ASSESSING BIOLOGICAL INVASION IN MAJOR PHILIPPINE PORTS USING ENVIRONMENTAL DNA METABARCODING

Lorraine Joyce M. Del Rosario[1], Lester Arvin S. Pascua[1], Karina Mae N. Delos Santos[1], Ardea M. Licuanan[1], Ambrocio Melvin A. Matias[1], Melody Anne B. Ocampo[2], Ian Kendrich C. Fontanilla[1], Benjamin M. Vallejo, Jr.[1]

[1]Institute of Biology, College of Science, University of the Philippines Diliman
[2]Department of Biology, College of Arts and Sciences, University of the Philippines Manila

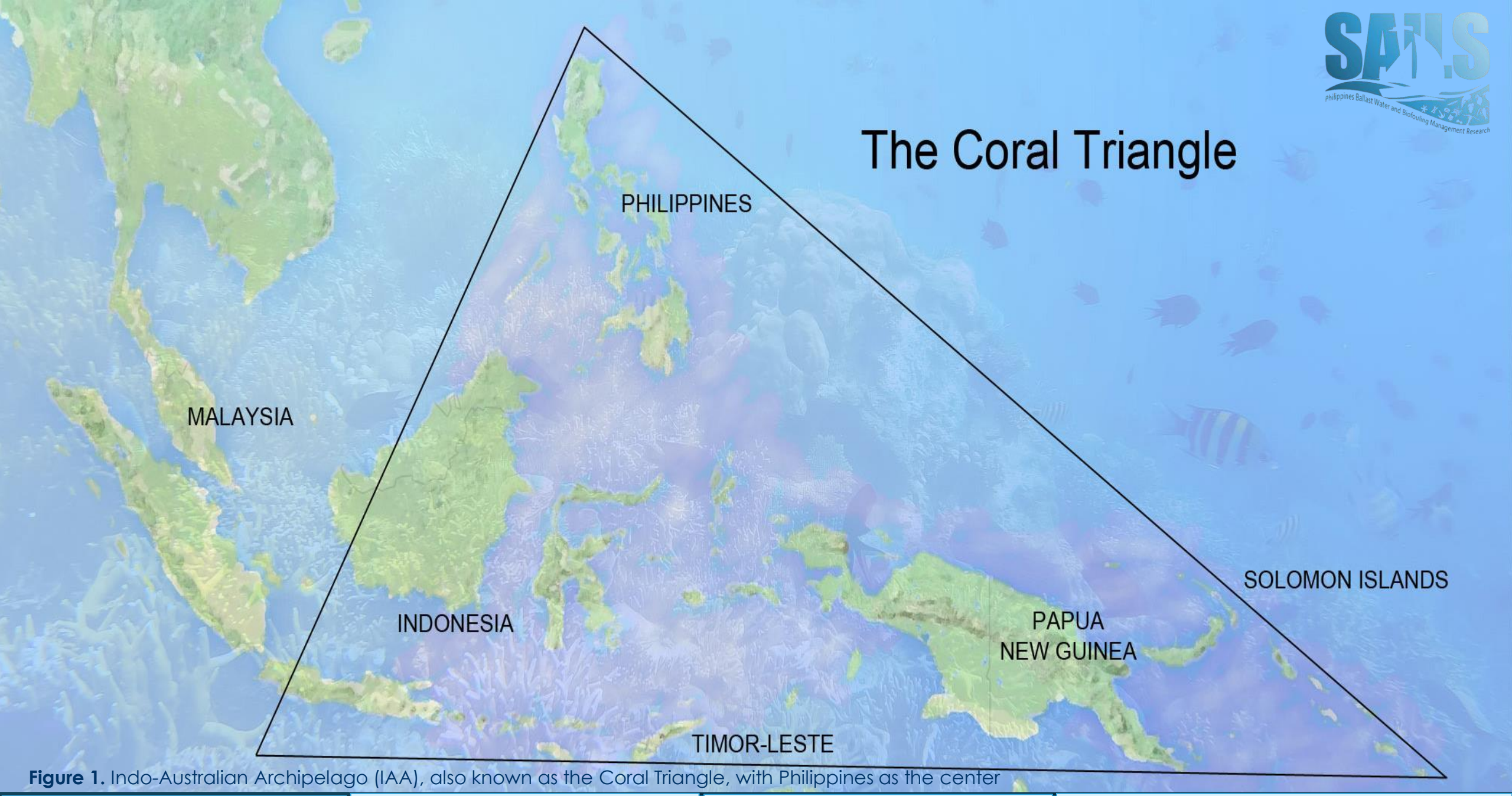


Figure 1. Indo-Australian Archipelago (IAA), also known as the Coral Triangle, with Philippines as the center

