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RESEARCH

An inter-site study of biofouling recruitment on static immersion panels in major ports of South East Asia and India

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ABSTRACT Limited knowledge of native marine biodiversity hinders effective biodiversity management to safeguard South and Southeast Asia's marine coastal environment against the threat of invasive species transfer through shipping. In particular, sessile marine biofouling organisms in South East Asian ports are poorly known. Through the support of the ASEAN-India Cooperation Project on the Extent of Transfer of Alien Invasive Organisms in South/South East Asia Region by Shipping, a coordinated effort to examine diversity of biofouling organisms in major port areas in Southeast Asia and India was made using polyvinylchloride (PVC) panels as recruitment surfaces in a static immersion study for a period of 12 months. Not surprisingly, the study revealed that fouling patterns differed between ports possibly as a result of dissimilar hydrographic conditions. However, there were also underlying similarities that reflected a regional uniformity in the composition of fouling communities. At the same time, the alien Caribbean bivalve *Mytilopsis sallei* was detected in Manila Bay (Philippines), Songkhla Port (Thailand) and Singapore. This is a first simultaneous biofouling survey involving scientists and government stakeholders from India and ASEAN nations of Brunei Darussalam, Indonesia, Lao PDR, Malaysia, Myanmar, Singapore, Thailand, Philippines and Vietnam.

1. INTRODUCTION

Biofouling, or the settlement of undesirable marine organisms on natural and man-made submerged bodies, accounts for significant operational and re-mediation costs in shipping (Schultz et al. 2011) and other coastal assets including desalination and power plants (Booy et al. 2017). The spread of non-native species to new marine environments via biofouling on commercial shipping vessels is a major transport pathway for species introduction (Eldredge and Carlton 2002; Coutts and Dodgshun 2007; Seebens et al. 2013). Such introductions may cause loss of marine biodiversity and irreparable damage to the marine ecosystem as a result of competition with native biota (Crowl et al. 2008).

The diversity of marine biota on submerged bodies was the subject of several papers published in India and Southeast Asia (SEA). In particular, detailed studies of biodiversity and seasonal variation of fouling at several ports and harbours in India have been undertaken (e.g. Gaonkar et al. 2010; Swami and Udhayakumar 2010; Pati et al. 2015; Nandhini and Revathi 2016) including Mumbai, India, which was selected as one of the six demonstration sites (including Dalian, China; Khark Is., Iran; Odessa, Ukraine; Saldanha, South Africa; Sepetiba, Brazil) in the Global Ballast Water Management Programme (GloBallast-IMO 2000) to reduce the transfer of non-native species via shipping ballast, and had garnered motivation in the study of biofouling community in ports and harbours in India. In SEA, Low et al. (1991) examined the seasonal variation of the dominant fouling species, *Perna viridis* (Asian green mussel) along the East Johor Straits, a shared waterway between Malaysia and Singapore, where shipyards and docks are located. Seasonal and successional colonization of macrofouling species affecting fish-cage nettings in Malaysia (Madin et al. 2009) were examined, while recent studies of biofouling and epibiotic diversity ('marine growth') on navigational buoys, marina pontoons and jetty pilings in Singapore (Ong and Tan 2012; Lee et al. 2013; Toh et al. 2017) also highlighted the potential of submerged structures to support a diversity of marine organisms.

Biofouling communities around vessel-frequented areas are therefore of particular interest, as man-made infrastructure (e.g. pilings, pontoons) in the port or marina may support a diversity of organisms. These may include non-native species that could have been introduced through arriving vessels, especially vessels operating between international and regional ports or marinas. Areas around ports and marinas have therefore been the subject of many invasive species surveys to examine species introduction and vector causes (Hewitt 2002; Hutchings et al. 2002; Gaonkar et al. 2010).

In a collective study of the potential negative impacts of non-native species on agriculture, human health and the environment (e.g. urban built-up areas) in SEA, Nghiem et al. (2013) estimated the total costs to around US$33.5 billion. However, the study focused on specific agricultural pests (e.g. golden apple snail), human diseases (e.g. SARS virus) and urban pests (i.e., pigeons and cats), where comprehensive data were more readily available. The model or estimated costs in the study did not consider the impacts of non-native species to the marine environment. Given that regional ports in SEA were identified as potential bioinvasion hotspots due to their high shipping intensity (Seebens et al. 2013), a better understanding of the impact of non-native marine species on the environment is needed. High marine biodiversity in the SEA region, coupled with a vast maritime area, has hindered progress (Peh 2010; Lee et al. 2013). This can be improved by a coordinated approach to examine biofouling organisms, where regional cooperation and communication can enhance monitoring efforts to manage the spread of non-native species. This is especially so in light of the 2011 Biofouling Guidelines for the Control and Management of Ship' Biofouling to minimize the Transfer of Invasive Aquatic Species (Biofouling Guidelines), where States are encouraged to 'provide ships with any available information on particular invasive aquatic species that may be present in a port and could attach to a ship as biofouling (in a timely manner)'.

