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Research Article

Status of six non-native marine species in the coastal environment of Hong Kong, 30 years after their first record

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Abstract

This is the first systematic assessment conducted on fouling communities to determine the current status of six non-native marine invertebrates that were first recorded in Hong Kong three decades ago. They include the solitary ascidian, *Ciona intestinalis*; the slipper limpet, *Crepidula onyx*; the bryozoan, *Bugula californica*; the Caribbean bivalve, *Mytilopsis sallei*; the Mediterranean mussel, *Mytilus galloprovincialis*; and the boring isopod, *Sphaeroma walkeri*. Field surveys were conducted at 31 locations during wet (2011) and dry (2012) seasons and included fouling communities on piers and mariculture zones from estuarine to oceanic zones. The sampling was conducted by using photo-quadrats, destructive quadrats and field observations. To detect temporal changes in the abundance and recruitment of the sedentary non-native species, three piers were monitored with permanent photo-quadrats and recruitment panels for a one year period. We examined the relationship between seawater quality data available for Hong Kong and the abundances of fouling and non-native species. Only four of the six target non-native species were recorded in this survey. The isopod *Sphaeroma walkeri* was common and widely distributed in fouling communities in Hong Kong, while *Ciona intestinalis*, *Crepidula onyx*, and *Mytilopsis sallei* were uncommon and mainly restricted to areas with intensive human activities and poor seawater quality. These findings suggest that near shore human activities and poor water quality could increase the risk of establishment of non-native species in Hong Kong's marine fouling communities.

Key words: distribution, fouling communities, invasive, invertebrates, monitoring, non-native, seawater quality

Introduction

Non-native species represent a biodiversity, social, and economic threat (Kuris and Culver 1999; Grosholz et al. 2000). Prevention and early detection of new introductions are crucial to avoid negative effects of invasive species in marine environments (Leung and Dudgeon 2008). Monitoring the abundance and distribution of non-native species, especially those with invasion histories, is necessary to assess invasion risk in order to prioritize management and research efforts (Thomsen et al. 2008).

Studies of non-native species within Asia are sparse (Seo and Lee 2009); therefore, their numbers are likely underestimated. As one of the world's busiest ports for commercial transport, Hong Kong is constantly exposed to non-native species

through ballast water and hull fouling (Chu et al. 1997). A recent study of marine invasion risk through ballast water, based on global shipping routes, showed Hong Kong to be one of the harbours with the highest probabilities of biological invasion in the world (Seebens et al. 2013). Mariculture activities and live seafood trade are widespread in Hong Kong, which also increase the chances of new introductions (Leung and Dudgeon 2008). However, due to the lack of appropriate biodiversity assessments and ecological studies, the knowledge on non-native marine species in Hong Kong remains very limited.

In Hong Kong, six marine invertebrate species were described as being non-native between 1972 and 1981 (Morton 1987). These species are the solitary sea vase tunicate, *Ciona intestinalis* (Linnaeus, 1767); the slipper limpet, *Crepidula*

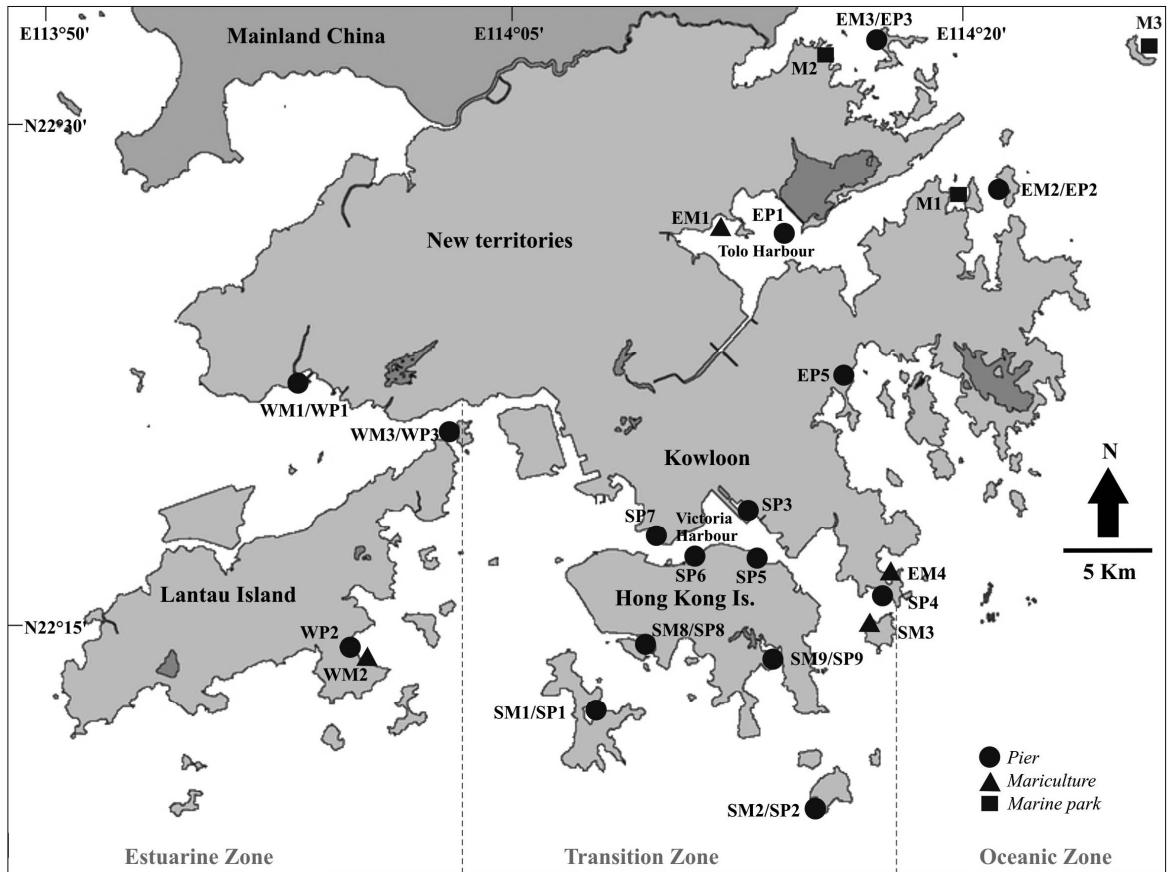


Figure 1. Map of sampling sites for marine non-native species on piers (circle), mariculture farms (triangle; i.e., fish and marina facilities) and piers within marine parks (square) in Hong Kong. See Table 1 for site names and their descriptions. Only circles are shown for sites where the pier and mariculture farm are in close proximity.