Biofouling and Ballast Water

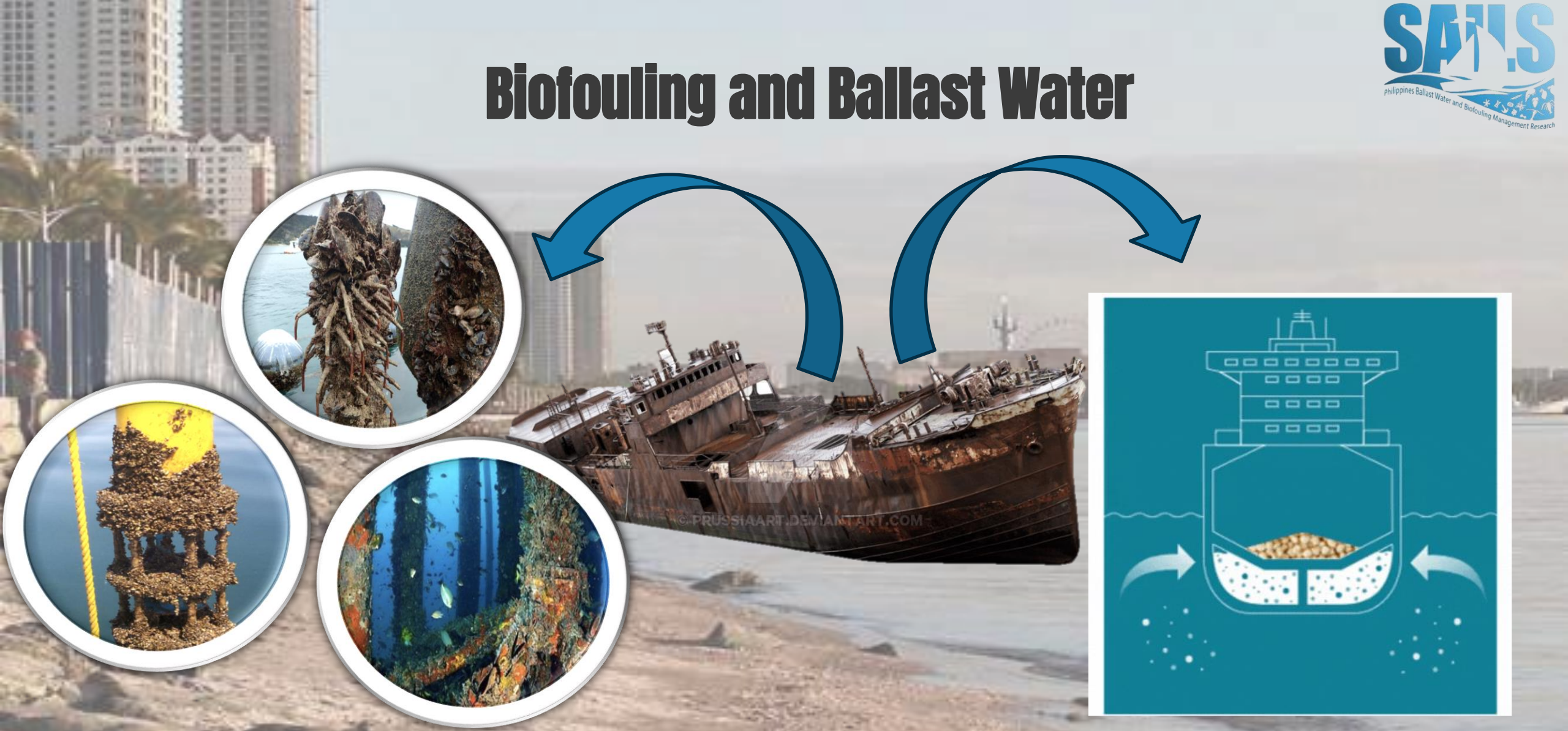


Figure 2. Biofoulers or hullfoulers

Figure 3. Ballast water as discharges from ships

Biological Invasion

- Marine Non-Indigenous Species (MNIS) can be harmful and become invasive, with impacts on native species

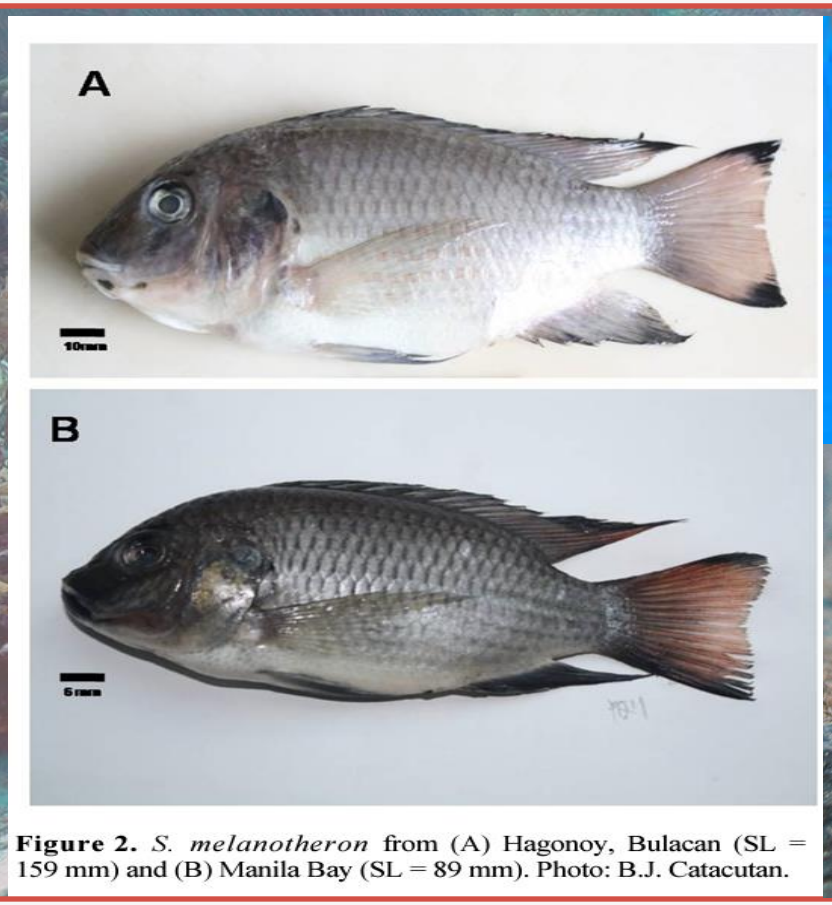


Figure 2. *S. melanotheron* from (A) Hagonoy, Bulacan (SL = 159 mm) and (B) Manila Bay (SL = 89 mm). Photo: B.J. Catacutan.

Figure 4. Non-indigenous Marine Species detected from Manila bay such as Invasive black-chin tilapia, a representative of a flowerhorn, Striped barnacle (*Balanus amphitrite*) and Charru mussel (*Mytella charruana*).

(Deagle et al. 2019; Ordoñez et al. 2015; Ocampo et al. 2014; Vallejo et al. 2017)

PRIMARY DRIVERS

HABITAT LOSS

INVASIVE SPECIES

OVEREXPLOITATION

POLLUTION

CLIMATE CHANGE ASSOCIATED WITH GLOBAL WARMING

INFLUENCERS

- Human population growth
- Increasing consumption
- Reduced resource efficiency

BIODIVERSITY LOSS

Traditional Surveying Method vs eDNA Metabarcoding

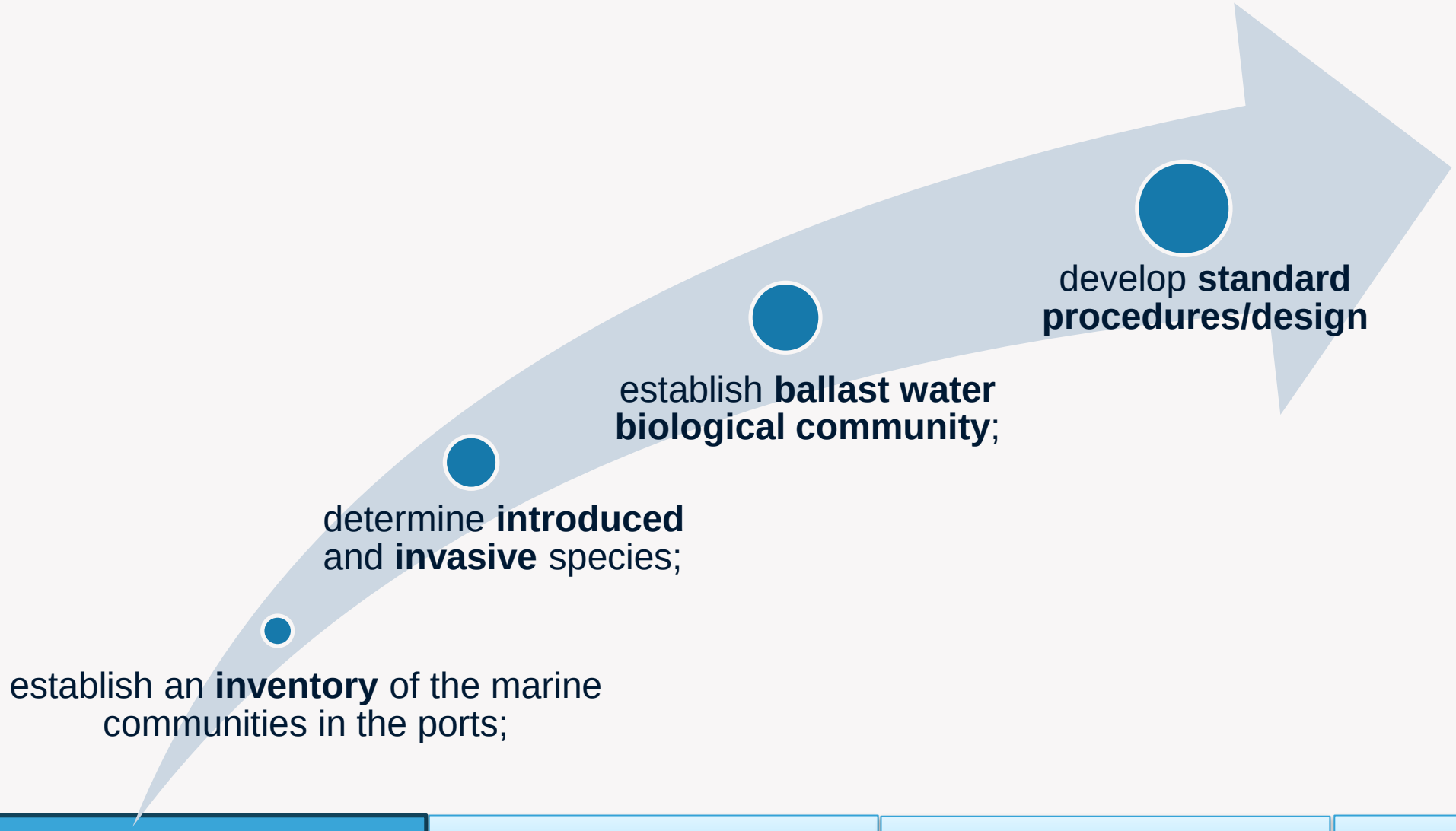
(a)

