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The Ecology of an Incipient Marine Biological Invasion: The Charru Mussel *Mytella charruana* d'Orbignyi, 1846 (Bivalvia: Mytilidae) in Manila Bay, Luzon, Philippines

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The study documents the initial colonization ecology of the Western Hemisphere's non-indigenous mytilid *Mytella charruana* in the Port of Manila, Manila Bay. As part of a monitoring effort to document fouling communities using PICES collectors, a recruitment pulse of *Mytella charruana* was detected in Jul 2014. The recruits have persisted and established in the port. Also noted was the possible recruitment competition with other indigenous and non-indigenous bivalve species. *Mytella* recruits during the onset of the southwest monsoon rainy season. Based on Canonical Correspondence Analysis of recruit abundances with water quality parameters, *Mytella*, the green mussel *Perna viridis*, *Musculista*, and *Brachidontes* have a lower salinity niche and recruits on *Amphibalanus* and *Hydroides* biogenic substrates. Also examined was the possible competition between *Mytella* and *Perna viridis*, since these species have been used for mariculture. *Perna* is traditionally cultured in Manila Bay, while *Mytella* is proposed as a new species for mariculture in the Philippines. Based on the results and its physiological ecology, *Mytella* is likely to have a competitive advantage over *Perna* in estuaries like Manila Bay.

Key words: estuaries, fouling communities, invasive species, mariculture, marine non-indigenous species, mussels

INTRODUCTION

The establishment of marine non-indigenous species (MNIS) to new locations and habitats is one of the most serious consequences of global anthropogenic environment change which may impact on food security. For ecologists and biogeographers, the introduction of MNIS provides an unprecedented opportunity to directly study the role of environmental factors in structuring the ecological community and subsequent range expansion (Carlton 1996; Betancur-R et al. 2011).

Understanding how a non-indigenous marine species becomes established and affects the existing ecological community is an important question in invasion biology. While a non-indigenous species may become established, it does not always follow that these species become invasive. How and when MNIS becomes invasive and modifies the recipient ecological community is a question of particular interest in the search for a possible biological

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control or environmental management strategy.

The researchers adopt the following definitions for when a MNIS becomes an invasive species (Chavanich et al. 2010) according to the International Oceanographic Commission sub Commission for the Western Pacific (WESTPAC). Invasive species are non-indigenous species whose introduction is due to human activities and:

1) its spread threatens the environment, economy or society,

2) is likely to cause economic or environmental harm or harm to human health, and

3) its establishment in natural or semi natural ecosystems is an agent of change and threatens biological diversity.

The definition excludes natural introductions, colonization, and distribution range expansions that are considered – in the broadest ecological sense – biological invasions.

There is strong evidence to show that species interactions play an important role in the structuring of natural communities. However, in the case of MNIS introductions, the initial effects may be indirect making it difficult to directly measure the net effects on the community. At the initial stage of invasion, effects may range from a decline in species abundance (due to direct competition or predation) to an increase in species abundance (facilitated by the invasion). These effects are usually a consequence of physical changes in the habitat. In many cases of biological invasion in aquatic habitats, the invaded ecosystem is stressed and subject to chronic anthropogenic stressors. Ship harbours and ports are an example. Two thirds of harbours in the world are located in anthropologically stressed estuaries resulting from urbanization of the watershed (Sylvester et al. 2013).

The Port of Manila on Manila Bay is the Philippines' biggest port. Manila's international ports – North and South Harbors – have recorded a combined total of 4,793 foreign ship calls in 2009, representing 73,000 tons and an estimated 13,000 hours of port service calls (PPA 2010). Manila Bay is almost completely surrounded by highly urbanized and rapidly urbanizing communities. It has many environmental issues ranging from land- and sea-based pollution (Prudente et al. 1994; Prudente et al. 1997; Sta. Maria et al. 2009), sedimentation, harmful algal blooms (Azanza et al. 2004), overexploitation of fishery resources (Munoz 1993), reclamation, land conversion and most recently, biological invasion (Chavanich et al. 2010; Jacinto et al. 2006b). Manila Bay is also used for mariculture and fisheries, and is the main shipping port of the country (PPA 2010). Thus, the risk of MNIS biological invasion is high.

