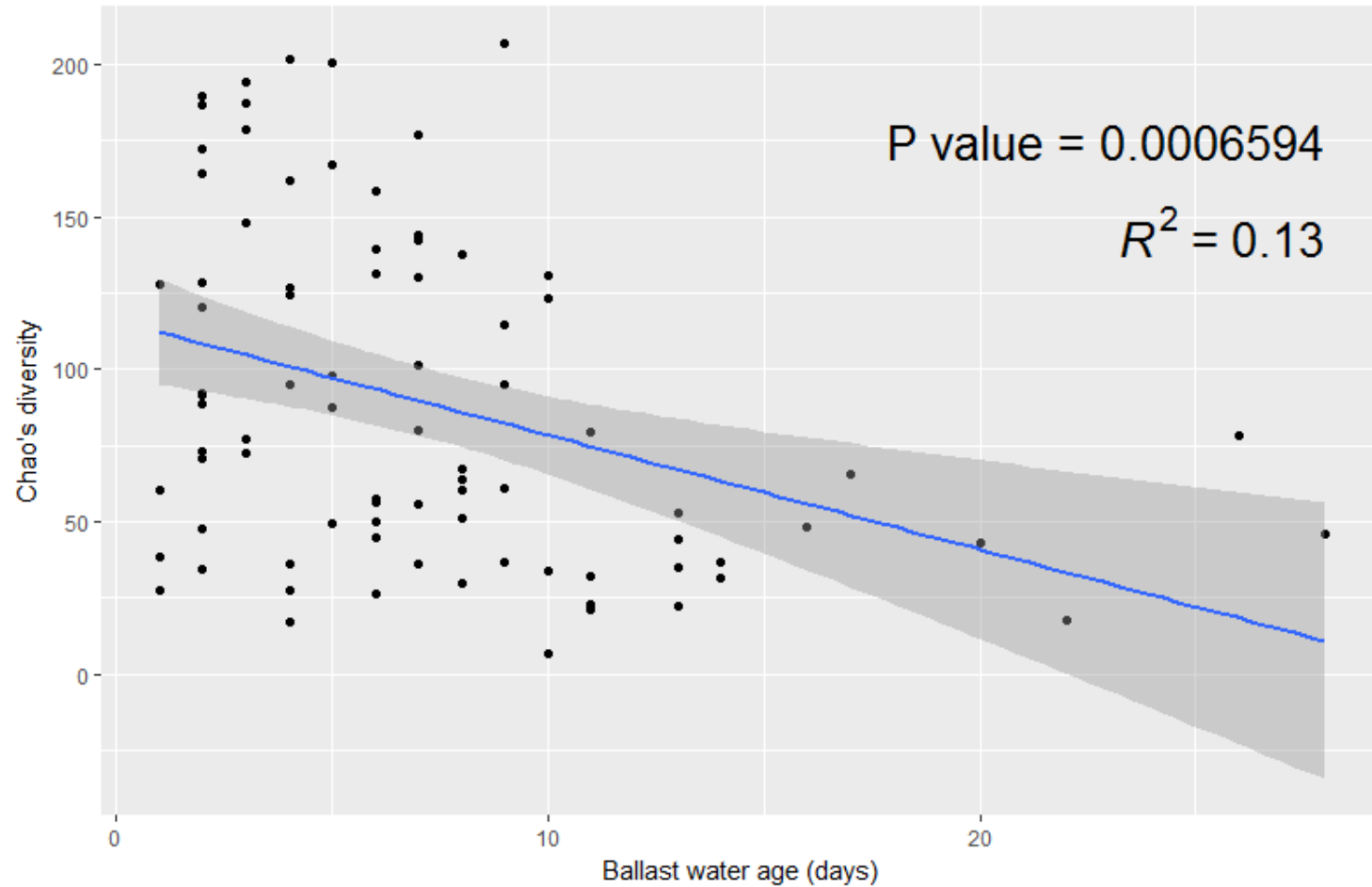
Effect of source region on community structure