(b)

Figure 5. Traditional method (a) vs Metabarcoding method using environmental DNA (b) (Templado et al. 2010)

Objectives of the Study

Using eDNA approach, the study aimed to:



Sampling Sites and Dates

Table 1. List of Philippine Ports Studied

Year 1 Ports	Code	Province
Manila Ocean Park	MOP	Manila
Cunanan Wharf	CW	Manila
Manila International Container Terminal	MICT	Manila
Matnog Port	MTG	Sorsogon
Cebu International Port	CIP	Cebu
Davao International Port	DIP	Davao
Year 2 Ports	**Code**	**Province**
Subic Ports	SUB	Zambales
Batangas Port	BAT	Batangas

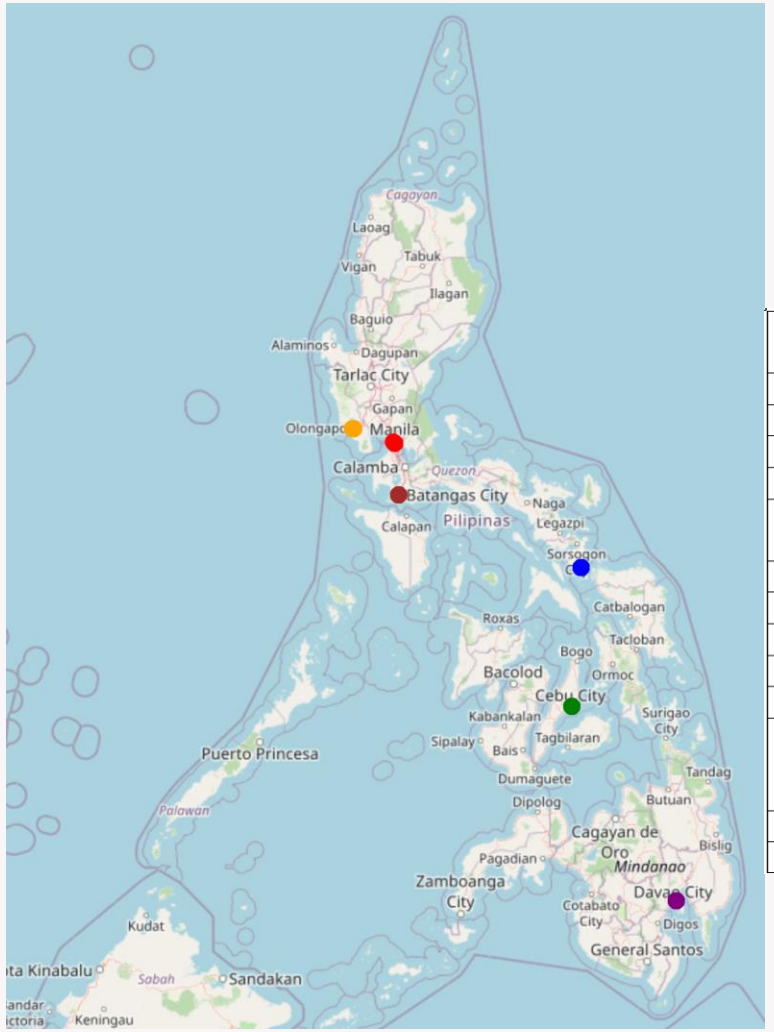


Table 2. Dates of retrieval of samples

YEAR 1 Retrievals	Manila	Matnog	Cebu	Davao
1st	2021-05-04	2021-05-12	2021-05-06	2021-05-07
2nd	2021-06-28	2021-07-13	2021-07-10	2021-07-09
3rd	2021-09-10	2021-09-09	2021-08-25	2021-09-07
4th	2021-10-26	2021-10-28	2021-10-21	2021-11-07

YEAR 2 Retrievals	Manila	Matnog	Cebu	Davao	Subic	Batangas
0th	2022-03-31	-	-	-	2022-04-01	2022-04-01
1st	2022-05-31	2022-05-29	2022-06-01	2022-05-30	2022-06-01	2022-06-02
2nd	2022-07-27	2022-07-28	2022-07-28	2022-07-28	2022-07-28	2022-07-29
3rd	2022-09-28	2022-09-30	2022-09-28	2022-09-29	2022-09-29	2022-09-30
4th	2022-11-28	2022-12-01	2022-11-28	2022-11-28	2022-11-29	2022-12-01

Ballast Water (BW)	Date
Ship #1	2023-06-08
Ship #2	2023-06-10

Figure 6. Mapped coordinates of sampling sites (OpenStreetMap)

Methods

Water Collection → Water Filtration → eDNA Extraction and Amplification → Next Generation Sequencing → Bioinformatics

Figure 7. Water collection of two litres with replicate from port

Figure 8. Filtration using glass microfiber filter papers (0.7µm pore size)

