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Biodiversity entering US ports via ballast water discharge

An analysis using High Throughput Sequencing

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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?

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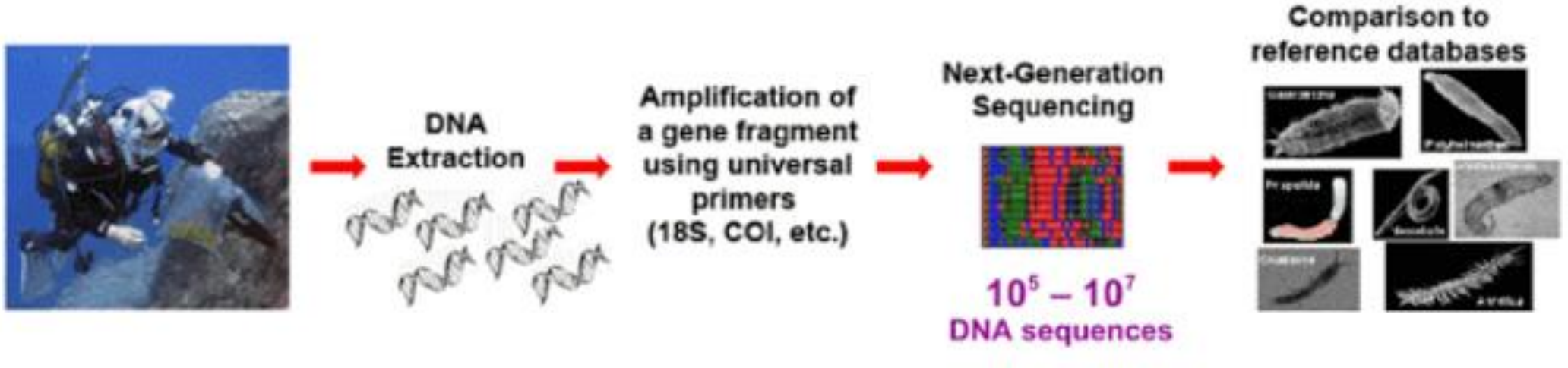
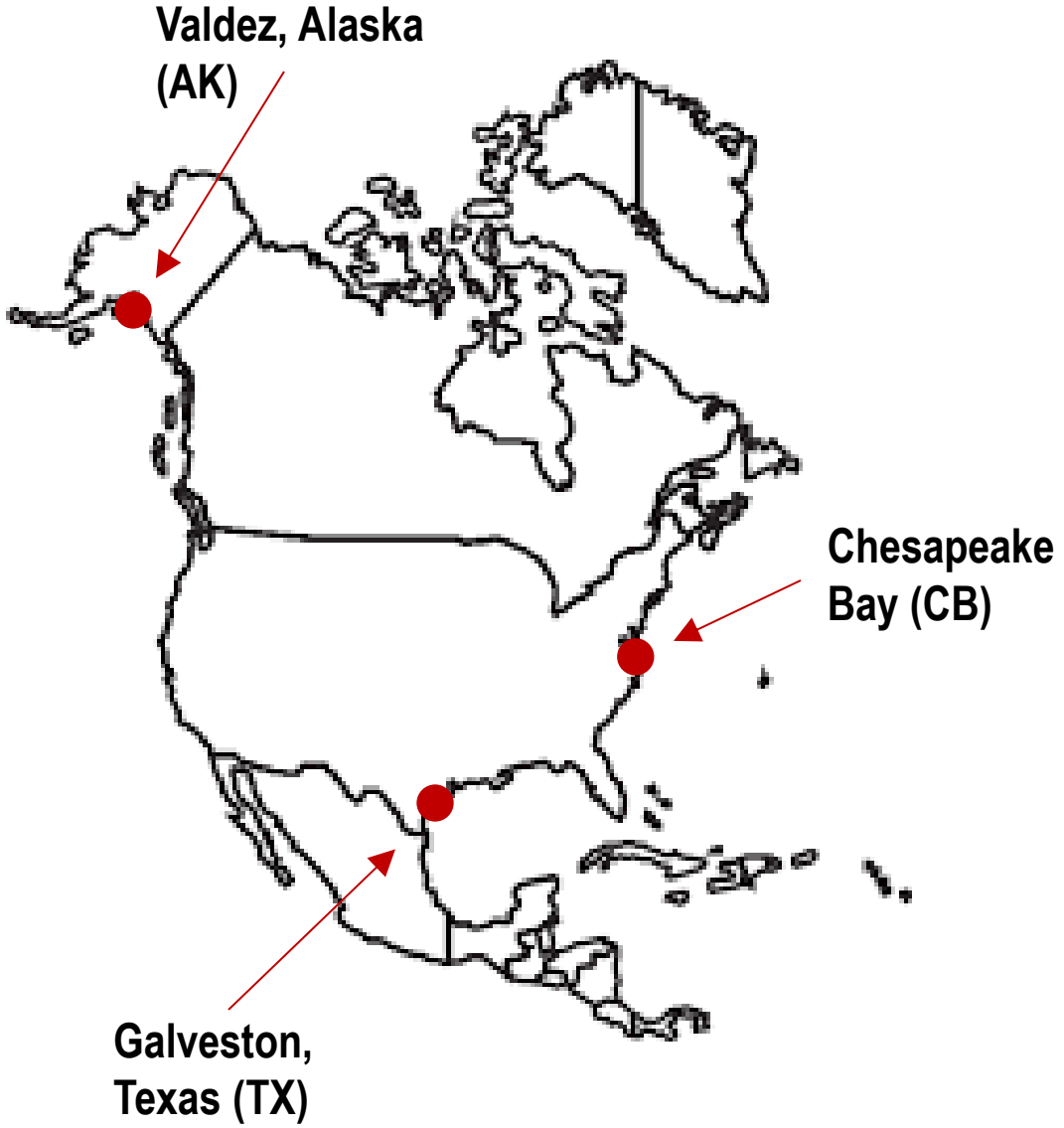
DNA Meta-barcoding