Effect of source region on community structure



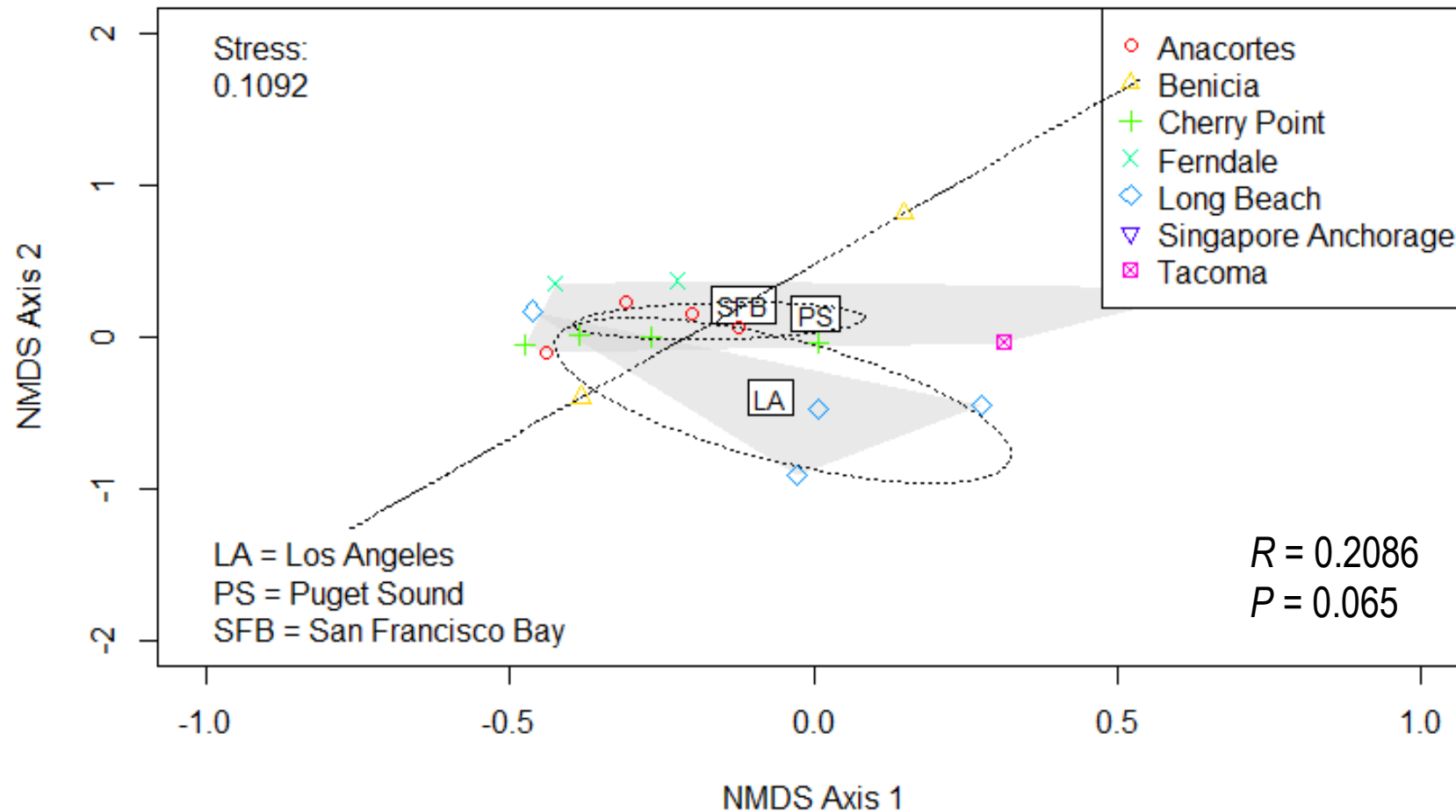
Effect of source region on community structure



