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RESEARCH

Marine Biofouling Communities of Manila South Harbor, Philippines

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KEYWORDS
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ABSTRACT An immersion experiment was conducted in the Manila South Harbor to document the development of sessile biofouling communities. Test panels were submerged below the sea surface in April 2012 for short- (one and three months) and long-term (one year) exposures in seawater, then fouling types and occurrences were scored based on digital images of panel surfaces. The short-term immersed panels were found with significant cover of soft fouling (undet.), slime, and the invasive *Balanus* (=*Amphibalanus*) *amphitrite*. These also filled the long-term immersed panels, although some fell off due to mortality from crude oil smothering. *Perna viridis*, native but also invasive, successfully established and then dominated the fouling cover by the 12th month (April 2013). Oysters, bryozoans (*Watersipora* sp.), colonial tunicates, polychaetes (*Hydroides* sp.), and green algae contributed minor to fouling cover. These fouling communities in the Manila South Harbor consisted of organisms that were cosmopolitan in port waters of SE Asia. A similar study must be carried out in other major ports of the country and then compared.

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1. INTRODUCTION

Biofouling is the colonization and accumulation of fast-growing aquatic organisms on dead or living surfaces and structures immersed in or exposed to the aquatic environment (Wahl 1989; Eguia and Trueba 2007). For the shipping industry, biofouling reduces ships' speeds and maneuverability and contributes to corrosion and deterioration processes (Myan et al. 2013; Bressy and Lejars 2014; Pati et al. 2015; Schwan et al. 2015). Indeed, ships' hulls and ballast tanks have become major vectors of invasive organisms (Gollasch et al. 2000; Lewis et al. 2003; Coutts and Dodgshun 2007). The translocation and introduction of these organisms into new areas have allowed them to move well beyond their natural range and made them evolve and develop dispersal mechanisms in order to spread and expand their populations, with most species becoming dominant (hence 'invasive') when environmental conditions were conducive and without predators (Deacutis and Ribb 2002; Burkholder et al. 2007). In the extreme, these invasive non-indigenous species dramatically altered ecosystem structure and biodiversity and caused environmental and health impacts (Bax et al. 2003; David and Percovič 2004; Molnar et al. 2008). Infestations of, for example, the zebra mussel *Dreissena polymorpha* in internal waterways of European freshwaters, Turkey, and the Great Lakes region, among others, have resulted to serious economic losses (Aldridge et al. 2003; Bobat et al. 2004; Burkholder et al. 2007; Sousa et al. 2011).

Among the most widespread and cosmopolitan fouling marine macro-organisms are shipworms *Teredo navalis*, striped barnacles *Balanus amphitrite* (=*Amphibalanus amphitrite*; WoRMS Editorial Board, 2017), blue (*Mytilus*) and brown (*Perna*) mussels, and oyster species (GSID 2018). Species that have become invasive include *Perna* spp., black striped mussel *Mytilopsis sallei*, Asian paddle crab *Charybdis japonica*, European shore crab *Carcinus maenas*, colonial tunicate *Didemnum vexillum*, North Pacific seastar *Asterias amurensis*, European fan worm *Sabella spallanzanii*, Bay barnacle *Amphibalanus improvisus*, and Wakame seaweed *Undaria pinnatifida*, among others (IMO 2017). A recent report indicated that there are already numerous non-indigenous species in countries of the Western Pacific region (Chavanich et al. 2010). For the Philippines, marine introductions since the 1970s served the aquaculture industry then subsequently became a consequence of the liberal trade (by aquarists) and ballast water exchange (Vallejo 2010). Marine invasive species—*B. amphitrite* (=*A. amphitrite*), *M. sallei*, *Brachidontes* sp., and *Mytella charruana* (=*strigata*; see Lim et al. 2018)—have been reported to occur in Manila Bay (Ocampo et al. 2014; Vallejo et al. 2017).

There are more species that can be potentially translocated by ship ballast water exchange and hull fouling. This study aims to document sessile fouling communities, using broad taxonomic categories, and determine their dynamics over a period of one year in the Manila South Harbor, one of the Philippines' busiest international ports that is among the most susceptible to invasion-related problems. Results of this study are deemed to serve as indication of the status of the port waters of the Manila South Harbor, including its risk as source of invasive organisms. Such

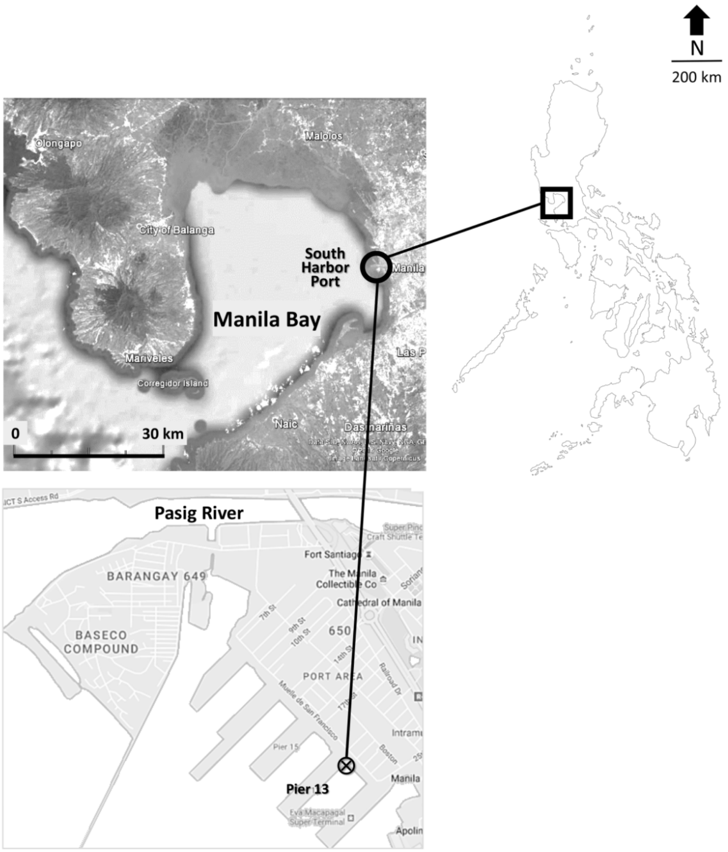

Figure 1. Location of the embarkation area (⊗) at Pier 13, Manila South Harbor, facing Manila Bay, Philippines, where the setups for the fouling experiment were submerged from April 2012 to April 2013. (Sources of images: Google Earth and Google Map).