onyx G.B Sowerby I, 1824; the bryozoan, *Bugula californica* Robertson, 1905; the Caribbean bivalve, *Mytilopsis sallei* (Récluz, 1849); the Mediterranean mussel, *Mytilus galloprovincialis* Lamarck, 1819; and the isopod, *Sphaeroma walkeri* Stebbing 1905. The introduction of these species was related to an increase of ship traffic into Hong Kong in the 1970's. By the early 1980's, these species were established in Victoria Harbour, Tolo Harbour, and Tsing Yi (Morton 1987), where *Ciona intestinalis* could reach densities of 44 individuals m^{-2} (Mak 1983), *Crepidula onyx* of 994 individuals m^{-2} (Morton et al. 1984), *Mytilopsis sallei* of 2,392 individuals m^{-2} (Huang and Morton 1983), *Mytilus galloprovincialis* of 5 individuals m^{-2} (Morton 1987), and *Sphaeroma walkeri* of 12,521 individuals m^{-2} (Mak et al. 1985). Most of the surveys for these non-native species were conducted sporadically in isolated

sites around Victoria and Tolo Harbour (Morton 1987, 1989a). Subsequent surveys also found *Sphaeroma walkeri*, *Crepidula onyx*, *Mytilopsis sallei* and *Ciona intestinalis* occurring in other coastal areas in Hong Kong (Huang et al. 1992; Huang et al. 1999), indicating that these species may have extended their range in Hong Kong during the last few decades.

The marine environment of Hong Kong is roughly divided into an estuarine zone in the west, oceanic zone in the east, and a transitional zone between these two (Figure 1). Seasonal fluctuations between dry and wet seasons strongly affect seawater characteristics such as temperature and salinity, which can fluctuate between 15 and 31°C, and between 11 and 34, respectively (EPD 2010). The shoreline is characterized by high human development and disturbances that include land reclamation, typhoon shelters, piers, mariculture

Table 1. Summary of sampling sites in Hong Kong and the presence of the non-native species *Ciona intestinalis* (*Ci*), *Mytilopsis sallei* (*Ms*), *Crepidula onyx* (*Co*) and *Sphaeroma walkeri* (*Sw*) found in this study using photo-quadrats (P), destructive quadrats (D) and field observation (O) as sampling methods.

Codes	Zone	Facility	Site names	Season	Presence of species			
					<i>Ci</i>	<i>Ms</i>	<i>Co</i>	<i>Sw</i>
WM1	E	Fish cage	Tuen Mun	W,D		D		D
WP1	E	Pier	Tuen Mun	W,D		D		D
WM2	E	Mariculture	Cheung Sha Wan	W,D				DO
WP2	E	Pier	Chi Ma Wan	W,D			O*	D
WM3	E	Mariculture	Ma Wan	W,D				D
WP3	E	Pier	Ma Wan	W,D		D	D	D
SM1	T	Mariculture	Sok Kwu Wan	W,D				DO
SP1	T	Pier	Sok Kwu Wan	W,D	O*			DO
SM2	T	Mariculture	Po Toi Island	W,D				D
SP2	T	Pier	Po Toi Island	W,D				D
SM3	T	Mariculture	Joss House Bay	W,D				DO
SP3	T	Pier	Kwun Tong	W,D	PD	PDO	D	PDO
EM1	O	Mariculture	San Mun Tsai	W,D				PDO
EP1	O	Pier	San Mun Tsai	W,D				D
EM2	O	Mariculture	Grass Island	W,D				D
EP2	O	Pier	Grass Island	W,D				D
EM3	O	Mariculture	Kat O	W,D				D
EP3	O	Pier	Kat O	W,D				D
M1	O	Pier, Marine Park	Hoi Ha Wan	W,D				
M2	O	Pier, Marine Park	Lai Chi Wo	W,D				D
M3	O	Pier, Marine Park	Tung Ping Chau	W,D	O*			D
Extra sites								
SM8	T	Marina	Aberdeen	D	PDO			
SM9	T	Fish cage	Tai Tam	D	PDO			
SP4	T	Pier	Joss House Bay	W,D	O*	D	D	DO*
SP5	T	Pier	Shau Kei Wan	D	D			DO
SP6	T	Pier	North Point	D	PDO			D
SP7	T	Pier	Tsim Sha Tsui	D			PDO	D
SP8	T	Pier	Aberdeen	D	P O			D
SP9	T	Pier	Tai Tam	D	DO			D
EM4	O	Mariculture	Po Toi O	D				D
EP5	O	Pier	Sai Kung	D				D

Zone: E= Estuarine (west), T= Transition (south), O= Oceanic (east).

Season: W= Wet season, D= Dry season.

* indicates that a species was observed in the field but not in the samples or the facility.

zones, marinas, sewage facilities, etc. (Morton 1989b). The prevalence of non-native species in semi-closed and disturbed systems, such as estuaries and bays, is well documented (Preisler et al. 2009). These systems are subjected to a range of human activities that could result in species introductions owing to high propagule supply of non-native species through shipping and mariculture activities (Ruiz et al. 1997). Many disturbed systems also have high artificial substrate availability for the development of fouling communities, which are considered an entry gate (i.e., predation refuge) for non-native species (Dumont et al. 2011b). Environmental stress, produced by anthropogenic and natural sources,

could alter seawater quality (e.g. oxygen levels, pollution, temperature and salinity) and give an advantage to non-native species that are generally more tolerant to abiotic stress than native species (Dethier and Hacker 2005; Piola and Johnston 2008; Lenz et al. 2011). Fouling communities in Hong Kong waters may have promoted the establishment and proliferation of non-native species. The current knowledge of these species in Hong Kong, however, is restricted to few ecological studies (Morton 1989a; Zhao 2002; Zhao and Qian 2002; Mackie et al. 2006), while the species' distributions and impacts are still unknown due to the lack of a systematic assessment.