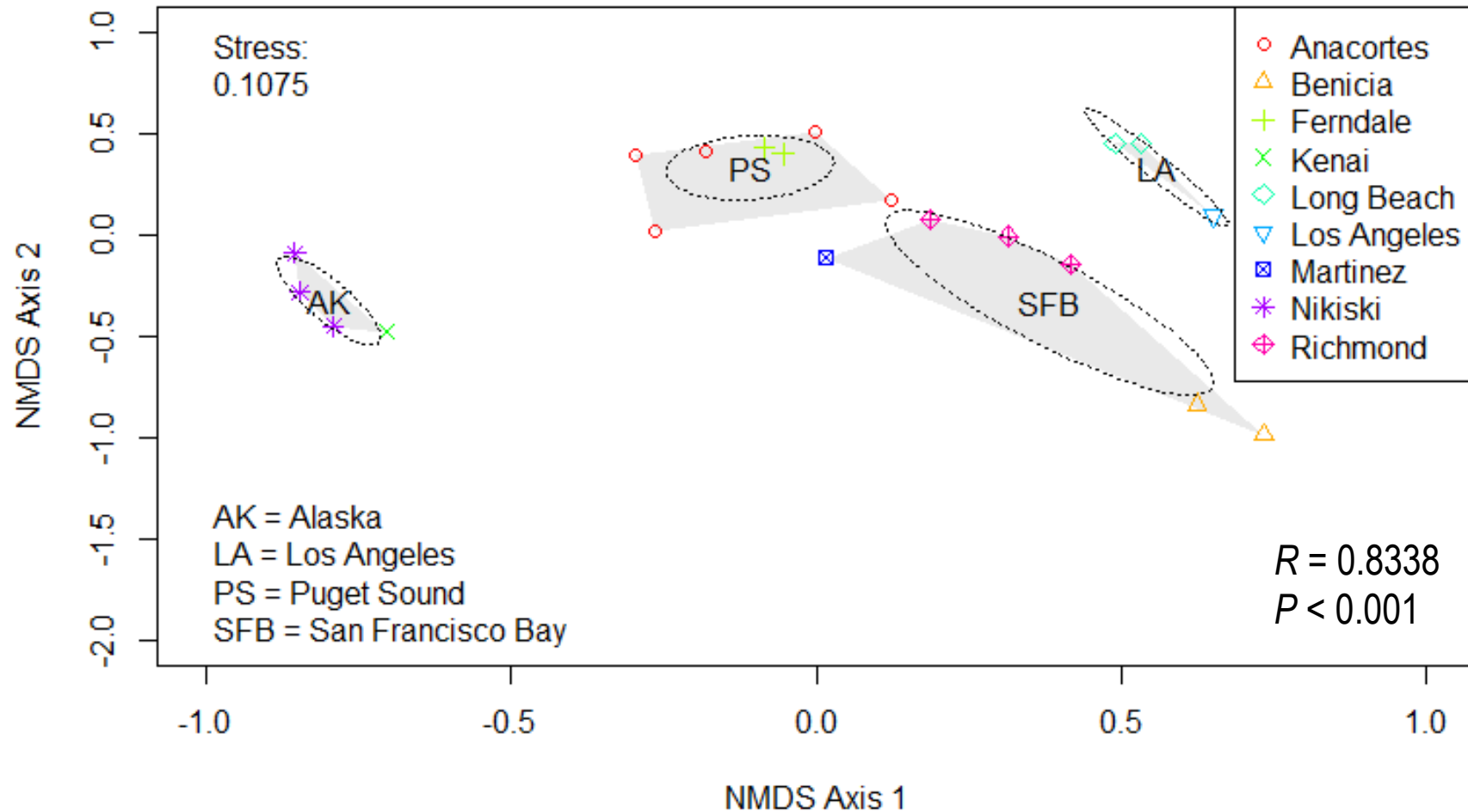
Effect of management on community structure