Through the support of the ASEAN-India Cooperation Project on Extent of Transfer of Alien Invasive Organisms in South/South East Asia Region by Shipping, biofouling surveys in major ports in Southeast Asia and India were carried out using polyvinylchloride (PVC) panels as recruitment surfaces in a short term static simultaneous immersion study. There were two main objectives: one, to understand the local marine flora and fauna, particularly in relation to biofouling on hard substrata in the vicinity of their port of study; two, to recognize the seasonal variation in the major and dominant fouling species present. This 'bottom-up' approach allowed capacity building in identification of fouling species and development and also raised awareness for invasive species transfer through shipping. The results from this study represented for the first time a joint survey carried out simultaneously with scientists and government stakeholders from India and ASEAN nations of

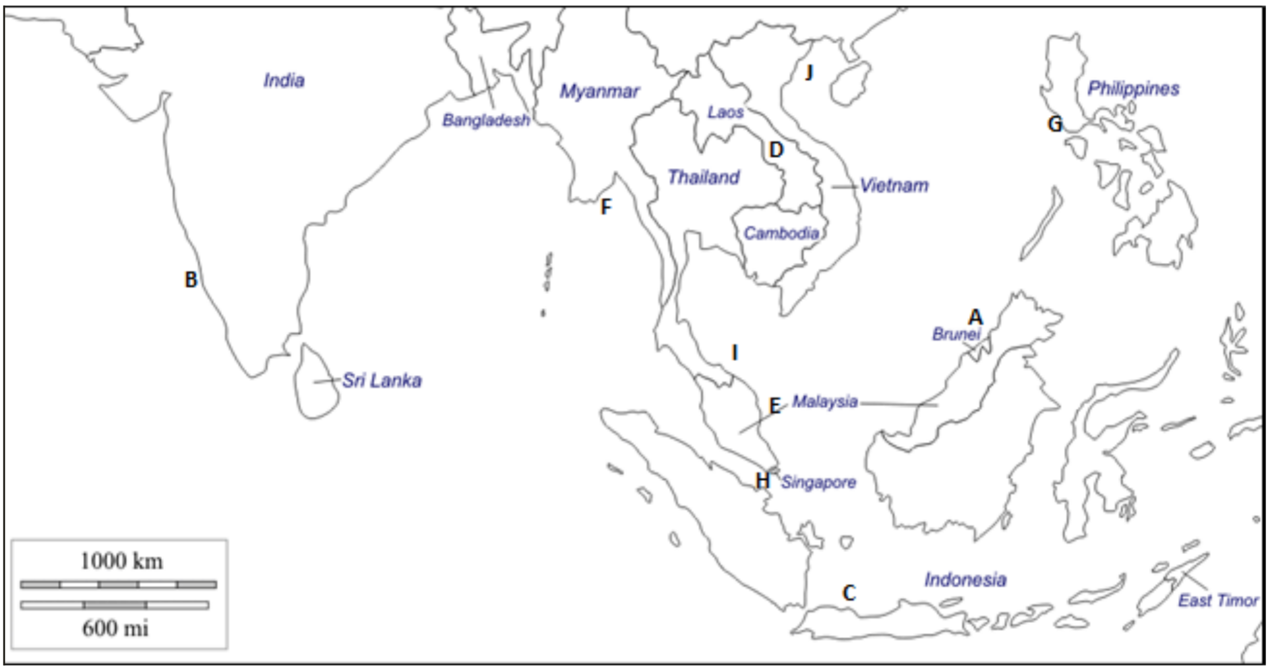

Figure 1. Port sampling locations in India and ASEAN countries. Countries are arranged in alphabetical order. (A) Muara, Brunei; (B) Goa, India; (C) Tanjong Priok, Indonesia; (D) Vientiane, Mekong River, Lao PDR; (E) Kertih, Malaysia; (F) Yangon, Myanmar; (G) Manila Bay, Philippines; (H) Singapore; (I) Songkhla, Thailand; (J) Hai Phong, Vietnam. Map downloaded from www.d-maps.com.

Table 1. Monthly fouling data collected from ports in India and ASEAN countries between May 2012 and December 2013. Diamonds indicate sampling months. Panels were examined after one month and replaced with a new panel each month.

Period	Muara, Brunei	Goa, India	Tanjong Priok, Indonesia	Kertih, Malaysia	Yangon, Myanmar	Manila Bay, Philippines	Singapore	Songkhla, Thailand	Hai Phong, Vietnam
May 2012	•	•				•	•	•	•
Jun 2012	•	•				•	•	•	•
Jul 2012	•	•				•	•	•	•
Aug 2012		•				•	•		•
Sep 2012	•	•			•	•	•		•
Oct 2012	•	•			•		•	•	•
Nov 2012	•	•			•	•	•	•	•
Dec 2012	•	•		•	•		•	•	•
Jan 2013	•	•		•	•		•		
Feb 2013	•	•		•			•		•
Mar 2013	•	•		•	•		•		
Apr 2013	•	•		•	•		•		
May 2013		•		•	•				
Jun 2013		•	•		•				
Jul 2013		•	•						
Aug 2013		•	•						
Sep 2013		•	•						
Oct 2013			•						
Nov 2013			•						
Dec 2013			•						

Table 2. Long term fouling data collected from ports in India and ASEAN countries between May 2012 and December 2013. Diamonds indicate sampling months. Panels were examined after one month, and the same panels were returned into the water and subsequently re-examined monthly.

Period	Muara, Brunei	Goa, India	Tanjong Priok, Indonesia	Kertih, Malaysia	Yangon, Myanmar	Manila Bay, Philippines	Singapore	Songkhla, Thailand	Hai Phong, Vietnam
May 2012		•				•	•	•	•
Jun 2012		•				•	•	•	•
Jul 2012		•				•	•	•	•
Aug 2012		•				•	•		•
Sep 2012		•				•	•		•
Oct 2012		•				•	•	•	•
Nov 2012		•				•	•	•	•
Dec 2012		•		•			•	•	•
Jan 2013		•		•			•		•
Feb 2013		•		•			•		•
Mar 2013		•		•			•		
Apr 2013	•	•		•			•		
May 2013		•		•					•
Jun 2013		•	•						
Jul 2013		•	•						
Aug 2013		•	•						
Sep 2013		•	•		•				
Oct 2013			•						
Nov 2013			•						
Dec 2013									

Brunei Darussalam, Indonesia, Lao PDR, Malaysia, Myanmar, Singapore, Thailand, Philippines and Vietnam.