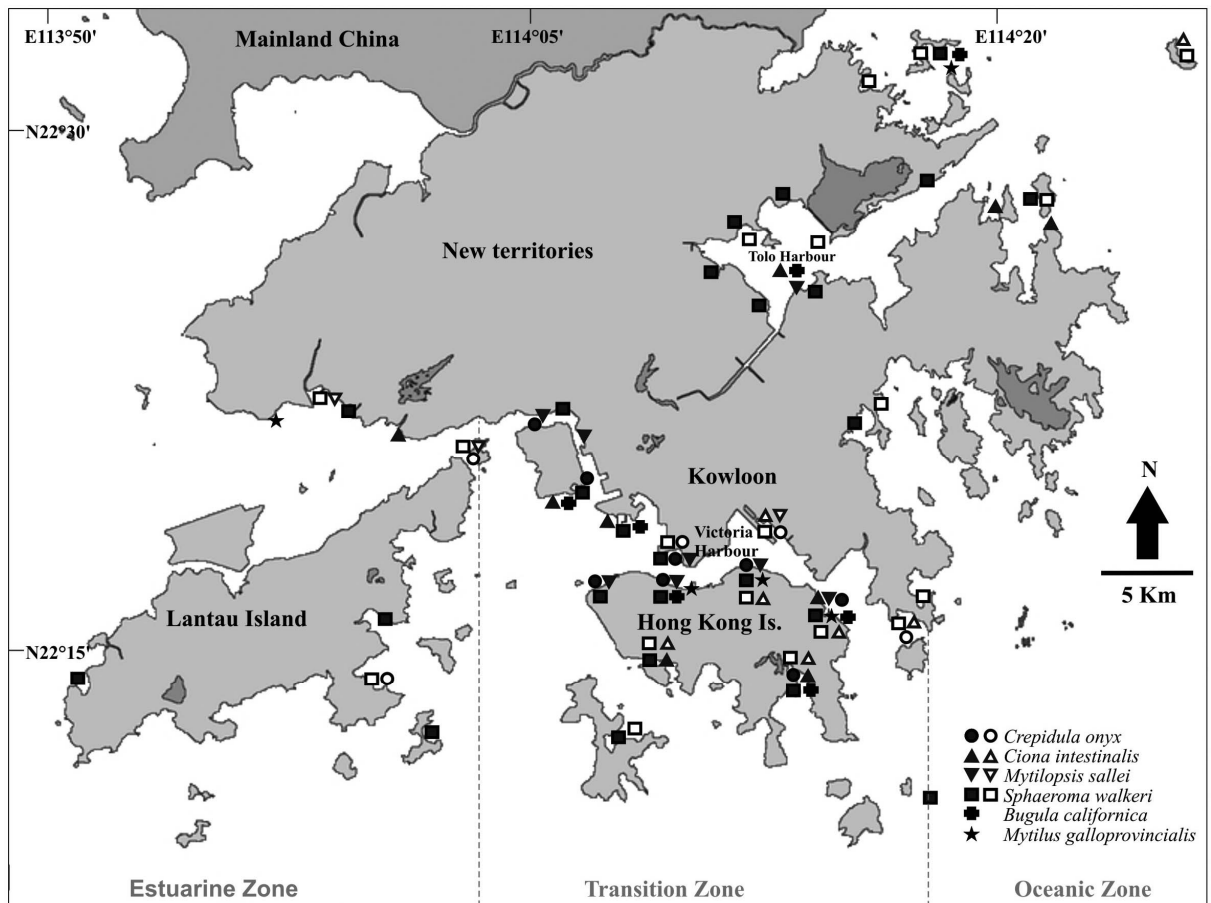


Figure 2. Map of the distribution of the marine non-native species recorded in the literature (black symbols) and in the present study (white symbols) in Hong Kong.

The objectives of this study were to assess the distribution and abundance of the six previously reported non-native species in marine fouling communities in Hong Kong waters and to investigate their relationship with seawater quality variables. The surveys were conducted in both dry and wet seasons, covering fouling communities from estuaries to oceanic zones, and in sites that extended into marine parks. We also examined the relationship between the abundance of non-native species and fouling communities with seawater quality measures obtained from the Marine Water Quality Programme operated by Environmental Protection Department of the Government of the Hong Kong Special Administrative Region (SAR).

Methods

Study sites

Thirty-one sites in Hong Kong that included piers, mariculture facilities (i.e., open-sea net cages and floating rafts for culturing fish), and marina facilities were sampled within estuarine, transitional, and oceanic zones (Figure 1, Table 1). Several of these sites were close to the areas where the six non-native species were reported previously (Figures 1, 2). The substrates sampled varied within and between sites and included concrete, metal, wood, plastic, and styrofoam surfaces (Table S1). Out of the 31 sites, three of the sampled piers, Chi Ma Wan (WP2), Sok Kwu

Wan (SP1) and Joss House Bay (SP4), were selected for regular monitoring of the target species for a 1-year period. These three sites were located in small bays with mariculture facilities.

Spatial and temporal sampling of non-native species and fouling communities

Fouling communities were sampled in the 31 sites (Table 1) to determine the distribution and abundance of six target non-native species: *Ciona intestinalis*, *Crepidula onyx*, *Bugula californica*, *Mytilopsis sallei*, *Mytilus galloprovincialis*, and *Sphaeroma walkeri*. Twenty-one of these sites (nine open access piers, three piers inside marine parks, and nine mariculture facilities) were sampled during both wet (2011) and dry (2012) seasons, while the 10 additional sites were surveyed only during the dry season (two mariculture facilities, one marina pontoon and seven open access piers). The surveys were conducted in both seasons due to the strong temperature and salinity fluctuations in the seawater (EPD 2010).

Twenty photo-quadrats (15 × 15 cm) and five destructive quadrats (15 × 15 cm) were randomly taken at intertidal, shallow subtidal (1–3 m), and deep subtidal (3–6 m) depths by SCUBA divers. The whole community (all layers) within each of the destructive quadrats was scraped using a metal scraper and saved in plastic bags. In the laboratory, the samples were transferred to, and stored in, plastic containers with 70% alcohol until examination. All piers were sampled at both intertidal and subtidal depths, while some sites could not be sampled at deep subtidal depth due to bad sea conditions or to a lack of water that deep (see Table S2). Floating facilities, such as open-sea-cage farms in mariculture zones and pontoon facilities, were sampled only at shallow subtidal depth, due to a lack of substrates for larval settlement at intertidal and deep subtidal depths. After sampling the quadrats, each site was visually explored for at least 15 minutes by SCUBA divers to check for the target non-native species.

We analysed each photo-quadrat using 50 random points with the software Coral Point Count with Excel extensions (CPCe) (Kohler and Gill 2006) to obtain the percentage cover of the main sedentary (including sessile) taxa (i.e., bryozoans, barnacles, bivalves, tunicates, polychaetes, sponges, and macroalgae) and the target sedentary non-native species on the superficial layer of fouling communities. For sorting and examination, we carefully washed the samples over a sieve (1

mm) and sorted them in a dissection tray. The total wet biomass of the fouling community, and the number and wet weight of the target non-native species (including *Sphaeroma walkeri*) were recorded. Wet biomass was measured after two minutes of air drying over a sieve.

Environmental characteristics and fouling communities

To examine how fouling communities and non-native species are affected by environmental variables, we obtained data of 24 seawater quality variables from the Marine Water Quality Programme operated by the Environmental Protection Department of Hong Kong SAR Government (Table S3; EPD 2010). The average of each variable (last five years until 2010) was calculated for the month and site that each fouling community was sampled.

Temporal changes and recruitment

We performed regular surveys in Chi Ma Wan (WP2), Sok Kwu Wan (SP1), and Joss House Bay (SP4) piers to determine whether the presence and abundance of the target non-native species changed throughout a year. To reduce disturbance on the community, only non-destructive photo-quadrats were used. Ten permanent, randomly-positioned, photo-quadrats (15×15 cm) for intertidal, shallow subtidal and deep subtidal depths (quadrat positions were marked using ropes and tags) were surveyed on the pier columns. Photographs of the quadrats were taken every second month for a 1-year period. Photo-quadrats were analysed using the software CPCe to yield the percentage cover of the target species.