Effect of management on community structure



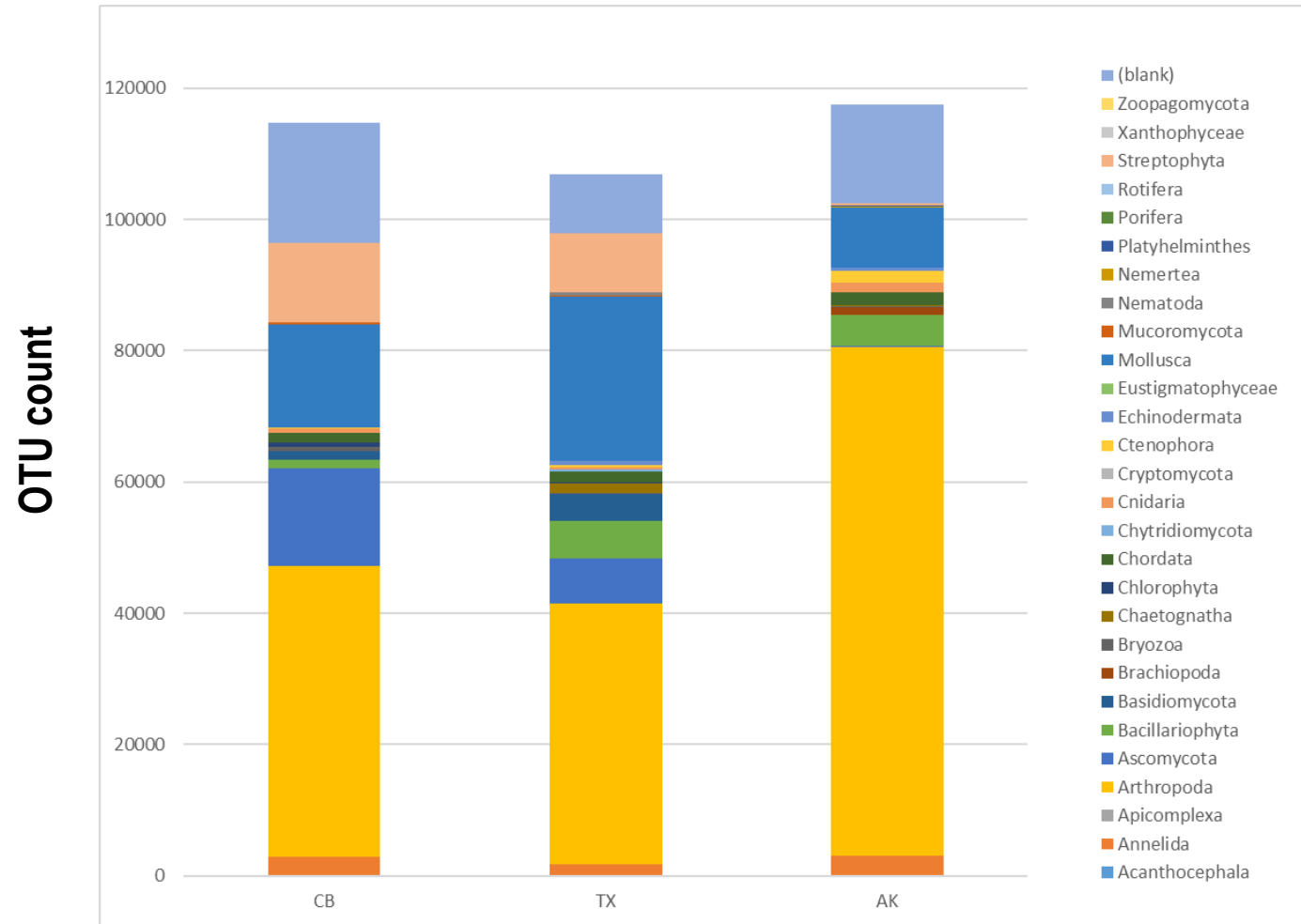
Effect of management on community structure