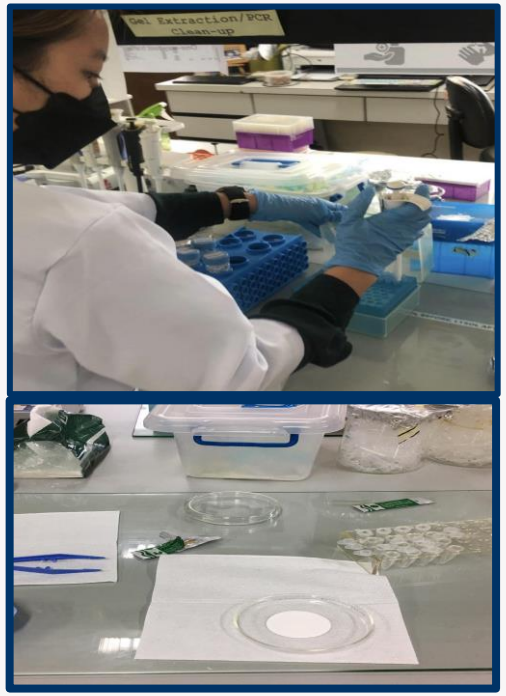


Figure 9. eDNA Extraction and Amplification Process using Uni-minibar primers

Figure 10. Illumina MiSeq System

Check sequence using Fastqc and summarize with multiQC

↓

Merge reads using bbmerge and trim merged reads using bbduk

↓

Run fastq_screen to map reads against known genomes using bowtie2

↓

Classify reads using Kraken2

Figure 11. Steps in Bioinformatics

I. Selected Philippine Ports

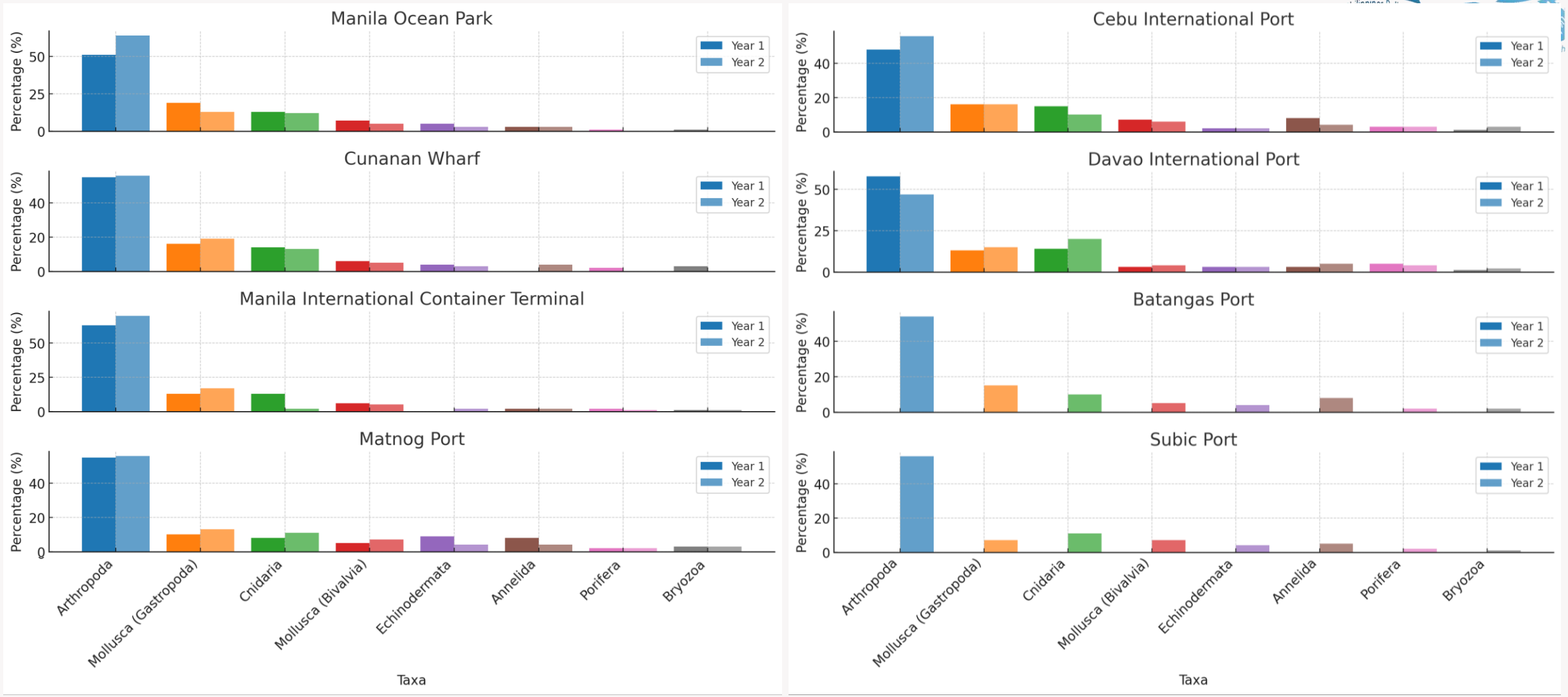


Figure 12. Relative abundance in terms of phyla composition per port (Year 1 and Year 2)

I. Selected Philippine Ports

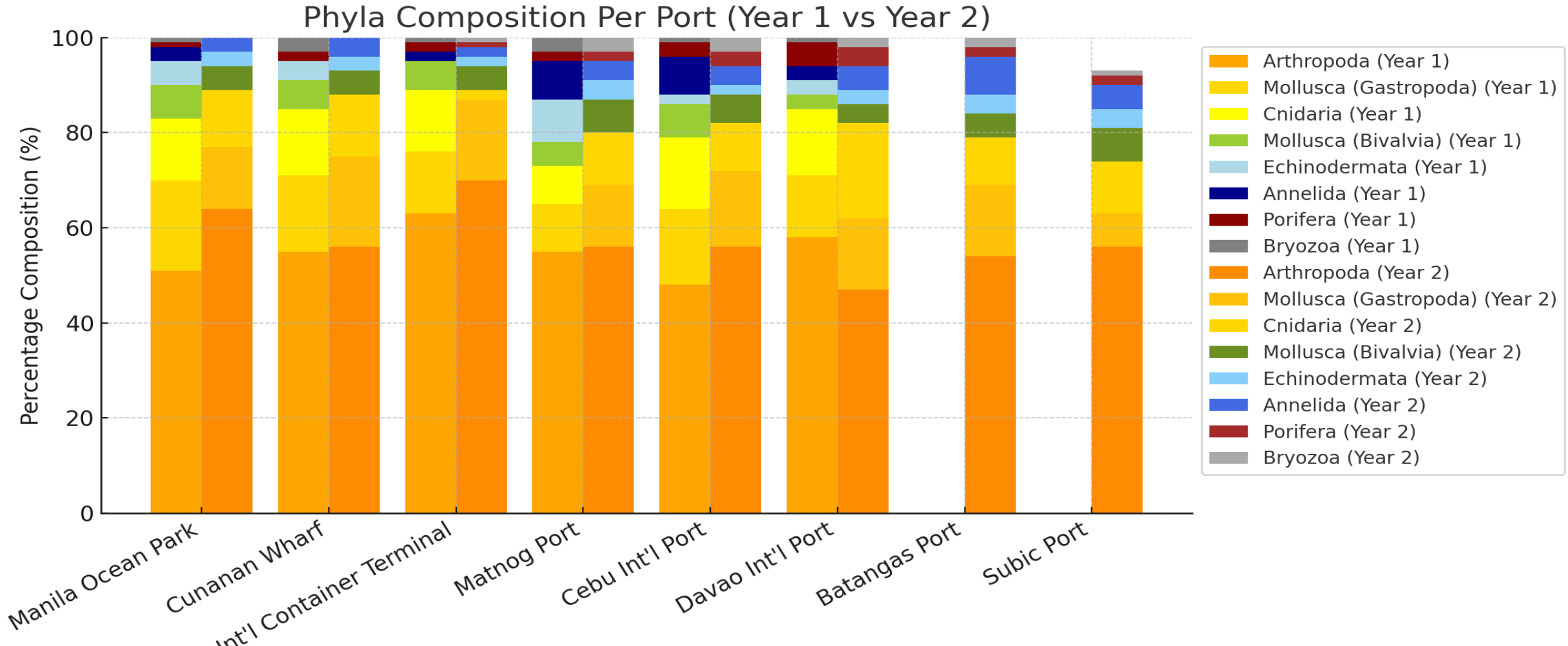


Figure 13. Summarized relative abundance in terms of phyla composition per port (Year 1 and Year 2)