Among the previously documented mollusc MNIS from Manila Bay are *Mytilopsis sallei, M. adamsi, Brachidontes exustus, B. pharaonis*, and *Crassostrea gigas* (Ocampo et al. 2014). *C. gigas* was introduced for mariculture but was never established as a viable population. There is no evidence to show that the listed MNIS are invasive since they occur at low abundances.

Rice and colleagues (2016) state that *Mytella charruana* was first recorded from Manila Bay in January 2014 near the towns of Kawit and Binakayan in Cavite Province, around 20 km from the port of Manila. Rice and colleagues (2016) also noted the presence of *Mytella charruana* from Tambac Bay in Lingayen Gulf, Pangasinan Province, 230 km north of Manila in 2015. *Mytella* has colonized many of the estuaries feeding the gulf.

Historically, Manila Bay has been a very biotically rich area that supported a productive fishery (Munoz 1993). Some remaining habitats, though much modified by reclamation, still support high levels of coastal biodiversity (Ocampo et al. 2015). The introduction of MNIS is likely to affect the biodiversity of these remaining habitats. Are environmental conditions in Manila Bay favorable to MNIS establishment and invasion? And if it were, what are the possible effects on the existing ecological community?

These questions are part of the continuing research and monitoring program of the Manila Bay environment with the Manila Ocean Park. The researchers have been monitoring the recruitment of fouling organisms since 2011, and have identified several non-indigenous bivalve species that have persisted although at very low abundances. A previous question was why some of these species, which are documented invasive in other Southeast Asian harbors, are not invasive in Manila Bay. To determine possible factors, water quality in the harbour have been intensively monitored as part of oceanarium operations as it draws water from the harbor. In Jul 2014, the researchers detected a recruitment pulse of a new species of blue mussel, which was genetically ascertained as *Mytella charruana* (Vallejo et al. 2017). The species was previously reported from Lingayen Gulf (Rice 2016; Rice et al. 2016). It gave the researchers a rare opportunity to capture the initial establishment of a potential invasive MNIS in the Port of Manila and study this in a systematic manner.

To help shed insight to these questions, the researchers described the initial establishment of new bivalve *Mytella charruana* in the fouling community of the South Harbor of the Port of Manila. Also determined was the possible competition dynamics of *Mytella* with indigenous mussel species and previously reported and established non-indigenous mussel species in Manila Bay. Further investigation covered the possible competitive dynamics of *Mytella* with the indigenous commercially maricultured green mussel *Perna viridis* (Rosell 1991), based on previous observations that marine foulers recruit onto *Amphibalanus* and *Hydroides* biogenic substrates.

Since the detection of an incipient biological invasion was within study monitoring and settlement detection for fouling organisms, the researchers were not able to report the size frequencies of *Mytella* at each sampling period but only their counts. Nonetheless, the researchers went to assess the invasion potential of the newly established *Mytella* based on information known about the life history and physiological ecology of this species.

MATERIALS AND METHODS

Sampling

The researchers used fouler collectors designed by the North Pacific Marine Science Organization (PICES) for ports in temperate East Asia, including Japan, China, and Korea (Fig. 1). These collectors were tested for use in ports in South East Asia as part of the International Oceanographic Commission sub-commission for the Western Pacific (IOC-WESTPAC) training programs on rapid assessment of marine non-indigenous species. The collectors were modified for use in tropical estuarine environments shown in Figure 1. Each collector has four recruitment plastic petri dishes each.

Seventeen of these collectors were deployed in the Port of Manila, South Harbor marina, around the Manila Ocean Park (MOP-SH) at 14°34'43.06"N, 120°58'18.69"E. Following the sampling strategy of Ocampo and colleagues (2014), these were tied to the jetties at five points in the MOP-SH deck for 16 months from Jan 2014 to Apr 2015 (Fig. 1).

For each of the 15 collectors, two of the dishes were collected from each collector at every bimonthly sampling schedule. The Oct 2014 and Dec 2014 fouler sampling periods were cancelled due to inclement weather, though water quality parameters were sampled. The remaining two plates for each sampler for this location were not replaced and served as temporal control plates. The control plates allowed for documentation and observation of sessile invertebrate community succession and establishment (Fig. 4). All benthic organisms were identified to the species level if possible, fixed in 10% formalin, and estimated for abundance in each plate. The remaining two samplers were sampled at 6-12 month intervals to determine ecological succession in the fouling community and as a temporal control.