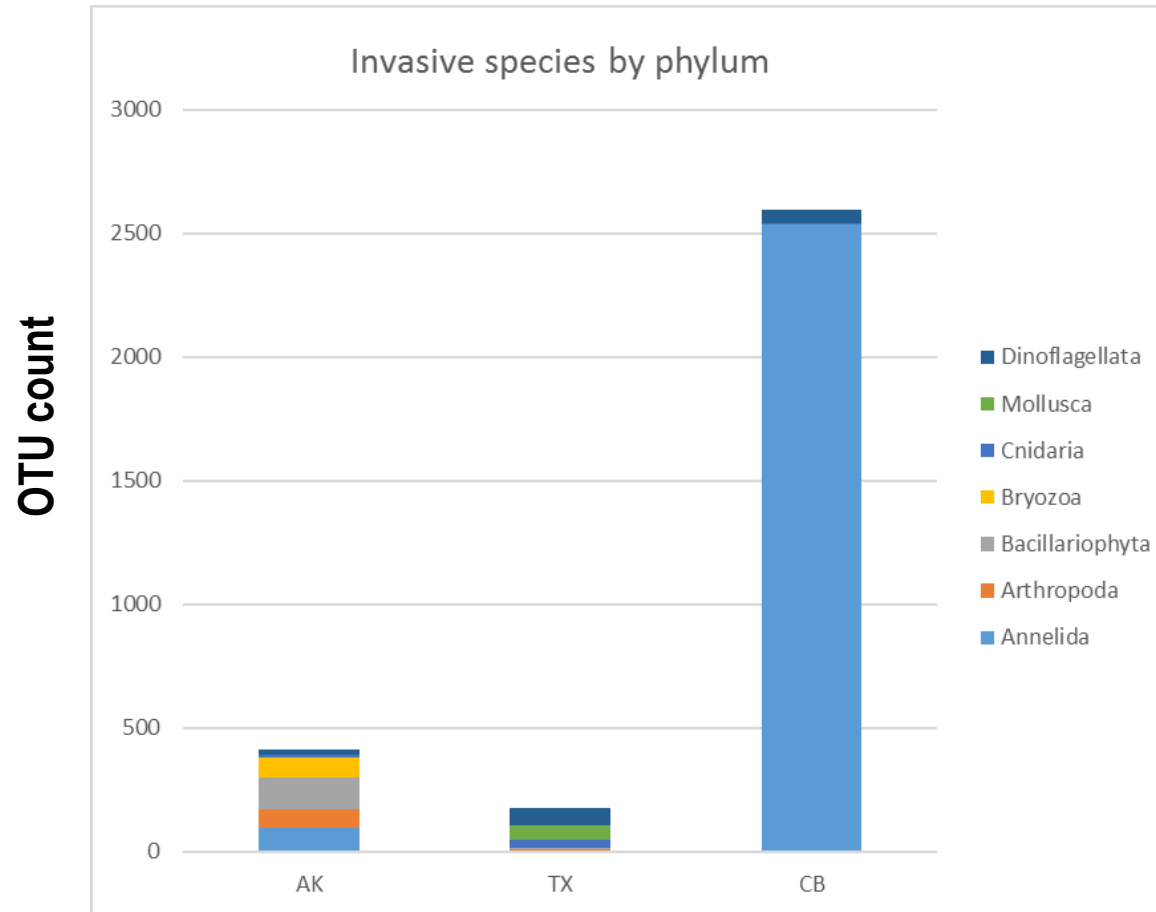
What's in the samples? How diverse are they? Are there any invasives?

DESCRIBING DIVERSITY

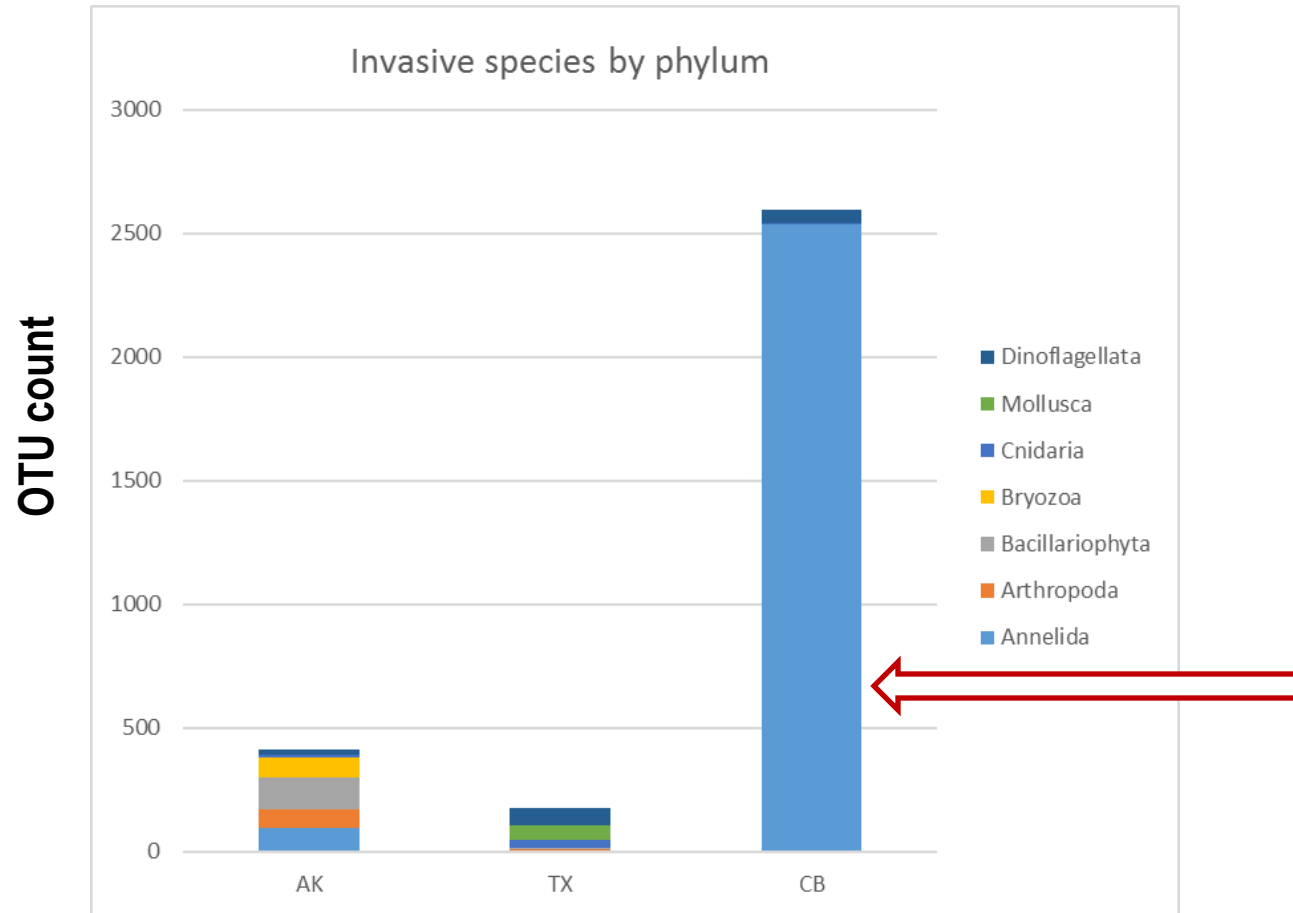
Total biodiversity (by Phylum)



