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ORIGINAL ARTICLE

Assessment of marine fouling communities in three sites across Batangas Port, Sta. Clara, Batangas City

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ABSTRACT

Background: Ports are susceptible to the arrival of non-indigenous species (NIS) which can subsequently become invasive through maritime traffic. In the Philippines, few studies have been conducted about macrofouling communities in marine vessel-frequented areas. Batangas Port is an international port serving the provinces of Cavite, Laguna, Batangas, Rizal, and Quezon, and docks vessels from areas around the globe. Baseline information of fouling communities in this area is essential as this can allow detection of NIS and alert the community about their presence. It can serve as basis for crafting of guidelines and mitigation measures for possible scenarios regarding the NIS' invasive potential.

Methodology: Three sampling sites in close proximity to Batangas Port in Brgy. Sta. Clara, Batangas City were studied using biofouling collectors. Fouler collector design was adapted from the North Pacific Marine Sciences Organization (PICES) design. Collectors were deployed in an unused pier, aquaculture pond and mangrove area from November 2014 to January 2015. Retrieved biofoulers were identified using taxonomic keys, and diversity indices were computed.

Results and Discussion: A total of 1044 individuals were collected in the plates submerged at the sampling sites. These organisms belonged to six phyla and at least nine classes. The community included bivalves, gastropods, polychaetes, and tunicates. A non-indigenous polychaete, *Eumida sanguinea*, was detected in the retrieval. Values of biodiversity indices indicate low biodiversity, and species evenness values are low as well.

Conclusion: The study generated a baseline listing of organisms in the port area and showed a roster of indigenous invertebrates. However, a NIS has been detected. The list is essential as it can be used to alert communities surrounding the port about the presence of NIS so that ecological, economic, and health impacts can be minimized, and monitoring be done regularly.

Introduction

Maritime trade and commerce have increased development of ports and other areas that are involved in product handling and transport. This increased development of man-made structures has been providing more areas for adhesion and growth of marine opportunistic organisms [1-3]. The colonization of these organisms on submerged surfaces is termed biofouling [4,5].

Biofouling is a multi-step process that begins with biochemical conditioning of the object once it comes into contact with water. As described by a study [6], the process begins when the surface immediately accumulates organic carbon residues. The accumulation of residues allows pioneering microorganisms to attach to it. The surface and the microorganisms form a biofilm. Bacteria and other colonizing microorganisms forming the biofilm secrete extracellular polymeric substances that alters the local surface chemistry. This alteration makes the surface conducive for recruitment of eukaryotic microorganisms. Further conditioning by these organisms will consequently allow settlement of macroorganisms.

Several studies have observed diversity of marine macrofouling organisms in different submerged substrates. Studies using panels in coastal areas in India have shown dominance of hydroids and barnacles in Karwar, while those in Kudankulam and Tamil Nadu exhibited a variation in abundance of polychaetes species during the pre-monsoon, monsoon and post-monsoon seasons [7,8]. Hydroids and barnacles have dominated structures at shallow waters in Sabah, Malaysia [9]. Mangrove trees have barnacles such as *Balanus sp* as dominant foulers in Kerala, India and Tongan Bay, China [10,11]. Some mangrove-fouling species of oysters impair the trees' physiological capacity [12]. Aquaculture pens are dominated by tunicates and hydroids [13]. For ports, an abundance of encrusting bryozoans, barnacles and calcareous polychaetes were reported in Songkla, Thailand [14] and port structures in Karambunai Bay, Malaysia showed numerous species of bivalves and maxillopods [9]. A survey in Myanmar ports at Yangon, Sittway and Myeik show high biodiversity of foulers [15].

Ports and areas nearby are of particular interest due to their potential to support non-indigenous species. These organisms can be introduced to the port via hull fouling or release of ballast water as vessels operate in local and international waters. When conditions in the port are conducive, these non-indigenous species may drastically increase in number and become invasive. *Mytilopsis sallei* has been reported in the Indo-West Pacific, and it has been invasive in Singapore, Malaysia. Hong Kong and Taiwan [16-18]. *Xenostrobus securis* is an Australian mytilid that has been reported in China and Hong Kong [18]. *Mytella strigata* has been seen in large densities in Thailand, Hong Kong, and the Philippines [19]. These invasive organisms cause significant ecological effects such as biodiversity and loss and change in ecosystem function. They also have economic impacts, as the cost of their eradication and control can entail millions in currency [1].