I. Selected Philippine Ports

Table 4. Total and Summary of number of detected species only from selected phyla

Phylum	No. of Species Detected
Annelida	89
Arthropoda	509
Bryozoa	29
Cnidaria	80
Echinodermata	43
Mollusca (Bivalvia)	59
Mollusca (Gastropoda)	177
Porifera	33
Other taxa	153
Total	**1,172**

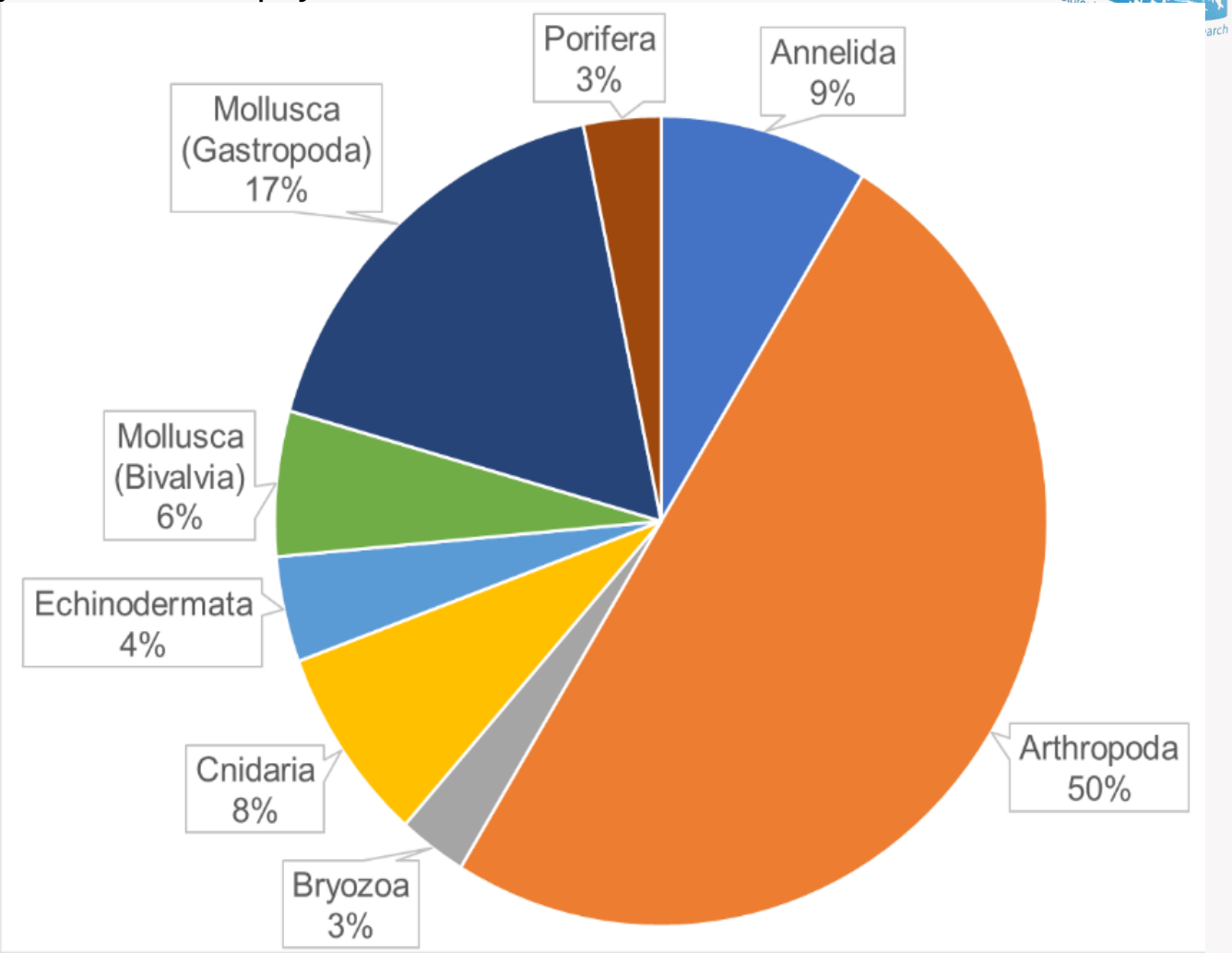


Figure 14. Summarized relative abundance across all ports

II. Ballast Water (BW) from Ship 1 and Ship 2

Table 5. Summary of a number of detected species only from selected phyla

	No. of Species Detected	
Phylum	**Ship #1**	**Ship #2**
Annelida	3	12
Arthropoda	72	112
Bryozoa	14	6
Cnidaria	15	31
Echinodermata	5	6
Mollusca (Bivalvia)	8	12
Mollusca (Gastropoda)	28	26
Porifera	6	2