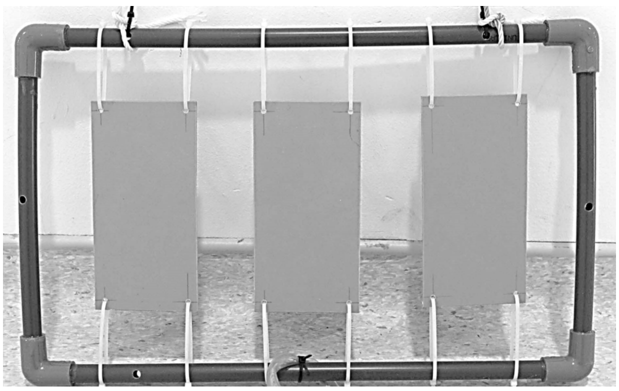

Figure 2. Setup used for the fouling experiment in the Manila South Harbor, Philippines, from April 2012 to April 2013. Three frames were deployed and then recovered monthly for photo-documentation. Frames 1 and 2 were replaced after one and three months, respectively; Frame 3 was submerged continuously for one year and recovered in early May 2013.

evidence is required to adjust measures implemented by port authorities in addressing the likelihood of the spread of invasive organisms, especially since the Ballast Water Management (BWM) Convention of 2004 has just entered into force on 8 September 2017 (PortCalls Asia 2017).

2. MATERIALS AND METHODS

2.1 Study site: Pier 13, Manila South Harbor

The Manila South Harbor (UN/LOCODE: PH MNS) is part of the Port of Manila, the chief international gateway for shipping in the Philippines (World Port Source 2005). It is located at 14°36.2'N and 120°58.0'E, faces Manila Bay directly, and is ~2 km from the mouth of the Pasig River estuary (Figure 1). A mixed diurnal tide regime occurs ranging from 0.4 m (neap) to 1.2 m (spring) (Jacinto et al. 2006). Climatic data from the Philippine Atmospheric, Geophysical and Astronomical Services Administration (PAGASA) indicated that cloud cover ranged from 3-8 oktas (mean 6.42 ± 0.04) during 2011 to 2013, while rainfall measured from 0.20-290.00 mm (mean 8.67 ± 0.95), and particularly high during the months of May until September. The port area of the Manila South Harbor is protected by a 3-km rock breakwater, enclosing ~600 ha of anchorage and five piers (Piers: 15, 13, 9, 5 and 3) (Migu 2009). Currently governed by the Philippine Ports Authority (PPA)—Port Management Office (PMO) for the National Capital Region (NCR) South (then Port District Office for Manila/Northern Luzon), the port has catered to between 2,330 and 7,172 domestic ships and from 1,370 to 2,101 foreign vessels annually for the years 2005 to 2015 (PPA Annual Reports, 2005-2015). The Asian Terminals, Inc. (ATI), through its General Stevedoring Division (GSD), handles the general cargo operations in the four berths of the port (World Port Source 2005). Pier 13 has a 400-m long berth protected by rubber fenders and a 9-m deep draft. The Pier has been assigned as special anchorage area for search and rescue (SAR) vessels of the Philippine Coast Guard, the auxiliary vessel BRP Pag-asa (AT-25) and various vessels of the Philippine Navy, and the research vessel M/V DA-BFAR. Although already earmarked for rehabilitation since 2011, then PPA-PDO Manila/Northern Luzon (currently PPA-NCR South) granted access to the embarkation/ disembarkation area of Pier 13 (Figure 1) for the experiment.

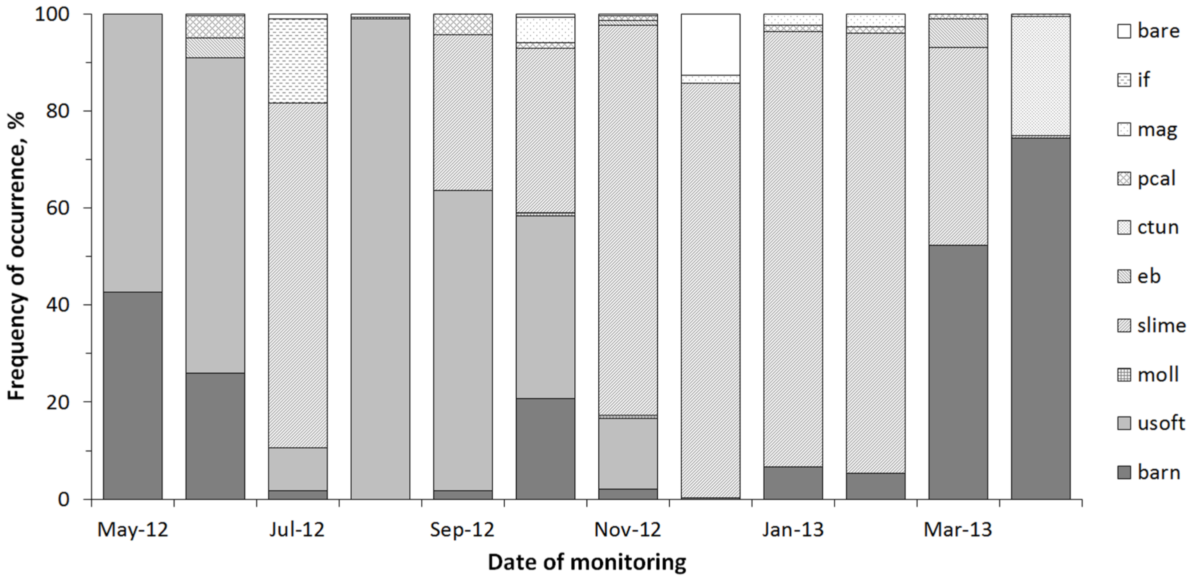

Figure 3. Composition of the fouling community and mean occurrence (in %) of organisms on panel surfaces submerged for one month (Frame 1) at Pier 13, Manila South Harbor, Philippines. Legend: *if*: incipient fouling; *mau*: undetermined macroalgae; *mab*: brown macroalgae; *mag*: green macroalgae; *pcal*: calcareous polychaetes; *ctun*: colonial tunicates; *eb*: encrusting bryozoans; *moll*: molluscs; *usoft*: undetermined soft fouling; *barn*: barnacles.