The Samples

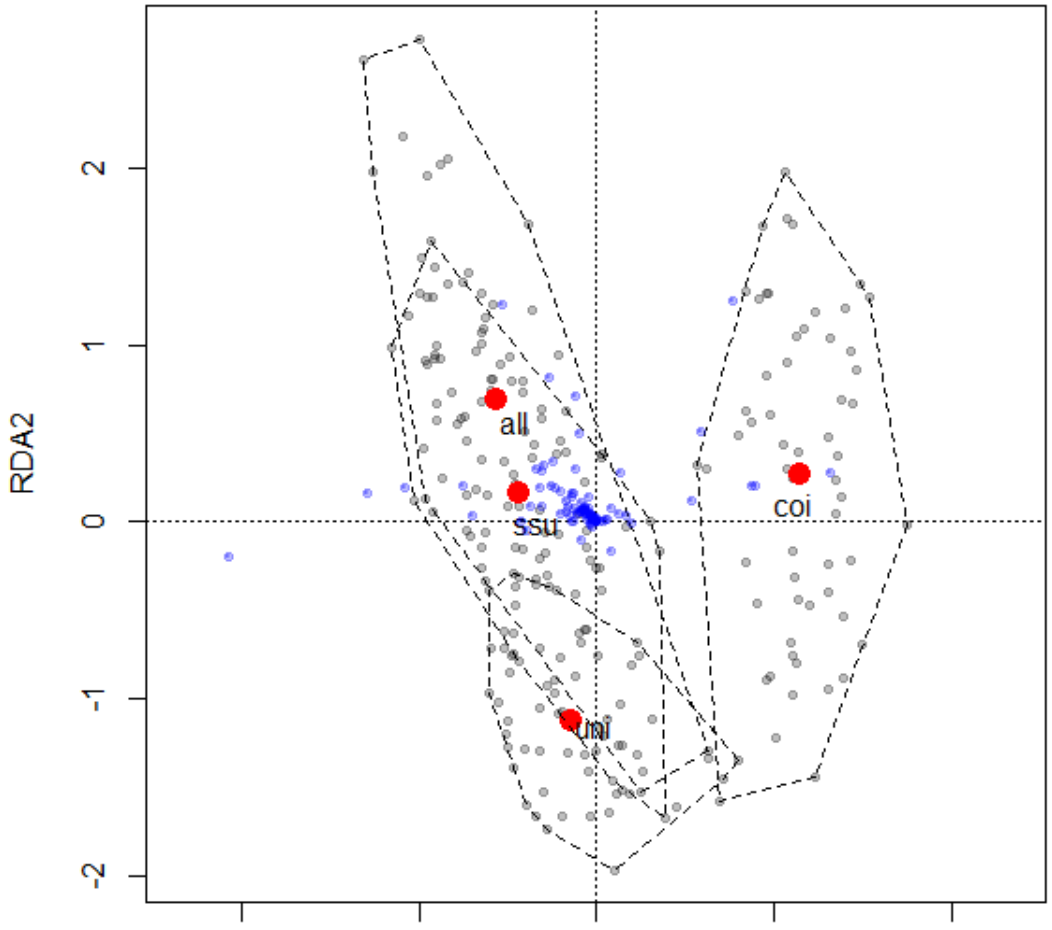
- Chesapeake: 51 samples sourced from 32 ports in 12 countries, mostly Europe and North America
- Texas: 45 samples from 32 ports in 16 countries, mostly North and South America
- Alaska: 47 samples from 14 ports, all in the US
- Sequence generated from 3 18S loci and COI



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

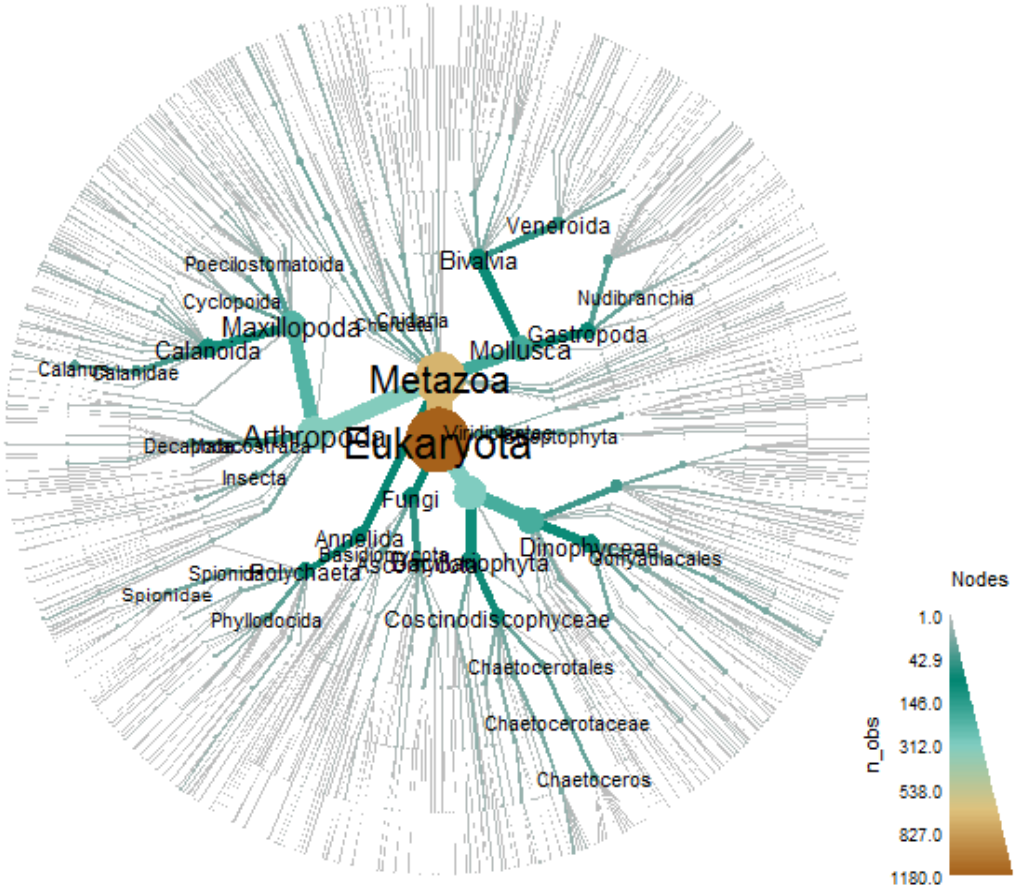
EXAMINING THE METHOD

Effect of primer on community structure

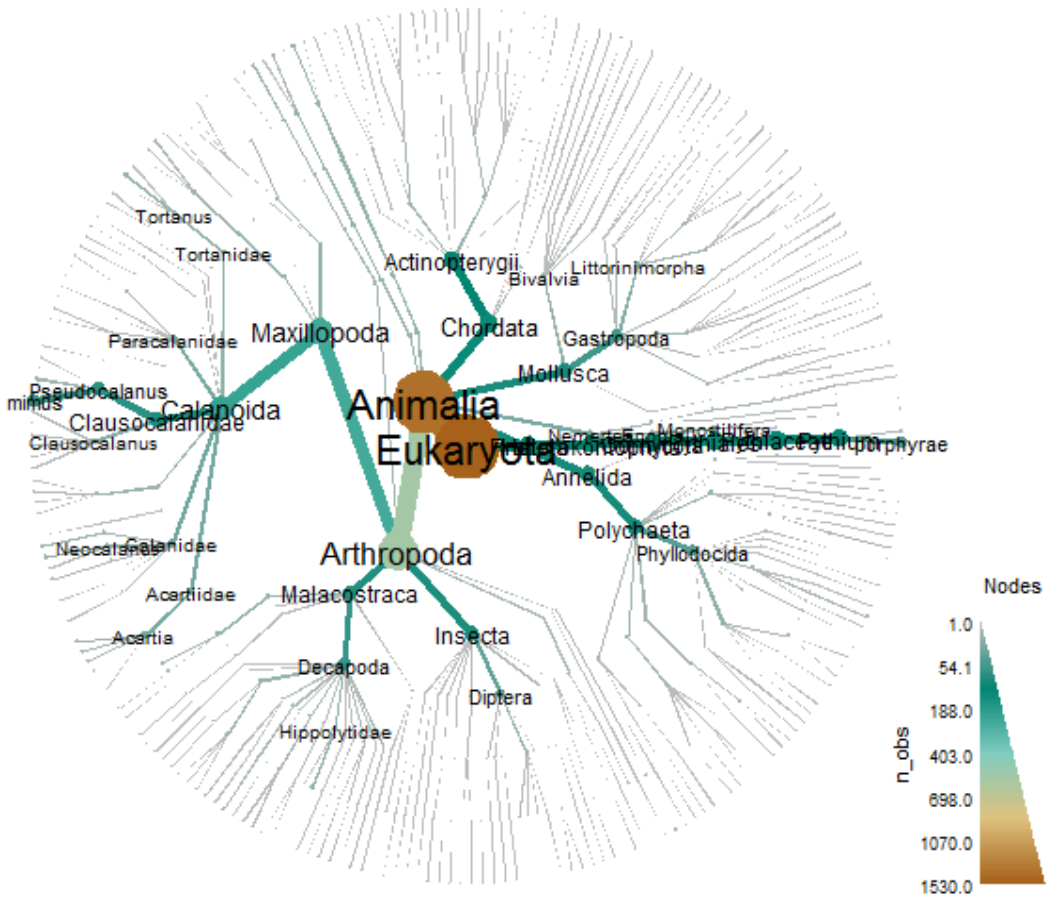


Effect of primer on community structure

SSFU (18S)