In the Philippines, few studies have been conducted about macrofouling communities in marine vessel-frequented areas. With the country's numerous ports across the islands, it is essential to assess these communities for preliminary profiling. Batangas Port, an international port serving the provinces of Cavite, Laguna, Batangas, Rizal, and Quezon, is a location of interest for macrofouling studies. It is situated in Sta. Clara, Batangas City, where aquaculture communities are also located. This study assessed the macrofouling communities along three different sites (marina, mangrove forest, and aquaculture pond) in Sta. Clara, Batangas City. The study sites are in close proximity to Batangas International Port and the Chevron Philippines Inc, which both serve as docks for international and local shipping vessels.

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Keywords: biofouling, non-indigenous species, invasive species, baseline study, port environments

Methodology

2.1 Study Area

Batangas Port is located in Barangay Sta. Clara, Batangas City, a coastal barangay located at Lat. 13° 45' 48" N, Long. 121° 2' 46" E, Northeast Coast of Batangas Bay, South of Luzon Island in Batangas City, Batangas (Figure 1). It is approximately 3 kilometers away from Chevron Philippines Inc. Brgy. Sta. Clara has a land area of 165.6232 hectares. Meetings with barangay officials were held several times for approval of conduct of study and determination of specific sampling sites.

Three sampling sites were used in the study: a small pier (13° 45' 45.49" N, 121° 2' 18.08" E), a mangrove area (13° 45' 57.78" N, 121° 2' 5.39" E) and an aquaculture pond (13° 45' 56.12" N, 121° 2' 15.17" E) all in close proximity to the port. The sites were chosen to represent the different environments around the port area that can be affected by impacts of port activities. The pier or marina is an unused structure at the western part of the port. The mangrove area is situated 172 meters from the Chevron Oil Company and 591.06 meters away from the marina. The mangrove forest is consisted mostly of rhizophoran mangroves. Water levels differ greatly during low tides (approximately 0.5 meters) and high tides (approximately 2 meters as described by a local). The aquaculture pond is located at a distance of 316.78

Figure 1. Map of Batangas Bay showing the location of Batangas International Port and Brgy. Sta. Clara on the coast of Batangas Bay (Map generated using simplemppr (www.simplemappr.net))