2. MATERIALS AND METHODS

2.1 Sampling locations

Ten ports and/or marina (sites) were selected for the panel static immersion to be carried out. These sites strategically represent major port areas where regional and transregional shipping activities were taking place, including infrastructure assets such as commercial (i.e., container), passenger and shipyard terminals. These sites were located (in alphabetical order of country) in Muara Port, Brunei Darussalam (Jolkifli and Wahab 2018); Goa, India (Desai et al. 2018); Tanjong Priok Port, Indonesia (Hadiyanto 2018); VTE Port, Lao PDR; Kertih Port, Malaysia (Hing et al. 2018); Yangon Port, Myanmar (Khaing 2018); Republic of Singapore Yacht Club, Singapore; Songkhla Port, Thailand (Puttapreecha et al. 2018); Manila Bay (South Harbor), Philippines (Nacorda et al. 2018); and Hai Phong Port, Vietnam (Figure 1). Of these sites, only VTE Port (Lao PDR) was located inland along the Mekong River. Access to ports was obtained from port administrators prior to commencement of immersion tests, while selection of a suitable immersion site for panel immersion was also subject to port approval (e.g., non-interference with vessels movement and personnel safety). As a permanent immersion platform was not readily available at all locations, it was necessary to modify immersion frames so that panels would be kept at a fixed

Figure 2. Sampling sites of ports/marina (pictures arranged alphabetically according to country); (A) Muara, Brunei; (B) Goa, India; (C) Tanjong Priok, Indonesia; (D) Vientiane, Lao PDR; (E) Kertih, Malaysia; (F) Yangon, Myanmar; (G) Manila Bay, Philippines; (H) Singapore; (I) Songkhla, Thailand; (J) Hai Phong, Vietnam.

immersion depth from sea surface regardless of tidal height (i.e., submersion of frames with attached floating buoys). In most cases, immersion of panels were carried out directly from permanent jetties, with the exception of panels submerged from an existing floating test platform in Singapore. Actual data from VTE Port (Lao PDR) was not included as the site consisted of freshwater body. However, it was included as a test site in the program to raise awareness and increase capacity building for test methods in invasive pest species management.

2.2 Monthly immersion test

To examine early stage (i.e., short immersion length) fouling colonization, settlement was recorded in a static immersion test using uncoated polyvinylchloride (PVC) sheets measuring 2 mm by 100 mm by 200 mm (three replicates). These materials, together with a simple PVC frame to secure the immersion plates were prepared in Singapore and sent to respective sites where they would be simultaneously immersed from May 2012. The plates were zip-tied to the PVC frames and immersed at a fixed depth of 0.8 m below sea surface at the site i.e., on floating structures/piers where the plates were maintained at a consistent depth. Every month, the frame was raised and fresh PVC plates were used to replace the one-month old plates, which was documented for fouling cover. Table 1 lists the months where fouling settlement was recorded at each site.

2.3 Long term immersion test

Long term cumulative fouling settlement observations to examine fouling development on a single set of PVC panels were also recorded. The fouling was documented every month from the same panels that were re-immersed after each observation. However, logistical and technical difficulties, including loss of panels due to unfavourable weather conditions prevented uninterrupted fouling settlement to be recorded. Table 2 shows the month(s) when long term fouling settlement for the respective sites were recorded.

2.4 Documentation of fouling

Upon inspection, the frames were raised and digital photographs of the plates were taken. Surface coverage and composition were obtained by a 100-point estimation method using Photogrid 1.0 according to ASTM D6990-05. 2.5 cm from the edge of the panel was discounted to reduce edge effect in settlement. Major fouling taxa (see Supplementary Table 1) at all sites were recorded for consistency and comparison. Fouling settlement (as percent cover) are shown in a stacked column detailing the organism taxa by month. For consistency, each taxon (e.g., barnacle) was shown in the same colour throughout, so that a dominant taxon may be visualised immediately. It will also be possible to monitor how the relative settlement cover have changed temporally, especially in a long term immersion period.

3. RESULTS

3.1 Port biofouling sampling

Cross-institution links and communication with regulatory boards and port authorities were established so that approval for immersion tests in the ports was obtained. This often necessitated the need for clarification of the impact of invasive pest species introduction on the environment and the role of vessels as a vector for transporting non-native species across ports in a shipping network. Thus, functional networks and communication were established for future monitoring and surveys to be done.

Several sampling difficulties were encountered which prevented commencement of the panel immersion at the sites simultaneously. This included gaining prior access to the port vicinity from the Port authorities and selection of a suitable immersion location on-site for panel immersion. Hence, three sites (Tanjong Priok, Kertih and Yangon) commenced the panel immersion at a slightly later date (Table 1). As there was no permanent test facility for immersion studies at the sites (except for a local marina in Singapore with an existing test platform for long term fouling studies), most of the immersion tests had to be designed on-site with approval from the port authorities. This may include temporary lines from the PVC frames to railings/mooring pins on the pier-side/jetty (Figure 2). In some cases, access to the actual sampling sites was limited from land (i.e. retrieval of frames from pier) and access by boats had to be carried out for panel inspection. This delayed some of the test sites to carry out immersion as planned.

