

How methods influence results: comparing morphological vs molecular identification of zooplankton for biodiversity studies and to answer ecological questions, and the possibility of combined methods

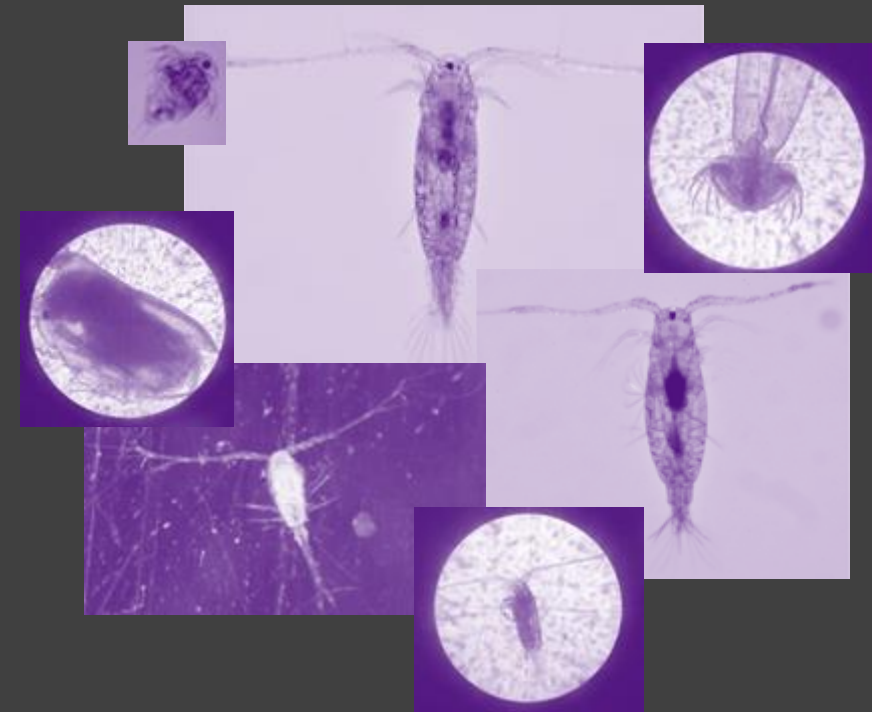
Catherine Fitzgerald

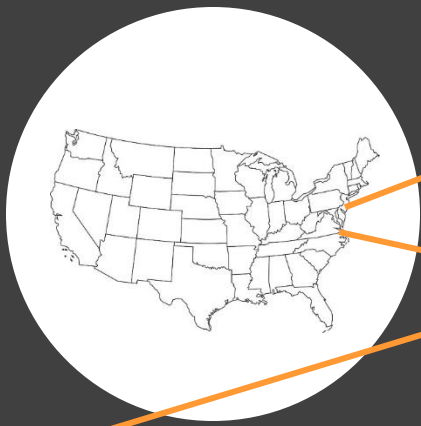
James J. Pierson

Louis V. Plough

University of Maryland Centers
for Environmental Science,
Horn Point Laboratory
Cambridge, Maryland, USA