At the same three sites selected above, the recruitment pattern of the six target species was examined monthly over a 1-year period. Polyvinyl chloride (PVC) panels (15 × 15 cm) attached vertically (orientation similar to the columns) to a suspended frame (2–3 meters deep) were used as artificial recruitment substrate. Each panel was roughened with sandpaper before submersion. Six panels per site were collected monthly after 4 weeks of submersion, examined in the laboratory under a dissecting microscope, and the number of individuals or colonies of the six target species was recorded for recruits greater than 2 mm in length. In the laboratory, photographs were taken of each panel in order to analyze the percentage cover of the species present based on 50 random points using the CPCe software.

Data analyses

Statistical analyses were performed separately for each depth sampled, facility, and season. Spatial and seasonal patterns of the fouling communities were analyzed using multivariate non-parametric statistics incorporated in the software PRIMER v6 and PERMANOVA+ for PRIMER (Clarke and Gorley 2006; Anderson et al. 2008). Permutational ANOVAs (9999 permutations) were performed to compare the percentage cover of main sedentary taxa (from photoquadrats) and to compare total biomass (destructive quadrats) of the fouling communities: (a) among oceanic zones (fixed, 3 levels) and sites (random, 3 levels nested within oceanic zone) for each artificial facility (pier and mariculture); and (b) between protection status (fixed, 2 levels – open access vs. marine park piers) and sites (random, 3 levels nested within protection). Multivariate analyses on the fouling communities were based on square-root transformed abundance data and Bray-Curtis similarity matrix. In these analyses the deep subtidal depth was excluded due to a lack of sites.

We used non-metric multidimensional scaling (nMDS) to visualize the similarity among fouling community assemblages (percentage cover of the main sedentary taxa only for shallow subtidal depth). The Linktree procedure was used to determine those taxa that could explain the main differences in the cluster ordination of the nMDS (Clarke and Gorley 2006), whereas the BVStep procedure was used to determine those taxa that explain 95% of the pattern in the nMDS (Clarke and Warwick 2001). Principal Components Analyses (PCAs) were used to determine the distance among sites according to their seawater quality variables, and such analyses were based on fourth-root transformed seawater quality data and Euclidean distance resemblance matrix according to the criteria of Clarke and Ainsworth (1993). Seawater variables were reduced to two components from the PCA, which explained more than 74% of the overall variation (Table S3). To interpret the PCA results, the seawater quality was described following the Environmental Protection Department criteria, which considers eastern seawaters (including marine parks) as good quality, and western (and some sites in Victoria Harbour as Kwun Tong typhoon shelter) as poor quality (EPD 2010). These analyses were performed separately for both facilities and seasons. BIO-ENV routine (Clarke and Ainsworth 1993) was used to correlate fouling community assemblages with seawater variables. This routine

used Spearman rank correlation method to correlate Bray-Curtis similarity matrix of the community with the Euclidean matrix of seawater variables. Then, it was also used to identify the best set of seawater variables that explained the correlation. The global BEST match permutation test (99 permutations by default) was used to determine the significance of each correlation.

Correlations between abundance of sedentary non-native species and seawater quality variables were not possible due to the low presence and abundance of the non-native species in the sampling sites. For the common non-native isopod *Sphaeroma walkeri*, however, the Spearman's rank order correlation could be performed to determine the relationship between the estimated abundance in the shallow subtidal depth (individuals m⁻²) and the distance of seawater quality variables per site (distance were obtained from PC1 of the seawater variables PCA) in both seasons.

Results

Composition of fouling communities

The composition of the main taxa (coverage) and biomass of fouling communities (by depth) in Hong Kong were not significantly different among coastal zones and between piers located inside and outside of marine parks (Tables S4, S5) in both seasons. However, fouling communities were strongly influenced by the location (site) of the pier and mariculture facilities (Tables S4, S5).

The biomass of fouling communities on piers and mariculture facilities varied between sites, with the highest wet biomass found on the intertidal zone in Chi Ma Wan pier (WP2) in the dry season (30,252 g m⁻², Table S2), while the lowest biomass was on the shallow subtidal zone in Po Toi pier (SP2) in the wet season (1,692 g m⁻², Table S2).

Distribution and abundance of non-native species

Only four of the six non-native species previously reported for Hong Kong were encountered in this study; *Mytilus galloprovincialis* and *Bugula californica* were not detected (Table 1). The sedentary species were not common in fouling communities on piers and mariculture structures in either season. However, the mobile isopod *Sphaeroma walkeri* was very common and abundant on most of the fouling communities sampled in both seasons (Table 1, Figure 2).

Table 2. Percentage of sites in which the non-native species *Ciona intestinalis* (Ci), *Mytilopsis sallei* (Ms), *Crepidula onyx* (Co) and *Sphaeroma walkeri* (Sw) were recorded using photoquadrats (PQ), destructives quadrats (DQ) and field observations (FO) as sampling methods in each sampling period.

Sampling methods	Total number of sites	Detection of species (%)			
		<i>Ci</i>	<i>Ms</i>	<i>Co</i>	<i>Sw</i>
Wet season					
PQ	22	0,0	4,5	0,0	13,6
DQ	22	4,5	9,1	4,5	90,9
FO	22	4,5	4,5	0,0	22,7
Dry season					
PQ	31	16,1	0,0	3,2	3,2
DQ	31	19,4	9,7	9,7	87,1
FO	31	19,4	3,2	3,2	19,4

Photo-quadrats recorded *Ciona intestinalis* in five sites and the largest percentage cover was 14.8% in the Aberdeen marina (SM8) during the dry season. *Mytilopsis sallei* was only detected in the intertidal zone of Kwun Tong pier (SP3) with a cover of 5.8% (wet season), while *Crepidula onyx* appeared only in the intertidal of Tsim Sha Tsui pier (SP7) with a cover of 1.2% (dry season). Although, the isopod *Sphaeroma walkeri* was recorded in the photo-quadrats (<1% cover), it was not considered in this instance because it is a mobile animal. Photo-quadrats did not record non-native species on mariculture facilities (Table 1).

Destructive quadrats detected more non-native species than the other methods across all sites (Table 2). *Ciona intestinalis* was found in Kwun Tong (SP3), Shau Kei Wan (SP5), North Point (SP6), Aberdeen (SM8) and Tai Tam (SM9 and SP9), with a maximum estimated abundance of 116 individuals m⁻² on Kwun Tong pier during the dry season (Table S2). The bivalve *Mytilopsis sallei* was abundant on Kwun Tong pier (SP3) with an abundance of 14,400 individuals m⁻² and wet biomass of 3,372 g m⁻². A few individuals also appeared in Tuen Mun (WM1 and WP1), Ma Wan (WP3) and Joss House Bay pier (SP4, Table S2). *Crepidula onyx* was collected in Ma Wan (WP3), Kwun Tong (SP3), Joss House Bay (SP4), and Tsim Sha Tsui (SP7). The isopod *Sphaeroma walkeri* was the most commonly found non-native species in Hong Kong, being present on nearly every pier and mariculture zone sampled (except in Hoi Ha Wan, M1), and it was the most abundant non-native organism in the mariculture zone in Tolo Harbour (EM1: 14,400 individuals m⁻², Table S2).

