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Marine fouling assemblage of South Harbor, Manila Bay, Philippines during 2021-2022 monitoring survey

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ABSTRACT

A port, being an interface of land and marine domains, is a unique anthropized coastal ecosystem. Ports have been altered to facilitate docking and berthing of ships and other vessels. Artificial structures replace natural geomorphologic features which are less heterogeneous. The structures, coupled with marine traffic, make port environments hostile to native species and open to non-indigenous (NIS) and invasive species. It is essential to conduct periodic biological surveys to effectively manage these species. In the Philippines, Manila Bay hosts the biggest international port, the Port of Manila. This port is at risk for biological invasions. This study reports the marine fouling assemblage in Manila Bay within a two-year period, from 2021-2022. The study used PICES collectors, which were deployed in a man-made structure along South Harbor. The collectors were retrieved four times a year, having a total of eight retrievals. Organisms on Petri plates were identified through morphological investigation, and DNA barcoding was used for an unidentified bivalve. Results show seven groups of fouling organisms, with barnacles, polychaetes and bivalves being most abundant. Barcoding results reveal that the unidentified bivalves are *Irus irus* and *Irus macrophylla*. They were described by Deshayes in 1854, citing Cebu, Samar and Maguindanao as type localities. However, no report of the species has been recorded in the Philippines since 1895. There is a need for a regular survey in Manila Bay to monitor previously recorded NIS, detect any new introductions, and determine if trends in the abundance of *Irus* spp. show potential for localized invasion.

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INTRODUCTION

Ports are regarded as highly altered coastal environments due to their exposure to intense shipping traffic and habitat degradation (Chapapria & Peris 2021, Agarwala & Saengsupavanich 2023). Globalization has resulted in an exponential increase in international trade and transaction of cargoes, with ninety percent of goods being transported by sea (UNCTAD 2021). Coastlines have been transformed to less heterogenous environments to accommodate vessels. The construction of ports fragments benthic habitats as structures replace soft and hard-bottom habitats (Floerl 2021, Aguilera 2018). The presence of physical and chemical barriers that comprise the infrastructures add to fragmentation (Boudouresque and Sempéré, 2017). Moreover, ports play a considerable role in introducing various types of pollutants to the environment (Bailey and Solomon 2004). Port activities contribute to air, water, noise, soil, sediments and light pollution as well as

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port environment, macrofoulers, barcoding, bioinvasion, *Irus* spp

hazardous toxins (Talley 2003, Anderson and Lee 2006, Elsahragty and Kim 2015, Sharma and Das 2019).

These alterations to the coastal habitat render the environment unsuitable and hostile for native species. As a consequence, native species diversity has been observed to be lower in these areas compared to that of natural habitats (Burt et al. 2011, Firth et al. 2016). These spaces become open to arriving non-indigenous species that are transported via hull fouling and ballast water. As these non-native organisms occupy available port structures, they can thrive and spread. Ports are then susceptible to biological invasions. Invasive species can pose a threat to native organisms, alter ecosystem processes and impact food security (Benson 2001, Bax et al. 2003, Rius et al. 2009).

Conducting studies about fouling biota in ports plays a significant role in assessing risks and creating management plans for biological invasions (Bishop 2011). Baseline and periodic monitoring studies document and detect existing biodiversity, and this is crucial in establishing protocols that will address the negative impacts caused by invasive species.

Biological invasions occur at local, regional and global scales (Wonham and Carlton 2005, Havel and Medley 2006). Spatial scaling can have impacts on processes involved in bioinvasions and their effects (Wonham and Carlton 2005). At the regional and global levels, spatial spread can be between or across specific biogeographic regions. At a local scale, invasive species may exist as indigenous and become invasive even within national and state boundaries. In island nations for example, a species can exist in some islands but not in some, as is the case in Japan (Watari et al. 2021); the spread of these species in areas where they did not previously exist in the nation can have profound impacts. At all scales, human agency allows the species to spread to areas where historically the species is not found and cause significant economic and ecological effects (Chavanich et al. 2010).

The Port of Manila is the largest international maritime container port facility in the Philippines. It is located in Manila Bay, an estuary with a coastline of 190 km and a total surface area of approximately 1,800 km^2. It has various ecosystems and exhibits high biodiversity and ecosystem services (Vallejo et al. 2019). The port's facilities include the Manila North Harbor, Manila South Harbor and Manila International Container Terminal. Per annum, it averages approximately 21,000 marine watercraft traffic and a footfall of 72 million passengers; in terms of capacity, it has container volume of 4.5 million TEUs and cargo tonnage of more than 75 million tons. In 2015, it was reported to have handled a total of 5 million TEUs (Menon 2021). This was a 14 percent increase from the previous year (ESCAP 2020), and the trend is expected to continue. The increase in volume of shipping activities in the bay increases the risk of introduction of potentially invasive marine (Ocampo et al. 2014).