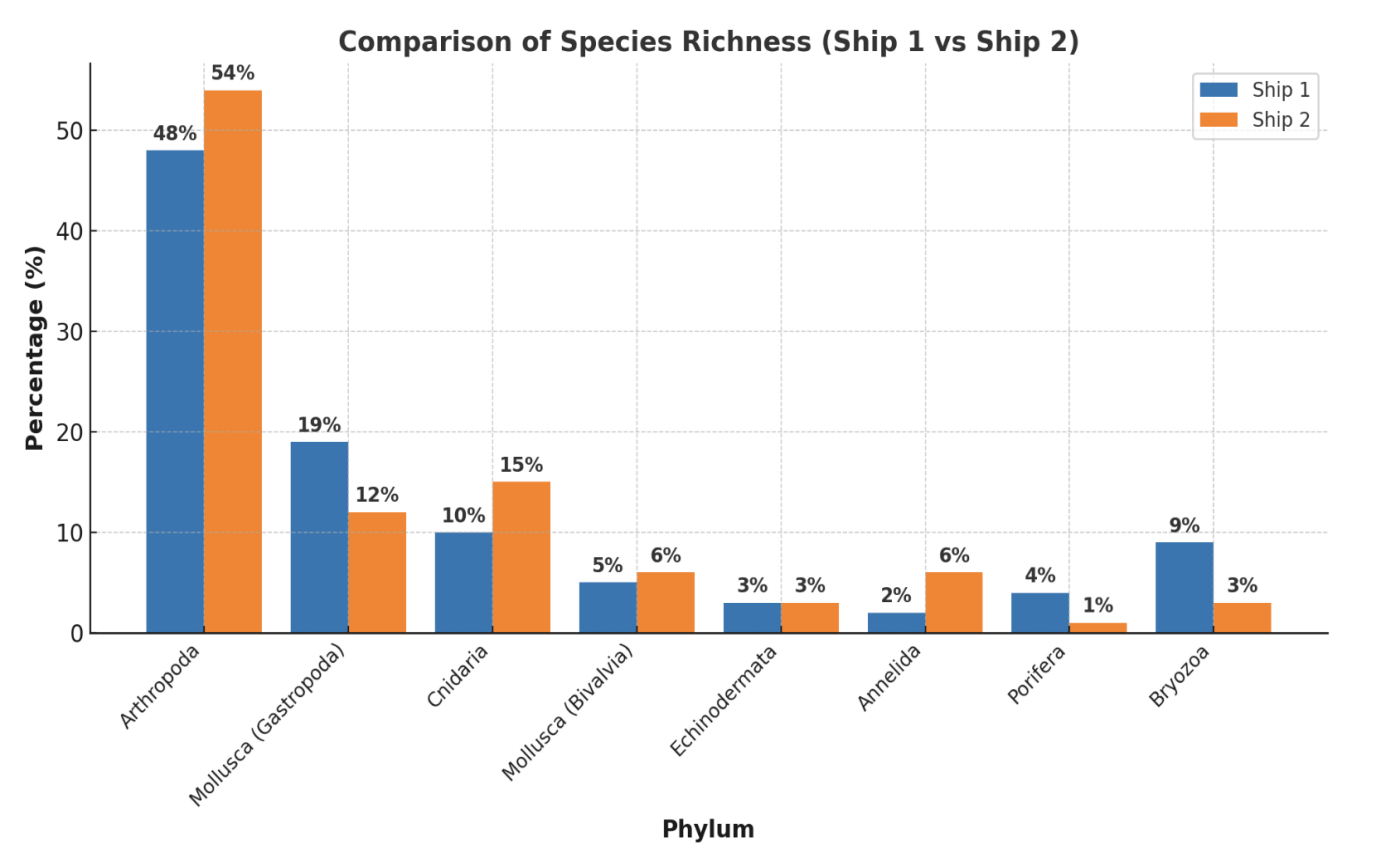
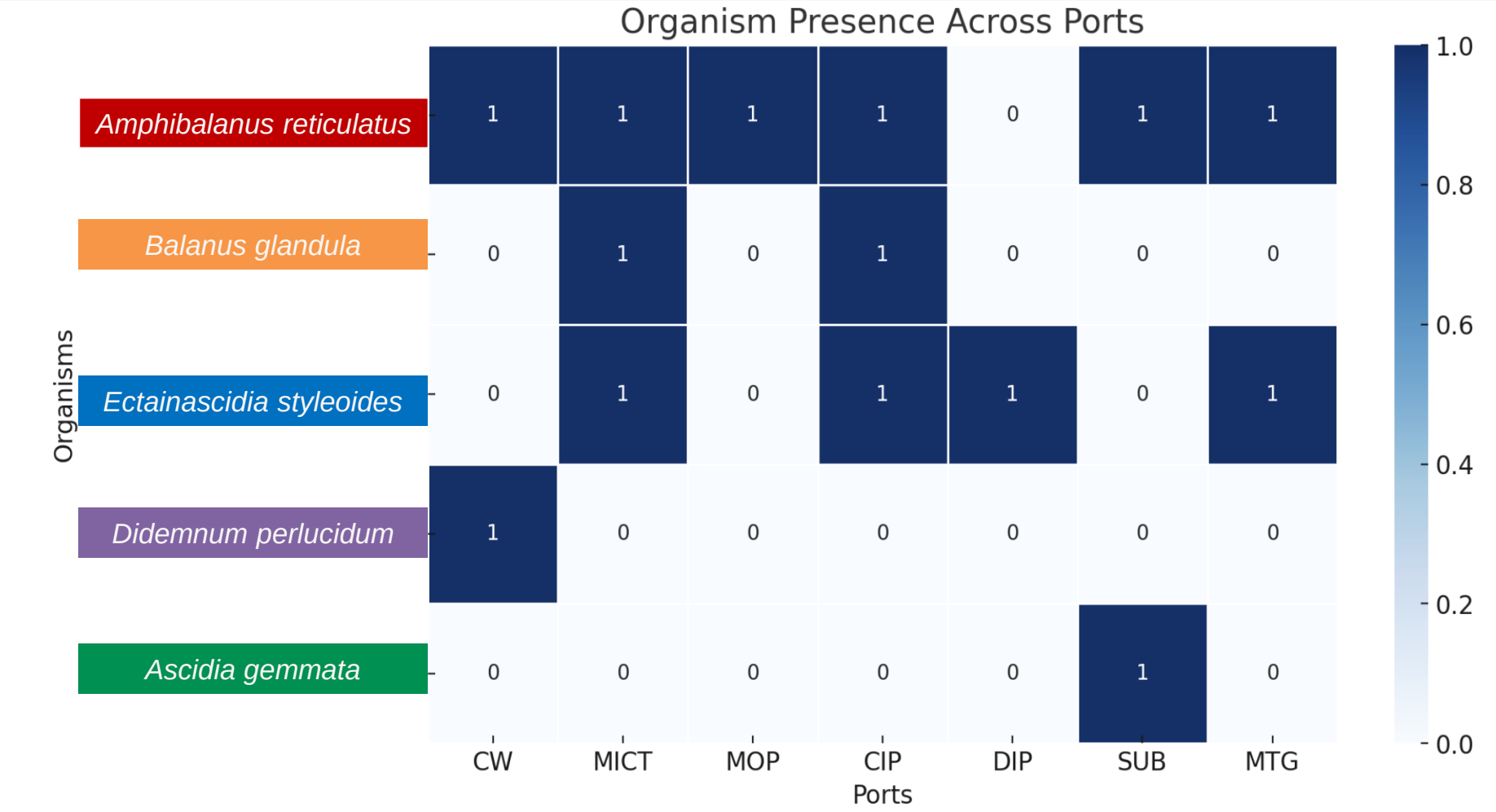


Figure 15. Ship 1 & Ship 2 Ballast Water Species Richness

III. Introduced Species: Potentially invasive to invasive



- **Figure 16** shows how *Amphibalanus reticulatus* dominated the ports. No introduced species were detected in Batangas port.
- *A. reticulatus* is recognized to be potentially invasive (Trivedi et al., 2021).
- *Balanus glandula* is an invasive species (Tricarico, 2016).
- According to World Register of Marine Species, *Ecteinascidia styeloides* and *Ascidia gemmata* are reported to be invasive.
- *Didemnum perlucidum is* invasive due to its rapid reproductive output. (Simpson, 2016).

Figure 16. Heat map generated based on the presence of introduced species per port.