2.2 Field deployments, photo-documentation, and retrievals

We adhered to the following protocol of the ASEAN-India Cooperation Project "Extent of transfer of alien invasive organisms in South/Southeast Asia region by shipping" (cf. Tan 2011) – Three frames (Figure 2), each fixed with three recruitment panels (size 10 x 20 cm) made of PVC, were submerged at 1 m depth at the embarkation area of Pier 13 on 24 April 2012. The submerged frames were spaced ~3 m apart and secured with cement blocks (as weights) and Styrofoam (as buoys). Subsequent observations of fouling organisms were done monthly by photo-documentation—digital photographs of each frame (back and front) were taken using a Digital Single-Lens Reflex (DSLR) Nikon D5000 camera. The back and front surfaces of individual panels were also photographed. To determine short-term fouling development, the panels were collected and replaced monthly (Frame 1) and quarterly (Frame 2); Frame 3 was retrieved after a year, in which the dynamics of fouling assemblages were examined. The retrieved panels were immersed in containers with ambient port water while in transit to the laboratory; these were preserved in 10% borax-buffered formalin in seawater after arrival.

2.3 Image processing and analyses

Using a photo-editing software, each image was cropped off 1.27 cm (0.5 in) from the panel edges, enhanced, color-autocorrected, image-resized, and then renamed (Lim and Chong 2012). Processed digital images were then scored for frequency of occurrence (as a proxy for surface cover) using PhotoGrid 1.0 Beta (Bird 2003) following the methods of Lim and Chong (2012). The software was set to record fouling types—i.e., silt, slime, incipient fouling (*if*), green/red/brown or undetermined macroalgae (*mag*, *mar*, *mab*, *mau*), Cnidaria (*cnid*), encrusting (*eb*) or arborescent bryozoans (*ab*), barnacles (*barn*), calcareous polychaetes (*pcal*), molluscs (moll), sponges (*sp*), colonial tunicates (*ctun*), and undetermined hard (*uhard*) or soft (*usoft*) fouling matter at 50 stratified random points on each photograph. The program was also set to access all photos successively in a folder for the scoring exercise. Scores were saved and then exported as comma-separated values; these were retrieved as data files in MS Excel for further processing and analyses.

3. RESULTS

3.1 Composition of fouling communities

At least half of all panel surfaces were covered by striped barnacles *Balanus* (=*Amphibalanus*) *amphitrite* after one month of immersion, while the cover of thick soft fouling (undet.) (*usoft*) matter smeared the remaining surfaces. Soft fouling matter included light-colored slime and dark brown sheath-like matter or soft mass with silt predominantly (i.e., with associated diatoms consisting of species of *Navicula*, *Nitzschia*, and *Pleurosigma*, among others, based on microscopic analysis). Other fouling types encoded were slime (transparent film on the surfaces), molluscs (*moll*; oysters and the green mussel *Perna viridis*), encrusting bryozoans (*eb*; Family Watersiporidae, *Watersipora* sp.), calcareous polychaetes (*pcal*; Family Serpulidae, *Hydroides* sp.), colonial tunicates (*ctun*), and filaments of green macroalgae (*mag*) as well as incipient fouling (*if*) from the short-term immersed panels. We noted the occurrence of sea spiders (Subphylum Chelicerata, Class Pycnogonida, Order Pantopoda); there were no arborescent bryozoans (*ab*), sponges (*sp*) or cnidarians (*cnid*) observed throughout the observation period. There were also a few points scored 'bare', e.g., areas scraped by the tether rope accidentally during retrievals.

3.2 Fouling communities on the short-term (one month)-immersed panels (Frame 1)

After a month of immersion, the panel surfaces were covered by two to seven biofouling types. Soft fouling (undet.) (usoft) occurred with *B. amphitrite* (*barn*) and *Watersipora* sp. (*eb*) in May and July 2012, respectively (Figure 3). Seven of the nine biofouling types were ob-

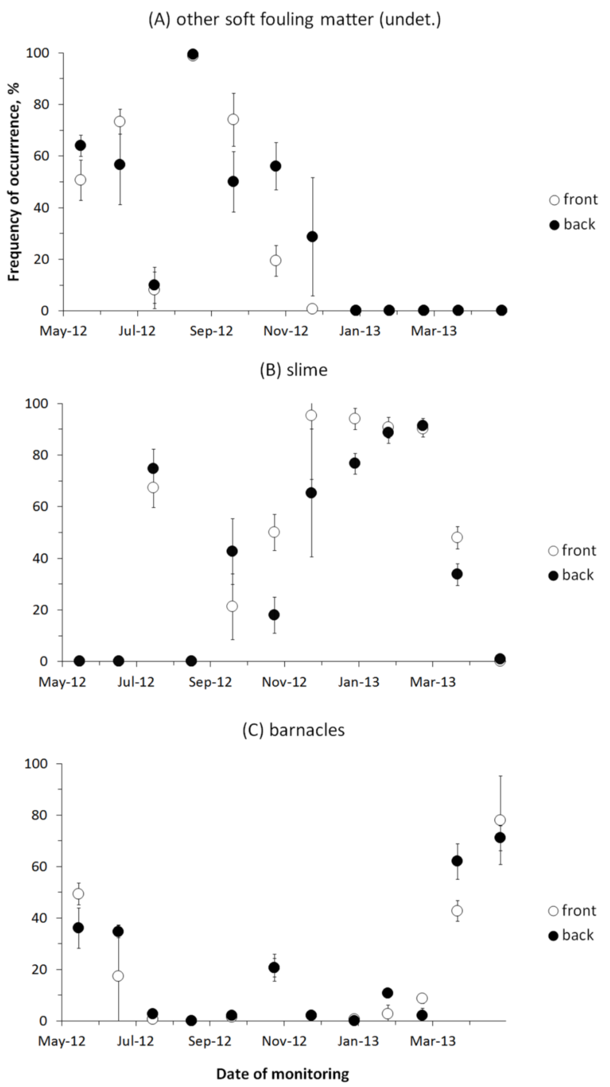

Figure 4. The mean cover (± SE, n=3) of major biofouling organisms—(A) other soft matter (undetermined), (B) slime, and (C) barnacles *Balanus* (=*Amphibalanus*) *amphitrite* on front and back surfaces of panels submerged for one month (Frame 1) at Pier 13, Manila South Harbor, Philippines.

served to occur on the panel surfaces in November 2012 (Figure 3), i.e., except for incipient fouling (*if*) and encrusting bryozoans (*eb*) (Figure 3). Three dominant biofouling types emerged—(a) soft fouling (undet.) (*usoft*) covered panel surfaces from 9 ± 4% (July) to 99 ± 1% (August) (mean 49 ± 7%, n=42) (Figures 3 and 4, A); (b) slime was apparent on the subsequent eight months (mean 65 ± 5%; n=72) and covered from 32 ± 6% (September 2012) to 91 ± 2% (February 2013) of the panel surfaces (Figures 3 and 4B); and (c) *B. amphitrite* was found every month on the surfaces but its cover was significant only in March 2013 (52 ± 6%) and April (91 ± 9%) (Figures 3 and 4C). The cover of each of these fouling types on either front or back surfaces of the panels were not consistently similar each month (Figure 4, A–C).