www.umces.edu/hpl



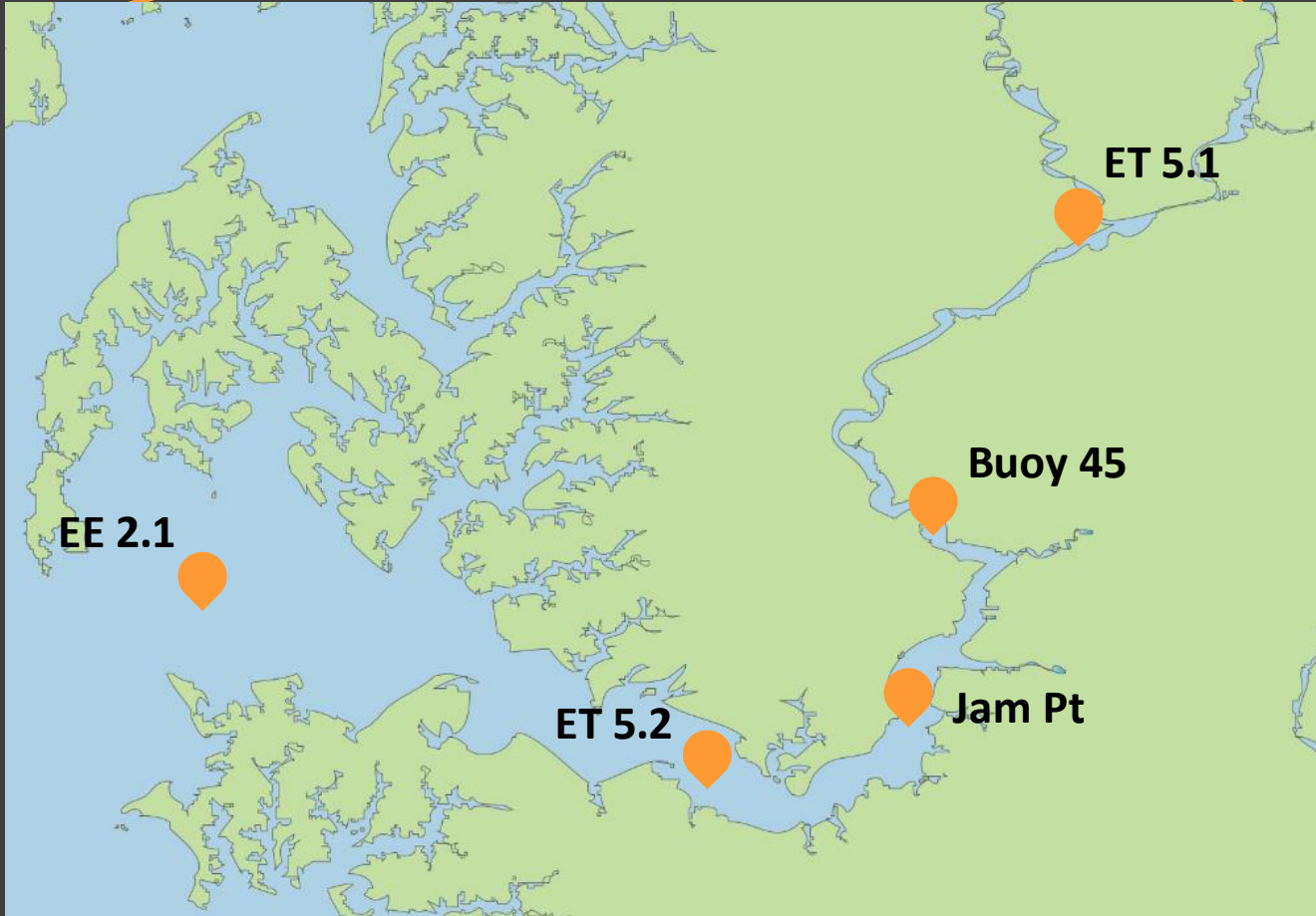


Sampling

5 sites

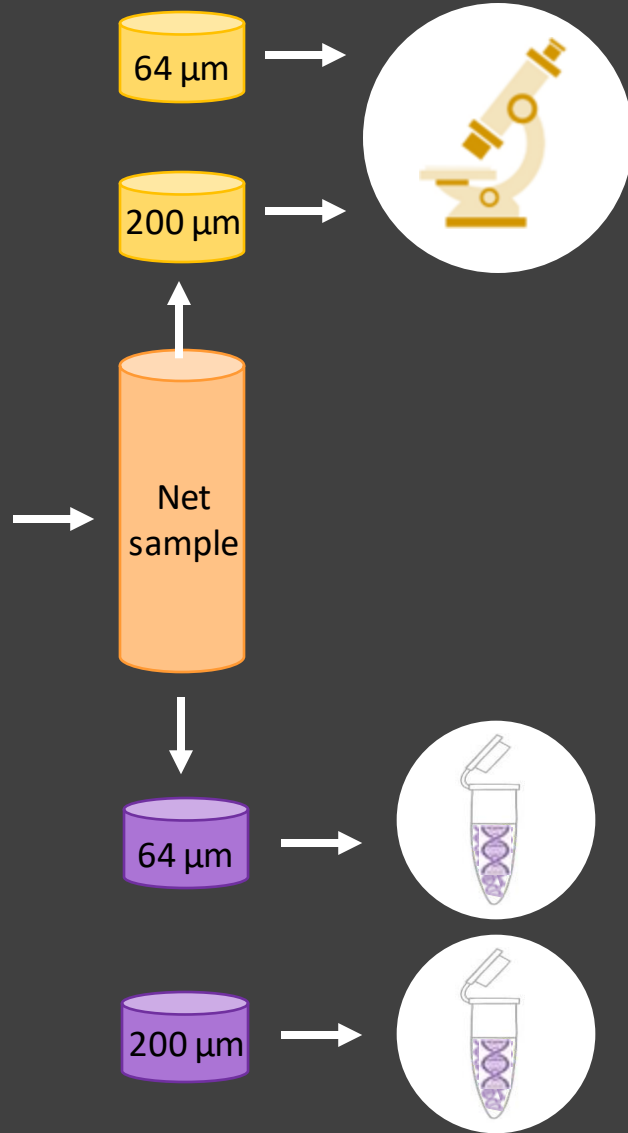
Salinity gradient

April-September, 2018-2019





Sampling



Physical parameters (CTD)

Salinity
Temperature
DO

Chla data from Eyes on the Bay

www.eyesonthebay.net

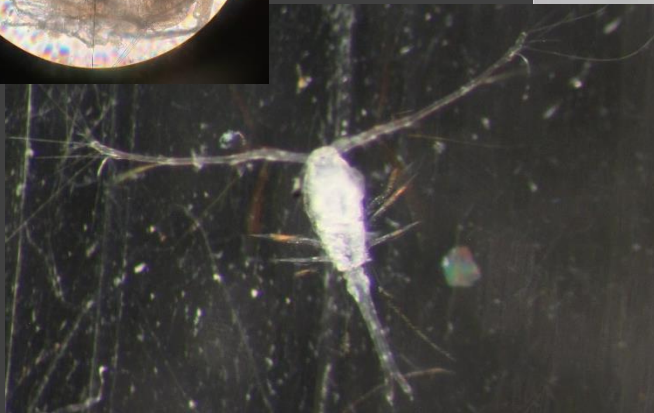
Zooplankton

- 1) Split in half
- 2) Size fractionated
 - 64 - 200 μm
 - >200 μm
- 3) Preserved two ways

Morphological process

Subsample of > 200 animals

Identified under dissecting scope



Morphological process

Phylum	Class	Order	Family	Genus	Species	Stage	Count
Arthropoda	Thecostraca	Balanomorpha	Balanidae	Balanus	Unknown	Nauplius	83
Arthropoda	Thecostraca	Balanomorpha	Balanidae	Balanus	Unknown	Cyprid	3
Arthropoda	Hexanauplia	Calanoida	Acartiidae	Acartia	tonsa	Adult fem	8
Arthropoda	Hexanauplia	Calanoida	Acartiidae	Acartia	tonsa	Adult mal	8
Arthropoda	Hexanauplia	Calanoida	Acartiidae	Acartia	tonsa	C5	4
Arthropoda	Hexanauplia	Calanoida	Acartiidae	Acartia	tonsa	C4	12
Arthropoda	Hexanauplia	Calanoida	Acartiidae	Acartia	tonsa	C3	11
Arthropoda	Hexanauplia	Calanoida	Acartiidae	Acartia	tonsa	C2	8
Arthropoda	Hexanauplia	Calanoida	Acartiidae	Acartia	tonsa	C1	11
Arthropoda	Hexanauplia	Calanoida	Acartiidae	Acartia	tonsa	N5 6	3
Arthropoda	Hexanauplia	Calanoida	Acartiidae	Acartia	tonsa	N4	5
Arthropoda	Hexanauplia	Calanoida	Acartiidae	Acartia	tonsa	N3	5
Arthropoda	Hexanauplia	Calanoida	Acartiidae	Acartia	tonsa	N1 2	3
Arthropoda	Hexanauplia	Calanoida	Acartiidae	Acartia	tonsa	Egg	15
Arthropoda	Hexanauplia	Calanoida	Temoridae	Eurytemora	carolleeae	C4	1
Arthropoda	Hexanauplia	Calanoida	Temoridae	Eurytemora	carolleeae	C3	5
Arthropoda	Hexanauplia	Calanoida	Temoridae	Eurytemora	carolleeae	C2	4
Arthropoda	Hexanauplia	Calanoida	Temoridae	Eurytemora	carolleeae	Nauplius	2
Annelida	Polychaeta	Unknown	Unknown	Unknown	Unknown	Larva	52
Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Egg	4
Rotifera	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	16
Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Larva	3
Arthropoda	Branchiopoda	Diplostraca	Podonidae	Pleopis	polyphemoide	Unknown	4
Mollusca	Bivalvia	Unknown	Unknown	Unknown	Unknown	Veliger	4

Taxonomic hierarchy based on resolution of identifications, and filled out according to NCBI

Non animal, non marine/freshwater taxa removed from data set (e.g. phytoplankton)

Metabarcoding process



AllGenetics.com

Subsample $\sim 1 \text{ cm}^3$

Marker gene: CO1

Leray CO1 primers

Illumina sequencing

Bioinformatics: DADA2 --> VSEARCH
(clustered @ 97%) --> LULU* --> OTUs



Metabarcoding process

BLCA Taxonomy Results														
OTU	K	%	P	%	C	%	O	%	F	%	G	%	S	%
655	Eukaryota	100	Oomycota	100	Peronosporales	68	Peronosporales	68	Peronosporaceae	68	Phytophthora	38	terrestris	13
209	Eukaryota	100	Arthropoda	100	Insecta	100	Diptera	83	Muscidae	22	Pintomyia	16	evansi	16
426	Eukaryota	100	Bacillariophyta	100	Coscinodiscophyceae	97	Thalassiosirales	52	Stephanodiscaceae	45	Skeletonema	29	menzelii	17
266	Eukaryota	100	Oomycota	71	Peronosporales	71	Peronosporales	71	Peronosporaceae	71	Phytophthora	71	hydropathica	18
78	Eukaryota	100	Oomycota	100	Pythiales	81	Pythiales	81	Pythiaceae	81	Globisporangium	45	humana	18
518	Eukaryota	100	Arthropoda	100	Insecta	78	Diptera	78	Calliphoridae	19	Caiusa	19	coomani	19
15	Eukaryota	100	Arthropoda	29	Demospongiae	23	Poecilosclerida	23	Microcionidae	19	Clathria	19	rugosa	19
631	Eukaryota	100	Arthropoda	84	Insecta	84	Lepidoptera	84	Erebidae	83	Neochera	40	inops	20
291	Eukaryota	100	Ascomycota	100	Sordariomycetes	36	Hypocreales	34	Teloschistaceae	21	Phaeoplacoma	21	campidia	21

Taxonomic assignment:

BLCA taxonomic hierarchy confidence
BLAST against custom ~5 million
sequence CO1 database (from NCBI-
January 2023)

Non animal, non marine/freshwater
taxa removed from data set (e.g.
turkey, algae, bacteria)

Metabarcoding process

BLAST Taxonomy Results			
ACC	Genus_Species	Percent ID	Match length
MH818747	Paracalanus_quasimodo_1267145	88	311
MT981118	Globisporangium_rhizosaccharum_222890	83	299
DQ356554	Boeckella_gracilipes_366523	79	282
MH136922	Phytophthora_macilentosa_1401296	86	303
DQ628627	Coelotes_terrestris_94022	76	273
KT276687	Eremobates_tuberculatus_1689386	81	291
MK817335	Lithodesmium_intricatum_1003076	84	298
HQ708780	Pythium_pectinolyticum_156629	97	312
MN343798	Zabrus_aurichalceus_2650838	86	304