COI



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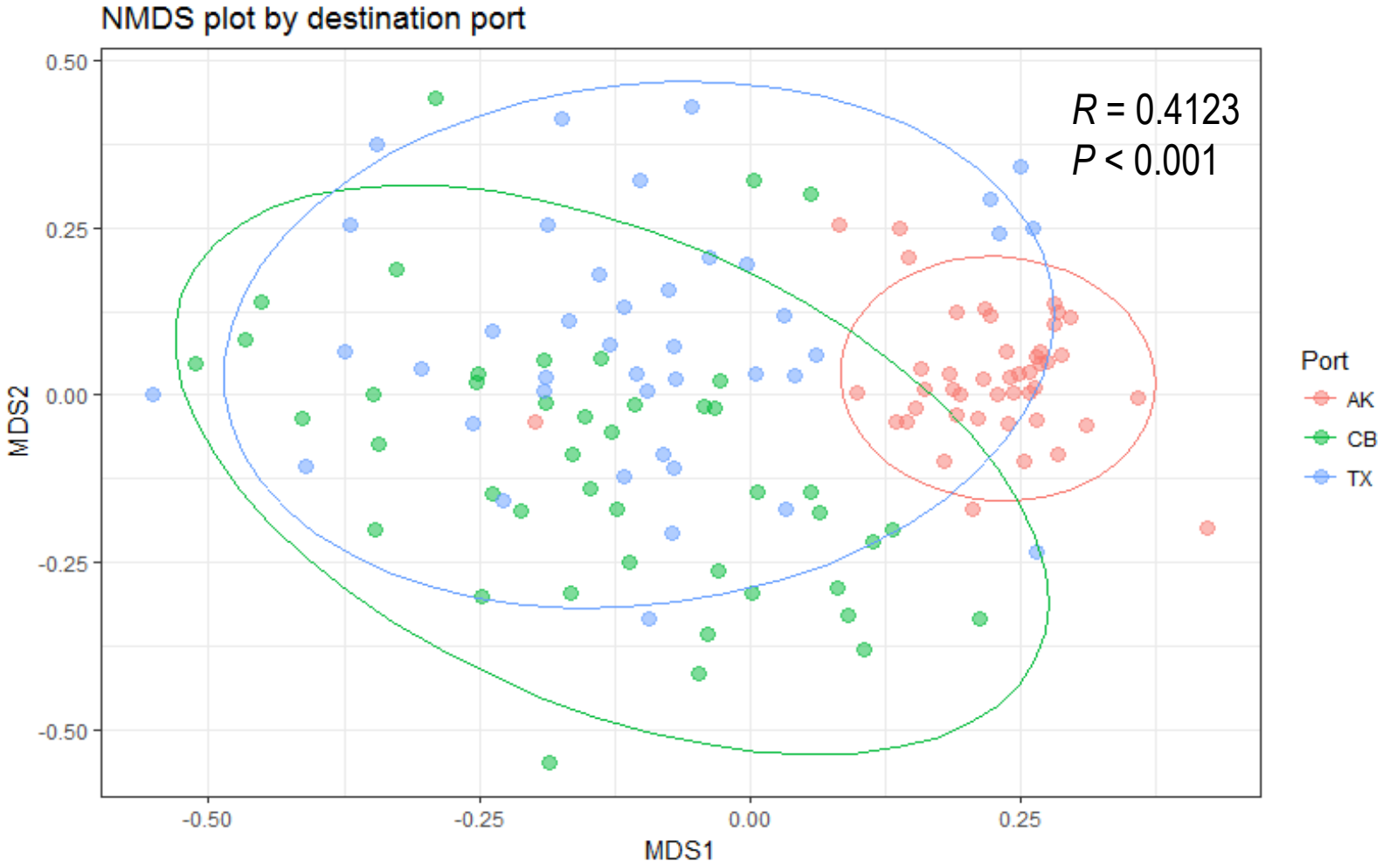
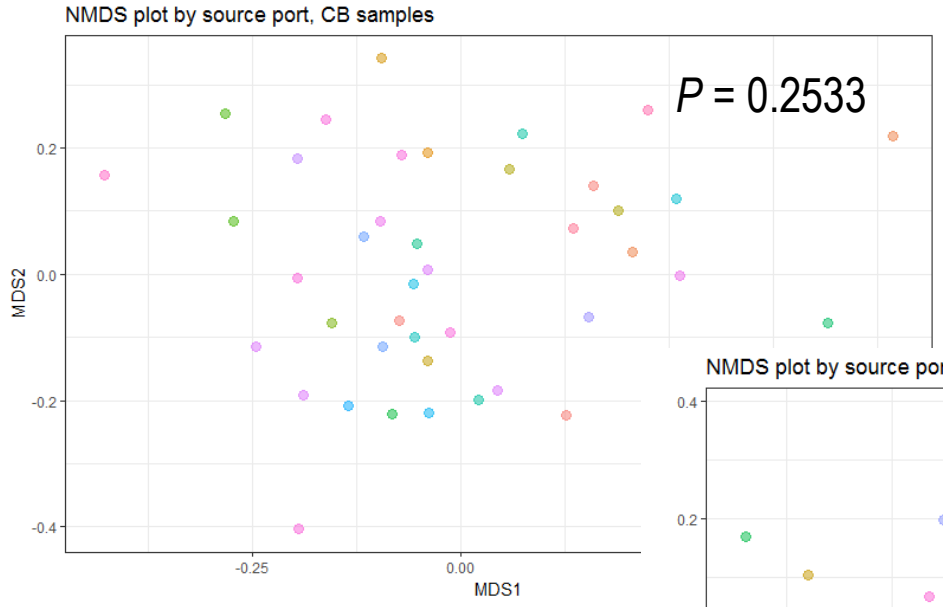
What are the drivers of differences in ballast water communities?