In the recent years, studies have documented communities of native, non-indigenous and invasive organisms in the port. A serpulid polychaete, *Hydroides elegans* has been recorded (Vallejo et al. 2019). It is a major tropical fouling marine worm that poses to populate cooler marine vessel port areas in Europe, Australia and North America due to warming temperatures. Bivalves have been given more attention, due to their economic and ecological importance to human settlements around Manila Bay, as bivalves are mainly a source of food and livelihood. *Mytilopsis sallei*, a bivalve closely related to invasive *Dreissena polymorpha*, was detected in 2014 (Ocampo et al. 2014). Its abundance though, has remained low in successive collections, unlike in Singapore, Thailand, Hong Kong and other neighboring countries where the populations formed mats in lagoons and harbors (Tan and Morton 2006, Morton 2009, Tan and Tay 2020). Another bivalve, *Brachidontes pharoanis* which originates from the Red Sea, was documented (Ocampo et al. 2014). The species is a migrant to the Mediterranean Sea due to maritime transport in the area (Galil 2007). *Mytella strigata*, a bivalve from the mytilid family, was detected and confirmed in 2017 (Vallejo et al. 2017). It is a native of the coasts of Central and South America (Lim et al. 2018) and it has expanded range over various parts of the Philippines (Fuertes et al. 2021). In a survey conducted in 2019 (Trinidad et al.), a new white bivalve was detected and was one of the most abundant in the study. The bivalve was not recorded in previous Manila Bay port studies (Ocampo et al. 2014, Ocampo et al. 2019). It was barcoded but did not get an identity match in the GenBank database (Trinidad et al. 2019), hence it was not conclusively identified.

To continue surveillance of Manila Bay, the present study was conducted in 2021 to 2022; it is the most recent in the bay. It aims to provide identification of groups of foulers in the bay for that period, with a focus on bivalves, using morphological method, and molecular method for the unidentified bivalve.

METHODOLOGY

Deployment and retrieval of PICES collectors

The study site was at the Manila Ocean Park of the Port of Manila in Manila Bay. There were five sampling points around the park (Figure 1).

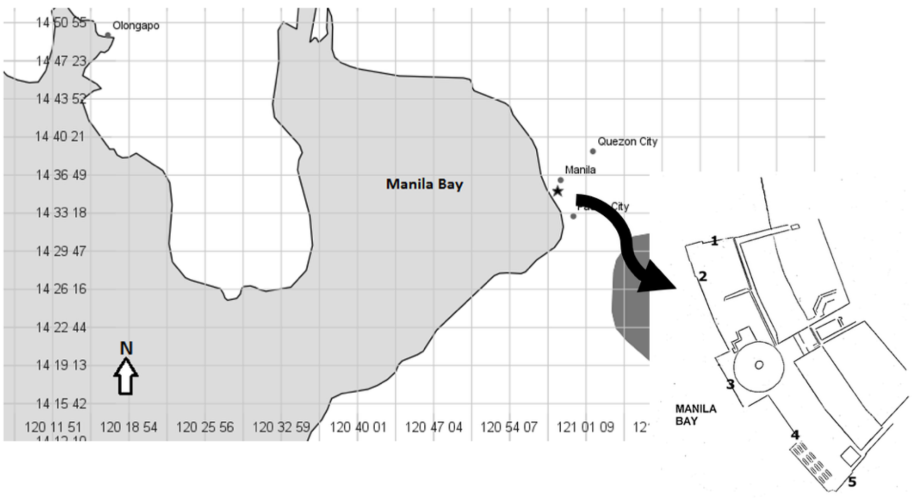

Figure 1: Location of study site represented by the star, and five sampling points in inset. Map generated through SimpleMappr (www.simplemappr.net)

Fouler collectors were used to gather samples in this study. Their design was from the North Pacific Marine Sciences Organization (PICES) collectors, which was modified for use of South East Asian countries (Ocampo et al. 2014, Vallejo et al. 2017, Vallejo et al. 2019). The collector is composed of a bucket lid of approximately 30 cm diameter, with four 9 cm diameter plastic petri plates attached to it. Each plate had an area of 64 cm^2. The plates were fastened to the bucket lid using cable ties. The fouling collectors were submerged in sea water for one week before usage. The center of the bucket lid was drilled with a hole where a 15 m nylon rope can pass through. One end of the nylon rope will be tied to a structure in the harbor, while at the other end of the nylon rope was a cement weight (≥2 kg) was attached. The purpose of the weight is to keep the collector submerged under water after its deployment.

Fifteen collectors were deployed around the Manila Ocean Park deck; three replicates were submerged for each of the five sampling points. The collectors were tied to the railings at the sampling five points, at a depth of at least 1 m from the surface of the water, from the lowest tide level. Water quality parameter measurements (pH, temperature, total dissolved solids, dissolved oxygen) of the sampling sites were taken. The fifteen collectors were used for one year for four retrievals. A new set of fifteen collectors was deployed for the second year.

The collectors were deployed from March 2021 until November 2022. Retrieval was done approximately every 60 days, removing one petri plate per collector. Photo documentation of the petri dishes was done after retrieval. The Petri dishes were sent to the laboratory for fouler identification and specimen storage. Ideally retrieval should be done exactly every sixty days. There was a discrepancy in the exact number of days in between retrievals due to logistical limitations for transportation and manpower brought about by the Covid pandemic.