Non-native biodiversity



Non-native biodiversity



Alitta succinea



Non-native biodiversity

PHYLUM	SPECIES	All18S	SSFU	Uni18S	COI	PHYLUM	SPECIES	All18S	SSFU	Uni18S	COI
Annelida	<i>Alitta succinea</i>	X		X		Cnidaria	<i>Diadumene leucolea</i>		X		
Annelida	<i>Hediste diversicolor</i>				X	Cnidaria	<i>Mnemiopsis leidyi</i>		X		
Annelida	<i>Myrianida pentadentata</i>			X	X	Cnidaria	<i>Obelia dichotoma</i>	X			X
Annelida	<i>Polydora cornuta</i>	X				Cnidaria	<i>Obelia geniculata</i>				X
Annelida	<i>Pseudopolydora paucibranchiata</i>	X	X	X		Cnidaria	<i>Obelia longissima</i>				X
Annelida	<i>Streblospio benedicti</i>	X				Dinoflagellata	<i>Alexandrium andersonii</i>		X		
Arthropoda	<i>Acartia longiremis</i>				X	Dinoflagellata	<i>Alexandrium monilatum</i>	X			
Arthropoda	<i>Acartia tonsa</i>	X	X	X	X	Dinoflagellata	<i>Alexandrium ostenfeldii</i>	X			
Arthropoda	<i>Callinectes bocourti</i>				X	Dinoflagellata	<i>Alexandrium tamarense</i>	X			
Arthropoda	<i>Eurytemora pacifica</i>	X	X			Dinoflagellata	<i>Alexandrium taylori</i>				X
Arthropoda	<i>Limnoithona tetraspina</i>	X				Dinoflagellata	<i>Ceratoperidinium falcatum</i>		X		
Arthropoda	<i>Megabalanus rosa</i>				X	Dinoflagellata	<i>Dinophysis caudata</i>		X		
Arthropoda	<i>Monocorophium insidiosum</i>				X	Dinoflagellata	<i>Gymnodinium aureolum</i>	X			
Arthropoda	<i>Oithona davisae</i>	X	X	X	X	Dinoflagellata	<i>Gymnodinium catenatum</i>		X		
Arthropoda	<i>Rhithropanopeus harrisii</i>				X	Dinoflagellata	<i>Karlodinium veneficum</i>	X	X		X
Bacillariophyta	<i>Coscinodiscus wailesii</i>		X			Dinoflagellata	<i>Levanderina fissa</i>		X		X
Bacillariophyta	<i>Ditylum brightwellii</i>	X	X			Dinoflagellata	<i>Phalacroma mitra</i>		X		
Bacillariophyta	<i>Heterosigma akashiwo</i>		X			Entoprocta	<i>Anguinella palmata</i>		X		
Bacillariophyta	<i>Mediopyxis helysia</i>	X				Entoprocta	<i>Barentsia benedeni</i>	X			
Bacillariophyta	<i>Rhizosolenia setigera</i>		X			Mollusca	<i>Balanus glandula</i>				X
Bacillariophyta	<i>Thalassiosira hendeyi</i>	X		X		Mollusca	<i>Chama macerophylla</i>	X			
Byozoa	<i>Conopeum seurati</i>	X				Mollusca	<i>Corambe obscura</i>	X			X
Byozoa	<i>Membranipora membranacea</i>		X	X	X	Mollusca	<i>Crassostrea virginica</i>		X		
Byozoa	<i>Styela clava</i>			X		Mollusca	<i>Crepidula onyx</i>				X
Chlorophyta	<i>Aegagropila linnaei</i>		X			Mollusca	<i>Martesia striata</i>	X	X		X
Chlorophyta	<i>Pterosperma cristatum</i>	X				Mollusca	<i>Mya arenaria</i>				X
Ciliophora	<i>Uronema marinum</i>	X				Mollusca	<i>Mytella charruana</i>		X		
Cnidaria	<i>Blackfordia virginica</i>	X				Mollusca	<i>Mytilopsis leucophaeata</i>	X			
Cnidaria	<i>Coryne eximia</i>			X		Mollusca	<i>Spurwinkia salsa</i>				X