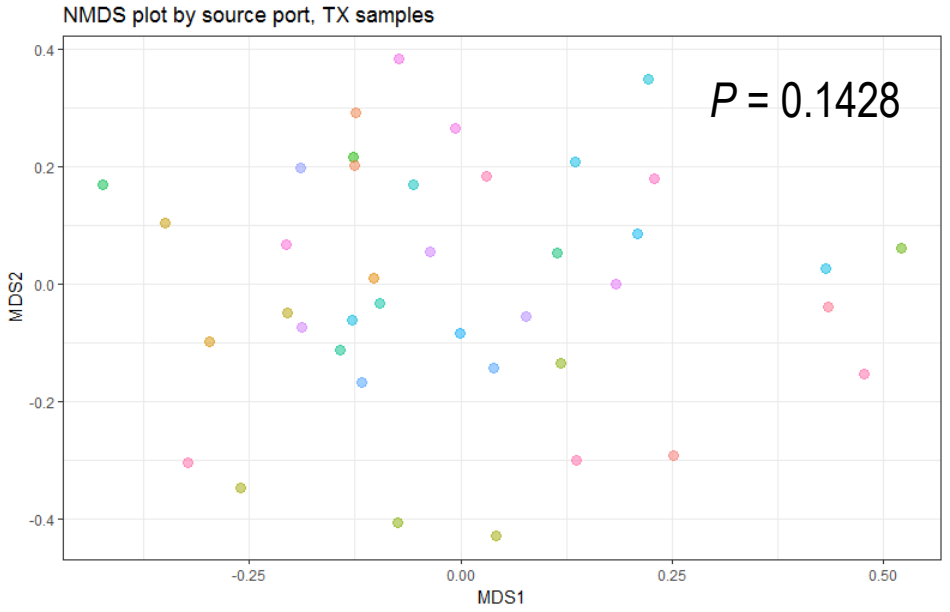
COMPARING DIVERSITY ACROSS SAMPLES

Effect of source region on community structure

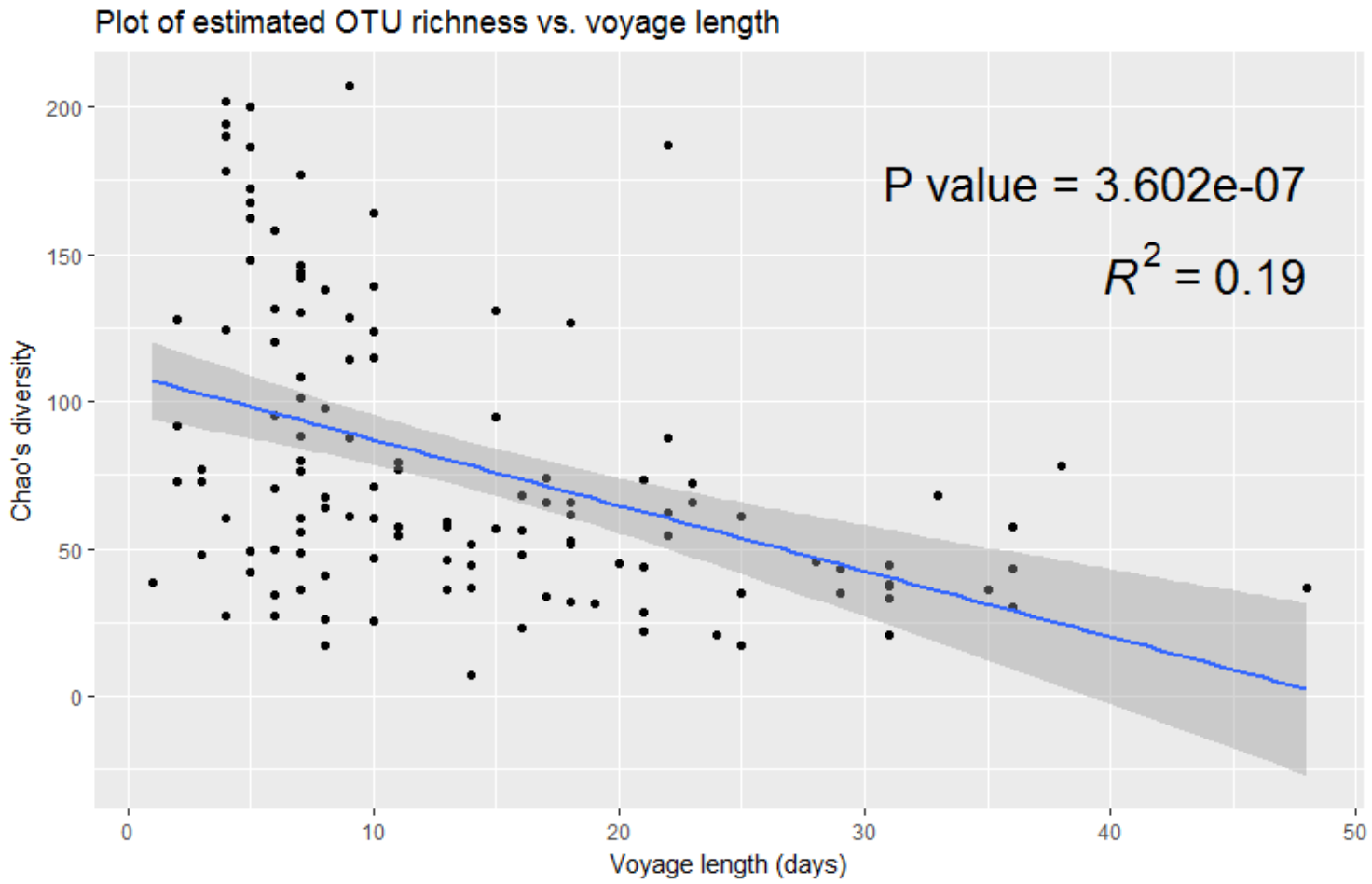


Effect of source region on community structure