Fouling organisms identification

Retrieved Petri plates were brought to the invertebrate laboratory of the Department of Biology, University of the Philippines Manila and the Biogeography, Environment, Evolution and Climate Laboratory of the Institute of Environmental Science and Meteorology, University of the Philippines Diliman. Each organism was removed from the Petri plate using forceps. They were placed in vials which contained 70% ethyl alcohol. The specimen were viewed under a Nikon stereomicroscope and were photo documented. Foulers, particularly bivalves, were identified to species level when feasible using taxonomic keys and online databases. Validation was done at both laboratories as well. White bivalves which were unidentified and suspected as *Irus* spp. were observed and morphological descriptions were taken note of. Samples were also preserved for DNA barcoding.

Determination of fouling organisms' relative abundance and biodiversity indices

Identified organisms and groups were counted, and the relative abundance computed using Microsoft Excel 365. Relative abundance is calculated by dividing the number of individuals of a specific species by the total number of individuals in the community, then multiplying by 100 to express it as a percentage. Biodiversity indices, such as Shannon-Wiener index and Simpson index per retrieval, were computed using Microsoft Excel 365.

DNA barcoding of suspected Irus spp specimen

DNA barcoding of the bivalve specimens was conducted at the DNA Barcoding Laboratory at the University of the Philippines Diliman Institute of Biology. Six samples from the pool of collected specimen in the sampling period were used for barcoding. DNA from the bivalve tissues were extracted using the Cetyltrimethylammonium Bromide (CTAB) protocol. Afterwards, DNA concentration was measured using NanoDrop™ 2000C.

The PCR reactions were carried out in 10µL volume consisting of the following: 1µl of 30-50 ng DNA template, 0.35 µL forward primer, 0.35 µL reverse primer, 5 µl MyTaq™ HS Red Mix 2x, and 3.2 µl nuclease-free water. The forwaard primer used to amplify the COI gene was LOBOF1: 5'KBTCHACAAAYCAYAARGAYATHGG-3' (Lobo et al. 2013), and reverse primers were either HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Vrijenhoek 1994) or LEPR1: 5'-TAAACTTCTGGATGTCCAAAAAATCA-3' (Hebert, 2004). The tubes with the PCR reaction mixtures were inserted into a thermocycler for amplification. The PCR amplification protocol of Lobo et al. (2013) was employed with modifications. Storage is at 4°C. PCR products were evaluated using agarose gel electrophoresis. The gel was done by putting together 0.5 g 1% agarose gel, 50 mL TBE loading buffer, and 3 µL GelRed® Nucleic Acid Gel Stain. The gel was then placed in the casting tray. Then, 5 µl of a 100 bp DNA ladder was first loaded into the well, followed by 2 µl of the samples on the succeeding wells. The gel ran for 25 minutes at 100 volts. Results were then visualized using a UV trans-illuminator. The remaining PCR products of the samples with positive bands were sealed and sent to Macrogen for bidirectional sequencing.

Once the sequences were received, they were assembled using Pregap4 version 4.10. The primer sequences were trimmed and the consensus sequences were generated using Gap4 version 4.10 (Staden et al., 2000). The assembled COI gene sequences were submitted for Basic Local Alignment Search Tool (BLAST) searches at the GenBank National Centre for Biotechnology Information (NCBI) to determine related sequences.

Morphological examination of suspected Irus spp

Suspected *Irus* spp specimen were placed in vials containing 70% ethyl alcohol. Shell orientation was observed, as well as external and internal features of the shell under a Nikon stereoscope. Observations were used for characterization and identification.

RESULTS

Identified foulers, relative abundances and biodiversity indices

There were eight retrievals of collectors from May 2021 to November 2022. The fouling groups contained in the plates during this period include arthropods, molluscs, annelids, cnidarians, bryozoans, platyhelminths, and chordates. Arthropods were most abundant, particularly the barnacles. Polychaetes and molluscs were second and third interchangeably, depending on retrieval date. The most abundant fouling species occurring during all retrievals was *Amphibalanus amphitrite* (Phylum Arthropoda), having a relative abundance ranging from 39.29% to 69.52% (Figures 2 and 3).

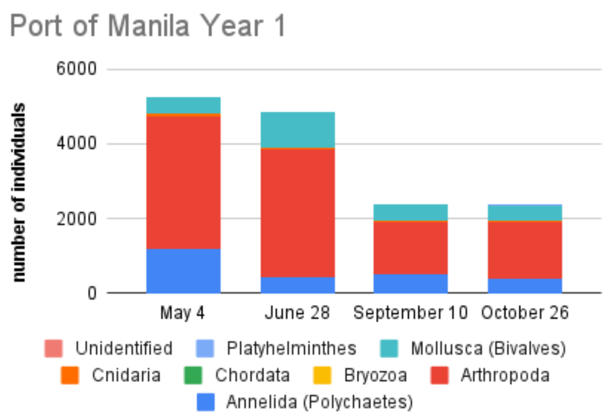

Figure 2: Relative abundance of macrofouling groups at the Port of Manila in Year 1 2021 (sample size 5268, 4862, 2364 and 2369 respectively).