Taxonomic assignment:

BLCA taxonomic hierarchy confidence
BLAST against custom ~5 million sequence CO1 database (from NCBI-January 2024)

Non animal, non marine/freshwater taxa removed from data set (e.g. turkey, phytoplankton, bacteria)

Metabarcoding process

Two data sets created from master taxonomy data set

Inclusive

> 80% confidence thresholds

Exclusive

> 95% confidence thresholds

BLCA Taxonomy Results														
OTU	K	%	P	%	C	%	O	%	F	%	G	%	S	%
655	Eukaryota	100	Oomycota	100	Peronosporales	68	Peronosporales	68	Peronosporaceae	68	Phytophthora	38	terrestris	13
209	Eukaryota	100	Arthropoda	100	Insecta	100	Diptera	83	Muscidae	22	Pintomyia	16	evansi	16
426	Eukaryota	100	Bacillariophyta	100	Coscinodiscophyceae	97	Thalassiosirales	52	Stephanodiscaceae	45	Skeletonema	29	menzelii	17
266	Eukaryota	100	Oomycota	71	Peronosporales	71	Peronosporales	71	Peronosporaceae	71	Phytophthora	71	hydropathica	18
78	Eukaryota	100	Oomycota	100	Pythiales	81	Pythiales	81	Pythiaceae	81	Globisporangium	45	humana	18
518	Eukaryota	100	Arthropoda	100	Insecta	78	Diptera	78	Calliphoridae	19	Caiusa	19	coomani	19
15	Eukaryota	100	Arthropoda	29	Demospongiae	23	Poecilosclerida	23	Microcionidae	19	Clathria	19	rugosa	19
631	Eukaryota	100	Arthropoda	84	Insecta	84	Lepidoptera	84	Erebidae	83	Neochera	40	inops	20
291	Eukaryota	100	Ascomycota	100	Sordariomycetes	36	Hypocreales	34	Teloschistaceae	21	Phaeoplacoma	21	campidia	21

Metabarcoding process

Two data sets created from master taxonomy data set

Inclusive

> 80% confidence thresholds

Exclusive

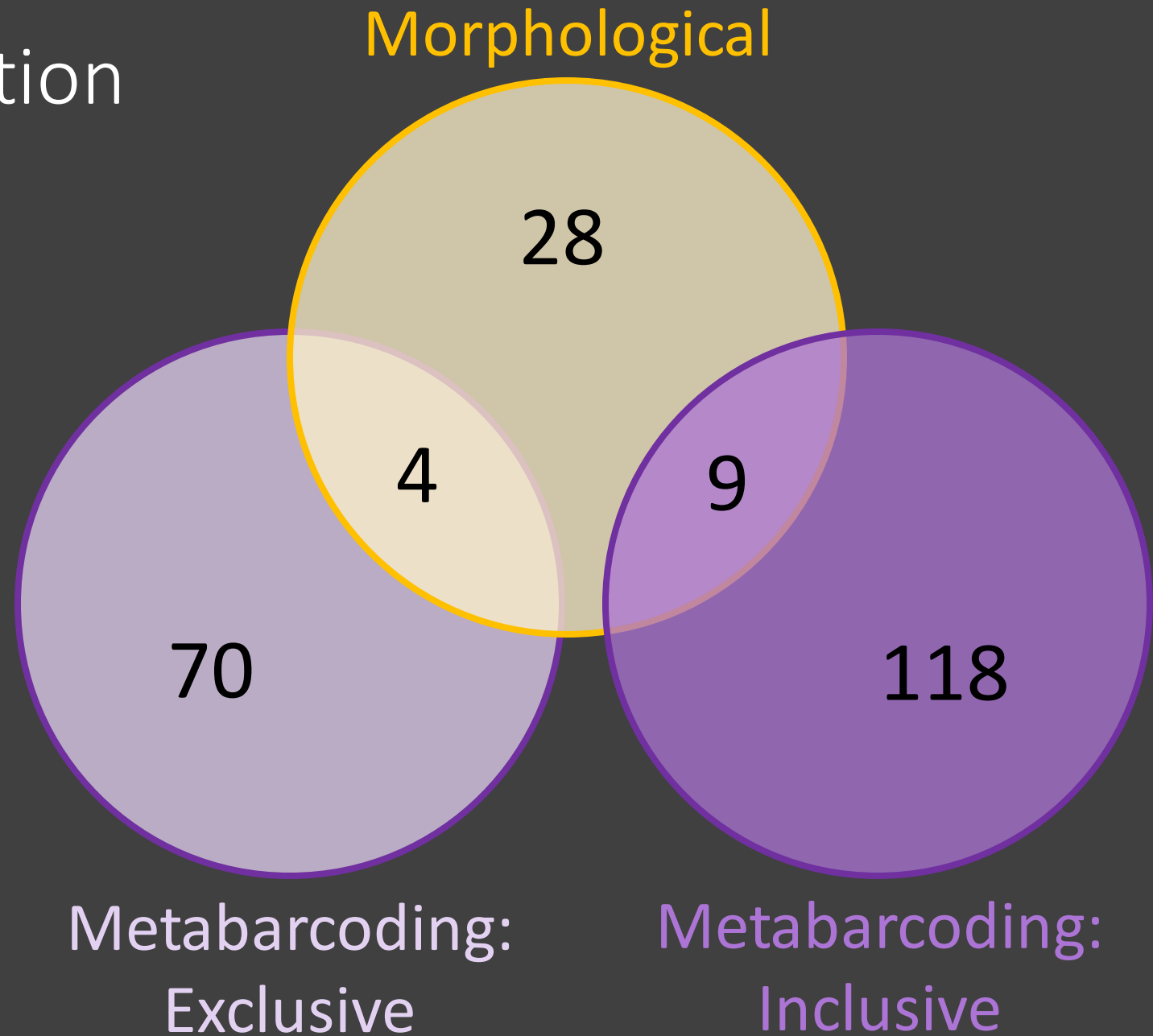
> 95% confidence thresholds

BLAST Taxonomy Results			
ACC	Genus_Species	Percent ID	Match length
MH818747	Paracalanus_quasimodo_1267145	88	311
MT981118	Globisporangium_rhizosaccharum_222890	83	299
DQ356554	Boeckella_gracilipes_366523	79	282
MH136922	Phytophthora_macilentosa_1401296	86	303
DQ628627	Coelotes_terrestris_94022	76	273
KT276687	Eremobates_tuberculatus_1689386	81	291
MK817335	Lithodesmium_intracatum_1003076	84	298
HQ708780	Pythium_pectinolyticum_156629	97	312
MN343798	Zabrus_aurichalceus_2650838	86	304

Points of comparison		Morphological	Metabarcoding		Finding agrees with literature?
			Inclusive	Exclusive	
Processing time		High	Low	Low	Yes
Processing cost		Low	High	High	--
Taxonomic resolution		Low	High	High	Yes
	Species richness	Low	High	Medium	Yes
Community	Composition	Very few taxa shared between data sets			Variable
	Dominance	Agreement on #1 rank. Top 10 are mostly within the same classes. Disagreement on rare taxa ranks. Metabarcoding sets more dominant than morphological			--
	Diversity	Low	High	Medium	Yes
Diversity	Environmental drivers of diversity	Temperature, station, month	Station	None	Variable
		Underestimated	Overestimated	Overestimated	Yes
Quantitative accuracy	Abundance	Total zoop: no significant patterns	Total zoop: sig diff between 2 stations, otherwise none	Total zoop: sig diff between additional 4 stations, otherwise none	Variable
		Specific taxa: agreement variable; disagreement not related to taxon dominance			Generally good agreement
	Environmental drivers of abundance	Total zoop: None	Total zoop: None	Total zoop: None	Variable
		Specific taxa: agreement variable; disagreement not related to taxon dominance			Generally good agreement