The six minor biofouling groups and their mean occurrences were as follows (Figure 3)— colonial tunicates (*ctun*, covered 24 ± 7% only in April 2013), incipient fouling (*if*, 9 ± 9% in June and July 2012), *Watersipora* sp. (*eb*, observed during four months with cover at 3 ± 1%), *Hydroides* sp. (*pcal*, recruited for eight months and covered 2 ± 2%), filamentous and bushy green algae (*mag*, covered 2.5 ± 2.5% for five months), and oysters (*moll*, ~1 ± 0%).

3.3 Fouling communities on the short-term (three months)-immersed panels (Frame 2)

After the deployment in April 2012, biofouling was initiated on new panels subsequently submerged in July, October, and January 2013. *Balanus* (=*Amphibalanus*) *amphitrite* (*barn*) occurred on the panel surfaces in May 2012 (93 ± 5%) and consistently dominated the surfaces until July (94 ± 2%) (quarterly mean 91 ± 2%, n=18) (Figure 5). In August, slime covered 68 ± 10% and a month later (September), the cover of soft fouling (undet.) (*usoft*) became significant at 70 ± 2%. Such soft fouling continued to cover most of the panel surfaces from November to January 2013 (quarterly mean 86 ± 5%, n=18), and on the setups immersed during the final quarter of the experiment, i.e., in February (88 ± 3%), and March (63 ± 3%) (Figure 5). The minor groups—*Watersipora* sp. (*eb*), oysters (*moll*), *Hydroides* sp. (*pcal*), colonial tunicates (*ctun*), and green algae (*mag*) increased the diversity of the biofouling communities per quarter and contributed from <1% to 18 ± 0.3% to fouling cover (Figure 5). The occurrence of green algae was noted only in March 2013.

3.4 Fouling communities on the long-term (one year)-immersed panels (Frame 3)

The barnacles accounted for significant cover on the panel surfaces initially and on the subsequent months (Figure 6), but some empty yet still cemented tests were observed starting June 2012. In July, crude oil was found to have contaminated the setups; oil clung to soft fouling matter (undet.) (*usoft*) and smothered the fouling communities. By August, crude oil contamination was less apparent, small recruits of *B. amphitrite* were observed, and the cover of larger barnacle individuals was coded the highest at 93 ± 3% (Figure 6). Thereafter, barnacle cover was observed to decrease from 86 ± 3% in September to mere 9 ± 3% by April 2013, with more barnacle cover observed on front than back surfaces of the panels (Figure 7A). Soft fouling (undet.) (*usoft*) cover increased from 19 ± 5% (September), to 88 ± 1% (January 2013), and then decreased to 66 ± 4% in March (Figure 3). *Perna viridis* occurred at <5% from June 2012 to February 2013 but was able to successfully establish on the panel surfaces in March (cover at 24 ± 4%) and April (91 ± 2%) (Figure 6). The occurrence of both soft fouling (undet.) and *P. viridis* were consistent on both front and back surfaces of the panels (Figure 7). Occasionally, *Watersipora* sp. (*eb*) occurred on the surfaces but covered only up to 7 ± 2% (November 2012); much less were the occurrences of colonial tunicates (*ctun*) at 3 ± 1% and *Hydroides* sp. (*pcal*) at <1% (Figure 6).

4. DISCUSSION

Albeit the generally broad taxonomic categories used in the scoring of sessile fouling macro-organisms, this study confirmed the presence of invasive species among the fouling communities in port waters of the Manila South Harbor—*Balanus* (=*Amphibalanus*) *amphitrite* and *Perna viridis*. Four barnacle species from this location have been described by Rosell (1973) and their presently accepted nomenclatures based on the WoRMS Editorial Board (2017) are—*Chthamalus stellatus stellatus* (Poli, 1791), accepted as *Chthamalus stellatus* (Poli, 1791); *Balanus amphitrite amphitrite* Darwin, 1854, accepted as *Amphibalanus amphitrite amphitrite* (Darwin, 1854); *B. a. hawaiiensis* Broch, 1922, synonym of *Amphibalanus amphitrite* Darwin, 1854

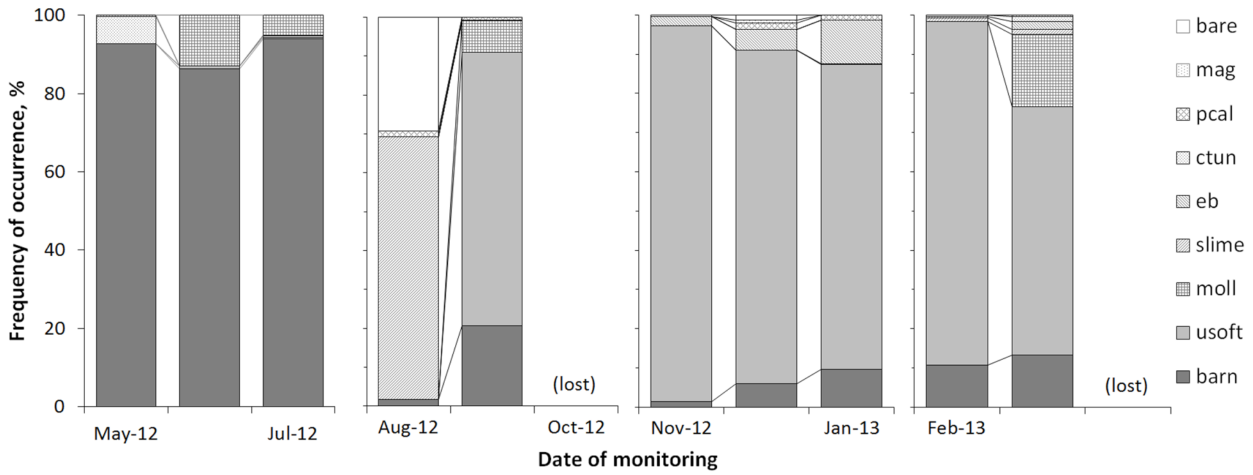

Figure 5. Composition of the fouling community and mean occurrence (in %) of organisms on panel surfaces submerged continuously for three months (Frame 2) at Pier 13, Manila South Harbor, Philippines. The frames were replaced in July and October 2012 and January 2013. Legend: *mag*: green macroalgae; *pcal*: calcareous polychaetes; *ctun*: colonial tunicates; *eb*: encrusting bryozoans; *moll*: molluscs; *usoft*: undetermined soft fouling; *barn*: barnacles.