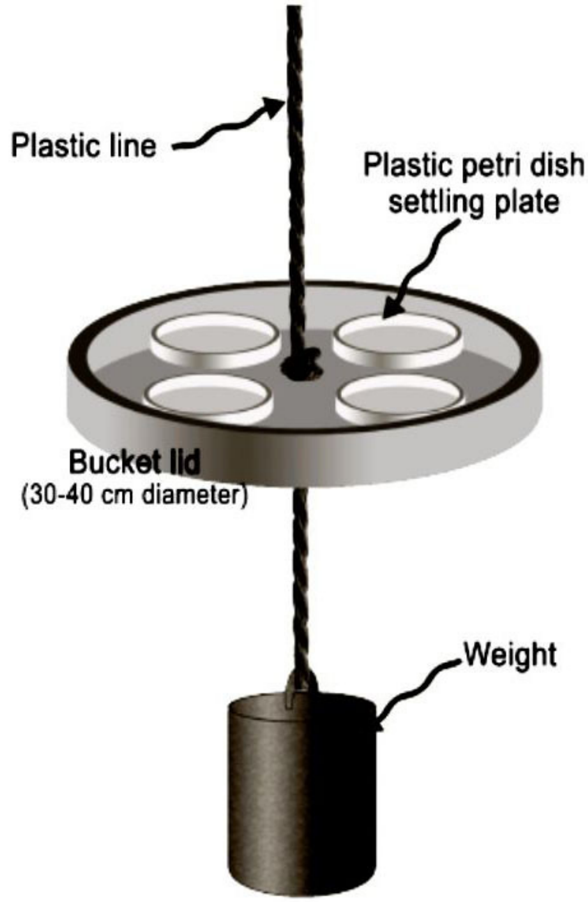

Figure 1. Design based on PICES fouling species recruitment collectors, modified for use in tropical environments and tested for use in harbors in Southeast Asia.

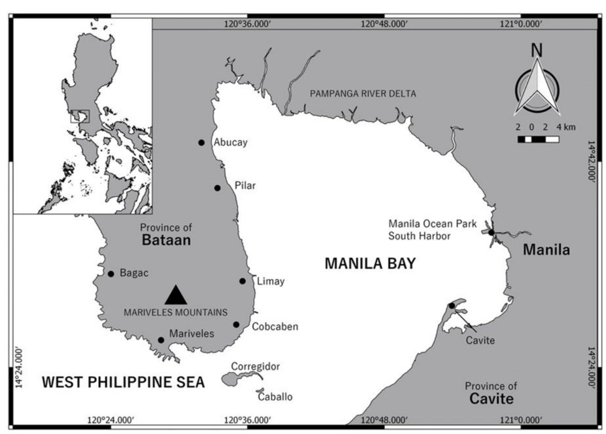

Figure 2. The study site in Manila Bay.

Physical and chemical water quality data for the sampling dates (i.e., salinity, temperature, NO_2-N, NH_4-N, NO_3-N, pH) of the sampling sites were obtained from the regular environmental monitoring activities of the curatorial department of the Manila Ocean Park.

Ecological community analyses

Specimens collected from the plates were identified in situ to the species level whenever possible based on Manila Bay identification keys (Ocampo et al. 2014; Ocampo et al. 2015). When further verification was needed, specimens collected were identified at the curatorial laboratory of Manila Ocean Park, the Institute of Environmental Science and Meteorology at the University of the Philippines Diliman, and at the University of the Philippines Los Baños. Mantle tissue samples were sent to the Institute of Biology at the University of the Philippines Diliman for DNA barcoding.

The frequencies and counts of each identified species over the year were estimated (Table 1). To determine initial patterns of MNIS establishment, the ecological community ordinations used were principal component analysis and detrended correspondence analysis. To relate the environmental variables with the frequencies of species counts at each sampling date, a canonical correspondence analysis (CCA) was used (Ter Braak et al. 1995; Ter Braak 1986). CCA aims to describe patterns of community variation along an environmental gradient and is a method of direct ordination (Ter Braak et al. 1995; Ter Braak 1986)). Furthermore, CCA is a technique that selects the linear combination of environmental variables that maximizes the species scores. Monte Carlo tests were used to determine the statistical significance of the environmental matrix in the CCA.

To determine if the abundances of *Amphibalanus* and *Hydroides* are significant in predicting the abundances of the mussel species, a MANOVA was done with the regressors as *Amphibalanus* and *Hydroides* and the nine mussel species as regressands.