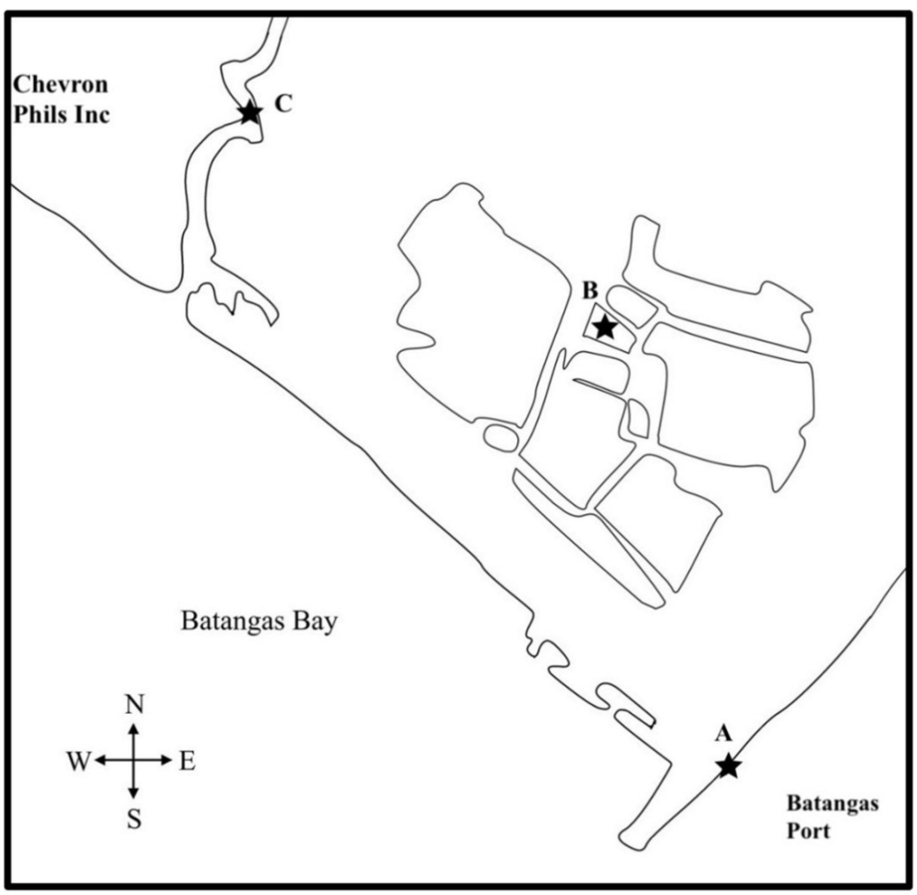

Figure 2. Map of Brgy. Sta. Clara, Batangas City showing the three sampling sites: A – marina/ pier, B – aquaculture pond, C – mangrove area.

meters from the mangrove area and 339.6 meters from the pier Most of the stock species in the aquaculture pond are small shrimps and tilapia (*Oreochromis niloticus*). The water levels were observed to vary with the tide.

2.2 Data Collection

2.2.1 Preparation of Fouler Collectors

The fouler collector used in this study was adapted from the design of the North Pacific Marine Sciences Organization (PICES) [20, 21] (Figure 3). Four recruitment plastic Petri plates, 10 millimeters in diameter and 15 millimeters in height, were fastened to one side of a 300-millimeter round plastic lid using plastic cable ties. The Petri plates, which served as the actual settling plate for the foulers were placed at equal distances. A hole was drilled in the middle of the bucket lid where a 5-meter nylon rope was passed through. One end of the rope was tied to railings and other stable structures in each sampling site, while the other end was tied to a cement weight which kept the fouler collectors submerged in water.

Figure 3. PICES fouling collector for deployment.

2.2.2 Deployment and Retrieval of Fouler Collectors

In each site, three sampling points were determined randomly. Random sampling was done by dividing each sampling site area into 16 square plots. Each plot was represented on a piece of paper, and using the fishbowl method sampling points were drawn.

Per sampling point, three fouler collectors were deployed. Deploying collectors meant tying the free end of the rope to a secure pole or structure in the site, then letting the other end with the collector submerge on the water. Three fouler collectors were deployed per sampling point. Each fouler collector was submerged 2 meters below the water surface in the marina, and 0.5 meters below the water surface in the mangrove forest and the aquaculture pond due to low water level. A total of 27 fouler collectors were deployed on November 2014. The geographical coordinates of each sampling site were recorded using Garmin GPSMAP 62sc.

On January 14, 2015, all 4 Petri plates per fouler collector were retrieved. They were submerged for 60 days, during the Northeast monsoon season. A total of 91 Petri plates were collected and photographed using Canon EOS Rebel T2i DSLR Camera. Each Petri plate was contained in a Ziploc bag containing formalin in saltwater water solution at 1:9 ratios.

2.2.3 Physico-Chemical Analysis of Water

The study was done from November 2014 to January 2015. Measurement of the physico-chemical parameters of water was performed on a weekly basis, taken in mid-afternoon. Grab water samples were collected per sampling point and immediately measured for their temperature, pH, total dissolved solids and dissolved oxygen using CyberScan Series 600 Water Quality Meter.

2.3 Specimen Identification

The collected Petri plates were brought to the invertebrate laboratory of the Department of Biology, University of the Philippines (UP) Manila Each organism was isolated from the Petri plate using forceps and placed in individual vials containing the formalin in saltwater solution at 1:9 ratios. The samples were viewed under a Leica ES2 stereomicroscope and photographed using Apple iPad Air 2. Each specimen was identified to the most specific level possible. Initial identification of the specimens was done using global online databases such as SeaLifeBase, Marine Species Identification Portal, and World Register of Marine Species. Validation was done at the Institute of Environmental Science and Metereology of UP Diliman and the Department of Biology of UP Manila.

2.4 Data Processing and Analysis

The abundance and relative abundance of identified species per sampling site was determined. Biodiversity of each sampling site was determined using the Shannon-Wiener index, Simpson index approximation, Species evenness and Hill numbers in the orders of 1 and 2. The indices were calculated using Biodiversity Pro Version 2 software and Microsoft Excel 2007 for Windows. Kruskal-Wallis test was used to compare the diversity values across the sites. Kruskal-Wallis test was performed using GNU-PSSP.