Some individuals of *Ciona intestinalis* were also observed in benthic rock crevices in Tung Ping Chau (close to M3) and inside of a mesh cage (mesh size about 5 cm) found suspended under Joss House Bay pier (SP4) during the wet season. Some small individuals of *Crepidula onyx* were also observed on the rocks under Chi Ma Wan (WP2) pier in the wet season but not on the pier itself.

Environmental variables and fouling communities

The assemblages (coverage) of the main sedentary taxa for fouling communities on piers in both seasons had > 50% similarity (Figures 3A and B). Some sites had above 70% similarity, but the order did not match with the coastal zone. This supports the results of the Permanova tests, which indicated that the difference among communities was affected by the sites instead of the coastal zones (Tables S4 and S5). The results of Linktree analyses suggested that the main cluster of the sites in the wet season (at 70% similarity, Figure 3A) had a higher coverage of macroalgae (>15.4 vs. <0 coverage) and barnacles (>21.7 vs. <10.7 coverage) when compared with the rest of the clusters, which could explain the differences between groups in wet season. In contrast, a higher coverage of macroalgae (>6.4 vs. <2.5 coverage) and lower coverage of sponges (<1.7 vs. >3.2 coverage) in the main cluster of sites could explain the differences in the dry season (Figure 3B). The results of BVStep routine analysis further indicated that 95% of the similarity among communities on piers in the wet season was explained by the presence of bryozoan, bivalves and macroalgae, whereas in the dry season the similarity was

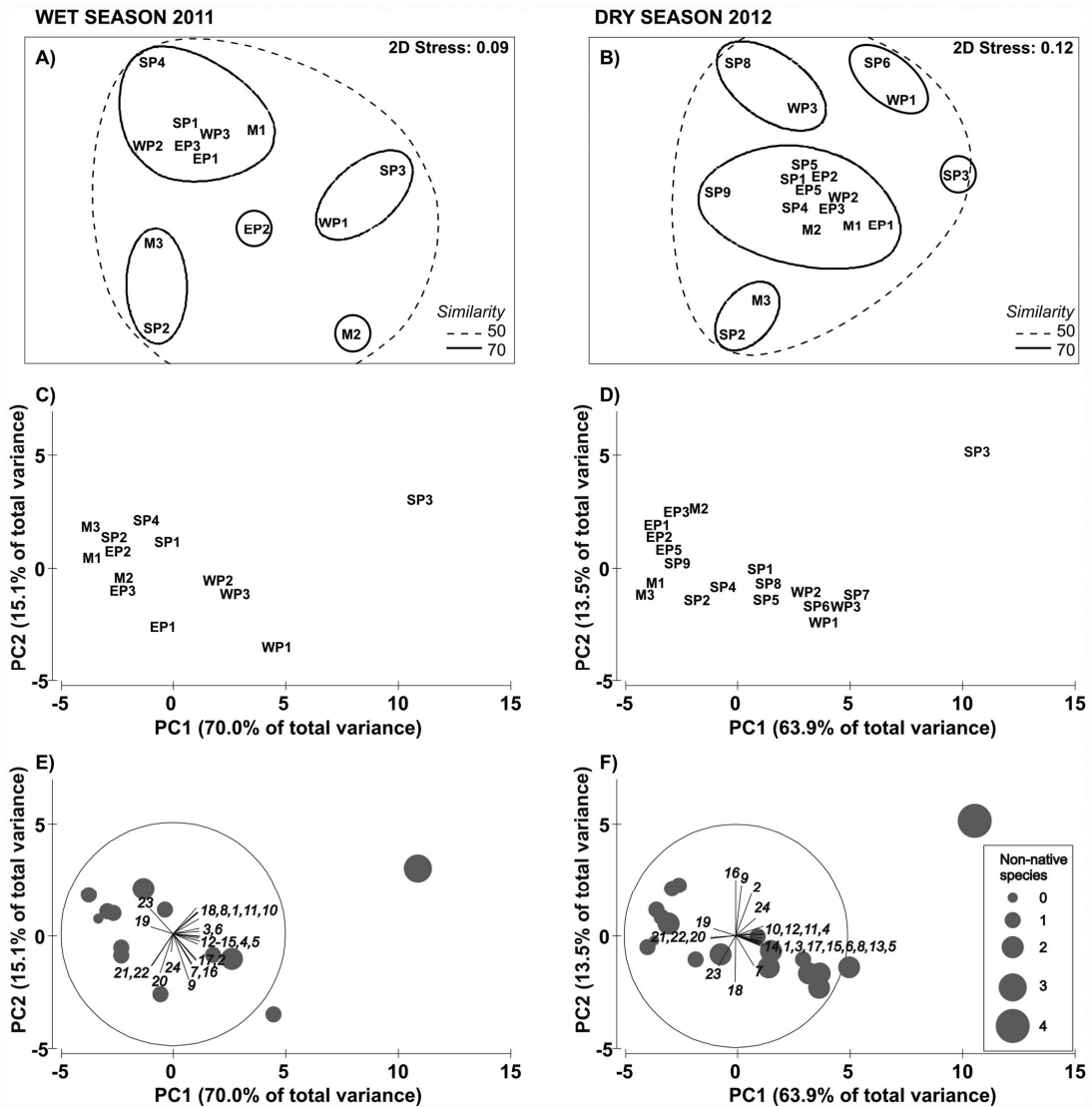


Figure 3. MDS and PCA ordinations of pier sites (including extra sites) for wet and dry season, based on: A and B) MDS for main sedentary taxa cover in shallow subtidal communities; C and D) PCA for average of the last 5 years of 24 seawater quality variables for the respective month in which each site was sampled; E and F) PCA to indicate the contribution of each seawater quality variable (eigenvectors and numbers) and the number of total non-native species found with destructive quadrats at each site. See Table 1 and 2 for site and seawater variable codes.

explained by the presence of polychaetes and tunicates.