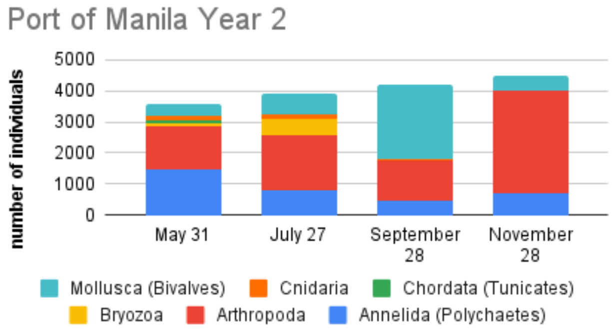

Figure 3: Relative abundance of macrofouling groups at the Port of Manila in Year 2 2022 (sample size 3574, 39907, 4180 and 4483 respectively).

The molluscs collected were all bivalves. Several bivalve species were detected throughout the entire sampling period. In relatively high abundances are *Mytella strigata*, *Brachidontes pharaonis*, *Perna viridis*, *Mytilopsis* sp., *Modiolus* sp. and suspected *Irus* spp. Suspected *Irus* spp. were detected in each of the eight retrievals. *Irus* spp. relative abundance throughout the entire sampling season ranged from 0.61% to 18.28%, having the least abundance during the first retrieval of year 1 and being most abundant in the third retrieval of year 2.

During the first year, *Irus* spp. composed 6.21% to 34.04% of the bivalve fouling community. Native *Perna viridis*. was the most abundant for the first retrieval (56.55%), another native *Modiolus* sp. for the second retrieval (66.49%), while invasive *Mytella strigata*. was the most abundant for the third and last retrieval (43.33% and 51.06%, respectively) (Figure 4).

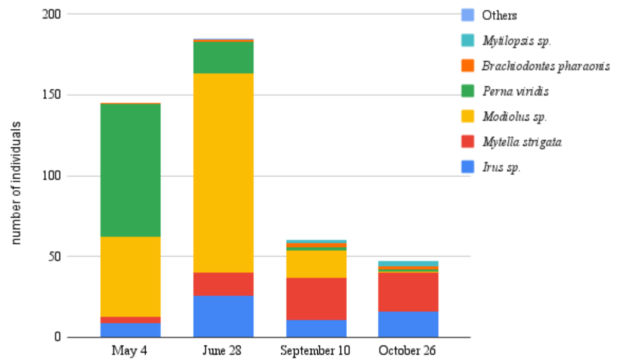

Figure 4: Relative abundance of bivalves collected at the Port of Manila in Year 1 (2021).

For the second year, *Irus* spp. were most abundant in the bivalve foulers in the second and fourth retrievals, with a relative abundance of 54.19% and 48.3%, respectively. It was least abundant on the first retrieval (11.81%) and in the third retrieval measured 28.98% of the community. *Modiolus* sp. dominated the first retrieval (80.31%) and *Mytella* sp. for the third retrieval (46.02%). *Irus* spp. increased in abundance during the second year (Figure 5). *Irus* spp. were only observed in the collectors at the Manila Ocean Park among all study sites in Manila Bay that are not included in this paper.

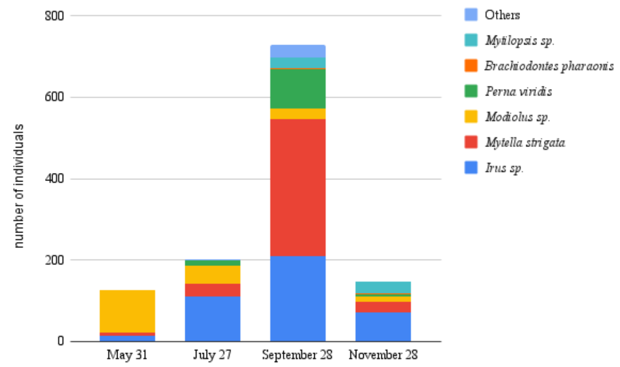

Figure 5: Relative abundance of bivalves collected at the Port of Manila in Year 2 (2022).

Biodiversity indices values were Shannon-Weiner diversity index and Simpson's diversity index for years 1 and 2 (Table 2 and 3). For both indices, the highest values in the eight retrievals were during June 2021, and the lowest during October 2021. Most of the values of Simpson's index though was close to 0.5, except for June 2021. The highest Shannon-Weiner index value was at 1.394, and the lowest at 0.791.

DNA Barcoding of Irus sp.

The COI gene was sequenced from six bivalve specimens. The sequences generated were subjected to BLAST to confirm species identity. Results revealed a 99.66% to 99.83% match for the collected bivalves and two species of *Irus* spp. Four of the samples (WB 1-4) were identified as *Irus irus*, while the other two were identified as *Irus macrophylla* (WB 5 and WB 6) (Table 1, Figures 8 and 9). The taxonomic classification of *Irus* spp. is as follows: class Bivalvia, infraclass Heteroconchia, superorder Imparidentia, order Venerida, family Veneridae, and genus *Irus*.