All statistical ecological analyses was done using PAST v. 3.0 (Hammer et al. 2001) and XlStat ecological package (Adinsoft 2010).

RESULTS

The range of temperatures recorded at the collection sites were from 25.9° C recorded during the cool northeast monsoon season in January and 31.3° C recorded during the May monsoon break and dry season. Salinities ranged from 21.8° C in August during the southwest monsoon rainy season to 31.4° C in May during the monsoon break. The average levels of standard water quality variables in the collection sites were as follows: pH (8.0), NH_4-N (0.297 mg/l), NO_2-N (0.439 mg/l), and NO_3-N (1.81 mg/l). Trends are shown in Figure 3.

Eight species of fouling mussels were recorded from collectors. Six species are non-indigenous to Manila Bay.

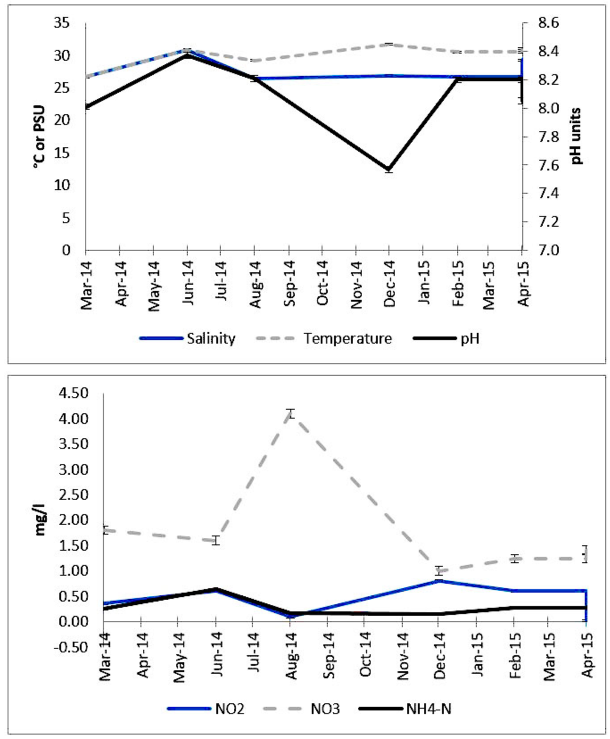

Figure 3. Trends in water quality parameters during the study period.

These are *Brachidontes exustus, B. pharoanis, Mytilopsis sallei, M. adamsi*, and *Mytella charruana*. The identity of *M. charruana* was confirmed by DNA barcoding by comparing Manila Bay sample DNA sequences from GenBank (Vallejo et al. 2017). The indigenous species are *Perna viridis, Modiolus metcalfei*, *and Musculista senhousia*. The indigenous and non-indigenous species are found with and attached to the barnacle *Amphibalanus amphitrite* and the tubeworm *Hydroides* sp. The barnacle and tubeworm form a habitat and recruitment matrix for the fouling mussels. Essentially, the mussel species are nestlers or species that require a biogenic recruitment substrate in order to establish themselves (Morton & Tan 2006). *Musculista* is a mudflat species but has been recorded on experimental fouling plates.

This habit is confirmed for *Modiolus, Musculista*, and *Mytella charruana*, which are significantly correlated with *Amphibalanus* abundance. *Perna viridis* is significantly correlated with *Hydroides* abundance. In the overall MANOVA model, *Mytella* abundances are marginally significantly predicted by *Amphibalanus* abundances. *Perna* abundances are significantly predicted by both biogenic substrate species. MANOVA results suggest that the variation of counts of the fouling bivalve community is a result of variations in *Amphibalanus* and *Hydroides* abundances, with *Hydroides* accounting for most of the variation in counts as evidenced by the Wilks Lambda statistic (Table 5).

Table 1. Summary statistics of abundance of mussel species and the *Amphibalanus* and *Hydroides* habitat matrix (n=162 plates).