Community composition

Jaccard's index	
Taxa- inc	Taxa- exc
5.8	3.7
6.5	4.7
4.9	2.3



Community composition

Polychaete larvae

Annelida, Polychaeta, Capitellidae, Capitellidae, Heteromastus, filiformis
 Annelida, Polychaeta, Capitellidae, Capitellidae, Mediomastus, ambiseta
 Annelida, Polychaeta, Orbiniidae, Orbiniidae, Leitoscoloplos, robustus
 Annelida, Polychaeta, Phyllodocida, Goniadidae, Glycinde, multidentis
 Annelida, Polychaeta, Phyllodocida, Nereididae, Alitta, succinea
 Annelida, Polychaeta, Phyllodocida, Nereididae, Laeoneis, culveri
 Annelida, Polychaeta, Phyllodocida, Nereididae, Unknown, Unknown
 Annelida, Polychaeta, Phyllodocida, Phyllodocidae, Hypereteone, heteropoda
 Annelida, Polychaeta, Spionida, Spionidae, Marenzelleria, neglecta
 Annelida, Polychaeta, Spionida, Spionidae, Polydora, cornuta
 Annelida, Polychaeta, Spionida, Spionidae, Polydora, websteri
 Annelida, Polychaeta, Spionida, Spionidae, Streblospio, benedicti
 Annelida, Polychaeta, Terebellida, Pectinariidae, Pectinaria, gouldii
 Arthropoda, Branchiopoda, Diplostraca, Bosminidae, Bosmina, freyi
 Arthropoda, Branchiopoda, Diplostraca, Bosminidae, Bosmina, longirostris
 Arthropoda, Branchiopoda, Diplostraca, Chydoridae, Chydorus, brevilabris
 Arthropoda, Branchiopoda, Diplostraca, Daphniidae, Daphnia, ambigua
 Arthropoda, Branchiopoda, Diplostraca, Daphniidae, Daphnia, parvula
 Arthropoda, Branchiopoda, Diplostraca, Podonidae, Pleopis, polyphemoides
 Arthropoda, Hexanauplia, Calanoida, Acartiidae, Acartia, tonsa
 Arthropoda, Hexanauplia, Calanoida, Diaptomidae, Skistodiaptomus, pallidus
 Arthropoda, Hexanauplia, Calanoida, Temoridae, Eurytemora, affinis
 Arthropoda, Hexanauplia, Calanoida, Temoridae, Eurytemora, Unknown
 Arthropoda, Hexanauplia, Cyclopoida, Cyclopidae, Acanthocyclops, americanus
 Arthropoda, Hexanauplia, Cyclopoida, Cyclopidae, Acanthocyclops, brevispinosus
 Arthropoda, Hexanauplia, Cyclopoida, Cyclopidae, Macrocyclus, albidus
 Arthropoda, Hexanauplia, Cyclopoida, Cyclopidae, Mesocyclops, edax
 Arthropoda, Hexanauplia, Cyclopoida, Cyclopidae, Mesocyclops, pehpeiensis
 Arthropoda, Hexanauplia, Harpacticoida, Miracidae, Schizopera, knabeni
 Arthropoda, Insecta, Diptera, Chaoboridae, Chaoborus, punctipennis
 Arthropoda, Insecta, Diptera, Chironomidae, Dicrotendipes, modestus
 Arthropoda, Insecta, Diptera, Culicidae, Ochlerotatus, Unknown
 Arthropoda, Insecta, Odonata, Calopterygidae, Calopteryx, maculata
 Arthropoda, Malacostraca, Decapoda, Palaemonidae, Palaemon, pugio
 Arthropoda, Malacostraca, Decapoda, Panopeidae, Eurypanopeus, depressus
 Arthropoda, Malacostraca, Decapoda, Panopeidae, Rhithropanopeus, harrisi
 Arthropoda, Malacostraca, Decapoda, Portunidae, Callinectes, sapidus
 Arthropoda, Malacostraca, Mysida, Mysidae, Neomysis, americana
 Arthropoda, Thecostraca, Balanomorpha, Balanidae, Amphibalanus, improvisus
 Arthropoda, Thecostraca, Balanomorpha, Balanidae, Amphibalanus, subalbidus
 Bryozoa, Gymnolaemata, Cheilostomata, Electridae, Conopeum, chesapeakeensis
 Bryozoa, Gymnolaemata, Cheilostomata, Electridae, Conopeum, tenuissimum
 Bryozoa, Gymnolaemata, Cheilostomata, Membraniporidae, Membranipora, tenuis
 Chordata, Actinopteri, Atheriniformes, Atherinopsidae, Membras, martinica
 Chordata, Actinopteri, Atheriniformes, Atherinopsidae, Menidia, beryllina
 Chordata, Actinopteri, Atheriniformes, Atherinopsidae, Menidia, menidia
 Chordata, Actinopteri, Blenniiformes, Blenniidae, Chasmodes, bosquianus
 Chordata, Actinopteri, Clupeiformes, Clupeidae, Brevoortia, Unknown
 Chordata, Actinopteri, Clupeiformes, Clupeidae, Dorosoma, cepedianum
 Chordata, Actinopteri, Clupeiformes, Engraulidae, Anchoa, mitchilli
 Chordata, Actinopteri, Moronidae, Moronidae, Morone, americana
 Chordata, Actinopteri, Moronidae, Moronidae, Morone, saxatilis
 Cnidaria, Anthozoa, Actiniaria, Diadumenidae, Diadumene, leucolena
 Cnidaria, Hydrozoa, Anthoathecata, Moerisiidae, Moerisia, inkermanica
 Cnidaria, Hydrozoa, Leptothecata, Blackfordiidae, Blackfordia, virginica
 Cnidaria, Scyphozoa, Semaestomeae, Pelagiidae, Chrysaora, Unknown
 Mollusca, Bivalvia, Cardiida, Tellinidae, Ameritella, mitchelli
 Mollusca, Bivalvia, Cardiida, Tellinidae, Limecola, petalum
 Mollusca, Bivalvia, Mytilidae, Dreissenidae, Mytilopsis, leucophaeata
 Mollusca, Bivalvia, Mytilidae, Mytilidae, Ischadium, recurvum
 Mollusca, Bivalvia, Venerida, Mactridae, Mulinia, lateralis
 Mollusca, Bivalvia, Venerida, Mactridae, Rangia, cuneata
 Mollusca, Gastropoda, Acteonidae, Acteonidae, Japonactaeon, punctostriatus
 Mollusca, Gastropoda, Cephalaspidea, Cylichnidae, Acteocina, canaliculata
 Mollusca, Gastropoda, Cephalaspidea, Haminoeidae, Haminoea, solitaria
 Mollusca, Gastropoda, Ellobiida, Ellobiidae, Melampus, bidentatus
 Mollusca, Gastropoda, Littorinimorpha, Cochliopidae, Littoridinops, tenuipes
 Mollusca, Gastropoda, Littorinimorpha, Littorinidae, Littoraria, irrorata
 Mollusca, Gastropoda, Nudibranchia, Facelinidae, Cratena, pilata
 Mollusca, Gastropoda, Nudibranchia, Onchidorididae, Corambe, obscura
 Mollusca, Gastropoda, Nudibranchia, Trinchesiidae, Tenellia, adpersa
 Platyhelminthes, Rhabditophora, Polycladida, Unknown, Unknown, Unknown
 Rotifera, Eurotatoria, Ploima, Brachionidae, Keratella, cochlearis
 Xenacoelomorpha, Acoela, Acoela, Anaperidae, Neochilidia, fusca