The lack of a permanent or dedicated test facility also affected the security and safety of the frames, where some of the long-term panels and frames were lost as a result of

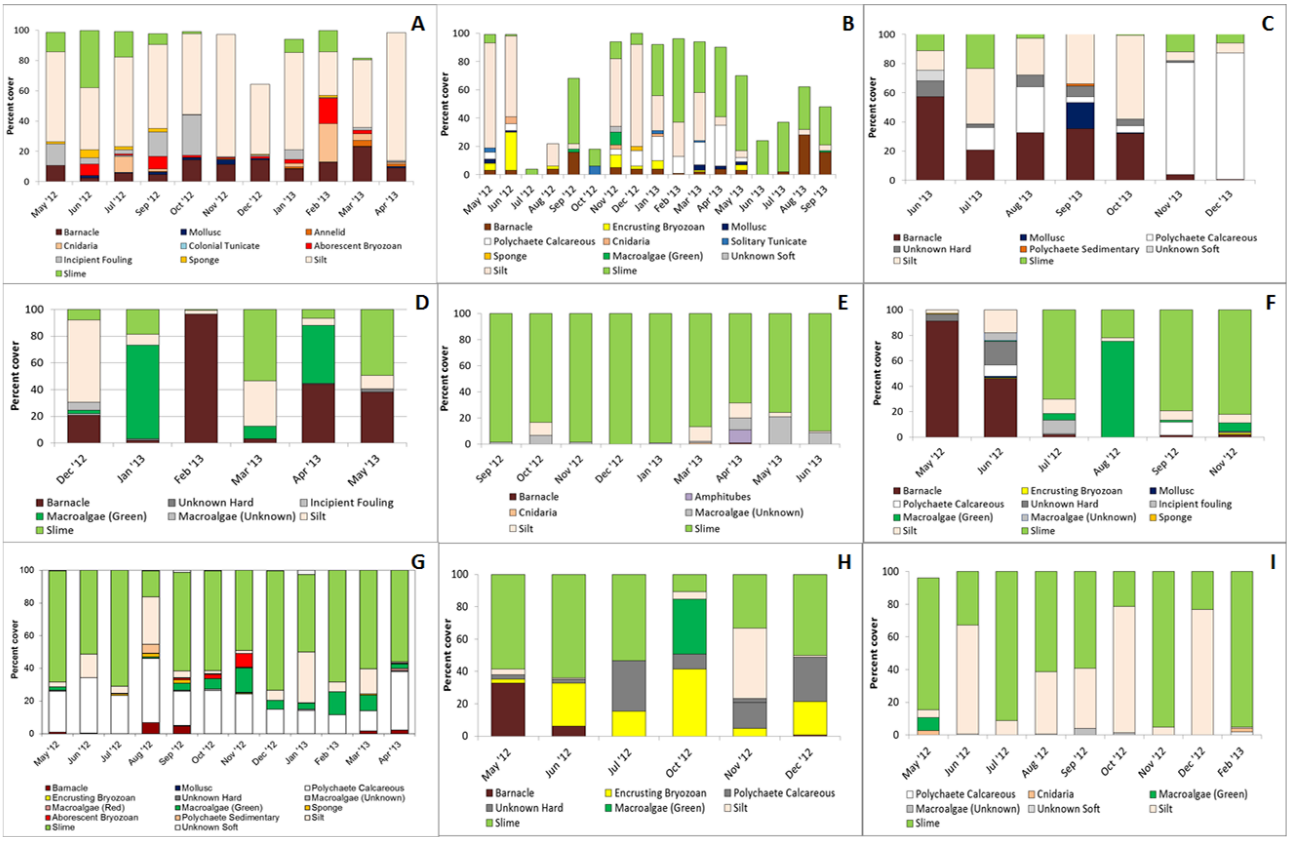

Figure 3. Monthly fouling composition at respective sites with periods shown: (A) Muara, Brunei; (B) Goa, India; (C) Tanjong Priok, Indonesia; (D) Kertih, Malaysia; (E) Yangon, Myanmar; (F) Manila Bay, Philippines; (G) Singapore; (H) Songkhla, Thailand; (I) Hai Phong, Vietnam. The legends are shown in each chart for easy reference, while the colours representing each taxon are marked the same.