	Amphibalanus	*Hydroides*	*Modiolus metcalfei*	*Mytilopsis sallei*	*Mytilopsis adamsi*	*Musculista*	*Brachidontes exustus*	*Mytella charruana*	*Brachidontes pharaonis*	*Perna viridis*
Total (N)	14388	876	2297	10	31	537	5	772	2	155
Mean (n)	88.81	5.4	14.17	0.061	0.19	3.32	0.03	4.76	0.0123	0.95
SE	5.91	1.6	2.7	0.027	0.16	0.609	0.0136	1.42	0.0087	0.344
Var	5665.35	417.02	1183.41	0.12	4.528	60.117	0.03	329.36	0.012	19.258
SD	75.26	20.42	34.4	0.346	2.127	7.753	0.1734	18.1483	0.11	4.388

The first recruits of *M. charruana* were first observed in the Jul 2014 sampling period. The abundance of *Mytella* peaked in Aug 2015 (Fig. 4) during the period of lowest salinity. This is the same sampling period when *Perna* and *Musculista* also peaked in abundances.

With the exception of *Mytella*, all other non-indigenous species have been reported in past studies in Manila Bay. *Mytella* was detected only in Jul 2014 and as of last sampling (Apr 2015) is still recruiting as 1-2 mm spats. *Mytilopsis* and *Brachidontes*, as reported in previous studies, remain in very low abundances. The high temporal variability in recruits suggests a recruitment pulse.

The total number of counts of each species is shown in Table 1.

Monte Carlo tests (N=1000 iterations) suggests that the CCA was significant (p=0.001) and that the first three axes can account for the variation in environmental parameters. CCA results suggest that NO_3, pH, and temperature are negatively loaded on the first axis, while salinity, NH_4-N, and NO_2 are positively loaded on this axis (Table 4). *Modiolus, Mytilopsis sallei,* and *M. adamsi* are positively loaded on the first axis, while *Perna* and *Mytella* are on the second axis. *Mytella charruana* is strongly positively loaded on the third axis. Degrees of variation influence by axis are as follows: first (48.64%), second (34.27%), and third (15.4%).

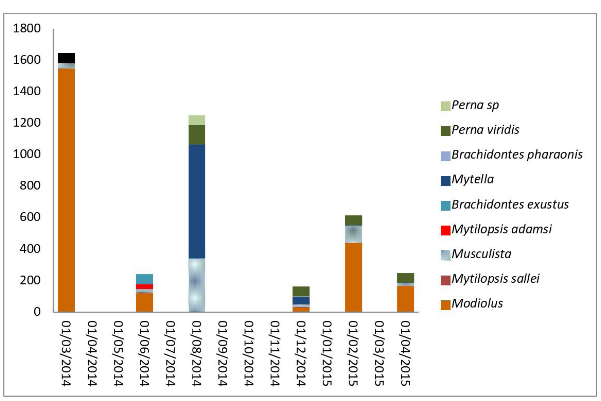

Figure 4. Abundance trends in the mussel species in this study.

Table 2. Loadings of the species on the first 3 CCA axes and the percentage variation accounted for by each axis.

	CCA Axes		
	F1	**F2**	**F3**
Amphibalanus	0.033	0.187	-0.086
Hydroides	-0.145	-1.590	-0.651
Modiolus	0.770	-0.432	0.533
Mytilopsis sallei	0.272	0.539	-0.618
Musculista	-1.027	-0.211	0.309
Mytilopsis adamsi	0.183	0.736	-1.094
Brachidontes exustus	-1.037	-0.927	-0.253
Mytella charruana	-1.746	-0.122	0.562
Brachidontes pharaonis	-0.022	0.738	-1.038
Perna viridis	-1.439	-0.818	0.224

Axis	Eigenvalue	%
1	0.24372	48.64
2	0.17323	34.57
3	0.077183	15.4

Table 3. Loadings of environmental factors on the CCA axes.

	F1	**F2**	**F3**
Salinity	0.149	0.107	-0.666
Temperature	-0.284	0.212	-0.902
NO_2	0.490	0.627	-0.534
NO_3	-0.835	-0.133	0.467
NH_4-N	0.295	0.257	-0.484
pH	-0.202	0.005	0.006

The CCA ordinations suggest that *Mytella*, *Perna*, and *Modiolus* have separate ecological niches with *Perna, Mytella, Musculista*, and *Brachidontes* able to tolerate lower pH, salinity, and higher NO_3. *Modiolus* prefers a higher salinity and higher NH_4-N and NO_2 levels. It is not surprising that *Musculista* – which is negatively loaded on the 1st axis and which has been observed in Hong Kong as a major fouling species – does well there in conditions of high turbidity and low salinity (Fong 2000).