Results

3.1 Physico-Chemical Parameters

Measurement of water quality parameters among the different study sites are summarized in Table 1. The mean pH values recorded in the marina and aquaculture pond were within the neutral ranges while the mean pH for the water obtained from the mangrove area was weakly acidic (6.9471 ± 0.9241). The pH measurements for all sites were within the acceptable pH range (6.0-8.5) for Philippine waters [22]. Mean dissolved oxygen levels were highest in the aquaculture pond (6.6133 ± 1.2007 mg/L) and lowest in the mangrove area (4.9788 ± 0.6451 mg/L). The dissolved oxygen values obtained for all the three sites reached the standard minimum level of 5 mg/L (DAO, 1990-34). The lowest recorded mean temperature was in the marina (27.3625 ± 0.7310 ˚C) and the highest mean temperature was in the aquaculture pond (28.9771 ± 1.9746 ˚C). Mean total dissolved solids was highest in the marina (47.0308 ± 0.3989 ppt), followed by the aquaculture pond (28.9179 ± 7.1734) and lowest in the mangrove area (7.8891 ± 2.7065 ppt).

Table 1. Water quality factors across three sites in Sta. Clara, Batangas City.

Water Quality Factors	Marina	Mangrove	Aquaculture Pond
pH	7.2788 ± 0.1103	6.9471 ± 0.9241	7.7079 ± 0.7717
Dissolved Oxygen (mg/L)	6.4563 ± 1.0231	4.9788 ± 0.6451	6.6133 ± 1.2007
Temperature (˚C)	27.3625 ± 0.7310	27.8958 ± 1.2275	28.9771 ± 1.9746
Total Dissolved Solids (ppm)	47.0308 ± 0.3989	7.8891 ± 2.7065	28.9179 ± 7.1734

3.2 Fouling organisms

A total of 1044 organisms (Table 2) belonging to six phyla and at least nine classes were collected across the three different sites. There were 606 individuals obtained from the marina, 277 individuals from the mangrove area, and 161 individuals from the aquaculture pond. No common organism was found for the three sites.

All collected foulers in the aquaculture pond were gastropods. Family Neritidae was the most abundant, with four species. Within the family, the most dominant species is *Clithon oualaniensis*.

The most abundant group are from the *Gammaridae* family in the marina and mangrove areas. Two polychaete families namely *Serpulidae* and *Nereididae* were second most abundant in the marina. In the mangrove site, the second most abundant family was *Ostreidae* followed by *Syllidae*.

Table 2. Abundance of organisms in the three sites in Sta. Clara, Batangas City.

Organisms	Abundance and relative abundance (%) per sampling point		
	Aquaculture pond	Mangrove	Marina/ Pier
Clithon oualaniensis	128 – 79	3 – 1.08	0
Neritina sp.	4 – 2.48	0	0
Neritina coromandeliana	3 – 1.86	0	0
Neritina waigiensis	1 – 0.62	0	0
Nerita squamulata	0	23 – 8.30	0
Balanus sp.	0	1 – 0.36	0
Isognomon sp.	0	1 – 0.36	0
Amphibalanus amphitrite	0	0	24 – 3.96
Ostrea sp.	0	46 – 16.61	1 – 0.17
Placuna placenta	0	0	1 – 0.17
Modiolus sp.	0	0	1 – 0.17
Eumida sanguinea	0	0	11 – 1.82
Mitridae family	21 – 13.04	0	31 – 5.12
Costellariidae family	3 – 1.86	0	0
Naticidae family	1 – 0.62%	0	0
Gammaridae family	0	169 – 61.01	276 – 45.55
Cypraeidae family	0	0	1 – 0.17
Sphaeromatidae family	0	5 – 1.81	0
Muricidae family	0	1 – 0.36	0
Syllidae family	0	22 – 7.94	0
Calyptraeidae family	0	0	5 – 0.83
Costellariidae family	0	0	2 – 0.33
Cardiidae family	0	0	1 – 0.17
Serpulidae family	0	0	99 – 16.34
Sabellidae family	0	0	9 – 1.49
Spionidae family	0	0	9 – 1.49
Terebellidae family	0	0	4 – 0.66
Dorvilleidae family	0	0	1 – 0.17
Syllidae family	0	0	1 – 0.17
Hesionidae family	0	0	1 – 0.17
Nereididae family	0	0	56 – 9.24
Tunicate	0	0	24 – 3.96
Ophiuroid	0	0	2 – 0.33
Turbellarian	0	1 – 0.36	1 – 0.17
Brachyuran crab	0	0	4 – 0.66
Penaeoidea shrimp	0	1 – 0.36	3 – 0.50
Echinoid	0	0	1 – 0.17
Unidentified bivalve	0	1 – 0.36	0
Unidentified Ostreidae species	0	3 – 1.08	33 – 5.44
Unidentified gastropod	0	0	4 – 0.66