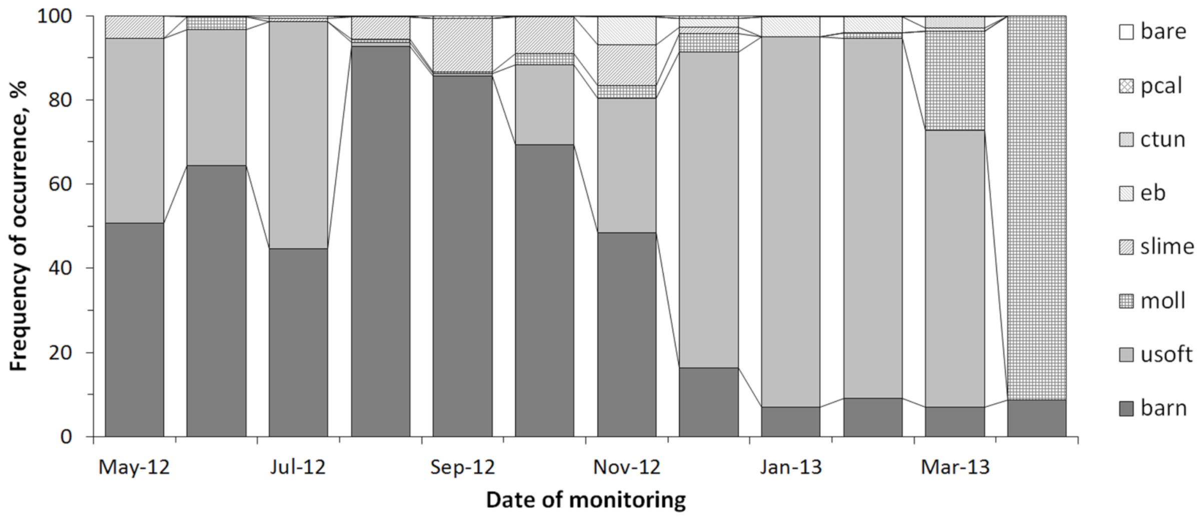

Figure 6. Composition of the fouling community and mean occurrence (in %) of organisms on panel surfaces submerged continuously for one year (Frame 3) at Pier 13, Manila South Harbor, Philippines. Legend: *pcal*: calcareous polychaetes; *ctun*: colonial tunicates; *eb*: encrusting bryozoans; *moll*: molluscs; *usoft*: undetermined soft fouling; *barn*: barnacles.

(GBIF Secretariat 2017); and, *B. variegatus cirratus* Darwin, 1854, accepted as *Amphibalanus variegatus* (Darwin, 1854). We could not, as yet, determine the accepted nomenclature for the fifth species described, *B. a. kondakovi* Tarazov and Zevina, 1957 from either WoRMS or GBIF. Nonetheless, we have identified the species observed for over a year on the panels as the cosmopolitan, competitive, and invasive *Balanus* (=*Amphibalanus*) *amphitrite*.

Like most crustaceans, *B. amphitrite* reproduced during the warm months and rapidly recruited to the benthos. Its establishment on the surfaces of the panels was an expected event in May 2012 in all the setups; barnacle cover appeared to increase in March and April 2013 in the monthly panels (Figures 3 and 4C), likely related to increasing water temperature. In the cold temperate region where the species is also known to occur (e.g., Peter the Great Bay, Sea of Japan), the processes of reproduction and benthic recruitment of *B. amphitrite* were reported to be facilitated by power plant thermal plumes, since these non-indigenous species naturally became physiologically disabled in the Bay's <12°C waters (Zvyagintsev and Korn 2003). After brief planktotrophic nauplii and cyprid stages, larvae settled on microbial and diatom biofilms (cf. Wahl 1989), often preferentially over unfilmed surfaces (Harder et al. 2001), then metamorphosed into cemented juveniles within 24 hours (WHOI 1952; Maruzzo et al. 2012). Since metamorphosis and subsequent growth depended on food concentrations and temperature (Thiyagarajan et al. 2003) as well as multiple physical cues from diatom exopolymers and biofilms (Patil and Anil 2005), we deduced that metamorphosis and early juvenile growth may have occurred earlier on the setups, since the ambient port waters of the Manila South Harbor were generally warm between April and May, and mixed phyto- and zooplankton communities were available as juveniles' food. However, the further growth of individual barnacles on the panel surfaces was observed to be limited, likely due to crowding, since these had quickly filled the panel surfaces already after one month. In the submerged state, other fouling organisms may also have competed with *B. amphitrite* for space. Calcagno et al. (1997, 1998) found that growth and

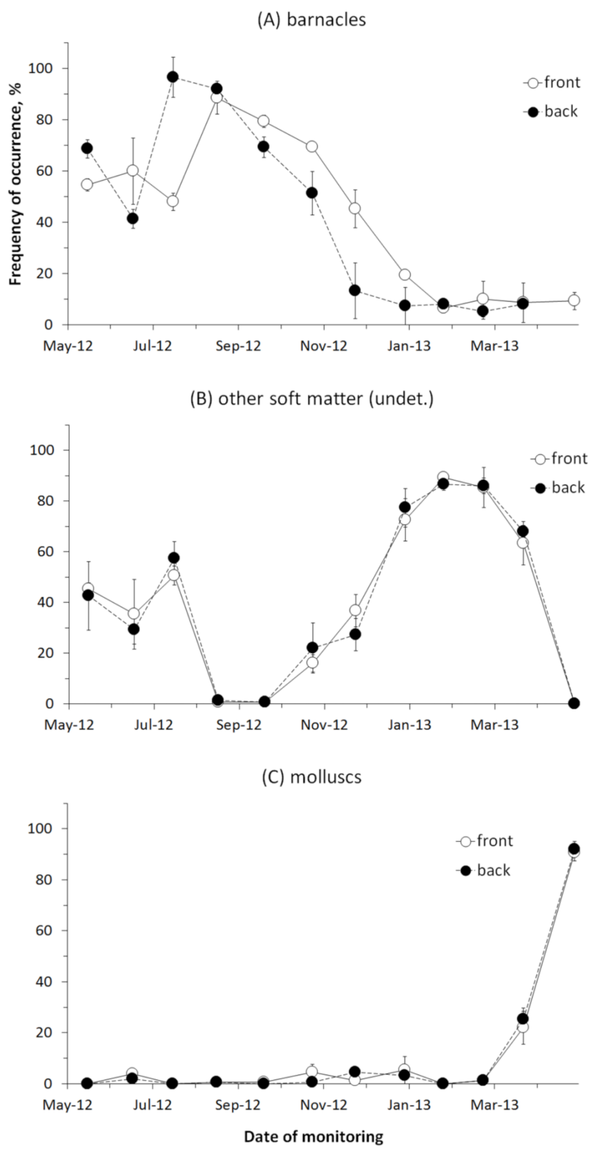

Figure 7. The mean cover (± SE, n=3) of major biofouling organisms—(A) barnacles *B. amphitrite*, (B) other soft matter (undetermined), and (C) mainly *P. viridis* on front and back surfaces of panels submerged for one year (Frame 3) at Pier 13, Manila South Harbor, Philippines.