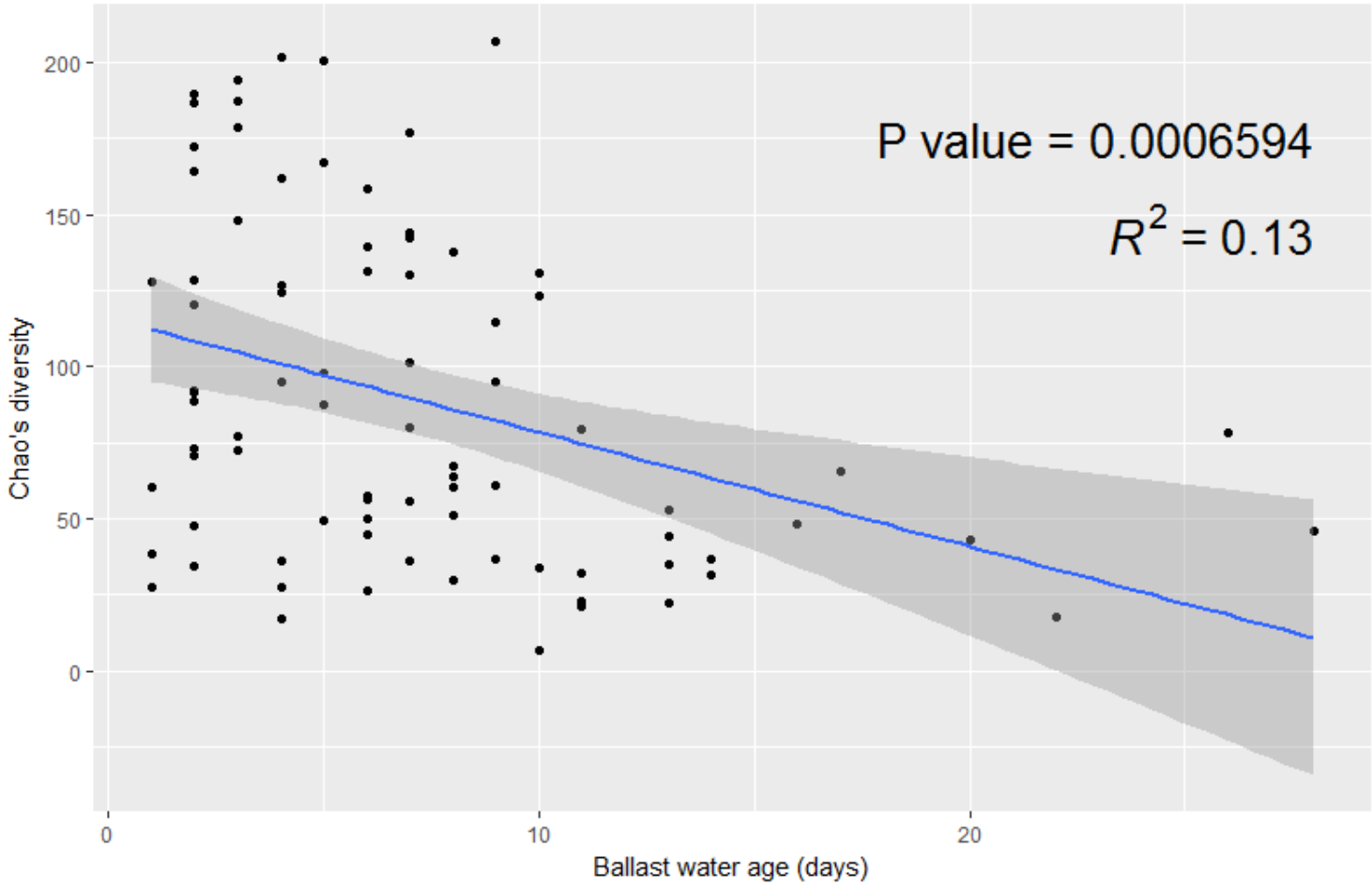




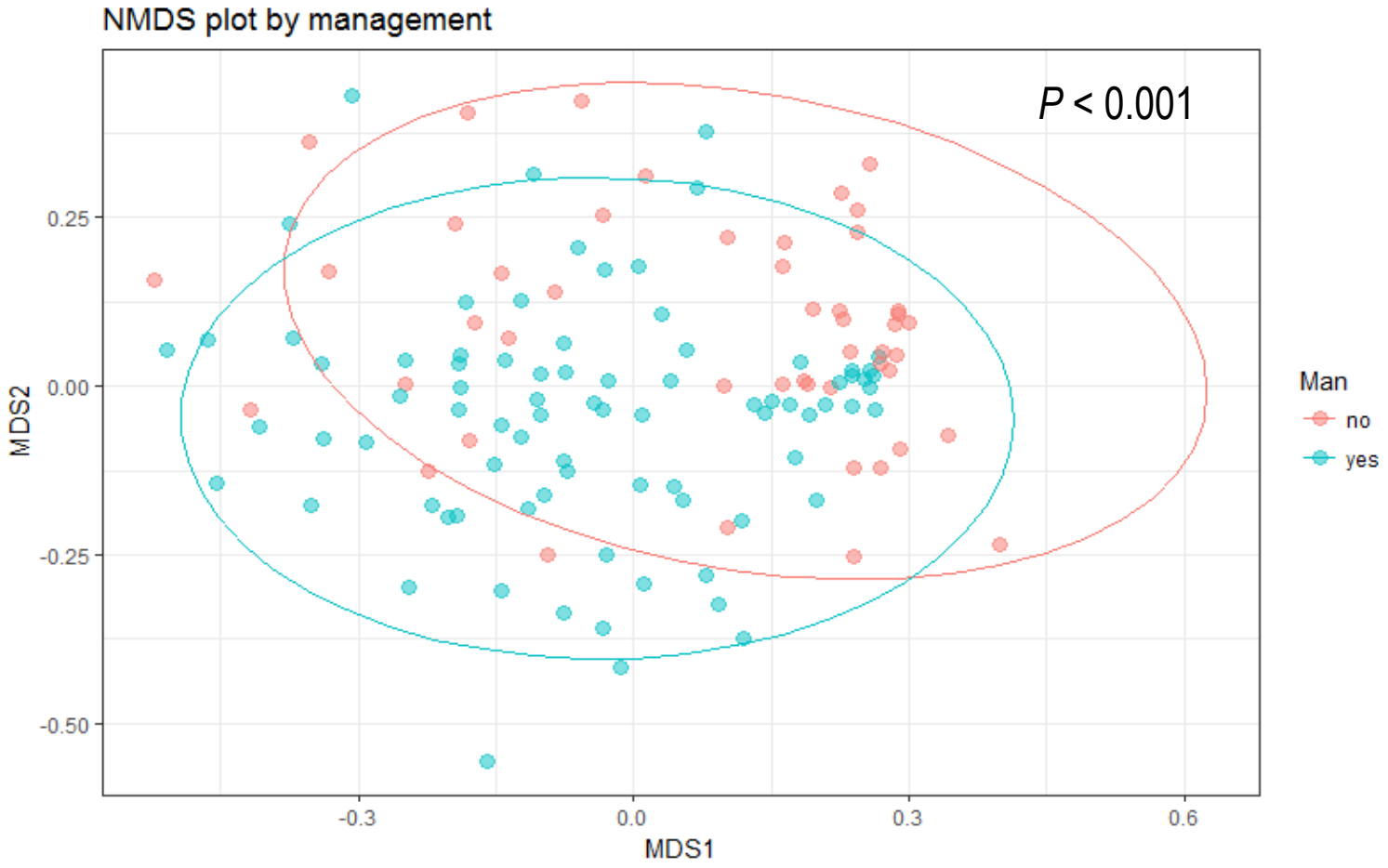
Effect of source region on community structure



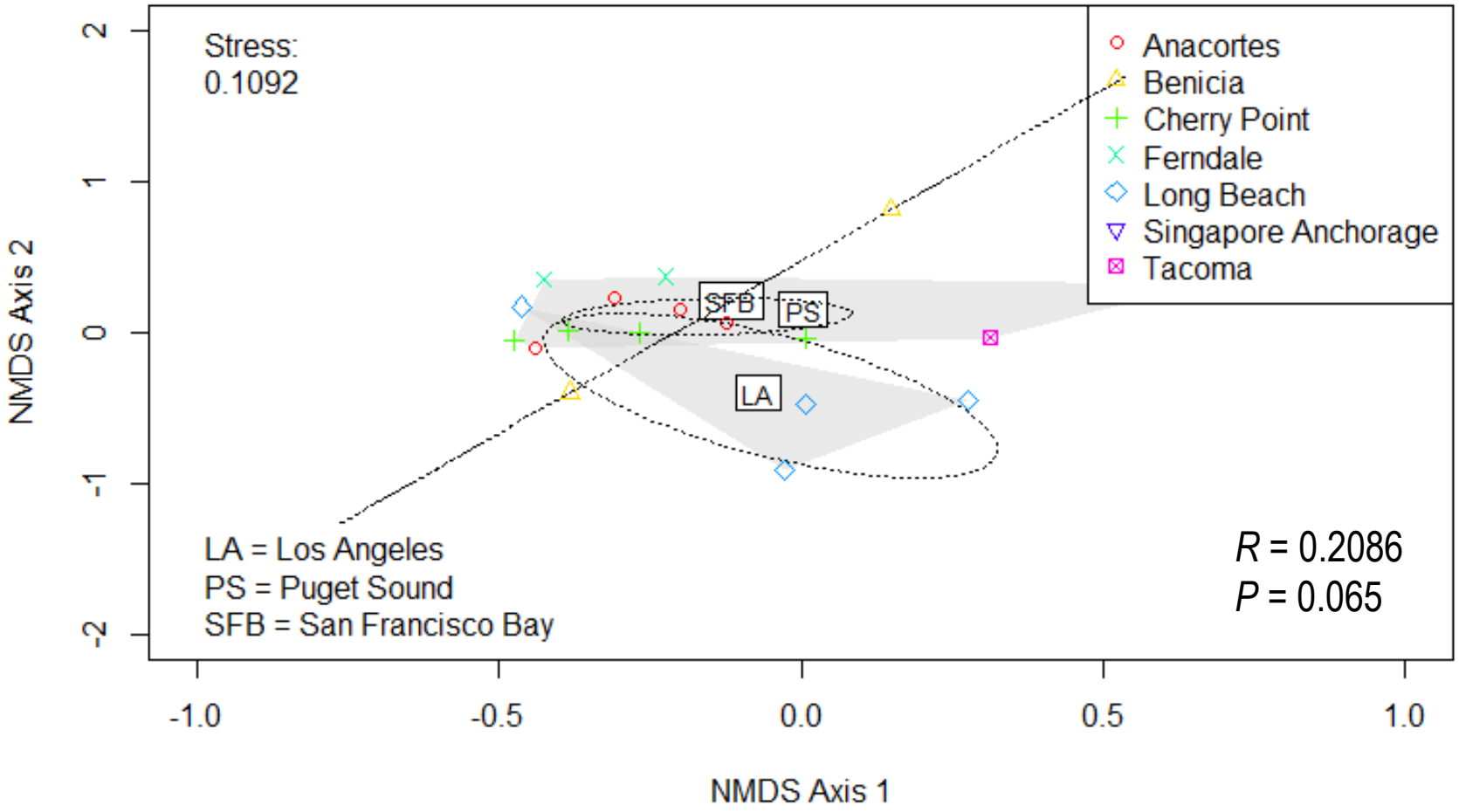
Effect of source region on community structure