PCAs for seawater variables indicated a gradient of seawater quality from eastern and marine parks sites to southern and western sites (Figures 3C and D). Two dimensional PCAs showed a good description of the seawater variables; in all cases the PC1 and PC2 explained more than 74% of the variance (Table S3). Most of the seawater measures contributed to PC1, with a higher

number of eigenvectors increasing from left to right, toward the western and some southern sites (Figures 3E and F). The low eigenvalues indicated that PC1 could not be explained by a single variable (Table S3). The gradient pattern coincides with the Hong Kong Environmental Protection Department criteria, which indicates that seawater quality decreases in western sites (and in Victoria Harbour). Kwun Tong pier (SP3) was the most dissimilar site for taxa and seawater quality (as it

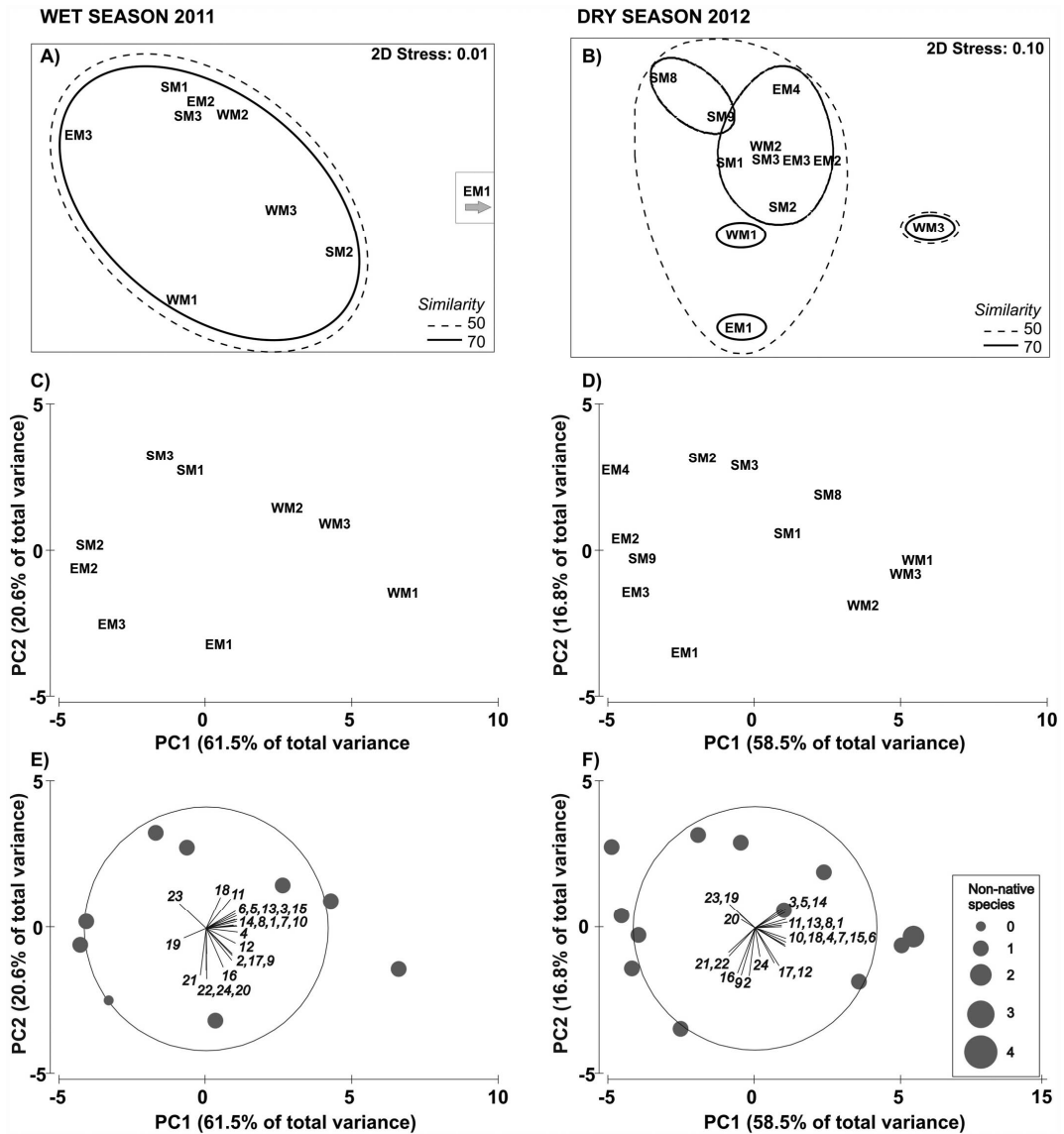


Figure 4. MDS and PCA ordinations of the mariculture sites for wet (left) and dry season (right), based on: A & B) MDS for main sedentary taxa cover in shallow subtidal communities; C and D) PCA for average of the last 5 years of 24 seawater quality variables for the respective month in which each site was sampled; E and F) PCA to indicate the contribution of each seawater quality variable (eigenvectors and numbers) and the number of total non-native species found with destructive quadrats at each site. See Figure 3 for more details. See Table 1 and 2 for site and seawater variable codes.

had the poorest seawater quality), and it was the only site where all four non-native species recorded were found (Figure 3E and F).

Bio-Env routines indicated that the correlation between fouling community assemblages on piers and seawater variables was not significant during the wet season ($r = 0.319$; $n = 13$; $P = 0.430$), but was significant in the dry season ($r = 0.601$; $n = 18$; $P = 0.030$). The best match result indicated

that salinity, faecal coliforms, chlorophyll-*a*, and total Kjeldahl nitrogen were the best predictors for the dry season.

The assemblages of main taxa for fouling communities on mariculture facilities during wet season shared 70% similarity, except for San Mun Tsai (EM1, Figure 4A). In dry season most of the communities shared 50% similarity, whereas at 70% similarity some sites clustered together but

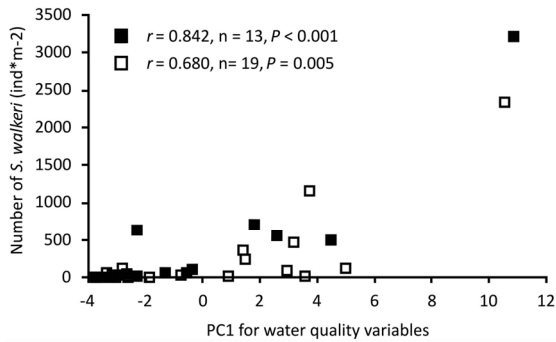


Figure 5. Scatter plot of the first PC axis score of the PCAs from the seawater quality for sampling sites and the average abundance of the non-native isopod *Sphaeroma walkeri* in piers at wet season 2011 (black square) and dry season 2012 (white square). PC1 broadly represents an axis from higher (left) to lower seawater quality (right). PC1 explains 70.0% of the total variance for wet season and 63.9% of the total variance for dry season.

did not match with the coastal zones (Figure 4B). The results of Linktree analysis indicated that in the wet season San Mun Tsai (at 50% and 70% similarity) had a higher coverage of bivalves (>34.9% vs. <14.8%) and barnacles (>31.6% vs. <18.0%), and a lower coverage of bryozoans (<0.0% vs. >19.0%) and tubicolous polychaetes (<4.1% vs. >5.5%) when compared with the other sites. In the dry season, Ma Wan (at 50% similarity) differed by a low coverage of bryozoans (<0.7% vs. >16.6%), whereas at 70% similarity the main clusters of the sites had a higher coverage of barnacles (>25.6% vs. <10.5%) and a lower coverage of bryozoans (<16.6% vs. 23.5%) when compared with the other sites. The results of BVStep routine analysis indicated that 95% of the pattern of the nMDS for mariculture sites in the wet season could be explained by the presence of sponges, bivalves, bryozoans, and tunicates, and in the dry season the pattern could be explained by the presence of bryozoans, tunicates, sponges, tubicolous polychaetes, and macroalgae.