abundance of *B. amphitrite* were highest at near the water surface (intertidal) and not at subtidal depths. *Balanus amphitrite* responded negatively to smothering by crude oil, which probably interfered with cirral activity during filter-feeding and eventually affected its overall motility (Morton and Wu 1977; Hashim 2010). The still cemented plates and crevices, however, offered rugose and uneven surfaces, as new sites for recolonization by primary (microbes), secondary (microalgae), and eventually tertiary fouling organisms (macroalgae and macrofauna).

Green mussels (*P. viridis*) apparently colonized and replaced barnacle cover towards the 12th month on the quarterly and long-term immersed setups (Figures 5 and 6C). *Perna viridis* similarly spawns during warm months and larval duration in the plankton can take up to 30 days prior to settlement in the benthos (Rajagopal et al. 2006), much longer compared to *B. amphitrite*. Spats have also been observed to be selective of settlement substrates (Nishida et al. 2003). In our setups, *P. viridis* attached their byssus threads firmly on the basibionts, e.g., biofilmed panel surfaces below the soft fouling (undet.) (*usoft*) cover

Table 1. Marine stowaway organisms that can be transported globally through ships' ballast water and hull fouling (GISD, 2017), of which some have thrived as non-indigenous species, became invasive, and/or established as constituents of fouling communities in e.g., the Philippines. Legend: [*], from Ocampo et al. (2014); [+], from Vallejo (2010); [#], in Philippine waters (among the invasive species; GISD 2017); [++], from Vallejo et al. (2017); [**], this study.

Kingdom Animalia
Phylum Annelida
Class Polychaeta
*Capitella capitata**
Dorvilleidae sp.*
Orbinia sp.*
Alitta succinea
Nereididae sp.*
Nereis sp.*
Polynoidae sp.*
Sabella spallanzanii
Ficopomatus enigmaticus
Hydroides sp.**
Polydora sp.*

Phylum Arthropoda
Class Hexanauplia
*Amphibalanus (=Balanus) amphitrite**, ***
Class Malacostraca
Penaeus (=Litopenaeus) stylirostris[+]
Penaeus (=Litopenaeus) vannamei[+]
Class Pycnogonida
Pantopoda sp.**

Phylum Bryozoa
Class Gymnolaemata
Schizoporella errata
*Bugula neritina**
Watersipora sp.**

Phylum Chordata
Class Actinopterygii
Pterapogon kauderni[+]
Lutjanus kasmira
Pterois volitans[#]
Class Ascidiacea
Didemnum spp.
Ascidiella aspersa
Ciona intestinalis
Styela clava
Styela plicata
Colonial tunicate (undet.)**

Phylum Cnidaria
Class Anthozoa
Actinia sp.*
*Anemonia manjano**
Carijoa riisei
Tubastraea coccinea
Phyllorhiza punctata

Phylum Ctenophora
Class Tentaculata
Mnemiopsis leidyi

Phylum Echinodermata
Class Asteroidea
Asterias amurensis
Acanthaster planci[#]

Phylum Mollusca
Class Bivalvia
Potamocorbula amurensis
Dreissena polymorpha
Mya arenaria
Arcuatula (=Musculista) senhousia
Brachidontes sp.*
Geukensia demissa
Perna perna
Perna viridis[#, **]
Mytilus galloprovincialis
Magallana (=Crassostrea) gigas[+]
Rangia cuneata
Mytella charruana[++]
Mytilopsis sallei[*, #, **]
Ostreoida sp.**
Class Gastropoda
Crepidula fornicata
Ergalatax sp.*
Rapana venosa

Phylum Platyhelminthes
Class Rhabditophora
Leptoplanidae sp.

Phylum Porifera,
Class Demospongiae
Mycale grandis

Kingdom Chromista
Phylum Myzozoa
Class Dinophyceae
Alexandrium minutum[+]
Gymnodinium catenatum[#]
Margalefidinium (=Cochlodinium) polykrikoides[+]

Phylum Ochrophyta
Class Phaeophyceae
Sargassum fluitans
Sargassum muticum
Undaria pinnatifida
Class Raphidophyceae
Chattonella marina[+]

Kingdom Plantae
Phylum Chlorophyta
Class Chlorophyceae
Codium fragile subsp. fragile (=tomentosoides)
Caulerpa taxifolia[#]
Caulerpa webbiana
Chlorophyceae sp.**

Phylum Rhodophyta
Class Florideophiceae
Acanthophora spicifera[#]
Kappaphycus spp.[#]
Polysiphonia brodiei
Gracilaria salicornia[#]
Gracilaria vermiculophylla (=verrucosa)[#]

and extant empty barnacle tests, hence, appeared embedded in the fouling community. Their presence in the fouling community possibly increased the rugosity of the settling surfaces and facilitated the recruitment of more *P. viridis* individuals, hence, colonizing most, if not all, basibiont surface. We did not observe *P. viridis* in the short-term immersed panels towards the 12th month (Figure 3), possibly because of the substantial cover of living *B. amphitrite*, slime, and to a lesser extent, colonial tunicates (*ctun*), *Watersipora* sp. (*eb*), and *Hydroides* sp. (*pcal*). It remains to be verified whether these fouling types, although minor, exhibited protective behavior or mechanisms against major fouling types, such as barnacles and mussels (Devi et al. 1998; Wahl et al. 1998; Wilsanand et al. 1999; da Gama et al. 2003).

Perna viridis, part of a thriving aquaculture industry in Manila Bay, has been used as bioindicator for marine pollution by monitoring levels of butyltins, organochlorines, and heavy metals in mussel tissues (Prudente et al. 1999). High levels of tributyltins were associated with port areas (i.e., from anti-fouling paints) and polychlorinated biphenyls (PCBs) were then found at relatively high concentrations in mussels from the Bay. In addition, *P. viridis* is regularly used in assays for paralytic shellfish poisoning (PSP) during harmful algal bloom (HAB) events that now recur in select sites around the country (Arcamo 2015). As a nuisance organism, these were observed as dense clumps on ship anchors and propellers berthed at Pier 13 during the study. In a separate survey of major fouling organisms conducted in April 2013, in which we scanned the ports of the Manila South Harbor and Manila North Harbor (the port catering to ships with domestic routes), we found *P. viridis* only in wharf stations within the Manila South Harbor.