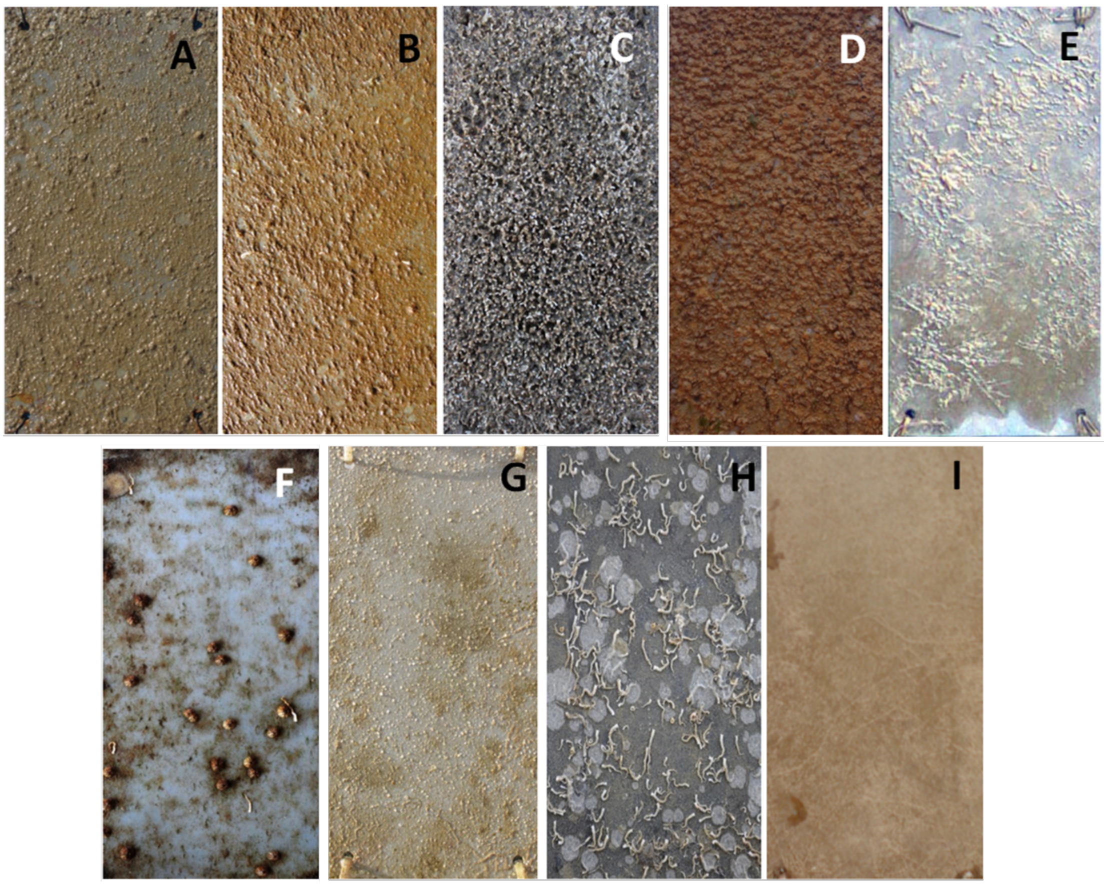

Figure 4. Monthly fouling composition of panels in Dec 2012, except Tanjong Priok (Dec 2013) and Manila Bay (Nov 2012). (A) Muara, Brunei; (B) Goa, India; (C) Tanjong Priok, Indonesia; (D) Kertih, Malaysia; (E) Yangon, Myanmar; (F) Manila Bay, Philippines; (G) Singapore; (H) Songkhla, Thailand; (I) Hai Phong, Vietnam.

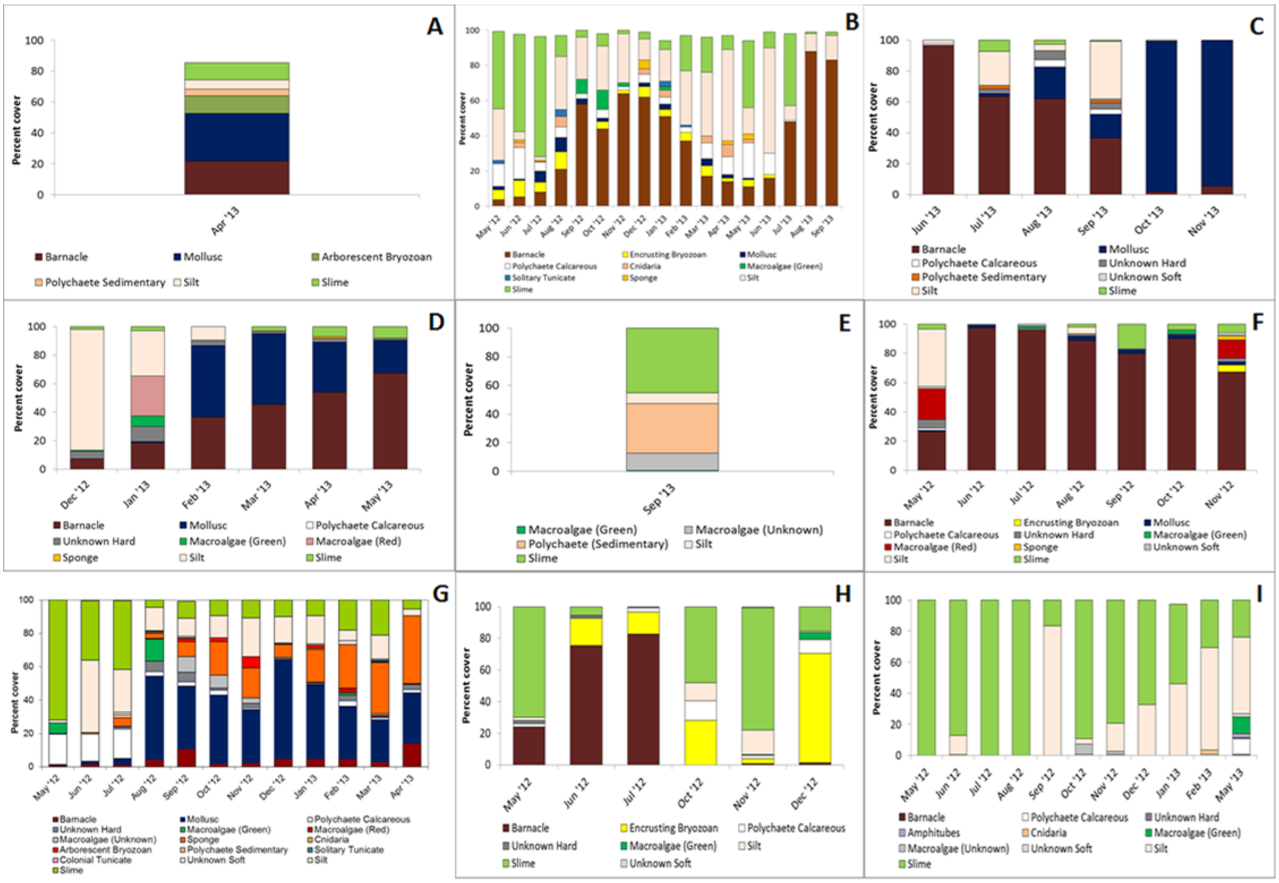

Figure 5. Long term fouling composition in respective sites with periods shown: (A) Muara, Brunei; (B) Goa, India; (C) Tanjong Priok, Indonesia; (D) Kertih, Malaysia; (E) Yangon, Myanmar; (F) Manila Bay, Philippines; (G) Singapore; (H) Songkhla, Thailand; (I) Hai Phong, Vietnam. The legends are shown in each chart for easy reference, while the colours representing each taxon are marked the same.

severe weather conditions at the ports (i.e. Kertih, Hai Phong, and Songkhla; Table 2). In particular, the early loss of panels (and replacement) delayed the commencement of the test at Kertih, Malaysia until these issues were rectified.