Morphological examination of Irus spp.

The white bivalves from the sampling points were identified using the morphological approach as *Irus* spp. The average length and height of the four samples barcoded as *Irus irus* were 13.5 mm and 8 mm, respectively. On the other hand, the average length and height of the two samples identified as *Irus macrophylla* through barcoding were 11.5 mm and 6 mm. There were no discernable differences in shell structure among the barcoded *Irus* samples. The shells were cream in color, with a solid brown to black discoloration that usually originates from the hinge to the posterior surface. In some samples, discoloration was scattered on the inner surface of the valves. In addition, brownish orange discoloration lines radiated from the hinge to the posterior margin. No nacreous layer can be found in the interior of the shell, hence there is no pearl-like luster appearance. The left and the right valves were equal in size (equivalve). The beak or umbo were placed beyond the shell midline; hence, the bivalve is inequilateral. The shell had an oblong outline with a narrower posterior side. The lunule was subtle and small and the escutcheon was extensive. The valves also have a distinct well-spaced concentric ruffle-like lamellae which becomes more elongated and ornate along the posterior margin. It had an intersecting radial and concentric linear pattern (cancellate pattern). The hinge plate bears three cardinal teeth and no lateral teeth. The sunken ligament was long, covering a third of the dorsal margin. The valve margin was smooth but appeared crenulate. The absence of morphological differences among the samples may be attributed to the sizes of the samples, being smaller than the adult, hence are juvenile (Baker and Mann 1997). Another factor is the environmental influence of a disturbed habitat like that of a port environment that might have affected the shell growth of the organisms (Cerdeira-Arias et al. 2024, Chahouri et al. 2023).

Figure 6: Average values of water quality parameters (a, temperature; b, pH level; c, conductivity; d, dissolved oxygen; e, total dissolved solids) from the sampling points in the Port of Manila, January to October 2021.

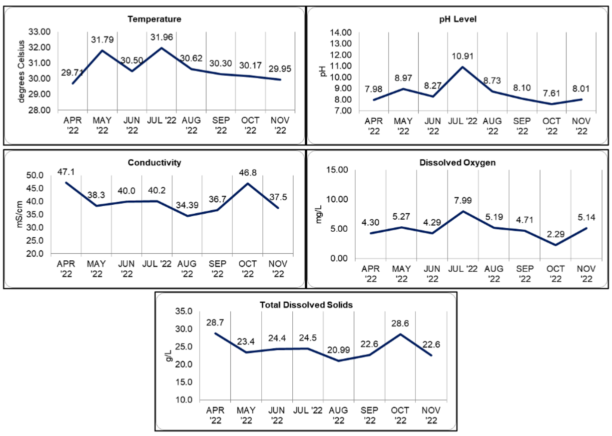

Figure 7: Average values of water quality parameters measured on the sampling points of the Port of Manila, April to November 2022.

Table 1: Genetic identities of white bivalves retrieved from Manila Bay.

Specimen ID	Closest Species	Family	GenBank ID	Identity Match (%)
WB 1	*Irus irus*	Veneridae	MN608355.1	99.83
WB 2	*Irus irus*	Veneridae	MN608354.1	99.83
WB 3	*Irus irus*	Veneridae	MN608355.1	99.66
WB 4	*Irus irus*	Veneridae	MN608355.1	99.66
WB 5	*Irus macrophylla*	Veneridae	OM292836.1	99.66
WB 6	*Irus macrophylla*	Veneridae	OM292836.1	99.83

Table 2: Biodiversity indices for Year 1.

Biodiversity Indices (2021)	May 4	June 28	September 10	October 26
Shannon-Wiener Diversity Index	0.865	0.862	0.986	0.935
Simpson's Diversity Index	0.483	0.466	0.565	0.519

Table 3: Biodiversity indices for Year 2.

Biodiversity Indices (2022)	May 31	July 27	September 28	November 28
Shannon-Wiener Diversity Index	1.284	1.394	0.957	0.791
Simpson's Diversity Index	0.663	0.705	0.565	0.433