Marine biofouling species are a subset of stowaway organisms that can be transported across the globe by shipping (GSID 2017) and the initial list of 15 taxa by Ocampo et al. (2014) came about after 60 days of experimental immersion in Manila Bay and may be expanded to include the organisms documented by this study after identifying these further to lower taxonomic levels. In our preliminary work, we documented *Mytilopsis sallei* from panels immersed between October and December 2011 and not in our samples obtained thereafter. Studies on aspects of its biology, ecology, and risk of invasion in port waters and nearby estuaries must be given priority (cf. Tan and Morton 2006), including the further examination of the soft fouling matter (undet.), since these host microalgae and bacteria associated with fouling communities.

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REFERENCES

Aldridge DC, Elliott P, Moggridge GD. 2003. The recent and rapid spread of the zebra mussel (*Dreissena polymorpha*) in Great Britain. Biological Conservation 119:253–261.

Arcamo SVR. 2015. HAB monitoring program of the Philippines. Paper presented at: Roundtable Discussion on Non-Communicable Diseases (NCDs) and other Health and Wellness Risks from the Marine Environment; 2015 Mar 31; Hotel Jen, Manila, Philippines. http://nast.ph/index.php/ downloads/category/61-ncd-s-and-marine-environment?download=205:hab-monitoring-program-of-the-philippines.

Bax N, Williamson A, Aguero M, Gonzalez E, Geeves W. 2003. Marine invasive alien species: a threat to global biodiversity. Marine Policy 27(4):313–323.

Bird CE. 2003. PhotoGrid: Ecological Analysis of Digital Photographs, Version 1.0. University of Hawai'i, Honolulu.

Bobat A, Hengirmen HO, Zapletal W. 2004. Zebra mussel and fouling problems in the Euphrates Basin. Turkish Journal of Zoology 28:161–177.

Bressy C, Lejars M. 2014. Marine fouling: An overview. The Journal of Ocean Technology 9(4):19–28.

Burkholder JAM, Hallegraeff GM, Melia G, Cohen A, Bowers HA, Oldach DW, Parrow MW, Sullivan MJ, Zimba PV, Allen EH, et al. 2007. Phytoplankton and bacterial assemblages in ballast water of U.S. military ships as a function of port origin, voyage time and ocean exchange practices. Harmful Algae 6:486–518.

Calcagno JA, López Gappa J, Tablado A. 1997. Growth and production of the barnacle *Balanus amphitrite* in an intertidal area affected by sewage pollution. Journal of Crustacean Biology 17(3):417–423.

Calcagno JA, López Gappa J, Tablado A. 1998. Population dynamics of the barnacle *Balanus amphitrite* in an intertidal area affected by sewage pollution. Journal of Crustacean Biology 18(1):128–137.

Chavanich S, Tan LT, Vallejo B, Viyakarn V, editors. 2010. Report on the current status of marine non-indigenous species in the Western Pacific Region. Bangkok: Intergovernmental Oceanographic Commission Sub-Commission for the Western Pacific (IOC/WESPAC). 61 p.

Coutts ADM, Dodgshun TJ. 2007. The nature and extent of fouling in vessel sea-chests: a protected mechanism for marine bioinvasions. Marine Pollution Bulletin 54:875–886.

da Gama BAP, Pereira RC, Soares AR, Teixeira VL, Yoneshigue-Valentin Y. 2003. Is the mussel test a good indicator of antifouling activity? A comparison between laboratory and field assays. Biofouling 19(1 Suppl):161–169.

David M, Perkovič M. 2004. Ballast water sampling as a critical component of biological invasions risk management. Marine Pollution Bulletin 49:313–318.

Deacutis CF, Ribb RC. 2002. Ballast water and introduced species: management options for Narragansett Bay and Rhode Island. Providence: Narragansett Bay Estuary Program and Rhode Island Department of Environmental Management. p. 1–48. http://www.nbep