III. Introduced Species Network

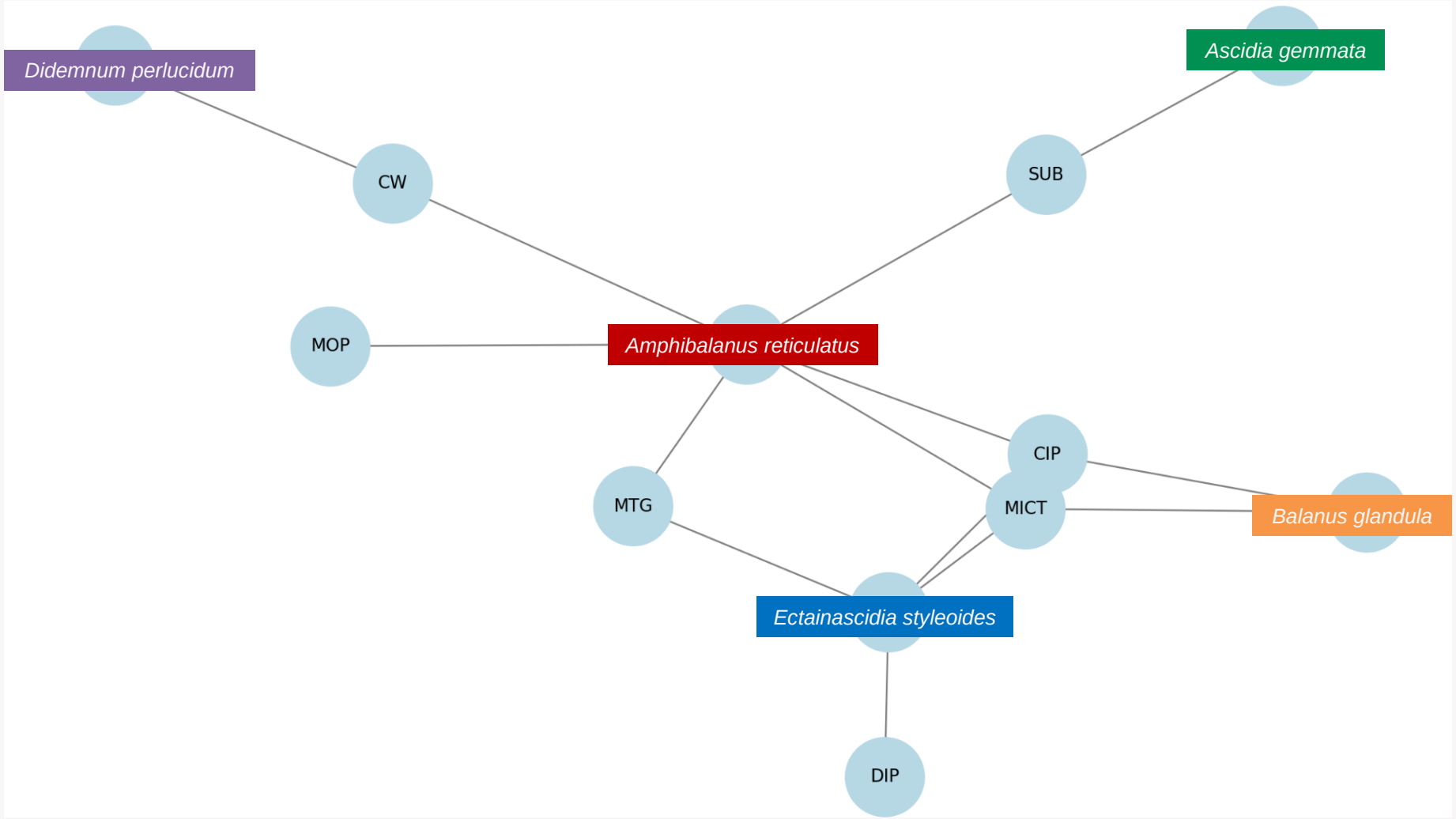


Figure 17 shows how *A. reticulatus* is connected to different ports. Given its widespread distribution and potential impacts, it is recognized as an introduced species with invasive potential in various regions. This has already been reported in Manila bay, using traditional methods (Rosell, 1973) and is known as a frequent fouler of ships and marine structures worldwide in warm subtropical-tropical waters.

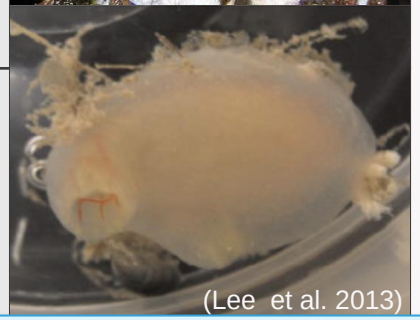
Figure 17. Network analysis of Introduced Species based from WRIMS and COL

III. Introduced Species Distribution

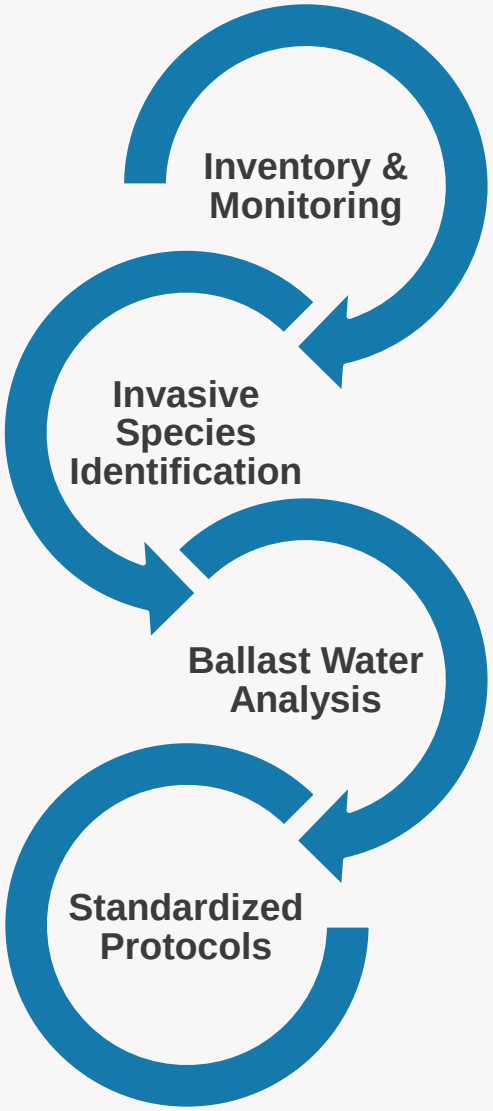
Table 6. List of Introduced Species and their distribution based from WRiMS and CoL

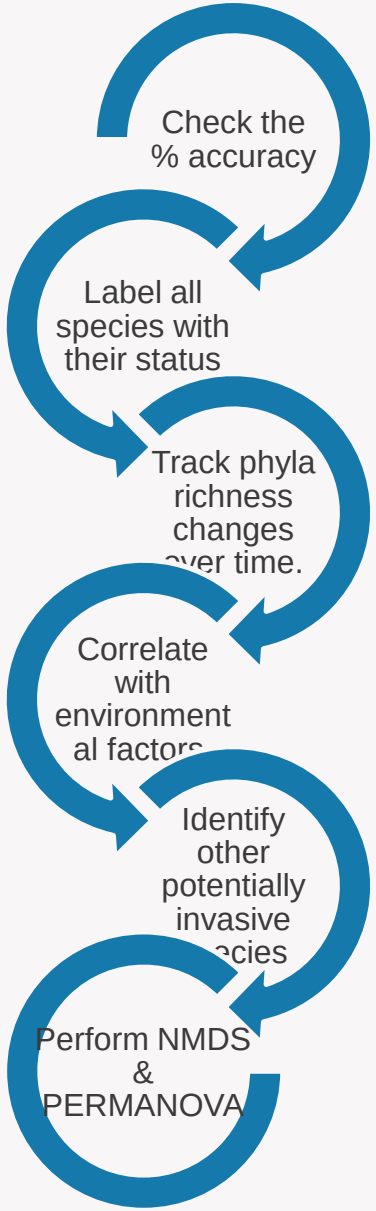
ID	Scientific Name	Authority	Introduced Localities based from WRiMS	Taxon ID	Alien Distribution based from Catalogue of Life	Representative Figures [Mostly from World Register of Marine Species (WoRMS)]
645	*Amphibalanus reticulatus*	(Utinomi, 1967)	Israeli part of the Mediterranean Sea - Eastern Basin, Belgian part of the North Sea, Gulf of Mexico, Gulf of California, Mexican part of the Gulf of California, Brazilian part of the South Atlantic Ocean, Belgian part of the North Sea, Australian part of the Coral sea	N/A	N/A	 (Trivedi et al. 2021)
1370	*Balanus glandula*	(Darwin, 1854)	Belgian part of the North Sea, Argentinean part of the South Atlantic Ocean, Japanese part of the North Pacific Ocean, Uruguayan part of the South Atlantic Ocean, Argentinean part of the South Atlantic Ocean, Belgian part of the North Sea, South African part of the South Atlantic Ocean	**N/A**	N/A	