The lack of baseline records and adequate taxonomic knowledge of fouling organisms in the ports, including the absence of trained personnel to handle organism identification, could have prevented effective inspection of the fouling organisms in some sites. This is especially beneficial if identification could be clarified on-site by the operator, given that post-process identification of the organisms on images collected may be subjected to the quality of the image taken.

3.2 Monthly fouling settlement

Monthly settlement (Figure 3) showed the presence of major taxonomic groups including e.g., Crustacean, Mollusc, Polychaete and Algae, in Muara (Brunei), Goa (India), Tanjong Priok (Indonesia), Kertih (Malaysia), Manila Bay (Philippines), Singapore and Songkhla (Thailand) throughout the immersion period (Table 1). Heavy sedimentation and biofilm (i.e., slime) were present on panels at sites in Yangon (Myanmar) and Hai Phong (Vietnam). In addition, the large surface cover (>50%) of barnacles (*Amphibalanus* sp.) and calcareous polychaetes (Serpulidae) on panels in Tanjong Priok (Figure 3) in Jun 2013 and Nov 2013; barnacles (>90%) in Kertih (Feb 2013) and barnacles (>90%) in Manila Bay (May 2013) and encrusting bryozoans (>40%) in Songkhla (from Jun 2012) were striking in their rapid colonization (Figure 3). Tubeworms (Spirorbidae less than 1 mm in diameter) were ubiquitous in Singapore throughout the immersion period. Panel fouling images for all the sites in Dec 2012, Manila Bay in Nov 2012 and Tanjong Priok in Dec 2013 (Table 1) are shown in Figure 4.

3.3 Long term fouling settlement

Panel immersion for all the sites started in May 2012, except for Tanjong Priok (May 2013), Kertih (Nov 2012) and Yangon (Aug 2012). Immersion at all the sites were carried out for at least six months, and up to a year (Table 2); inspection of the fouling on the panel was carried out monthly. New PVC panels were used for replacement midway in Songkhla due to a loss of immersion plates on-site in Aug and Sep 2012 (Figure 5). Only the final settlement cover were recorded at Muara (one year duration) and Yangon (one year duration) due to a lack of monthly records taken.

Fouling settlement at the end of the long term immersion revealed that several taxonomic groups were common at the different sites. These included 1) barnacles (possibly *Amphibalanus* sp.) at Muara (Brunei), Goa (India), Tanjong Priok (Indonesia), Kertih (Malaysia), Manila Bay (Philippines), Singapore and Songkhla; 2) molluscs; Ostreidae at Muara (Brunei), Kertih (Malaysia), Singapore and 3) bivalves (*Perna viridis*) at Tanjong Priok (Indonesia).

4. DISCUSSION

4.1 Inter-site study of biofouling settlement – Building a monitoring program

Vessel biofouling is one of the most successful vectors for alien species transfer (Bax et al. 2003, Coutts and Dodgshun 2007; Molnar et al. 2008). In particular, crusta-