In mariculture sites, PCAs also indicated a seawater quality gradient from marine parks and eastern sites to western sites (Figures 4C and D). Seawater variables contributed mostly to PC1 with a clear loading toward western sites (Figures 4E and F). Non-native species were less commonly found on mariculture facilities, where only one site in the west had two non-native species (WM1: Tuen Mun).

The results of Bio-Env routines indicated that the correlations between mariculture fouling

assemblages and seawater quality measures were not statistically significant in both wet ($r = 0.483$; $n = 9$; $P = 0.470$) and dry ($r = 0.577$; $n = 12$; $P = 0.130$) seasons. However, the abundance of the non-native isopod *Sphaeroma walkeri* on piers was significantly correlated with the decrease of seawater quality for both wet ($r = 0.842$; $n = 13$; $P < 0.001$) and dry ($r = 0.680$; $n = 19$; $P = 0.001$) seasons (Figure 5), while the abundance of isopods on mariculture facilities was not significantly correlated with seawater quality in wet ($r = 0.617$; $n = 9$; $P = 0.077$) and dry ($r = 0.214$; $n = 12$; $P = 0.505$) seasons.

Temporal changes and recruitment monitoring of non-native species

The non-native species were not recorded in the permanent quadrats in any of the three piers through the one year period. However, the bryozoa *Bugula neritina*, and the tubeworm *Hydroides elegans* were widespread and abundant. On the monthly recruitment panels, only one juvenile individual of the tunicate *Ciona intestinalis* was found in March 2012 in Joss House Bay Pier (SP4). Tubeworms and encrusting bryozoans were the most common and abundant organisms recruiting on the panels during the year.

Discussion

Distribution and abundance of non-native species

The distribution of the four non-native invertebrates described in this assessment (*Ciona intestinalis*, *Crepidula onyx*, *Mytilopsis sallei* and *Sphaeroma walkeri*) suggests that they have not expanded their distributions since the 1980's. The highest number and abundance of non-native species are still in the transitional zone, especially in semi-enclosed and protected areas around Victoria Harbour and Hong Kong Island (Morton 1987). It has been proposed that the low salinity in the estuarine zone (during wet season) and the strong wave actions in the oceanic zone (especially during the monsoon) could limit their distribution and/or abundance (Mak et al. 1985; Morton 1987; Zhao 2002). Semi-enclosed or protected systems with limited circulation could reduce gamete and larvae dispersal, which might facilitate the establishment of a local population from a small number of individuals (Preisler et al. 2009).

The sedentary, non-native species, *Ciona intestinalis*, *Mytilopsis sallei* and *Crepidula onyx*, were commonly found on fouling communities in

Victoria Harbour in the 1970's and 1980's (Hon 1978; Morton et al. 1984), which differs from the present finding. Seawater in Hong Kong varies according to the season and location; temperature (14 to 28°C between dry and wet season) and salinity (34 to 21 between dry and wet season in the estuarine zone) fluctuations were evident in our sampling sites. The solitary ascidian *Ciona intestinalis*, native to North Atlantic and Mediterranean Sea, is widely introduced in temperate regions but rarely found in subtropical or tropical regions (Carver et al. 2006; Zhan et al. 2010; Dias et al. 2013). It can tolerate wide temperature (-1 and 30°C) and salinity (12 and 40) ranges, but the combination of high temperature (above 21°C) and low salinity (below 18) may become stressful and affecting their survival (Carver et al. 2006; Vercaemer et al. 2011), which could explain the higher presence in the dry season in this survey.

The slipper limpet *Crepidula onyx* is native to the Pacific coast of North America and has been introduced to the Atlantic coast of North America and also to Asia (Arakawa 1980; Tseng and Huang 1987; Choe and Park 1992; Huang et al. 1992; Holm 2006). *Crepidula onyx* has been found in Victoria Harbour throughout the wet season, which indicates that it can cope with seawater conditions of Hong Kong (Morton et al. 1984), but it is affected negatively by salinity lower than 15 (Zhao 2002).

The bivalve *Mytilopsis sallei* is a tropical estuarine species, native to the Caribbean and Central America, and it has been introduced to several locations into the Indo-Pacific region and in the Mediterranean Sea (Morton 1981; Puyana 1995; Tan and Morton 2006; Galil and Bogi 2009; Wong et al. 2011). *Mytilopsis sallei* can tolerate high temperatures (18-30°C) and wide salinity (2-40) variations (Raju et al. 1975; Morton 1981; Morton 1989a; Puyana 1995; Tan and Morton 2006). Although *Mytilopsis sallei* has been recorded recruiting in both seasons in Hong Kong (Morton 1989a), its distribution is restricted and does not constitute the threat that it poses in other locations within its introduced distribution, such as India, Singapore or China (Morton 1981; Lin and Yang 2006; Tan and Morton 2006).

The marine isopod *Sphaeroma walkeri*, suggested to be native to the northern Indian Ocean, is widely distributed from tropical to warm-temperate regions (Carlton and Iverson 1981; Harrison and Holdich 1984; Mak et al. 1985; Sing and Sasekumar 1994; Galil 2011; Cai and Teo 2012). Although the South China Sea could

represent the northern limit of its natural distribution in the Pacific region, it is considered introduced to Hong Kong due to its absence in previous surveys on fouling communities (Mak et al. 1985). *Sphaeroma walkeri* has been well established in Hong Kong since the 1980's from estuarine to oceanic zones, and it is still more abundant in the transitional zone, especially in protected and enclosed bays (Mak et al. 1985). The abundance of the isopod *Sphaeroma walkeri* on piers seems to increase when the seawater quality decreases. Recent studies have demonstrated an increase in the abundance of non-native species with abiotic stressors such as pollution (Dafforn et al. 2009; Crooks et al. 2011; McKenzie et al. 2011). Non-native species directly benefit from the environmental conditions of these degraded habitats since they could be more tolerant to environmental stresses than native species (Piola and Johnston 2009; Groner et al. 2011; Lenz et al. 2011). They could also benefit indirectly due to changes in the food chain, competition, and predation that are affected by the degradation of their environment (Adams 2005). The fact that this relationship with water quality was not found with mariculture facilities in the current study could be due to the regular maintenance of these facilities by fish farmers (i.e., cleaning the submerged structures on a regular basis).