Table 6. List of Introduced Species and their distribution based from WRiMS and CoL (Continuation)

ID	Scientific Name	Authority	Introduced Localities based from WRiMS	Taxon ID	Alien Distribution based from Catalogue of Life	Representative Figures [Mostly from World Register of Marine Species (WoRMS)]
4368	*Ecteinascidia styeloides*	(Traustedt, 1882)	French part of the Mediterranean Sea - Western Basin	54319649	Mediterranean Sea - Western Basin, Mediterranean Sea, French Exclusive Economic Zone	
4108	*Didemnum perlucidum*	(Monniot F., 1983)	Gulf of Mexico, **Philippines**, Indonesia, United States Exclusive Economic Zone, Australian part of the Indian Ocean, Australian part of the Indian Ocean, Costa Rican part of the North Pacific Ocean, Madeiran part of the North Atlantic Ocean, Galapagos part of the South Pacific Ocean	54319483	**Philippines**, Texas, Panama, Indonesia, North Atlantic Ocean, North Pacific Ocean, Gulf of Mexico, **Philippine Sea**, United States Exclusive Economic Zone, United States Exclusive Economic Zone (Hawaii), Northern Marianas and Guam Exclusive Economic Zone, Pacific Coast	
1052	*Ascidia gemmata*	(Sluiter, 1895)	Indian part of the Laccadive Sea	N/A	N/A	 (Lee et al. 2013)

TAKEAWAYS





FUTURE DIRECTIONS

Acknowledgement

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REFERENCES:

- (2024, January 24). COL. https://www.catalogueoflife.org/
- Azanza, R. V., Alino, P. M., Cabral, R. B., Juinio-Menez, M., Pernia, E. M., Mendoza, R. U., & Siriban, C. S. (2017). *Valuing and Managing the Philippines' Marine Resources toward a Prosperous Ocean-Based Blue Economy.* Public Policy.
- Bailey SA. 2015. An overview of thirty years of research on ballast water as a vector for aquatic invasive species to freshwater and marine environments. Aquatic Ecosystem Health & Management.18(3):261-268.
- Deagle BE, Jarman SN, Coissac E, Pompanon F, Taberlet P. 2014. DNA metabarcoding and the cytochrome C oxidase subunit I marker: Not a perfect match. Biology Letters. 10(9):20140562.
- Lee, S. S., Teo, S. L., & Lambert, G. (2013). New records of solitary ascidians on artificial structures in Singapore waters. Marine Biodiversity Records, 6. Meusnier, I., Singer, G. A., Landry, J.-F., Hickey, D. A., Hebert, P. D., & Hajibabaei, M. (2008). A universal DNA mini-barcode for biodiversity analysis. *BMC Genomics*(9), 214. doi:https://doi.org/10.1186/1471-2164-9-214
- Mannion P D, Upchurch P, Benson RB., Goswami A. 2014. The latitudinal biodiversity gradient through deep time. Trends in Ecology & Evolution, 29(1):42-50.
- Miller EC, Hayashi KT, Song D, Wiens JJ. 2018. Explaining the ocean's richest biodiversity hotspot and global patterns of fish diversity. Proceedings of the Royal Society B: Biological Sciences, 285(1888):20181314.
- Ocampo MA, Oliva IW, Tan R, Su GS, Vallejo Jr B, Manubag ML. 2014. Assessing the marine fouling community in a man-made marina at Manila Bay. Advances in Environmental Biology, 8(13):597-601.
- Ordoñez, JF, Asis, AM, Catacutan B, Dela Pena J, Santos MD. 2016. First report on the occurrence of invasive black-chin tilapia Sarotherodon melanotheron (Ruppell, 1852) in Manila Bay and of Mayan cichlid Cichlasoma urophthalmus (Gunther, 1892) in the Philippines. BioInvasions Records. 4(2):115-124.
- Nakano D, Strayer DL. 2014. Biofouling animals in freshwater: Biology, impacts, and ecosystem engineering. Frontiers in Ecology and the Environment, 12(3):167-175.
- Rosell, N. C. (1973). Some thoracic barnacles (Crustacea; Cirripedia) of Manila Bay. Kalikasan, Philipp. J. Biol, (2), 69-95.
- Simpson, T. S., Wernberg, T., & McDonald, J. I. (2016). Distribution and localised effects of the invasive ascidian Didemnum perlucidum (Monniot 1983) in an urban Estuary. PLOS ONE, 11(5), e0154201.
- Templado, José & Paulay, Gustav & Gittenberger, Adriaan & Meyer, C. 2010. Sampling the marine realm.
- Tricarico, E., Junqueira, A. O., & Dudgeon, D. (2016). Alien species in aquatic environments: A selective comparison of coastal and inland waters in tropical and temperate latitudes. Aquatic Conservation: Marine and Freshwater Ecosystems, 26(5), 872-891. Trivedi, J. N., Doshi, M., Patel, K. J., & Chan, B. K. (2021). Diversity of intertidal, epibiotic, and fouling barnacles (Cirripedia, Thoracica) from Gujarat, northwest India. ZooKeys, 1026, 143-178. Vallejo BM, Aloy AB, Ocampo M, Conejar-Espedido J, Manubag LM. 2019. Manila Bay ecology and associated invasive species. Impacts of Invasive Species on Coastal Environments. 145-169.
- Wood, D. E., & Salzberg, S. L. (2014). Kraken: ultrafast metagenomic sequence classification using exact alignments. *Genome Biol*(15), R46. doi:https://doi.org/10.1186/gb-2014-15-3-r46
- (n.d.) Nemesis. https://nemesis.nisbase.org/
- (n.d.). Smithsonian Tropical Research Institute. https://panamabiota.org/
- (n.d.). WRiMS - World Register of Introduced Marine Species. https://www.marinespecies.org/introduced
- (n.d.). WoRMS - World Register of Marine Species. https://www.marinespecies.org/

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Do you have any questions? Let's connect.

LORRAINE JOYCE MOULIC DEL ROSARIO
DNA Barcoding Laboratory, Institute of Biology
University of the Philippines Diliman
Quezon City 1101
Metro Manila, Philippines

lmdelrosario2@up.edu.ph
: lorraine.delrosario