Table 4. MANOVA results showing the biogenic recruitment substrates *Amphibalanus* and *Hydroides* species and how they affect fouling bivalve abundances on recruitment plates. Significant correlations are in bold font.

MANOVA						
Tests on independent variables						
		Wilks lambda	F	df1	df2	p
Amphibalanus		**0.891**	**2.052**	**9**	**151**	**0.03735**
Hydroides		**0.715**	**6.687**	**9**	**151**	**4.96E-08**
Tests on dependent variables						
		R^2	F	df1	df2	p
Modiolus		**0.04579**	**3.815**	**2**	**159**	**0.02407**
Mytilopsis sallei		0.001339	0.1066	2	159	0.899
Musculista		**0.08379**	**7.27**	**2**	**159**	**0.000952**
Mytilopsis adamsi		0.001364	0.1086	2	159	0.8972
Brachidontes exustus		0.165	15.71	2	159	5.96E-07
Mytella charruana		0.03106	2.548	2	159	0.08139
Brachidontes pharaonis		0.0008247	0.06562	2	159	0.9365
Perna viridis		**0.05363**	**4.505**	**2**	**159**	**0.0125**
Modiolus sp1		**0.04096**	**3.396**	**2**	**159**	**0.03597**
Regression coefficients and statistics						
		Coeff.	Std.err.	t	p	R^2
Modiolus	Constant	5.2764	4.2038	1.2551	0.21127	
	Amphibalanus	**0.09145**	**0.03544**	**2.5802**	**0.01077**	**0.03847**
	Hydroides	0.14429	0.13064	1.1045	0.27105	0.00584
Mytilopsis sallei	Constant	0.076214	0.043377	1.757	0.08084	
	Amphibalanus	-0.00014	0.00036	-0.3847	0.70094	0.000872
	Hydroides	-0.00037	0.00134	-0.2727	0.78543	0.000409
Musculista	Constant	1.0973	0.92844	1.1819	0.239	
	Amphibalanus	**0.01974**	**0.00782**	**2.5223**	**0.01264**	**0.03284**
	Hydroides	**0.08578**	**0.02885**	**2.9733**	**0.00340**	**0.04713**
Mytilopsis adamsi	Constant	0.10442	0.26603	0.3925	0.69521	
	Amphibalanus	0.00102	0.002243	0.45462	0.65001	0.001322
	Hydroides	-0.00067	0.008267	-0.08104	0.93551	0.000056
Brachidontes exustus	Constant	0.018847	0.019832	0.95034	0.34338	
	Amphibalanus	-0.07305	0.000167	-0.43881	0.66139	0.002546
	Hydroides	0.003428	0.000616	5.5613	1.11E-07	0.16396
Mytella charruana	Constant	0.87734	2.2348	0.39258	0.69515	
	Amphibalanus	**0.04032**	**0.01884**	**2.1399**	**0.03388**	**0.02698**
	Hydroides	0.05677	0.06944	0.8175	0.41485	0.00315
Brachidontes pharaonis	Constant	0.014447	0.013851	1.0431	0.2985	
	Amphibalanus	-0.00145	0.000117	-0.12512	0.90059	0.00075
	Hydroides	-0.00015	0.00043	-0.34539	0.73026	0.000726
Perna viridis	Constant	-0.02143	0.53407	-0.04012	0.96805	
	Amphibalanus	**0.00858**	**0.00450**	**1.9065**	**0.05839**	**0.01923**
	Hydroides	**0.0399**	**0.01659**	**2.404**	**0.01736**	**0.03199**
Modiolus sp1	Constant	-0.02749	0.02714	-1.013	0.31262	
	Amphibalanus	**0.00059**	**0.00022**	**2.5948**	**0.01035**	**0.04087**
	Hydroides	**-0.0001**	**0.000843**	**-0.12093**	**0.9039**	**0.00035**

Mytilopsis appears to be more positively correlated with the salinity and NH_4-N and NO_2 environmental variables than the other mussel species. *Mytilopsis* has a wide range of salinity tolerance but becomes easily established in turbid estuaries with high levels of nutrients (Morton 1981, 1989). At the Hong Kong Harbour, the increase in its numbers were observed during high salinity periods but at the Singapore Harbour, it became established at brackish water salinities (Tan & Morton 2006).