Insect larvae

Crab larvae

Fish eggs or larvae

Cnidarian larvae

Bivalve larvae

Gastropod larvae

Community composition

Annelida, Polychaeta, Unknown, Unknown, Unknown, Unknown
 Arthropoda, Arachnida, Trombidiformes, Halacaridae, Unknown, Unknown
 Arthropoda, Branchiopoda, Diplostraca, Bosminidae, Bosmina, Longirostris
 Arthropoda, Branchiopoda, Diplostraca, Podonidae, Pleopis, polyphemoides
 Arthropoda, Branchiopoda, Diplostraca, Sididae, Diaphanosoma, Unknown
 Arthropoda, Branchiopoda, Diplostraca, Sididae, Unknown, Unknown
 Arthropoda, Hexanauplia, Calanoida, Acartiidae, Acartia, tonsa
 Arthropoda, Hexanauplia, Calanoida, Temoridae, Eurytemora, carolleae
 Arthropoda, Hexanauplia, Calanoida, Unknown, Unknown, Unknown
 Arthropoda, Hexanauplia, Cyclopoida, Cyclopidae, Trocyclops, Unknown
 Arthropoda, Hexanauplia, Cyclopoida, Oithonidae, Oithona, colcarva
 Arthropoda, Hexanauplia, Cyclopoida, Unknown, Unknown, Unknown
 Arthropoda, Hexanauplia, Harpacticoida, Ameiridae, Stygonitocrella, Unknown
 Arthropoda, Hexanauplia, Harpacticoida, Unknown, Unknown, Unknown
 Arthropoda, Hexanauplia, Poecilostomatoida, Ergasilidae, Ergasilus, Unknown
 Arthropoda, Hexanauplia, Unknown, Unknown, Unknown, Unknown
 Arthropoda, Malacostraca, Decapoda, Panopeidae, Rhithropanopeus, harrisii
 Arthropoda, Thecostraca, Balanomorpha, Balanidae, Balanus, Unknown
 Arthropoda, Thecostraca, Balanomorpha, Chthamalidae, Chthamalus, Unknown
 Bryozoa, Unknown, Unknown, Unknown, Unknown, Unknown
 Foraminifera, Unknown, Unknown, Unknown, Unknown, Unknown
 Mollusca, Bivalvia, Unknown, Unknown, Unknown, Unknown
 Mollusca, Gastropoda, Unknown, Unknown, Unknown, Unknown
 Mollusca, Unknown, Unknown, Unknown, Unknown, Unknown
 Nematoda, Unknown, Unknown, Unknown, Unknown, Unknown
 Platyhelminthes, Unknown, Unknown, Unknown, Unknown, Unknown
 Rotifera, Eurotatoria, Ploima, Brachionidae, Brachionus, angularis
 Rotifera, Eurotatoria, Ploima, Brachionidae, Brachionus, calyciflorus
 Rotifera, Eurotatoria, Ploima, Brachionidae, Brachionus, caudatus
 Rotifera, Eurotatoria, Ploima, Brachionidae, Brachionus, rubens or plicatilis
 Rotifera, Eurotatoria, Ploima, Brachionidae, Brachionus, Unknown
 Rotifera, Eurotatoria, Ploima, Brachionidae, Brachionus, urceolaris
 Rotifera, Eurotatoria, Ploima, Brachionidae, Keratella, gracilentata
 Rotifera, Eurotatoria, Ploima, Brachionidae, Keratella, Unknown
 Rotifera, Eurotatoria, Ploima, Notommatidae, Notommatia, Unknown
 Rotifera, Eurotatoria, Ploima, Synchaetidae, Synchaeta, Unknown
 Rotifera, Unknown, Unknown, Unknown, Unknown, Unknown