3.3 Diversity indices

Shannon-Wiener Index, Simpson's Index, Species evenness, and Species richness of the three sampling sites are summarized in Table 3. Highest species diversity of 1.947 and species evenness of 0.5908 was recorded at the marina. Simpson's index values ranged from 0.02524 to 06505, showing low diversity in the community. Species evenness ranged from 0.3861 to 0.5908 and are low, as shown by the number of individuals in the three environments varying greatly from one species to another. Shannon-Wiener index values were low for all three sites indicating low diversity, as typical values for this index in ecological studies are from values of 1.5 to 3.5 [23]. The low diversity may be attributed to the anthropogenic activities done at the sites, which cause changes to the areas and make them unsuitable for more species occurrence.

Discussion

This study provides a baseline documentation of the community of macro-organisms present in areas around Batangas Port, as well as the detection of a non-indigenous species. Various studies have highlighted the importance of establishing baseline studies and construction of species profiles of marine ecosystems, as this may be used for monitoring their status over time [24,25]. Macrofouling community composition in the waters in Brgy. Sta. Clara include barnacles, gastropods, bivalves, and polychaetes, and this is commonly reported by several studies [26-29]. Although the data may not be representative of the current community of the port due to its year of data collection, this listing establishes the baseline information of species in the area and can be used as foundation of subsequent sampling and analyses.

Table 3. Diversity indices across three sites in Sta. Clara, Batangas City.

Diversity Indices	Aquaculture Pond	Mangrove	Marina
Shannon -Wiener Index (H)	0.7514	1.3	1.947
Simpson Index Approximation (λ)	0.6505	0.4137	0.2524
Species evenness (E)	0.3861	0.5067	0.5908

In the aquaculture pond, a gastropod species was most abundant. *Clithon oualaniensis* is commonly distributed in tropical or subtropical Indo-pacific fresh or brackish water [30]. They are commonly abundant in coastal ecosystems. These snails are small organisms that feed on algae are vulnerable to water movement and prefer slow-moving, sheltered and shallow waters [31]. *C. oualaniensis* snails were observed to be sensitive to reductions in salinity levels in water and as such has been suggested to be used as indicator organisms for salinity of aquatic ecosystems [32].

Both mangrove and marina communities were dominated by amphipods. Gammarid amphipods benthic crustaceans can live in freshwater, brackish and marine environments. The abundance of these amphipods that in both areas can be attributed to their natural habitat being intertidal zones [33]. These species are highly motile which may explain their presence in the adjacent marine and mangrove waters [34]. They are also regarded as bioindicators of pollution and their dominating presence in the community may suggest contaminated waters [35]. One species belonging to the family, *Eriopisa chilkensis* from the Cochin estuary, southwest coast of India, was reported by Aravind et al. [36] as organic pollution tolerant. In other studies of mangrove ecosystems, however, barnacles and polychaetes are the dominant fouling species in the communities [37-39].

Gammarids are an ecologically important group of benthic crustaceans. They play a role in the energy flow and nutrient cycling, as they can be herbivores [40], carnivores [41] or detritivores [42]. As such, they have a significant impact on the transfer of carbon in the food chain. They also serve as keystone species in some marine and aquatic ecosystems [41]. They constitute a significant diet source for fish and waterbirds. Gammarid amphipods are successful invaders and some have become NIS in different parts of the globe due to their wide trophic range, high reproductive capacity and migration ability [41].