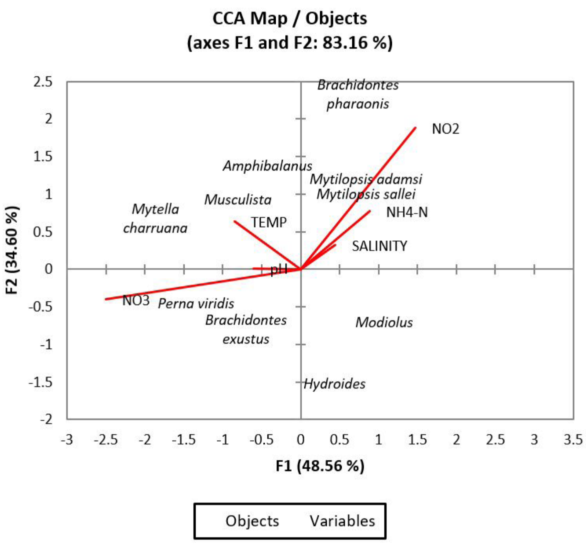

Figure 5. CCA biplot of the abundances of the bivalve species and two biogenic substrate fouling species and environmental factors.

DISCUSSION

Possible invasion vectors and effects to Manila Bay mariculture

The major introduction vectors for *Mytella* are likely via ballast water or by translocation for mariculture (Spinuzzi et al. 2013). As in the case of the previously reported non-indigenous bivalves *Mytilopsis* and *Brachidontes* (Chavanich et al. 2010, Ocampo et al. 2014), these species have likely been introduced by ballast water or from foulers on ship hulls (Rice et al. 2016). These species have been reported only from areas closest to and at the Manila South Harbor and North Harbor. Also, these species have not been recorded in high abundances in previous studies on invasive species in Manila Bay. Their persistence in the harbor marin – as observed by ocean park staff and the Philippine Coast Guard – is likely due to repeated introductions via ballast water. Mature gonadal individuals have not been recorded from the PICES collectors.

In contrast, *Mytella* may have been introduced by translocation as part of mariculture activities. There is no report of *Mytella* mariculture in Manila Bay. However, there are reports that the species has been farmed in Lingayen Gulf, 250 km north of Manila Harbor (Rinoza 2015; Rice 2016; Rice et al. 2016). In Manila Bay and Lingayen Gulf, the traditional bivalve aquaculture is centered on the green mussel *Perna viridis*, slipper oyster *Crassostrea iredalei*, and to a lesser extent on the brown horse mussel *Modiolus*. *Perna viridis* and *Crassostrea iredalei* are important mariculture species in coastal Philippines.

Perna and *Crassostrea* mariculture in Manila Bay is in decline due to declining environmental quality as the Manila Bay watershed rapidly urbanizes (Jacinto et al. 2006a, b). *Mytella* quickly established itself in this polluted environment. There is some evidence to show that non-indigenous species introduction has resulted in a negative effect on *Perna* settlement (Bownes & McQuaid 2006). This has been observed in *Mytilus* and *Perna perna* settlement in South African rocky reefs. This needs to be further investigated and several hypotheses can be proposed. These are all related to characterizing the ecological niche of the non-indigenous and indigenous species.

The community ecology of *Mytella* as a non-indigenous and possibly invasive species

It can be presumed that the mussels in this study are all non-selective filter feeders, and are extremely tolerant of salinity changes in intertidal to estuarine environments. What may structure their communities are related to the time of spawning and recruitment to suitable substrate (Bayne 1976). Thus, this study focuses on inferring the community environmental dynamics of recruitment of these mussel species as fouling organisms.

MANOVA results suggest that variations in the settlement of the mussel species in this study are dependent on the variations of *Amphibalanus* and *Hydroides* counts. *Amphibalanus* serves as a biogenic recruitment substrate for *Mytella, Musculista, Modiolus*, and *Perna*. If *Mytella* is able to colonize the plates right after the establishment of the *Amphibalanus* habitat matrix, then they can compete with *Perna* recruits. This is the likely scenario on Manila Bay since the *Perna* recruitment and settlement happens at the mid part of the rainy season from June to September. At the Hong Kong Harbour, a high gonadosomatic index (GSI) was observed in *Perna viridis* before the onset of the monsoon season and by September, the GSI was lowest thus indicating post spawning condition (Cheung 1993). Similar recruitment patterns were also observed in Malaysia (Al-Barwani et al. 2007) and in India (Rajagopal et al. 1998). Thus, it is possible that *Mytella* and *Perna* are in competition for recruitment space since they recruit within the same rainy season, with *Mytella* possibly recruiting at least 30 days earlier and thus having an advantage.