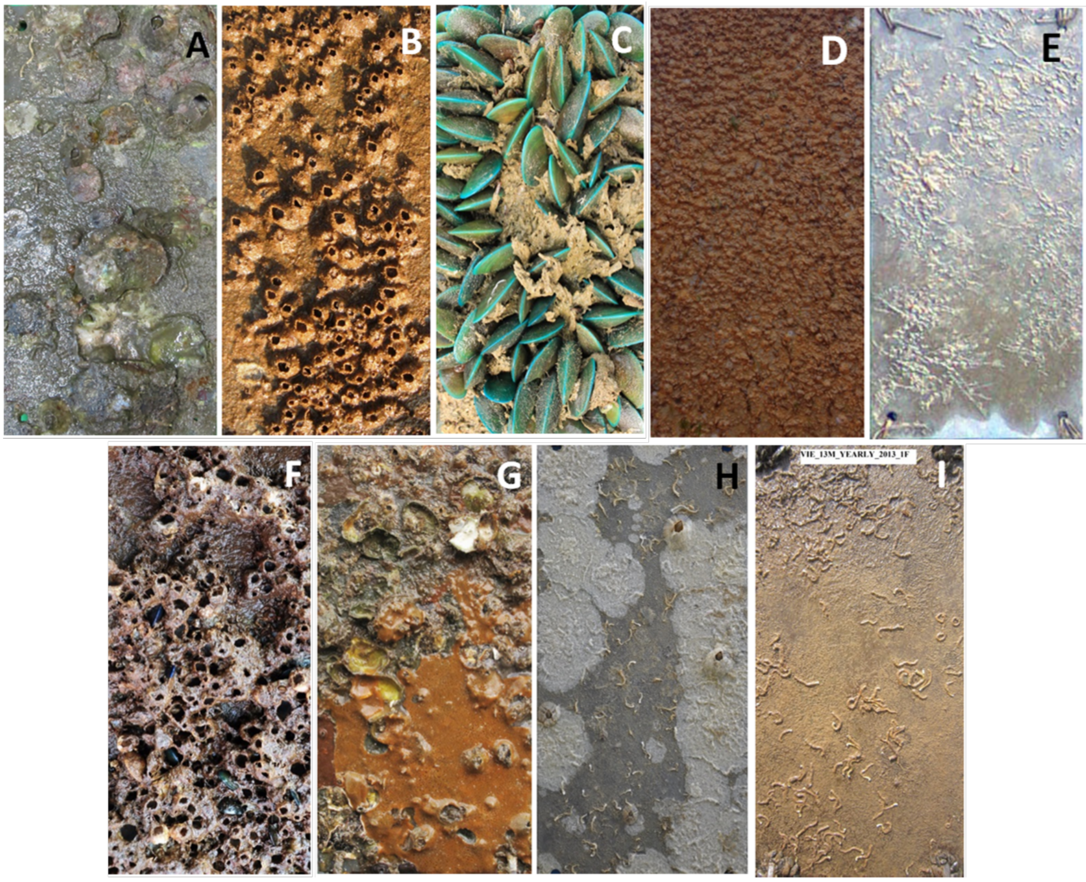

Figure 6. Long term fouling settlement at the end of immersion test at (A) Muara, Brunei; (B) Goa, India; (C) Tanjong Priok, Indonesia; (D) Kertih, Malaysia; (E) Yangon, Myanmar; (F) Manila Bay, Philippines; (G) Singapore; (H) Songkhla, Thailand; (I) Hai Phong, Vietnam.

ceans and molluscs, which are often found as sessile biofouling (e.g., barnacles and bivalves) are widely represented in many global invasive databases (Cohen and Carlton 1998; Eldredge and Carlton 2002; Molnar et al. 2008). To mitigate the transfer of alien invasive organisms by shipping, a knowledge of prevailing or baseline biofouling community in the environment (e.g. port area) facilitates detection of alien species which may pose a risk of infesting vessels while in port. Timely detection of biofouling pests will enable greater success of mitigation methods for vector management such as those outlined in Floerl et al. (2005), e.g. prevention of species exposure to vectors. This is also in line with the Biofouling Guidelines (Article 9.2), where port states are encouraged to communicate the presence of high risk species to in-coming vessels.

Figure 7. The Caribbean dreissenid bivalve *Mytilopsis sallei* at Songkhla Port.

The results of the inter-site study of biofouling settlement enabled each site to identify dominant, rapid colonising organisms (Figure 3). The monthly settlement showed that barnacles (*Amphibalanus* sp.) were common in all sites except Yangon (Myanmar) and Hai Phong (Vietnam). In particular, barnacle cover on immersion plates was almost complete in Kertih, Malaysia (Feb 2013) and Manila Bay, Philippines (May 2012). Serpulid tubeworms had high abundance (>80% cover) in Tanjong Priok, Indonesia (Nov and Dec 2013). Molluscs (i.e. oysters, mussels and other bivalves) were not common on the monthly plates, where the frequent changing (disturbance) of plates likely prevented these organisms from settling or detected.

The heavy monthly fouling settlement at Tanjong Priok (Indonesia), Kertih (Malaysia), Manila Bay (Philippines) and Songkhla (Thailand) was reflected in the long term cumulative fouling at these sites. These sites experienced rapid (almost) complete hard cover consisting of barnacles and molluscs (Figure 5C, D, F, H). Even though a fresh set of panels at Songkhla were replaced in Aug 2012, they were rapidly colonized by encrusting bryozoans. It is likely that Goa (India) and Singapore were experiencing a more gradual increase in panel cover (Figure 5B and G) unlike the other sites, where the panels were rapidly taken over by a dominant group in a single month. In particular, *P. viridis* at Tanjong Priok (Figure 6C) swiftly settled over the earlier barnacle cover from Sep to Oct 2013 (Figure 5C). Even though monthly images of the long term panels were not recorded at Muara (Brunei), monthly fouling at the site (Figure 3A) and the final image taken after a year (Figure 6A) suggested that the recruitment was also gradual. Panels at

Yangon (Myanmar) and Hai Phong (Vietnam) consisted largely of soft-bodied organisms like algae and annelid worms, although there was also ~10% cover of serpulid tubeworms at the end of the test in Hai Phong (Vietnam).

The identification of a dominant taxa (or species) can allow for greater chance of detection of high-risk species by port personnel. Timely port monitoring can further inform vessel owners of the risk of exposure to prevent introduction of these organisms to their vessels. For example, the presence of the invasive Caribbean bivalve, *Mytilopsis sallei* in Songkhla Port (Figure 7), Singapore (e.g., monsoon canals; Tan and Morton 2006) and Manila Bay South Harbor, should raise awareness of the potential risk of these mussels hitch-hiking on vessels. In addition, the recent records of another alien invasive mussel, *Mytella strigata* (as *charruana* d'Orbigny, 1846) in 2014 in Manila Bay (Vallejo et al. 2017), and 2016 in Singapore (Lim et al. 2018) pointed out the need for urgent regional attention towards mitigating species transfer.

4.2 Establishing functional networks and communication with port agencies

The ASEAN-India Cooperation Project on Extent of Transfer of Alien Invasive Organisms in South/South East Asia Region by Shipping was incepted in December 2010 with the goals of building capacity in invasive species awareness and robust scientific sampling methods for vessel ballast water and port biofouling organisms. The project aimed to create key partnerships and dialogue between regulatory agencies, shipping industry and personnel to foster functional networks and communication, while promoting regional cooperation in sampling, monitoring and communication of national strategies in invasive pest management. This is to align regional interests, identify limitations and foster development and integration of National Action Plans in South/South East Asia with global environmental instruments such as the 2004 International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWM Convention). The methods and results in this paper focused mainly on the study of biofouling diversity in ports located in South/South East Asia, where the risk of invasive species transfer by vessels in the SEA region (Kaluza et al. 2010; Seebens et al. 2013) remains a key concern, both as recipient and donor ports.