Foraminifera

Nematoda

Platyhelminthes

Rotifers

Diplostraca

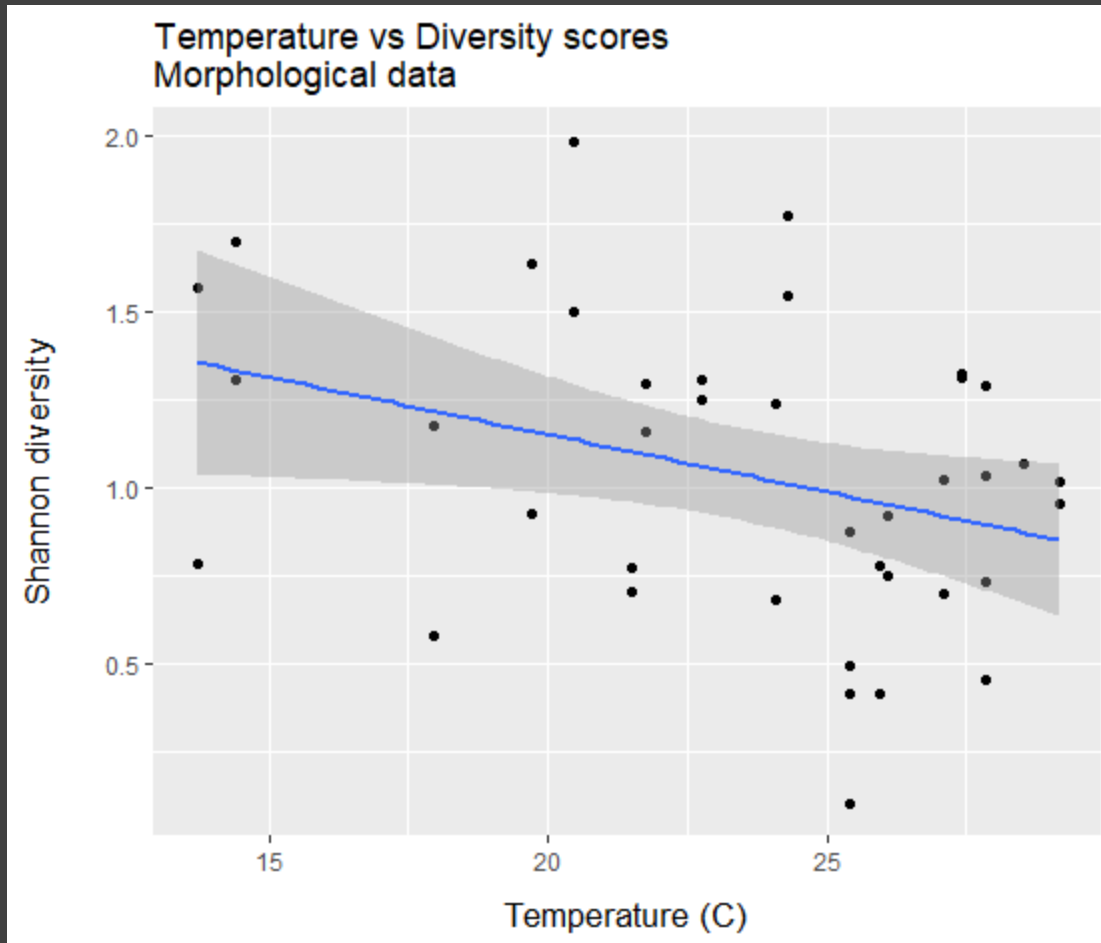
Cyclopoida

Bryzoa

Rotifer

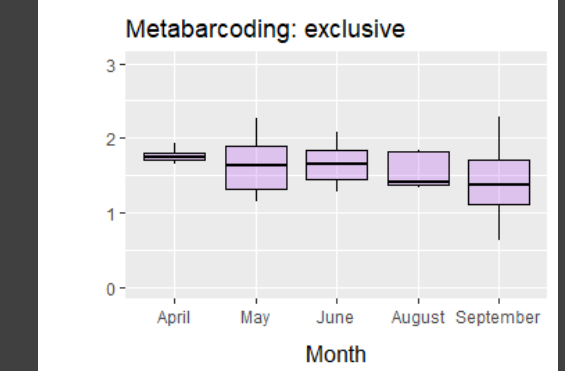
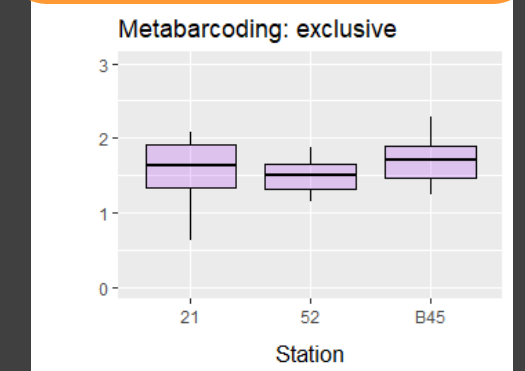
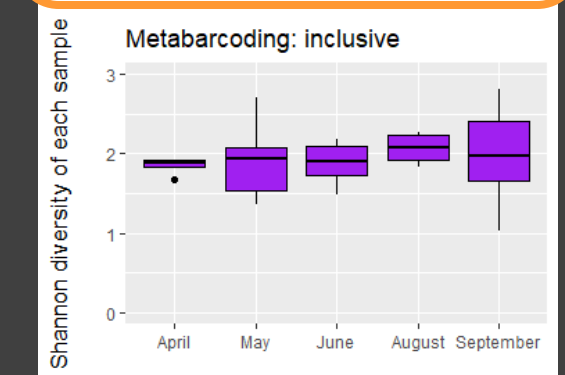
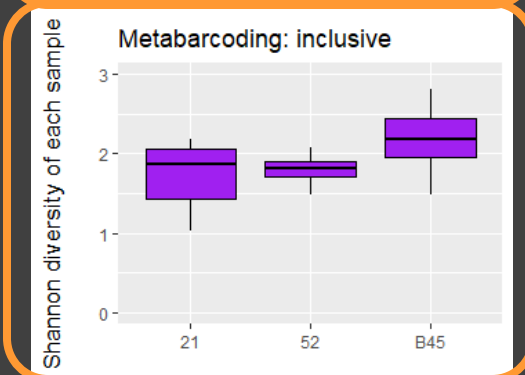
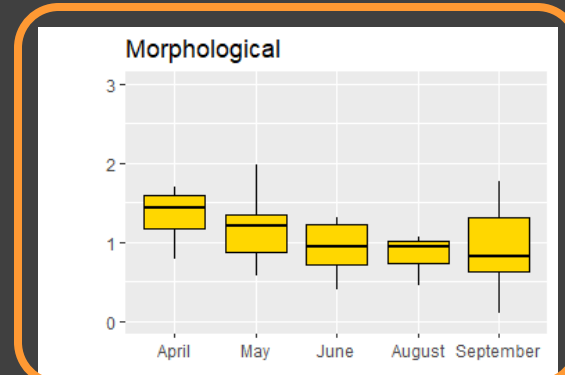
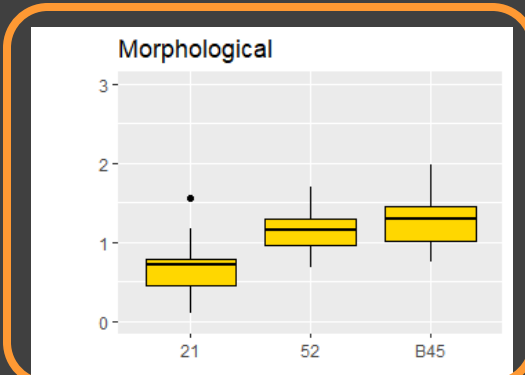
Annelida, Polychaeta, Capitellidae, Capitellidae, Heteromastus, filiformis
 Annelida, Polychaeta, Capitellidae, Capitellidae, Mediomastus, ambiseta
 Annelida, Polychaeta, Orbiniidae, Orbiniidae, Leitoscoloplos, robustus
 Annelida, Polychaeta, Phyllodocida, Goniadidae, Glycinde, multidentis
 Annelida, Polychaeta, Phyllodocida, Nereididae, Alitta, succinea
 Annelida, Polychaeta, Phyllodocida, Nereididae, Laeonereis, culveri
 Annelida, Polychaeta, Phyllodocida, Nereididae, Unknown, Unknown
 Annelida, Polychaeta, Phyllodocida, Phyllodocidae, Hypereteone, heteropoda
 Annelida, Polychaeta, Spionida, Spionidae, Marenzelleria, neglecta
 Annelida, Polychaeta, Spionida, Spionidae, Polydora, cornuta
 Annelida, Polychaeta, Spionida, Spionidae, Polydora, websteri
 Annelida, Polychaeta, Spionida, Spionidae, Streblospio, benedicti
 Annelida, Polychaeta, Terebellida, Pectinariidae, Pectinaria, gouldii
 Arthropoda, Branchiopoda, Diplostraca, Bosminidae, Bosmina, freyi
 Arthropoda, Branchiopoda, Diplostraca, Bosminidae, Bosmina, longirostris
 Arthropoda, Branchiopoda, Diplostraca, Chydoridae, Chydorus, brevibris
 Arthropoda, Branchiopoda, Diplostraca, Daphniidae, Daphnia, ambigua
 Arthropoda, Branchiopoda, Diplostraca, Daphniidae, Daphnia, parvula
 Arthropoda, Branchiopoda, Diplostraca, Podonidae, Pleopis, polyphemoides
 Arthropoda, Hexanauplia, Calanoida, Acartiidae, Acartia, tonsa
 Arthropoda, Hexanauplia, Calanoida, Diaptomidae, Skistodiaptomus, pallidus
 Arthropoda, Hexanauplia, Calanoida, Temoridae, Eurytemora, affinis
 Arthropoda, Hexanauplia, Cyclopoida, Cyclopidae, Acanthocyclops, americanus
 Arthropoda, Hexanauplia, Cyclopoida, Cyclopidae, Acanthocyclops, brevispinosus
 Arthropoda, Hexanauplia, Cyclopoida, Cyclopidae, Macrocyclops, albidus
 Arthropoda, Hexanauplia, Cyclopoida, Cyclopidae, Mesocyclops, edax
 Arthropoda, Hexanauplia, Cyclopoida, Cyclopidae, Mesocyclops, pehpeiensis
 Arthropoda, Hexanauplia, Harpacticoida, Miracidae, Schizopera, knabeni
 Arthropoda, Insecta, Diptera, Chaoboridae, Chaoborus, punctipennis
 Arthropoda, Insecta, Diptera, Chironomidae, Dicrotendipes, modestus
 Arthropoda, Insecta, Diptera, Culicidae, Ochlerotatus, Unknown
 Arthropoda, Insecta, Odonata, Calopterygidae, Calopteryx, maculata
 Arthropoda, Malacostraca, Decapoda, Palaemonidae, Palaemon, pugio
 Arthropoda, Malacostraca, Decapoda, Panopeidae, Eurypanopeus, depressus
 Arthropoda, Malacostraca, Decapoda, Panopeidae, Rhithropanopeus, harrisii
 Arthropoda, Malacostraca, Decapoda, Portunidae, Callinectes, sapidus
 Arthropoda, Malacostraca, Mysida, Mysidae, Neomysis, americana
 Arthropoda, Thecostraca, Balanomorpha, Balanidae, Amphibalanus, improvisus
 Arthropoda, Thecostraca, Balanomorpha, Balanidae, Amphibalanus, subalbidus
 Bryozoa, Gymnolaemata, Cheilostomata, Electridae, Conopeum, chesapeakeensis
 Bryozoa, Gymnolaemata, Cheilostomata, Electridae, Conopeum, tenuissimum
 Bryozoa, Gymnolaemata, Cheilostomata, Membraniporidae, Membranipora, tenuis
 Chordata, Actinopteri, Atheriniformes, Atherinopsidae, Membras, martinica
 Chordata, Actinopteri, Atheriniformes, Atherinopsidae, Menidia, beryllina
 Chordata, Actinopteri, Atheriniformes, Atherinopsidae, Menidia, menidia
 Chordata, Actinopteri, Blenniiformes, Blenniidae, Chasmodes, bosquianus
 Chordata, Actinopteri, Clupeiformes, Clupeidae, Brevoortia, Unknown
 Chordata, Actinopteri, Clupeiformes, Clupeidae, Dorosoma, cepedianum
 Chordata, Actinopteri, Clupeiformes, Engraulidae, Anchoa, mitchilli
 Chordata, Actinopteri, Moronidae, Moronidae, Morone, americana
 Chordata, Actinopteri, Moronidae, Moronidae, Morone, saxatilis
 Cnidaria, Anthozoa, Actiniaria, Diadumenidae, Diadumene, leucolena
 Cnidaria, Hydrozoa, Anthoathecata, Moerisiidae, Moerisia, inkermanica
 Cnidaria, Hydrozoa, Leptothecata, Blackfordiidae, Blackfordia, virginica
 Cnidaria, Scyphozoa, Semeaeostomeae, Pelagiidae, Chrysaora, Unknown
 Mollusca, Bivalvia, Cardiida, Tellinidae, Ameritella, mitchelli
 Mollusca, Bivalvia, Cardiida, Tellinidae, Lamecolina, petalum
 Mollusca, Bivalvia, Mytilidae, Dreissenidae, Mytilopsis, leucophaeata
 Mollusca, Bivalvia, Mytilidae, Mytilidae, Ischadium, recurvum
 Mollusca, Bivalvia, Venerida, Mactridae, Mulinia, lateralis
 Mollusca, Bivalvia, Venerida, Mactridae, Rangia, cuneata
 Mollusca, Gastropoda, Acteonidae, Acteonidae, Japonactaeon, punctostriatus
 Mollusca, Gastropoda, Cephalaspidea, Cylichnidae, Acteocina, canaliculata
 Mollusca, Gastropoda, Cephalaspidea, Haminoeidae, Haminoea, solitaria
 Mollusca, Gastropoda, Ellobiidae, Ellobiidae, Melampus, bidentatus
 Mollusca, Gastropoda, Littorinimorpha, Cochliopidae, Littoridinops, tenuipes
 Mollusca, Gastropoda, Littorinimorpha, Littorinidae, Littoraria, irrorata
 Mollusca, Gastropoda, Nudibranchia, Facelinidae, Cratena, pilata
 Mollusca, Gastropoda, Nudibranchia, Onchidorididae, Corambe, obscura
 Mollusca, Gastropoda, Nudibranchia, Trinchetiidae, Tenellia, adpersa
 Platyhelminthes, Rhabditophora, Polycladida, Unknown, Unknown, Unknown
 Rotifera, Eurotatoria, Ploima, Brachionidae, Keratella, cochlearis
 Xenacoelomorpha, Acoela, Acoela, Anaperidae, Neochilidia, fusca