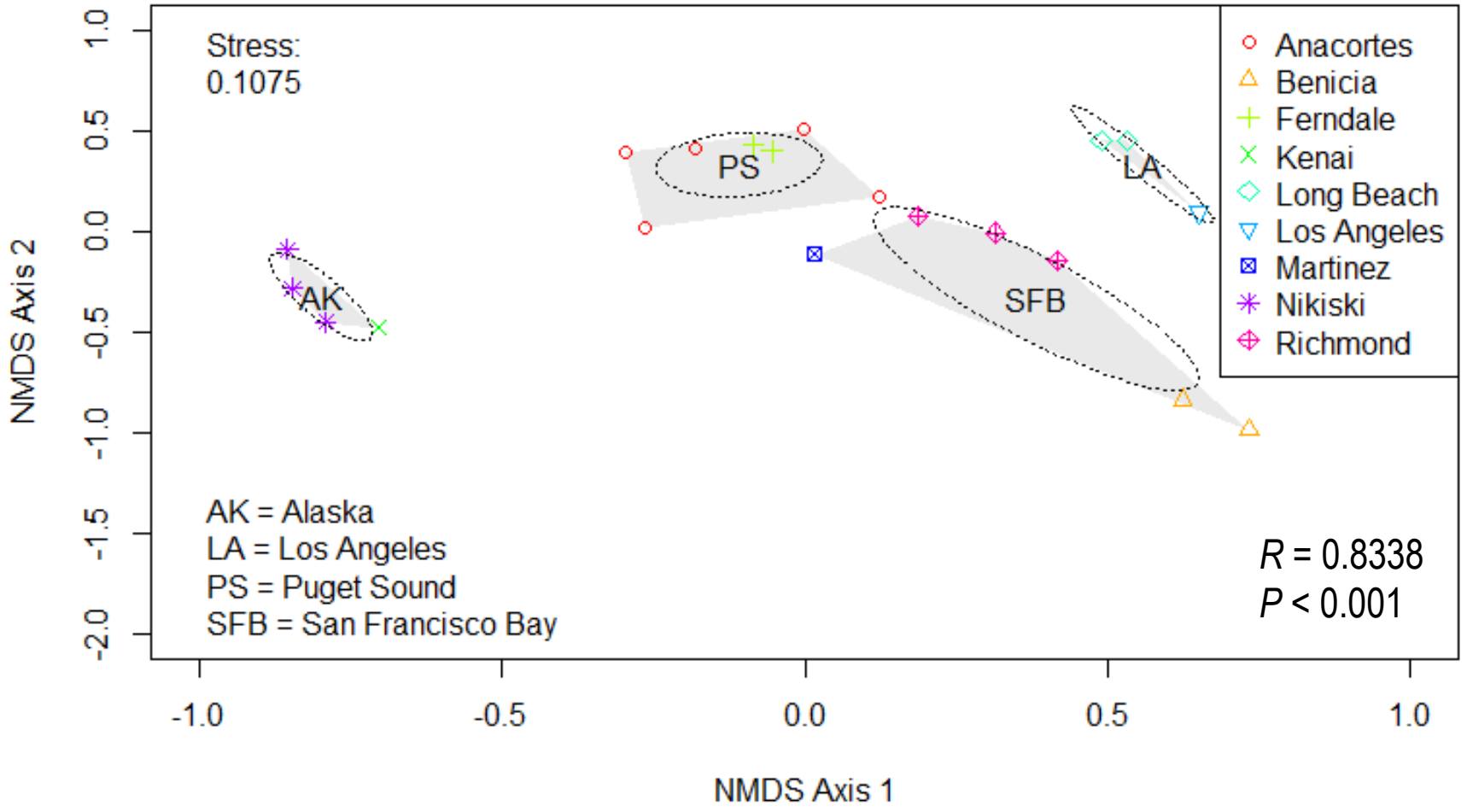
Effect of management on community structure