The biofouling study using immersion plates demonstrated that the selection of a secure and safe location will be advantageous for the safe inspection of plates, preventing loss of panels (or potential personnel injury) while accessing the plates with ease. This was the case, given that loss of panels were reported early in Kertih and Songkhla, as a result of strong sea conditions damaging ropes and frames securing the plates; while some of the sites had to be accessed by boat or treading in the water. This hindered timely inspection and maintenance of the frames or plates whenever necessary, but more importantly, may cause injurious accident while attempting to access the frames. The site should therefore be accessible safely and conveniently by personnel, cause no interference with vessel or port activity and be in an area where the biofouling diversity may likely be representative in the port (e.g., a location that is too shallow may not capture organisms (e.g., larvae) in the water column); however, this will most often require some baseline knowledge of the fouling onsite, which may not always be known without prior access. Therefore, coordination with port agencies will be key to facilitating a suitable site for any monitoring programs to be carried out. Nonetheless, all the sites benefited from communication exchanges leading up to the commencement and duration of the study, with all the sites acknowledging technical support from the port authorities, which will bode well for future phase-in programmes. Likewise, settlement data could be accurately captured if personnel undertaking the collection of the information have knowledge or recognition of biofouling organisms, which will be useful when inter-site comparison are made on a common exchange platform. General awareness and confidence in invasive pest monitoring, using a port baseline biofouling recruitment and direct training of port authorities and personnel, were raised in this program. These are key drivers to build a framework for functional processes in invasive pest monitoring and management (e.g., Piola and McDonald 2012).

4.3 Future steps – regional integration and action plans

The outcome of the study here highlighted the groundwork made to facilitate a baseline study of biofouling status at the respective sites, and allowed for the first time, a systematic way to record and share the information on a common platform such as the website set up by the National Institute of Oceanography, Goa (see below for link to website). Across regional sites, a long term monitoring program will greatly benefit from harmonization of methods for biofouling inspection, for example in the use of biofouling recruitment plates or traps, and collection of data when information are shared on a regional platform. Continued training are also beneficial for overall confidence in organism identification.

While these remain early stages in formulating a specific regional plan to manage invasive species transfer by shipping, one of the secondary outcomes was that sites were able to examine the physical environment and hydrology in the port areas, as well as gaining an understanding of the vessel traffic patterns within the region. These observations are critical to understanding the implications for invasive species, sedimentation, vessel movement, and threat of pest invasion to natural wildlife reserves nearby (e.g. Cat Ba Biosphere Reserve and Red River Delta Biosphere Reserves, Vietnam).

The baseline information on biofouling community can form part of a National/Regional Port Baseline Repository (http://bampi.nio.org/Final%20MainAsean/species.htm), collecting long term fouling records that aid in knowledge of seasonal recruitment for organisms (e.g. bivalves). These can support vector management policies and strong cooperation among regulatory bodies (e.g., ship owner and port agency) which are necessary when managing alien invasive organism transfer or incursion via shipping (Strayer 2009; Piola and McDonald 2012). While there are no formal global instruments currently aimed at biofouling control in shipping to manage invasive species transfer (unlike the BWM Convention), the Biofouling Guidelines (IMO) prescribe responsible practices in shipping operation to prevent invasive species transfer by biofouling. Several regional and national biosecurity doctrines are reviewed by Dahlstrom et al. (2010) and are similarly guided by the IMO principles (e.g. inspection of vessel biofouling and maintenance of hull cleanliness). The Ministry for Primary Industries (New Zealand) has also specifically crafted biofouling standards (e.g., Craft Risk Management Standard), on vessels to manage invasive species introduction. Port states in South/SEA can do well to adopt and align interests with these instruments. One example may be to mandate vessel inspection and cleaning when biofouling recruitment of certain species are known to be high (e.g. *Perna viridis*; Piola and McDonald 2012).

Risk assessments involving shipping network and volume have also continuously highlighted the potential threat faced by ports as a result of connectivity (e.g., Liu and Tsai 2011; Keller et al. 2011; Lo et al. 2012). With the support from port authorities, future studies in South/South East Asia may also utilize vessel movement patterns within/beyond the region, based on the eco-region concept (Spalding et al. 2007), as an aid to implement vector management plans to prevent risk of invasive organisms transfer (Lim et al. 2017).

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Supplementary Table 1. Definitions of biofouling taxonomic groups.

Fouling Type	Definition
Silt	Absorbed detritus and early stage biofilm/slimes
Slime	Thicker biofilms containing microalgae, cyanobacteria and low form algae
Incipient fouling	Recently settled, early/juvenile stage macrofouling
Algae (Green)	Fully established macroalgae
Algae (Red)	Fully established macroalgae
Cnidaria	Attached forms of hydrozoans (hydroids, etc.)
Encrusting bryozoans	Colonial animals forming an encrusting layer over the surface. eg. *Membranipora* sp
Arborescent bryozoans	Upright, bush-like colonies eg. *Bugula* sp
Barnacles	A hard shelled crustacean that cements itself permanently to a substrate, and is difficult to remove. The outer shell is generally white in color and shaped like a truncated cone
Polychaetes, calcareous	Calcerous tubeworms (Serpulidae, Spirorbidae)
Polychaetes, sedimentary	Worms that may form a soft sedimentary tube which becomes cemented to the substrate
Molluscs	Typical examples include oysters, mussels, vermitids
Sponges	Colonial organisms, often brightly colored. Maybe be encrusting or erect
Tunicates	Colonial or solitary form of sea squirts
Unknown hard	
Unknown soft	