Diversity



Multiple linear models			
Parameter	Data set	p-value	slope
Temperature	Morpho	0.00857	-0.076
	Meta: Inc	0.53	-0.01
	Meta: Exc	0.12	-0.05
Salinity	Morpho	0.13	-0.059
	Meta: Inc	0.82	0.01
	Meta: Exc	0.79	0.01
Dissolved oxygen	Morpho	0.45	-0.059
	Meta: Inc	0.42	-0.06
	Meta: Exc	0.25	-0.10
Chlorophyll a	Morpho	0.39	-0.01
	Meta: Inc	0.52	-0.01
	Meta: Exc	0.47	-0.01
Station	Morpho	2.03e⁻⁶	0.029
	Meta: Inc	0.0033	0.018
	Meta: Exc	0.13	0.01
Month	Morpho	0.00119	-0.003
	Meta: Inc	0.64	0.00
	Meta: Exc	0.08	-0.01

Diversity

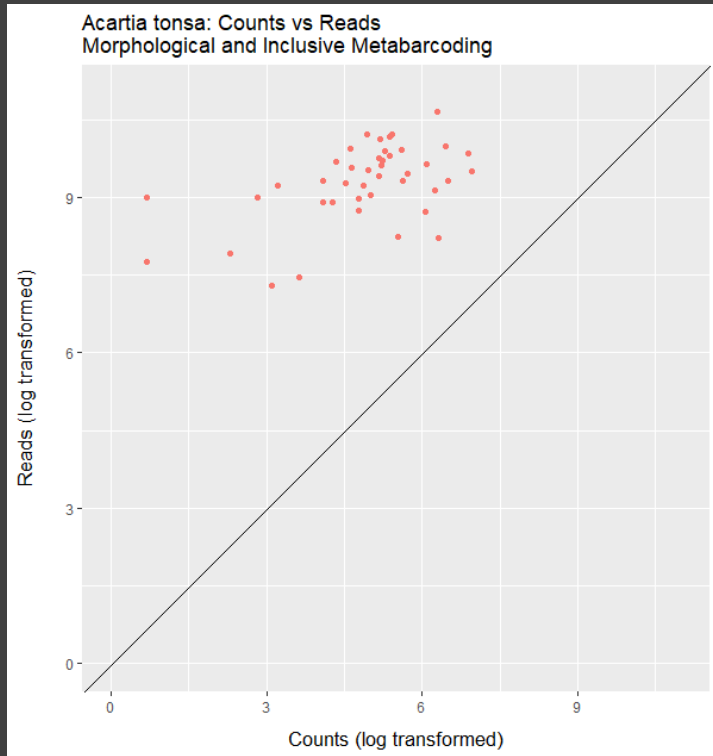


Multiple linear models

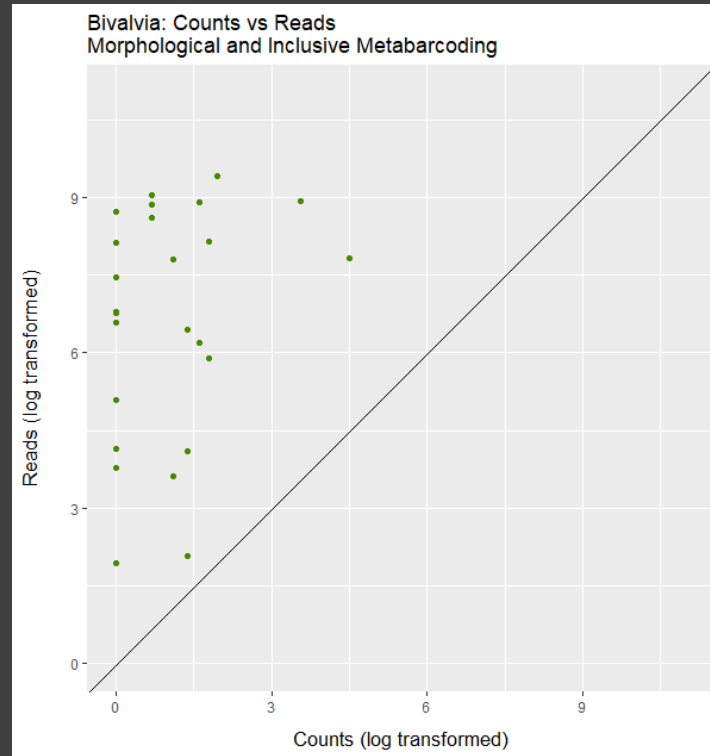
Parameter	Data set	p-value	slope
Temperature	Morpho	0.00857	-0.076
	Meta: Inc	0.53	-0.01
	Meta: Exc	0.12	-0.05
Salinity	Morpho	0.13	-0.059
	Meta: Inc	0.82	0.01
	Meta: Exc	0.79	0.01
Dissolved oxygen	Morpho	0.45	-0.059
	Meta: Inc	0.42	-0.06
	Meta: Exc	0.25	-0.10
Chlorophyll a	Morpho	0.39	-0.01
	Meta: Inc	0.52	-0.01
	Meta: Exc	0.47	-0.01
Station	Morpho	2.03e^-6	0.029
	Meta: Inc	0.0033	0.018
	Meta: Exc	0.13	0.01
Month	Morpho	0.00119	-0.003
	Meta: Inc	0.64	0.00
	Meta: Exc	0.08	-0.01