Non-native biodiversity

Martesia striata



Sequences producing significant alignments:

Select: [All](#) [None](#) Selected:0

Alignments Download GenBank Graphics Distance tree of results						
	Description	Max score	Total score	Query cover	E value	Ident
<input type="checkbox"/>	Martesia striata voucher BivAToL-250 cytochrome c oxidase subunit I (COI) gene, partial cds; mit	414	414	99%	4e-112	100%
<input type="checkbox"/>	Martesia striata isolate MTHS-37 cytochrome oxidase subunit I (COI) gene, partial cds; mitochon	335	335	97%	3e-88	94%
<input type="checkbox"/>	Martesia striata cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	333	333	100%	1e-87	93%
<input type="checkbox"/>	Martesia striata isolate MTHS-35 cytochrome oxidase subunit I (COI) gene, partial cds; mitochon	329	329	97%	2e-86	94%
<input type="checkbox"/>	Selenops bifurcatus isolate 893ESSVic cytochrome c oxidase subunit I (COI) gene, partial cds; n	117	117	58%	1e-22	83%
<input type="checkbox"/>	Selenops bifurcatus isolate 882SSNMNH cytochrome c oxidase subunit I (COI) gene, partial cds;	117	117	58%	1e-22	83%
<input type="checkbox"/>	Turbo cidaris mitochondrial partial COI gene for cytochrome oxidase I, isolate TCID.RUF.1	115	115	80%	5e-22	78%
<input type="checkbox"/>	Echinolittorina radiata haplotype Dm04 cytochrome oxidase subunit I (COI) gene, partial cds; mit	111	111	64%	6e-21	81%
<input type="checkbox"/>	Selenops bifurcatus isolate 967NicJic cytochrome c oxidase subunit I (COI) gene, partial cds; mit	111	111	58%	6e-21	82%
<input type="checkbox"/>	Selenops bifurcatus isolate 964NicJic cytochrome c oxidase subunit I (COI) gene, partial cds; mit	111	111	58%	6e-21	82%

Non-native biodiversity

Hediste diversicolor



Sequences producing significant alignments:

Select: [All](#) [None](#) Selected:0

Alignments Download GenBank Graphics Distance tree of results						
	Description	Max score	Total score	Query cover	E value	Ident
<input type="checkbox"/>	Hediste diversicolor voucher WCH_0118 cytochrome oxidase subunit 1 (COI) gene, partial cds; m	405	405	100%	3e-109	99%
<input type="checkbox"/>	Alitta succinea voucher SERCINVERT0102 cytochrome oxidase subunit 1 (COI) gene, partial cds	394	394	100%	6e-106	98%
<input type="checkbox"/>	Alitta succinea voucher SERCINVERT0507 cytochrome oxidase subunit 1 (COI) gene, partial cds	388	388	100%	3e-104	98%
<input type="checkbox"/>	Alitta succinea voucher SERCINVERT0101 cytochrome oxidase subunit 1 (COI) gene, partial cds	383	383	100%	1e-102	97%
<input type="checkbox"/>	Hediste diversicolor voucher WCH_0266 cytochrome oxidase subunit 1 (COI) gene, partial cds; m	383	383	100%	1e-102	97%
<input type="checkbox"/>	Alitta succinea voucher SERCINVERT0504 cytochrome oxidase subunit 1 (COI) gene, partial cds	375	375	99%	2e-100	97%
<input type="checkbox"/>	Alitta succinea voucher SERCINVERT0505 cytochrome oxidase subunit 1 (COI) gene, partial cds	372	372	100%	3e-99	96%
<input type="checkbox"/>	Alitta succinea voucher SERCINVERT0506 cytochrome oxidase subunit 1 (COI) gene, partial cds	372	372	100%	3e-99	96%
<input type="checkbox"/>	Alitta succinea voucher SERCINVERT0508 cytochrome oxidase subunit 1 (COI) gene, partial cds	372	372	100%	3e-99	96%
<input type="checkbox"/>	Alitta succinea isolate DNAS-4E-32786 cytochrome c oxidase subunit I (COI) gene, partial cds; m	315	315	80%	4e-82	98%



Non-native biodiversity

Hediste diversicolor



Results Summary

[Download](#)

Query ID	Best ID	Search DB	Tree	Top %	Graph	Low %
OTU853	<i>Alitta succinea</i>	COI SPECIES DATABASE		100.00		82.69

Query: OTU853

Top Hit: Annelida Polychaeta - Phyllodocida - *Alitta succinea* (100%)

Search Result:

A species level match could not be made, the queried specimen is likely to be one of the following:

Alitta succinea
Alitta limbata
Alitta saltoni
Hediste diversicolor
Alitta sp. 2

For a hierarchical placement - a neighbor-joining tree is provided:

[TREE BASED IDENTIFICATION](#)

Challenges for identifying invasive species

- How should we interpret differences between loci?
- Importance of assessing accuracy of taxonomic assignment
- Which reference lists should be used?
- Should there be a sequence abundance threshold?

Potential Applications of HTS

- Understanding patterns of biotic connectivity
- Investigating the variables that determine the rate of biodiversity accumulation in recipient ports
- Assessing the effects of ballast water management practices (exchange or treatment)
- Early detection to prioritize future surveillance efforts?

STOP BALLAST WATER INVASIONS



PERFORM OPEN OCEAN BALLAST EXCHANGE IF SAFETY PERMITS

KEEP RECORDS OF BALLASTING OPERATIONS

REDUCE INVASIONS VIA HULL AND ANCHOR FOULING

MINIMIZE BALLASTING IN PORTS AND COASTAL AREAS

AVOID BALLAST UPTAKE AT NIGHT


AVOID BALLAST UPTAKE IN "HOT SPOTS"

INVASIVE SPECIES

ASIAN CLAM
Asian clams now dominate San Francisco Bay, having replaced other bivalves and altered the estuary's delicate food web.



NORTHERN PACIFIC SEASTAR
This voracious predator from Japan threatens Tasmannian shellfish industry.



CHOLERA BACTERIA
Ballast water brought this deadly waterborne disease to the Gulf of Mexico from South America in the early 1990s.



TOXIC DINOFLAGELLATES
Japanese dinoflagellates, microscopic organisms, invaded Australian waters causing paralytic shellfish poisoning and closure of shellfish beds in 1986.



CHINESE MITTEN CRAB
Hijacking Chinese mitten crabs, introduced to SF Bay in the early 1960s, have clogged California's water delivery facilities and fish salvage operations.



NORTH AMERICAN COMB JELLY
The voracious, Atlantic jellyfish introduced to the Black Sea and Sea of Azov in the late 1980s has decimated anchovy fisheries.



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