Effect of management on community structure



Effect of management on community structure



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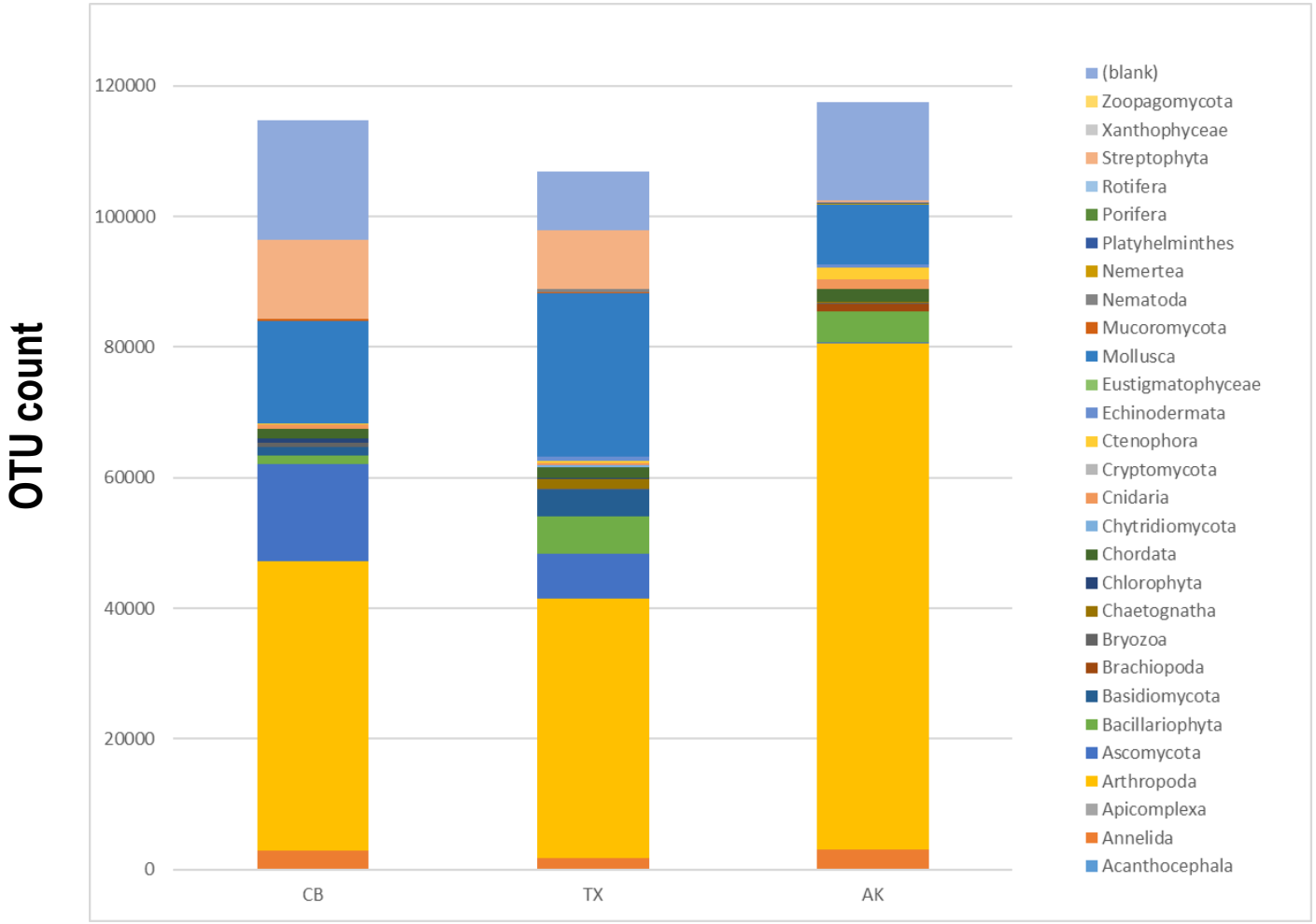
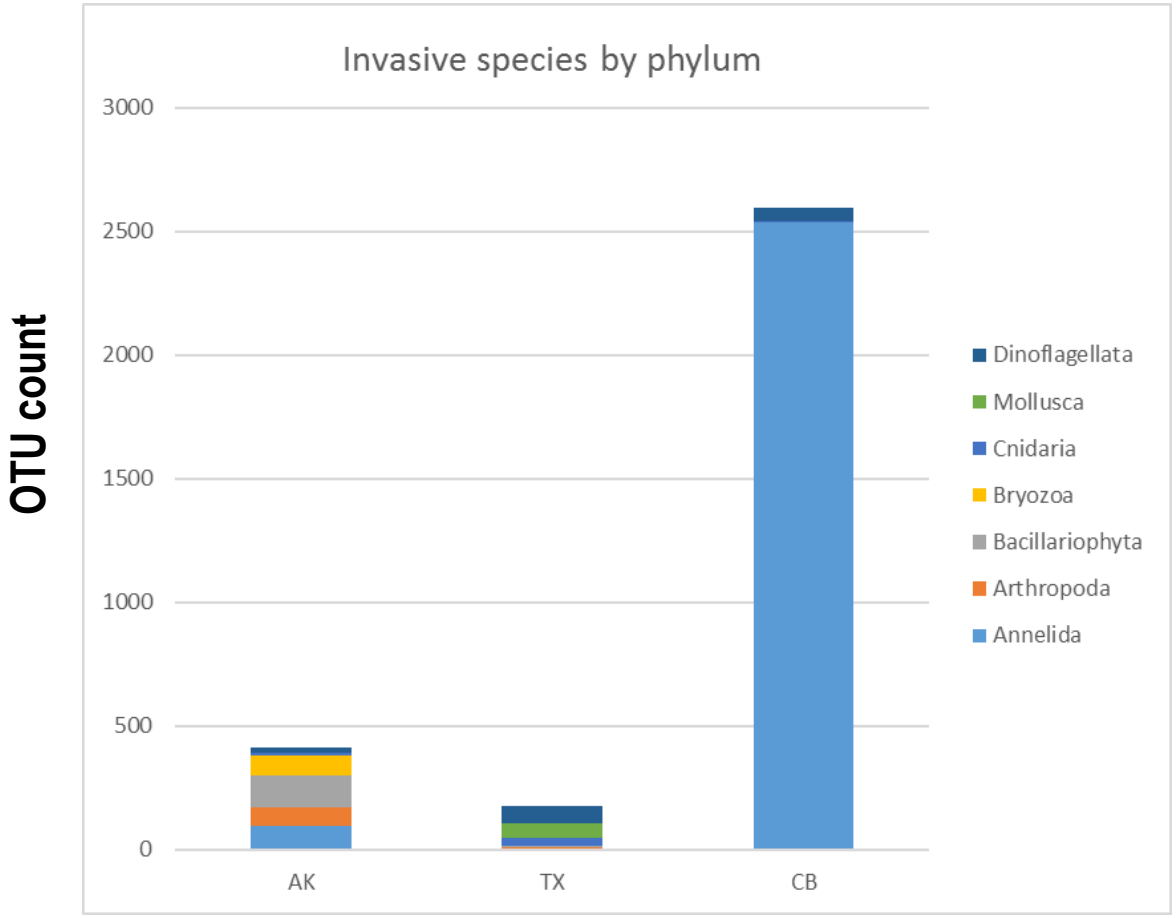
What's in the samples? How diverse are they? Are there any invasives?

DESCRIBING DIVERSITY

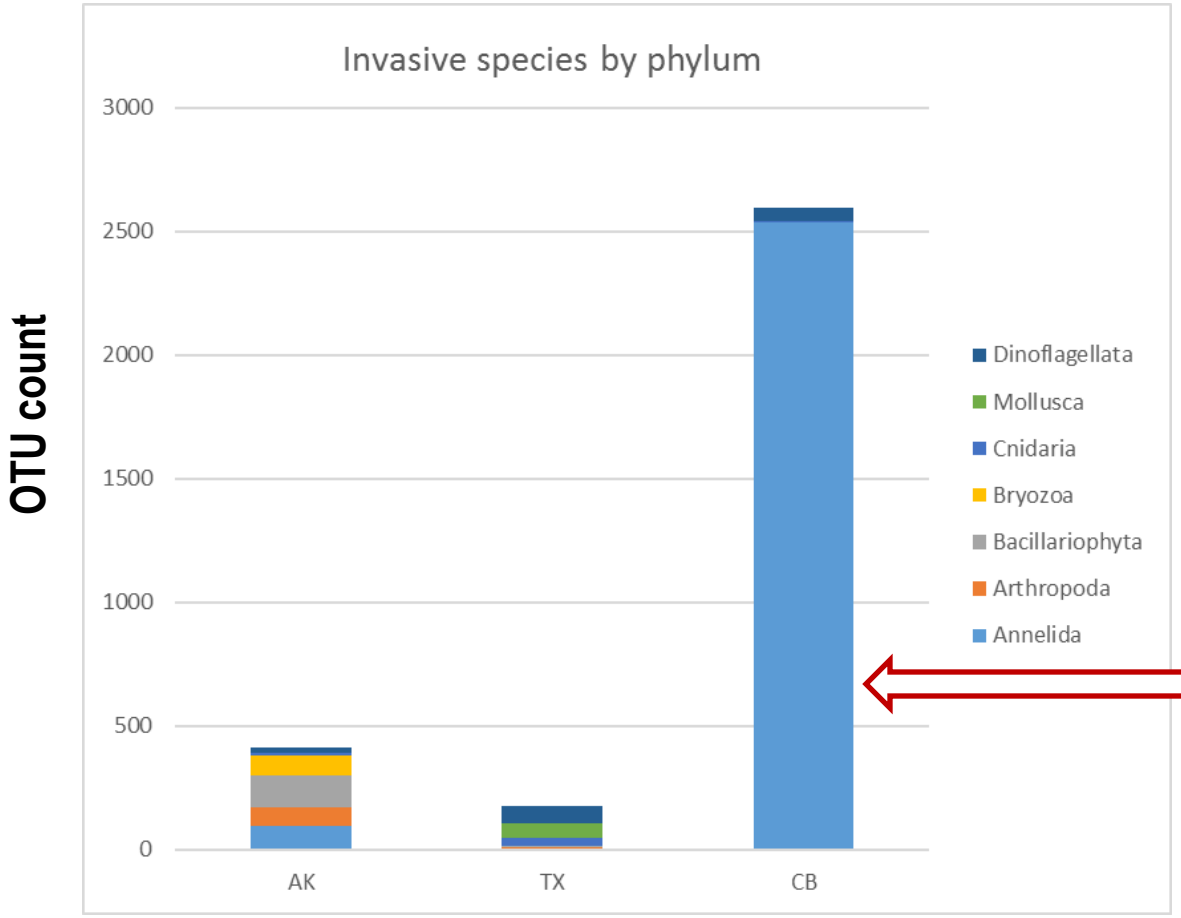
Total biodiversity (by Phylum)