.org/publications/other/ballast/BallWaterIntro-SpeciesRpt.pdf.
Devi P, Vennam J, Naik CG, Parameshwaran PS, Raveendran TV, Yeshwant KS. 1998. Antifouling activity of Indian marine invertebrates against the green mussel *Perna viridis* L. Journal of Marine Biotechnology 6:229–232.
Eguia E, Trueba A. 2007. Application of marine biotechnology in the production of natural biocides for testing on environmentally innocuous antifouling coatings. Journal of Coatings Technology and Research 4(2):191–202.
Global Biodiversity Information Facility (GBIF) Secretariat. 2017. *Balanus amphitrite* subsp. *hawaiiensis* Hiro, 1937. GBIF Backbone Taxonomy; [accessed 2017 Nov 26]. https://doi.org/10.5072/hufs9m.
Global Invasive Species Database (GISD). 2018. Downloaded from http://193.206.192.138/gisd/search.php on 12-07-2018.
Gollasch S, Lenz J, Dammer M, Andres HG. 2000. Survival of tropical ballast water organisms during a cruise from the Indian Ocean to the North Sea. Journal of Plankton Research 22:923–937.
Harder TN, Thiyagarajan V, Qian PY. 2001. Effect of cyprid age on the settlement of *Balanus amphitrite* Darwin in response to natural biofilms. Biofouling 17(3):211–219.
Hashim AA. 2010. Effect of sublethal concentrations of fuel oil on the behavior and survival of larvae and adults of the barnacle *Balanus amphitrite amphitrite* (Darwin). Turkish Journal of Fisheries and Aquatic Sciences 10:499–503.
[IMO] International Maritime Organization. 2017. Common hull fouling invasive species. International Maritime Organization. [accessed 2017 Oct 17]. http://www.imo.org/en/OurWork/Environment/Bio-fouling/Pages/Common-Hull-Fouling-Invasive-Species.aspx.
Jacinto GS, Azanza RV, Velasquez IB, Siringan FP. 2006. Manila Bay: environmental challenges and opportunities. In: Wolanski E, editor. The environment in Asia-Pacific harbours. Dordrecht: Springer. p. 309–328.
Lewis PN, Hewitt CL, Riddle M, McMinn A. 2003. Marine introductions in the Southern Ocean: an unrecognized hazard to biodiversity. Marine Pollution Bulletin 46:213–223.
Lim CS, Chong CSE. 2012. Estimating surface coverage of biofouling with PhotoGrid 1.0 and CPCe 4.0. Paper presented at: ASEAN-India Cooperation Project "Extent of transfer of alien invasive organisms in South/SE Asia region by shipping" Training Workshop II (Identification methods); 22 February 2012; Visakhapatnam, India.
Lim JY, Tay TS, Lim CS, Lee SSC, Teo SLM, Tan KS. 2018. *Mytella strigata* (Bivalvia: Mytilidae): an alien mussel recently introduced to Singapore and spreading rapidly. Molluscan Research 38(3):170–186.
Maruzzo D, Aldred N, Clare AS, Høeg JT. 2012. Metamorphosis in the cirripede crustacean *Balanus amphitrite*. PLoS ONE 7(5):e37408.
Migu. 2009. Featured Philippine Ports: The Manila South Harbor. Pinoy Shipspotter's Travel Guide; [accessed 2016 Jun 27]. https://mcgutib.wordpress.com/2009/06/27/featured-ports-pmo-south-harbor.
Molnar JL, Gamboa RL, Revenga C, Spalding MD. 2008. Assessing the global threat of invasive species to marine biodiversity. Frontiers in Ecology and the Environment 6:485–492.
Morton B, Wu RSS. 1977. The toxic effects of hydrocarbons upon the naupliar and adult stages of *Balanus* (Crustacea: Cirripedia). Marine Pollution Bulletin 8(10):232-236.
Myan FWY, Walker J, Paramor O. 2013. The interaction of marine fouling organisms with topography of varied scale and geometry: a review. Biointerphases 8(1):30.
Nishida A, Ohkawa K, Ueda I, Yamamoto H. 2003. Green mussel *Perna viridis* L.: attachment behaviour and preparation of antifouling surfaces. Biomolecular Engineering 20:381–387.
Ocampo MA, Oliva IW, Tan R, Su GS, Vallejo 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.
Pati SK, Rao MV, Balaji M. 2015. Spatial temporal changes in biofouling community structure at Visakhapatnam harbor, east coast of India. Tropical Ecology 56(2):139–154.
Patil JS, Anil AC. 2005. Influence of diatom exopolymers and biofilms on metamorphosis in the barnacle *Balanus amphitrite*. Marine Ecology Progress Series 301:231–245.
Philippine Ports Authority (PPA)–Annual Report. c2005–2015. Philippine Ports Authority; [accessed 2017 Nov 23]. http://www.ppa.com.ph/?q=annual-report.
PortCalls Asia. 2017. BWM Convention in force; Jamaica and Malta latest signatories. PortCalls Asia; [accessed 2017 Nov 24]. https://www.portcalls.com/bwm-convention-force-jamaica-malta-latest-signatories.
Prudente M, Ichihashi H, Kan-atireklap S, Watanabe I, Tanabe S. 1999. Butyltins, organochlorines and metal levels in green mussel, *Perna viridis* L. from the coastal waters of the Philippines. Fisheries Science 65:441–447.
Rajagopal S, Venugopalan VP, Van der Velde G, Jenner HA. 2006. Greening of the coasts: a review of the *Perna viridis* success story. Aquatic Ecology 40:273–297.
Rosell NC. 1973. Some thoracic barnacles (Crustacea: Cirripedia) of Manila Bay. Kalikasan, The Philippine Journal of Biology 2:69–95.
Schwan I, Brasil AC, Neves D, Dias M. 2015. The invasive worm *Hydroides elegans* (Polychaeta - Serpulidae) in southeastern Brazil and its potential to dominate hard substrata. Marine Biology Research 12(1):96–103.
Sousa R, Pilotto F, Aldridge DC. 2011. Fouling of European freshwater bivalves (Unionidae) by the invasive zebra mussel (*Dreissena polymorpha*). Freshwater Biology 56:867–876.
Tan KS. 2011. Extent of transfer of alien invasive organisms in South/SE Asia region by shipping. Paper presented at: ASEAN-India Cooperation Project "Extent of transfer of alien invasive organisms in South/SE Asia region by shipping" Training Workshop I (Standardization of sampling methods for ballast water and hull fouling; Experimental design; Preservation techniques; Collation of ballast water reporting form data; Application of self-validating electronic ballast water reporting form, Data management and its utility in risk assessment); 20 July 2011; St. John's Island National Marine Laboratory, Tropical Marine Science Institute, National University of Singapore.
Tan KS, Morton B. 2006. The invasive Caribbean bivalve *Mytilopsis sallei* (Dreissenidae) introduced to

Singapore and Johor Bahru, Malaysia. The Raffles Bulletin of Zoology 54:429–434.

Thiyagarajan V, Harder T, Qiu JW, Qian PY. 2003. Energy content at metamorphosis and growth rate of the early juvenile barnacle *Balanus amphitrite*. Marine Biology 143:543–554.

Vallejo B. 2010. Philippines. In: Chavanich S, Tan LT, Vallejo B, Viyakarn V, editors. Report on the Current Status of Marine Non-Indigenous Species in the Western Pacific Region. Bangkok: Intergovernmental Oceanographic Commission Sub-Commission for the Western Pacific (IOC/WESPAC). p. 31–33.

Vallejo B, Conejar-Espedido J, Manubag L, Artiaga KCC, Damatac II AM, Imperial ICVJ, Itong TAB, Fontanilla IK, Cao EP. 2017. First record of the Charru mussel *Mytella charruana* d'Orbignyi, 1846 (Bivalvia: Mytilidae) from Manila Bay, Luzon, Philippines. BioInvasions Records 6(1):49–55.

Wahl M. 1989. Marine epibiosis. I. Fouling and anti-fouling: some basic aspects. Marine Ecology Progress Series 58:175–189.

Wahl M, Kröger K, Lenz M. 1998. Non-toxic protection against epibiosis. Biofouling 12(1–3):205–226.

[WHOI] Woods Hole Oceanographic Institution. 1952. The principal fouling organisms. In: Marine fouling and its prevention. Annapolis: US Naval Institute. p. 118–164.

Wilsanand V, Wagh AB, Bapuji M. 1999. Antifouling activities of marine sedentary invertebrates on some macrofoulers. Indian Journal of Marine Sciences 28:280–284.

World Port Source. 2005. Port of Manila. [accessed 2016 Nov 23]. http://worldportsource.com/ports/review/PHL_Port_of_Manila_1947.php.

WoRMS Editorial Board. 2017. World Register of Marine Species. [accessed 2017 Nov 25]. https://doi.org/10.14284/170.

Zvyagintsev AY, Korn O. 2003. Life history of the barnacle *Balanus amphitrite* Darwin and its role in fouling communities of Peter the Great Bay, Sea of Japan. Russian Journal of Marine Biology 29(1):41–48.