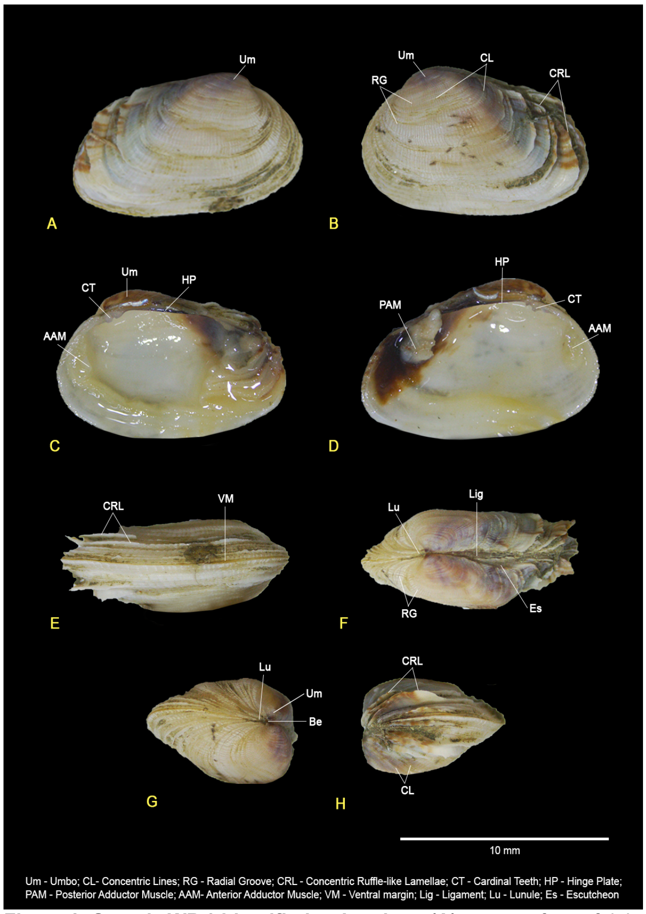

Figure 8: Sample WB 3 identified as Irus irus. (A) outer surface of right valve **(B)** outer surface of left valve **(C)** inner surface of right valve **(D)** inner surface of left valve **(E)** ventral view **(F)** dorsal view **(G)** anterior view **(H)** posterior view.

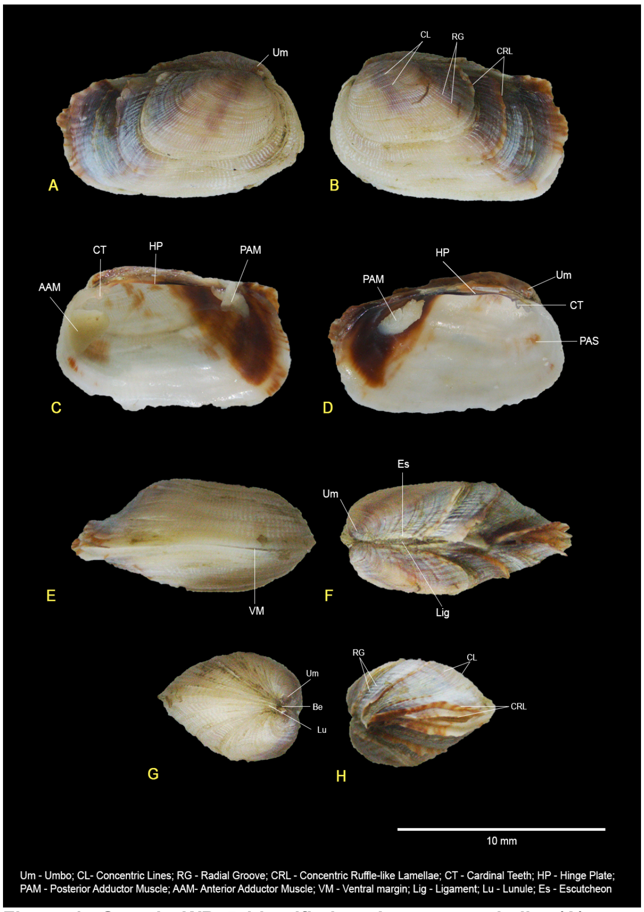

Figure 9: Sample WB 5 identified as Irus macrophylla. (A) outer surface of right valve **(B)** outer surface of left valve **(C)** inner surface of right valve **(D)** inner surface of left valve **(E)** ventral view **(F)** dorsal view **(G)** anterior view **(H)** posterior view.

DISCUSSION

The study provides the most recent data on fouling assemblages in South Harbor, Manila Bay. This study is essential, as the conduct of periodic surveys allows the monitoring of the introduction and spread of non-indigenous and invasive species. Surveys serve as a vital tool in creating and supporting effective management policies for these species (Rey et al. 2020).

The fouling groups observed in the study include arthropods, molluscs, annelids, cnidarians, bryozoans, platyhelminths, and chordates. The arthropod *Amphibalanus amphitrite*, the most abundant organism in all retrievals, is a common fouling species native to the Indo-Pacific region and distributed in tropical and subtropical waters (Galil 2011). As it is a common fouler, it is used as model species for invertebrate studies (Marechal & Hellio 2011) and test species for biofouling studies and antifouling paints (Kojima et al. 2019). *A. amphitrite* has spread to other tropical and temperate regions. In areas where it is considered invasive, such as Florida, USA, its presence can lead to a decrease in the growth and settlement of eastern oyster *Crassostrea virginica* (Bordeaux et al. 2009) and competes with native *Amphibalanus* species (Bordeaux et al. 2009).

Annelids and molluscs account for the second and third most abundant group in the retrievals, their ranks interchanging depending on the retrieval period. Annelids were comprised solely of polychaetes. Polychaetes are among the most abundant and diverse organisms in most marine benthic habitats. Polychaetes are observed to be an environmentally tolerant group, as they can adapt to exposure to pollutants such as organic matter and heavy metals (Dean 2015). The absence, presence or change in their species composition of a community can indicate the environmental quality and pollution status of a marine environment. (Nasi et al. 2018, Sobczyk et al. 2021).