Non-native biodiversity



Non-native biodiversity



Alitta succinea

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Non-native biodiversity

PHYLUM	SPECIES	All18S	SSFU	Uni18S	COI
Annelida	*Alitta succinea*	X		X	
Annelida	*Hediste diversicolor*				X
Annelida	*Myrianida pentadentata*			X	X
Annelida	*Polydora cornuta*	X			
Annelida	*Pseudopolydora paucibranchiata*	X	X	X	
Annelida	*Streblospio benedicti*	X			
Arthropoda	*Acartia longiremis*				X
Arthropoda	*Acartia tonsa*	X	X	X	X
Arthropoda	*Callinectes bocourti*				X
Arthropoda	*Eurytemora pacifica*	X	X		
Arthropoda	*Limnoithona tetraspina*	X			
Arthropoda	*Megabalanus rosa*				X
Arthropoda	*Monocorophium insidiosum*				X
Arthropoda	*Oithona davisae*	X	X	X	X
Arthropoda	*Rhithropanopeus harrisii*				X
Bacillariophyta	*Coscinodiscus wailesii*		X		
Bacillariophyta	*Ditylum brightwellii*	X	X		
Bacillariophyta	*Heterosigma akashiwo*		X		
Bacillariophyta	*Mediopyxis helysia*	X			
Bacillariophyta	*Rhizosolenia setigera*		X		
Bacillariophyta	*Thalassiosira hendeyi*	X		X	
Byozoa	*Conopeum seurati*	X			
Byozoa	*Membranipora membranacea*		X	X	X
Byozoa	*Styela clava*			X	
Chlorophyta	*Aegagropila linnaei*		X		
Chlorophyta	*Pterosperma cristatum*	X			
Ciliophora	*Uronema marinum*	X			
Cnidaria	*Blackfordia virginica*	X			
Cnidaria	*Coryne eximia*			X	

PHYLUM	SPECIES	All18S	SSFU	Uni18S	COI
Cnidaria	*Diadumene leucolena*		X		
Cnidaria	*Mnemiopsis leidyi*		X		
Cnidaria	*Obelia dichotoma*	X		X	
Cnidaria	*Obelia geniculata*			X	
Cnidaria	*Obelia longissima*			X	
Dinoflagellata	*Alexandrium andersonii*		X		
Dinoflagellata	*Alexandrium monilatum*	X			
Dinoflagellata	*Alexandrium ostenfeldii*	X			
Dinoflagellata	*Alexandrium tamarense*	X			
Dinoflagellata	*Alexandrium taylori*			X	
Dinoflagellata	*Ceratoperidinium falcatum*		X		
Dinoflagellata	*Dinophysis caudata*		X		
Dinoflagellata	*Gymnodinium aureolum*	X			
Dinoflagellata	*Gymnodinium catenatum*		X		
Dinoflagellata	*Karlodinium veneficum*	X	X	X	
Dinoflagellata	*Levanderina fissa*		X	X	
Dinoflagellata	*Phalacroma mitra*		X		
Entoprocta	*Anguinella palmata*		X		
Entoprocta	*Barentsia benedeni*	X			
Mollusca	*Balanus glandula*				X
Mollusca	*Chama macerophylla*	X			
Mollusca	*Corambe obscura*	X			X
Mollusca	*Crassostrea virginica*		X		
Mollusca	*Crepidula onyx*				X
Mollusca	*Martesia striata*	X	X		X
Mollusca	*Mya arenaria*				X
Mollusca	*Mytella charruana*		X		
Mollusca	*Mytilopsis leucophaeata*	X			
Mollusca	*Spurwinkia salsa*				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
Martesia striata voucher BivAToL-250 cytochrome c oxidase subunit I (COI) gene, partial cds; mit	414	414	99%	4e-112	100%
Martesia striata isolate MTHS-37 cytochrome oxidase subunit I (COI) gene, partial cds; mitochon	335	335	97%	3e-88	94%
Martesia striata cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	333	333	100%	1e-87	93%
Martesia striata isolate MTHS-35 cytochrome oxidase subunit I (COI) gene, partial cds; mitochon	329	329	97%	2e-86	94%
Selenops bifurcatus isolate 893ESSVic cytochrome c oxidase subunit I (COI) gene, partial cds; n	117	117	58%	1e-22	83%
Selenops bifurcatus isolate 882SSNMNH cytochrome c oxidase subunit I (COI) gene, partial cds;	117	117	58%	1e-22	83%
Turbo cidaris mitochondrial partial COI gene for cytochrome oxidase I, isolate TCID.RUF.1	115	115	80%	5e-22	78%
Echinolittorina radiata haplotype Dm04 cytochrome oxidase subunit I (COI) gene, partial cds; mit	111	111	64%	6e-21	81%
Selenops bifurcatus isolate 967NicJic cytochrome c oxidase subunit I (COI) gene, partial cds; mit	111	111	58%	6e-21	82%
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
Hediste diversicolor voucher WCH_0118 cytochrome oxidase subunit 1 (COI) gene, partial cds; m	405	405	100%	3e-109	99%
Alitta succinea voucher SERCINVERT0102 cytochrome oxidase subunit 1 (COI) gene, partial cds	394	394	100%	6e-106	98%
Alitta succinea voucher SERCINVERT0507 cytochrome oxidase subunit 1 (COI) gene, partial cds	388	388	100%	3e-104	98%
Alitta succinea voucher SERCINVERT0101 cytochrome oxidase subunit 1 (COI) gene, partial cds	383	383	100%	1e-102	97%
Hediste diversicolor voucher WCH_0266 cytochrome oxidase subunit 1 (COI) gene, partial cds; m	383	383	100%	1e-102	97%
Alitta succinea voucher SERCINVERT0504 cytochrome oxidase subunit 1 (COI) gene, partial cds	375	375	99%	2e-100	97%
Alitta succinea voucher SERCINVERT0505 cytochrome oxidase subunit 1 (COI) gene, partial cds	372	372	100%	3e-99	96%
Alitta succinea voucher SERCINVERT0506 cytochrome oxidase subunit 1 (COI) gene, partial cds	372	372	100%	3e-99	96%
Alitta succinea voucher SERCINVERT0508 cytochrome oxidase subunit 1 (COI) gene, partial cds	372	372	100%	3e-99	96%
Alitta succinea isolate DNAS-4E-32786 cytochrome c oxidase subunit I (COI) gene, partial cds; m	315	315	80%	4e-82	98%

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Non-native biodiversity
Hediste diversicolor

Results Summary Download

Query ID	Best ID	Search DB	Tree	Top %	Graph	Low %
OTU853	*Alitta succinea*	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

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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?

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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?

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