Abundance

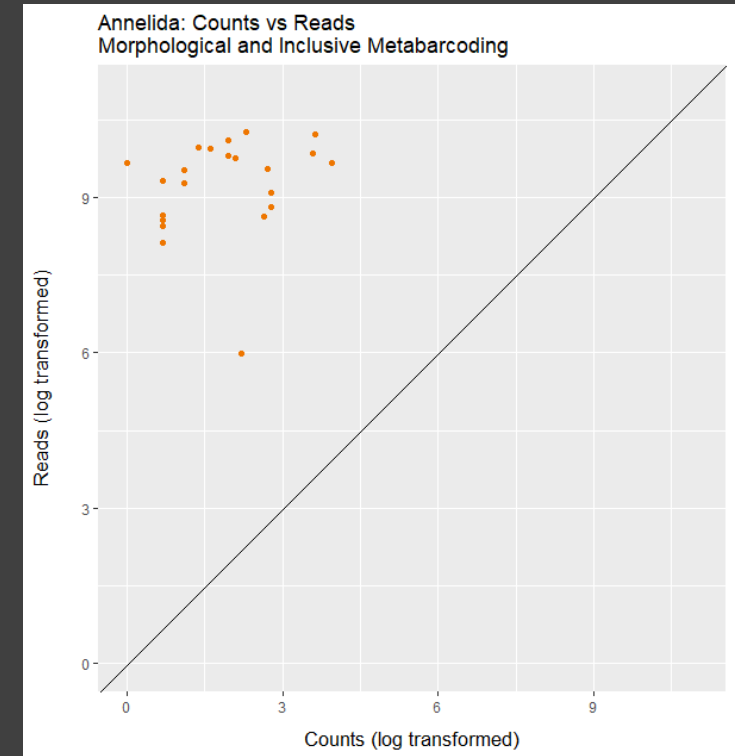
Acartia tonsa



Bivalvia



Annelida



Abundance

Acartia tonsa

Multiple linear models		
Parameter	Data set	p-value
Size fraction	Morpho	0.0085
	Meta: Inc	No significant difference
	Meta: Exc	
Station	Morpho	No significant difference
	Meta: Inc	0.00672
	Meta: Exc	0.00672
Year	Morpho	No significant difference
	Meta: Inc	
	Meta: Exc	
Month	Morpho	0.00971
	Meta: Inc	0.02598
	Meta: Exc	0.02598
Temperature	Morpho	0.0412
	Meta: Inc	0.01388
	Meta: Exc	0.01388
Salinity	Morpho	No significant difference
	Meta: Inc	0.02878
	Meta: Exc	0.02878
Dissolved oxygen	Morpho	No significant relationship
	Meta: Inc	
	Meta: Exc	
Chlorophyll a	Morpho	No significant relationship
	Meta: Inc	
	Meta: Exc	

Bivalvia

Multiple linear models		
Parameter	Data set	p-value
Size fraction	Morpho	No significant difference
	Meta: Inc	
	Meta: Exc	
Station	Morpho	No significant difference
	Meta: Inc	
	Meta: Exc	
Year	Morpho	No significant difference
	Meta: Inc	
	Meta: Exc	
Month	Morpho	No significant difference
	Meta: Inc	0.02371
	Meta: Exc	0.02371
Temperature	Morpho	No significant difference
	Meta: Inc	0.0389
	Meta: Exc	0.0388
Salinity	Morpho	No significant relationship
	Meta: Inc	
	Meta: Exc	
Dissolved oxygen	Morpho	No significant relationship
	Meta: Inc	
	Meta: Exc	
Chlorophyll a	Morpho	No significant relationship
	Meta: Inc	
	Meta: Exc	

Annelida

Multiple linear models		
Parameter	Data set	p-value
Size fraction	Morpho	No significant difference
	Meta: Inc	
	Meta: Exc	
Station	Morpho	No significant difference
	Meta: Inc	No significant difference
	Meta: Exc	0.00583
Year	Morpho	No significant difference
	Meta: Inc	No significant difference
	Meta: Exc	0.00204
Month	Morpho	No significant difference
	Meta: Inc	No significant difference
	Meta: Exc	0.01978
Temperature	Morpho	No significant relationship
	Meta: Inc	
	Meta: Exc	
Salinity	Morpho	No significant relationship
	Meta: Inc	
	Meta: Exc	
Dissolved oxygen	Morpho	No significant relationship
	Meta: Inc	
	Meta: Exc	
Chlorophyll a	Morpho	No significant relationship
	Meta: Inc	
	Meta: Exc	

So what?

Next Steps

- 1) Leveraging strengths: metabarcoding biodiversity, morphological sex and stage information
- 2) Run comparisons on strength-leveraged data
- 3) Run it all again with: relative abundances, biovolume, biomass



Field work, zooplankton metabarcoding: Dr. Jamie Pierson, Dr. Louis Plough, Ben Lee

Cost data: Courtney Atkinson and Addie Bowie

Chlorophyll a data: Eyes on the Bay www.eyesonthebay.net

Committee:

Dr. Jamie Pierson
Dr. Louis Plough
Dr. Ann Bucklin
Dr. Mike Roman
Dr. Ryan Woodland
Dr. Dave Richardson

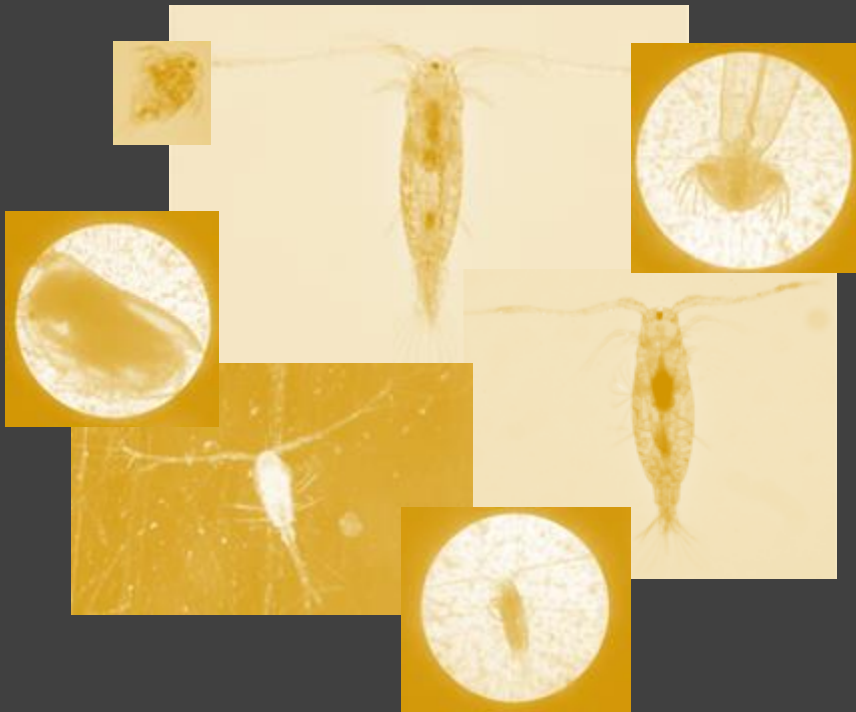
Dr. Heather Cunningham
Interns: Sofiah Ali, Graham Alston, Stephanie D'Elia, Lauren Ervin, Miles Bateson, Kacey Kaub, Maya Skirka

Contact

Catherine Fitzgerald: cfitzgerald@umces.edu

James J. Pierson: jpierson@umces.edu

Louis V. Plough: lplough@umces.edu



University of Maryland Centers
for Environmental Science,
Horn Point Laboratory
Cambridge, Maryland, USA
www.umces.edu/hpl