Molluscs were composed entirely of bivalves. They are more closely monitored as bivalves in Manila Bay are a source of food and livelihood (Bayate et al. 2016). Moreover, they are observed to be one of the most invasive assemblages of marine organisms, causing serious environmental, ecological and economic damages (Tan & Morton, 2006, Crespo et al. 2015, Moura et al. 2017, Vallejo et al. 2017, Biju Kumar et al. 2019, Muhdati et al. 2024). Their ability to form byssus allows them to attach on substrates on rocky shores but at the same heavily foul man-made surfaces and form mats over large areas. Also, as bivalves are filter feeders, they can deplete large amounts of particulate organic matter. This can cause a drastic decrease in the availability of food for indigenous species; their presence in large numbers may also can also affect metabolic functions, life cycle and growth of indigenous species (Sousa et al. 2009).

Mytilopsis sp. and *Mytella strigata,* were observed in all retrievals. *M. sallei* is a Carribean mussel that has been invasive in many parts of the Indo-Pacific region (Tan and Morton 2006, Morton 2009, Tan and Tay 2020). In Singapore and Malaysia, it was observed to establish a broad, expansive, heavily populated band on the concrete walls and floor of monsoon drains with its mat stretching up to several kilometers inland from the sea (Tan and Morton 2006). In the Philippines, *M. sallei* has not been found in high abundances. This may be because the green mussel *Perna viridis* and oyster *Magallana bilineata* reproduces and establishes earlier than *M. sallei* (Vallejo et al. 2019). *Mytella strigata*, a bivalve detected in 2017 (Vallejo et al.) in Manila Bay and in other Philippines areas in subsequent years (Fuertes et al. 2021), has become part of the community coastal diet along with *Perna viridis.* The species has also been seen in India, accumulating in protective walls, bridges and pillars in its Kerala coast (Biju Kumar et al. 2019). *Perna viridis* and *Modiolus* sp were also observed in most of the retrievals, both are indigenous and commonly consumed (Salinas and Rosario 2016). *Perna viridis* is widely cultivated in Manila Bay.

The study confirms the presence of *Irus* spp. in Manila Bay. *Irus* spp is a venerid clam that has been reported and described in 1854 by Deshayes through species synonyms from shell collections, the synonym *Venerupis macrophylla* for *Irus macrophylla,* and *Venerupis pulcherrima* and *Venerupis derelicta* for *Irus irus*. The collections for these species cited the Philippines as the species' type locality, Cebu for *Irus macrophylla* (de Elera 1895), and Samar (de Elera 1895) and Maguindanao (Deshayes 1854) for *Irus irus*. However, there had been no known reports of the two species in the Philippines after their description by Deshayes and publication by de Elera.

Irus irus has also been reported as native to the Mediterranean Sea, Black Sea, and Northeast Atlantic from the United Kingdom and Ireland (Osturk et al. 2014, Hayward and Ryland 1990, Gofa et al. 2001). It has been found in the 1980s in China and Hong Kong (Morton 1983). In Singapore, *Irus irus* has been recorded in the Johor Strait at Kranji Dam (Tan and Low 2013), commonly encountered in mangroves and muddy conditions. They also attach to structures in piers, buoys and rafts (Morton 1983). South Africa has reported its presence in Sta. Lucia estuary in 2012 (Nel et al.). *Irus macrophylla*, on the other hand, is also located in Tanzania and Madagascar according to databases. It has also been recorded in the Gulf of Thailand (Sartori et al. 2008). There was no indication in the reports, though, if the species' presence were likely introductions.

There has been no historical record of *Irus* spp. and its synonyms in Manila Bay prior to the barcoding of a morphologically similar specimen in 2019 (Trinidad et al.). There have been no reports of the species in other ports in the Philippines as well. Their current presence in the bay may be likely due to their transport by ballast water or fouled vessel hulls of a local ship coming from islands in the Philippines where they were first documented in the 1800s, or from an international vessel coming from their other native habitats. It is also possible that *Irus* spp has been present but was not detected due to very low counts; however, past surveys have not reported *Irus* spp and cannot support this.

The increasing abundance of *Irus* spp in the sampling periods at the Port of Manila may show the possibility of localized domestic invasion, as historically exhibited by *Perna viridis*, whose initial distribution in the Philippines may have been limited to Manila Bay and Bacoor Bay (Aypa 1978). It then spread throughout the country through translocation for aquaculture, as the places in which it was subsequently recorded are embayments with a long history of aquaculture, such as Sapian Bay in Aklan (Aypa 1978, Guerrero et al. 1983). Biological features of the *Irus* spp may contribute to its invasive potential. It possesses a well-developed adult-shell byssus, which is characteristic of its subfamily Tapetinae. Its adult stout byssal attachment allows the organism to adhere well into substrates, making it a successful fouler. The byssus also enables it to be a nestling species in the rocky intertidal zone; it is able to find its space by inserting itself in crevices and cracks in the substrate. It can be found nestled under rocks, on piers, buoys and rafts, finding a unique niche (Morton 1983). Its shell is usually distorted in form as the organism adjusts into the cavity it occupies and avoids predation. This species is highly adaptable and inhabits intertidal areas, down to a depth of 40 meters (Huber 2010). The species is also a generalist (Huber 2010), another characteristic of a successful invasive. Moreover, the wide range of temperature and salinity of the water bodies where it is native of suggests that it exhibits tolerance to a wide range of values of these environmental factors. It can live in waters as low as 12 ppt as found in the Black Sea, and up to 39.7 ppt as in the Mediterranean Sea (Poulos 2023). It can also thrive in a wide range of temperature, from 21°C of the Black Sea in summer (Krivoguz et al. 2021) and 29°C of the Mediterranean Sea (Poulos 2023). Manila Bay's range of temperatures during the sampling period at the sampling sites was from 27 to 33 °C. Salinity measurements were from 20 to 31 ppt. With the favorable marine environmental conditions, it is likely that *Irus* spp. can establish and form populations in Manila Bay during its breeding season.