The species *Bugula californica* and *Mytilus galloprovincialis* were not found in the present study. *Bugula californica* seems to be native to the Pacific coast of America from British Columbia, Canada, to the Galapagos Islands, and introduced to Japan, Korea and China (Morton 1987; Tseng and Huang 1987; Lamb and Hanby 2005). This species is widely distributed in East China Sea and it has been also recorded in the South China Sea. Since the 1980's there have been no additional records of *Bugula californica* in Hong Kong (Hon 1978; Mak 1983; Huang and Li 1986), but it has been recorded in Hainan (Zheng et al. 1984), which suggests that it could be present in Hong Kong and subtropical coast of China. The mussel, *Mytilus galloprovincialis*, native to the Mediterranean Sea has a temperate distribution, invading the north and south hemisphere (Hilbish et al. 2000; Mead et al. 2011). In Asia it has been recorded in Japan, China and Hong Kong (Arakawa 1980; Lee and Morton 1985; Li et al. 2011). *Mytilus galloprovincialis*, like its congener, *M. edulis*, is abundant and restricted to the northern-temperate coast of China (Tseng and Huang 1987). In the 1980's, *Mytilus galloprovincialis* was found in several locations from estuarine to

oceanic zones in Hong Kong (Lee and Morton 1985; Morton 1987). Its low density in piers suggested that those individuals were members of a pioneer population (Lee and Morton 1985). The finding of this survey indicates that *Mytilus galloprovincialis* probably failed to establish in Hong Kong.

Temporal changes and recruitment monitoring of non-natives species

The absence of non-native species in permanent quadrats and recruitment panels (except one individual of *Ciona intestinalis* found in Joss House Bay) during the one-year study period indicates that these species are not common on these fouling communities, which could be due to a low propagule supply and lack of available bare space in the established community (Clark and Johnston 2009). The appearance of a few individuals of *Ciona intestinalis* inside a mesh cage (field observations) under one of the piers could suggest that the non-native species recruited but failed to establish in the recruitment panels. Previous studies have shown that predation could restrict the establishment of *Ciona intestinalis* in similar type of panels (Dumont et al. 2011a; Dumont et al. 2011b).

Composition of fouling communities

The assemblages of the main sedentary taxa and biomass of the fouling communities were influenced by the location of the sampling sites. Studies have demonstrated that factors, such as shading, depth, surface composition, substrate orientation, type of habitat and biotic interactions can influence the assemblage of the fouling communities (Glasby 1999; Glasby 2000; Glasby and Connell 2001; Dumont et al. 2011b). Considering that most of the fouling species are suspension-feeders (Astudillo et al. 2009); the water circulation and the suspended food availability in each site should also influence the assemblage composition. In the present study, there is a clear seawater-quality gradient from eastern and marine parks to western sites (and some in Victoria harbour). The Kwun Tong typhoon shelter located beside residential and industrial areas presents one of the poorest seawater-quality sites (EPD 2010), which could explain the dissimilarity of the fouling assemblage and also the presence of the non-native species. Previous surveys in fouling communities in Hong Kong, reported that barnacles, bryozoans, and bivalves are the most dominant

taxa (Huang and Lin 1990; Huang et al. 1992). Some common species in local fouling communities include the bryozoans, *Bugula neritina* and *Sinoflustra amoyensis*; the bivalves, *Perna viridis*, *Brachidontes variabilis*, *Barbatia virescens*, *Irus irus*, *Neotrapezium sublaevigatum*; and the tunicates, *Styela plicata* and *Styela canopus* (Huang and Lin 1990; Huang et al. 1992; Huang et al. 1999). About 340 species have been reported in fouling communities for Hong Kong (Huang 2003), however, the origin of those species is still unclear.

Conclusions and further research

The sampling method plays a decisive role in understanding the distribution and abundance of non-native species. Photo-quadrats allow a higher number of replicates and sites, but it is useful only for superficial layers which underestimates the abundance of small animals covered by others (e.g. *Mytilopsis sallei*). Destructive quadrats include all the layers of the fouling community, which increases the detection of non-native species and also gives more information of the community and species (e.g. biomass, number of individuals, etc.). However, the sampling and analysis of the samples require more effort, which limits the number of replicates and sites. Field observations may provide non-objective data, but they are useful to validate the findings obtained from the photo- and destructive-quadrats (after preservation).

Since Hong Kong is one of the busiest harbours in the world and it is under constant human disturbance, we expected non-native species to be abundant and widely distributed. Nonetheless, their distribution has remained similar to the distribution reported in 1980's (Morton 1987). The only non-native species well established and abundant was the isopod *Sphaeroma walkeri*, however, there is no information available regarding its ecology and impact on marine benthic communities. The non-native bryozoan *Bugula californica* and mussel *Mytilus galloprovincialis* were absent in this study, suggesting that they failed to establish. Our results also suggest that the distribution and abundance of non-native species in Hong Kong could increase with low seawater quality. Further studies are needed to establish causality between environmental and/or biotic factors on the establishment of non-native species.

Though the current study found that the distribution and abundance of the known non-native species were limited, the number of non-

native species in Hong Kong has been clearly underestimated. The introductions of the non-native species reported in this survey were related with the increase of trade ships entering to Hong Kong in late 1970's (Morton 1987). A recent study based in ballast water and global ship routes predicts that Hong Kong has the highest risk of biological invasion in its marine environments (Seebens et al. 2013). Other international harbours in the world present much higher numbers of non-native species, which can easily exceed a hundred (Carlton and Geller 1993; Ruiz et al. 2000; Mead et al. 2011). For example in South Africa until 2009, 22 species were considered as non-native, but in 2011 after a re-assessment a total of 86 introductions and 36 cryptogenic species were recorded (Mead et al. 2011). These studies in tandem indicate that an assessment of the origin of the species recorded for Hong Kong is necessary to determine the status of non-native species in the marine environment of Hong Kong.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Dates, surface observed, and coordinates of facility locations sampled in this study.

Table S2. Number of individuals (ind m⁻² ± SE) and biomass (g m⁻² ± SE) of fouling communities and non-native species per site and depth for dry and wet season in coastal marine environments of Hong Kong.

Table S3. Principal component (PC) analysis loadings of seawater characteristics, eigenvalues and the percentage of variance it explains in parentheses.

Table S4. Results of permutational ANOVAs of the main taxa percentage cover of the fouling communities in Hong Kong: A) Comparison among oceanic zones and B) Comparison between piers in marine parks and non-marine parks (protection status). The analyses were done separately for each facility, depth and season.

Table S5. Results of permutational ANOVAs of the total biomass of fouling communities in Hong Kong: A) Comparison among oceanic zones and B) Comparison between piers in marine parks and non-marine parks (protection status). The analyses were done separately for each facility, depth and season.

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