The second most abundant group of foulers in the marina is the polychaete, particularly of families *Serpulidae* and *Nereididae*. A study of a marina in Manila Bay [20] showed polychaetes as second most abundant foulers as well, particularly those of the families *Spionidae* and *Nereididae*. Polychaetes are highly abundant and diverse in most marine habitats, especially in benthic communities [42]. According to Relini *et al.* [44], the presence of polychaetes may indicate the presence of an environmental stressor, particularly pollution. Similarly, Dafforn *et al.* [45] has reported an increase in the richness and abundance of polychaetes in an anthropogenically modified estuarine in Australia as a positive response to increased nutrient availability.

Diversity indices across the three sites differ. The marina shows high diversity and evenness. A study has shown that urban places like ports can support higher diversity, depending on the level of disturbance [46]. The aquaculture pond, on the other hand, has low diversity and evenness. This can be attributed to controlled growing of few specific types organisms as food resource in the area. Aquaculture has been causing homogenization of species in marine systems [47]. The mangrove area values are between those of the two other sites, although this area is expected to have richer biodiversity. The values in this study can be attributed to negative impacts of port activities such as pollution to the mangrove area, as mangroves are sensitive to environmental changes [48].

Eumida sanguinea is the non-indigenous species identified in this survey. This polychaete is natively distributed in the Northern Hemisphere [49], including the northern Iberian Peninsula [50]. It is found on bottoms with sand, gravel and rocks from a few to a hundred meters depth, and in estuaries and coastal environments [51]. It can be found closely associated with another tubeworm, *Lanice conchilega*, which has been observed to share with its food resources [52]. A study [53] considers the species to have potential for larger scale dispersal due to egg size and its larvae having planktotrophic development. Outside the Northern Hemisphere, it has been found in Brazil, Gulf of Mexico, the Caribbean and New Zealand [54, 55, 56, 57]. Its invasive potential, however, has not been studied.

The presence of a non-indigenous species in the marina can be attributed to maritime shipping, as it is the major vector of the transport of these organisms. The organisms can be carried by vessels through hull fouling and ballast water intake. Hulls carry viable adhering organisms, and when the vessel reaches the port of destination, the organism can be released and can survive. *Mytilopsis sallei*, for example, was transported to Darwin Harbor, Australia by fouling a yacht [1]. Ballast water, on the other hand, may harbor viable larvae as it is taken in by the vessel for stability at the port of origin. Once released at the port of destination, it can survive in the port waters and become invasive, like in the case of *Dreissena polymorpha*, the zebra mussel which invaded the Great Lakes in the 1980s [58].

Non-indigenous and invasive species are recognized to have ecological and economic impacts. Ecological effects of NIS on ecosystems include biodiversity loss, alteration of food webs, physical habitat disruption, and import of parasites and diseases. In mangroves, invasive species can interfere with propagule dispersal and seedling establishment of mangrove trees [59]. A study in [60] showed physiological impairment of mangroves due to oyster biofouling, damaging their capacity to accrue sediments. In aquaculture, invasive species can cause physical damage and mechanical interference to the functions of cultivated organisms, compete with their food, bring diseases, restrict water exchange in the environment and damage cages and nets [61] . In ports, fouled structures are damaged which can cost a significant amount of money in clean up [1]. Hence, it is essential to have knowledge of prevailing or baseline biofouling community in the environment (e.g. port and aquaculture areas). This facilitates detection of non-indigenous and potentially invasive species, and mitigates the transfer of these organisms to different parts of the country.

Conclusions

Areas in close proximity to Batangas International Port were surveyed for their macrofouling community. A total of 1044 individuals were collected in the collecting plates which were submerged at three sampling points in three sampling sites, namely an aquaculture pen, a small pier and a mangrove area. These organisms belonged to six phyla and at least nine classes. The community included bivalves, gastropods, polychaetes, and tunicates. A non-indigenous polychaete, *Eumida sanguinea*, was detected in the retrieval. Non-indigenous species have the capacity to be invasive. The generated list can be updated as ports and nearby areas are prone to the arrival of non-indigenous and potentially invasive species. Communities surrounding the area can be alerted about their presence so that ecological, economic, and health impacts can be minimized. Moreover, detection and awareness about these species will enable mitigation methods for management of ships and ports to be crafted, as well policies for invasive species.

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