The diversity indices of the fouling groups show that, during Year 1, the highest values were obtained during the second retrieval (June 2021). Values increased from the first sampling period to the second and went a downward trend to the fourth. During Year 2, the highest values peaked in the third retrieval (September 2022), then decreasing at the fourth. The biodiversity index values were higher in Year 1 compared to Year 2.

The Shannon Weiner indices for years 1 and 2 were highest in June 2021 and September 2022, hence these are the months with the highest species diversity present in the community. The higher value of the Shannon Weiner index shows an increase in species diversity. Both months are within the southwest monsoon season. The monsoon season is from June to October. Simpson's index range from 0 to 1, with 1 indicating no diversity. During Year 1, retrieval in June showed a value of 0.705, the highest that year, *Amphibalanus amphitrite* has the highest count during that time, dominating the sample. Year 2 values ranged from 0.466 to 0.565, all close to 0.5. Simpson's diversity index of 0.5 indicates a moderate level of diversity, which indicated a variety of species present, and that the distribution of individuals across those species is not perfectly even.

The variation in the values of the biodiversity indices is common in marine assemblages as they undergo seasonal fluctuations (Madkour and El-Shoubaky 2007, Rueda et al. 2008, Marimuthu et al. 2023). Environmental conditions accompanying the changes in seasons and other pressures in the port can play a role in the variability of the benthic community composition. A study in Manila Bay (Trinidad et al. 2019) showed community composition changes during the northeast monsoon season. Another study in Taiwan showed a difference in species richness during the northeast monsoon and southwest monsoon seasons (Lin and Shao 2002). A study in a Mediterranean port showed that the abundance and mortality of harbour benthic communities are linked to disturbances related to seasonality, such as reduced salinity and increased organic matter (Betti et al. 2025).

RECOMMENDATIONS

With globalization and heavy marine traffic in the Port of Manila, periodic surveys are recommended, as they are essential in monitoring previously recorded non-indigenous and invasive species and detecting new introductions. There are suspected introductions of other species of *Mytilopsis*, and a non-indigenous *Modiolus*. With the increasing abundance of *Irus* spp, it is also important to examine the effect of the presence *Irus* spp. with populations of other bivalves, such as the indigenous *Perna viridis* and *Modiolus* sp. and invasive *Mytella strigata*. In depth studies about the reproduction, ecology and community interactions of *Irus* spp. in Manila Bay can be done.

CONCLUSION

The study, with the use of PICES collectors, showed the assemblage of fouling organisms in the Port of Manila, Manila Bay for 2021-2022. Results show seven groups of fouling organisms, namely arthropods, molluscs, annelids, cnidarians, bryozoans, platyhelminths, and chordates. Barnacles, polychaetes and bivalves were the most abundant organisms. Non-indigenous bivalves were observed, such as *Mytella strigata*, *Mytilopsis* sp and *Brachidontes pharaonis*. Unidentified bivalves were barcoded revealing two species: *Irus irus* and *Irus macrophylla*. These species were not detected in previous surveys, and the current survey show increasing abundances. They were described by Deshayes in 1854, citing Cebu, Samar and Maguindanao as type localities. However, no report of the species has been recorded in the Philippines since 1895. There is a need to survey ports in Manila Bay on a regular basis. This will allow monitoring of previously recorded NIS, detection of any new introductions, and determine if trends in the abundance of *Irus* spp. show potential for localized invasion.

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CONFLICT OF INTEREST

The authors affirm that they do not hold any conflicts of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

Melody Anne Ocampo, as the corresponding author, contributed to the conception of experimental design, experiment supervision, project leadership, acquisition of funding, conducted a thorough review and critical revision of the manuscript, and ensured that all listed authors granted their approval before submitting the manuscript. Richard Casiguran, John Victor Marcelino and June Lucille Bacay were responsible for specimen sampling, processing, safe keeping, and initial interpretation of data. Anne Pauline Anover and Aryan Arenas were responsible for data analysis and interpretation, contributed to the initial gathering, processing, and interpretation of data, and drafted the initial manuscript. Ian Kendrich Fontanilla and Benjamin Vallejo Jr made critical and essential inputs in the conception of the design of the experiment, resources, acquisition of funding, manuscript review, and review of the final paper to be submitted.

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