Modiolus, on the other hand, recruited during the first half of the year with major settlement happening from March through June. *Modiolus* is likely not in direct competition with *Mytella* and *Perna*, since it recruits at an earlier season and occupies a higher pH and salinity niche as shown in the CCA.

Will *Mytella* become an invasive species in Manila Bay?

This is possible if *Mytella* follows an invasive biological strategy similar to that of *Mytella* and *Perna viridis* in the Tropical Atlantic and Caribbean. In a study reporting the establishment of *Perna viridis* in Florida, USA, study spat were first collected in Sep 1998 at the latter months of the summer wet season. The estimated age of the spat was 9 weeks (2-8 mm length), and this was estimated from the larval duration of 3 weeks and 6 weeks of post-settlement growth.

The *Mytella* collected from Manila Bay in Jul 2014 were at 5-8 mm in length. If the life history and growth parameters of this population are similar to *Perna*, then the first introduction or spawning of *Mytella* in the South Harbor occurred in late-Apr 2014 or early-May 2014 just before the onset of the southwest monsoon rainy season. Introduction is very likely via ballast water or through fouled ship hulls. Given the adaptability of mytilids to estuarine and coastal conditions and their high reproductive rates in eutrophic conditions, it is extremely likely that *Mytella* will be established and be an invasive in Manila Bay. Its abundance on the PICES collectors increased as compared to other non-indigenous species which have remained at low abundances.

Rice and colleagues (2016) did experiments on the salinity tolerances of *M. charruana* collected from Lingayen Gulf estuaries. They recorded high mortalities with specimens transferred from a salinity of 5-35 but lesser mortalities at lower salinities. When mussels were conditioned at 30 and transferred to lower salinities, survival rates were higher. This suggests that *Mytella* is more adaptable to lower salinities and can tolerate salinity changes below 35. *Mytella* can survive higher salinities up to 55 if acclimation is gradual and within a 5 day period. Above 55 increasing mortalities were observed and total mortality at 65.

The salinity tolerance experiments suggest that *Mytella* can survive low salinities associated with increased freshwater run-off resulting from the southwest monsoon rainy season. This season lasts from June to September on the western coast of Luzon. As recruitment was observed in the first two months of the rainy season, one can conclude that the onset of the rainy season in June is the trigger for spawning and recruitment. But based on the evidence presented in this study, *Mytella* recruited in greater numbers than *Perna* on the same *Amphibalanus* biogenic substrate. Thus there might be competition with *Perna* for space. *Perna* abundances are however also determined by *Hydroides* substrates. *Perna* may have a competitive advantage since they can colonize both substrates but it recruits at a later time period. *Perna* has a narrower range of salinity tolerance (19-44) than *Mytella* and is less adaptable to longer periods of low salinities. The current environmental conditions of Manila Bay are likely to be more favorable to *Mytella* colonization rather than *Perna*. The ecological ordinations corroborate the experimental laboratory findings. *Mytella* is more negatively associated with the first CCA axis, which represents salinity trends and implies that populations are likely to be established in low salinity environments.

The CCA ordinations

Whether *Mytella* will displace *Perna viridis* in Manila Bay needs to be further investigated. However, MNIS that are tolerant of salinity fluctuations in warm water conditions usually have the advantage (Sylvester et al. 2013). In subtropical Jacksonville, Florida, USA where both species are non-indigenous and invasive, *Perna* has a competitive advantage over *Mytella* at lower temperatures (<15° C) and at a higher salinity (35) (Yuan et al. 2010). On the other hand, *Mytella* has a competitive advantage over *Perna* at lower salinities and at warm temperatures (> 22° C) (Yuan et al. 2016). Manila Bay temperatures did not go below 25° C during the study period, thus it is more likely that *Mytella* will have a competitive advantage. It is recommended that further studies on the life cycle, growth, recruitment, and reproduction of both *Perna* and *Mytella* be conducted to ascertain if there is recruitment limitation and competition between the two species that may allow for habitat segregation. This also is necessary for any possible strategy to manage the mariculture of *Perna* and *Mytella*. There are recommendations that *Mytella* can be cultured during the low salinity southwest monsoon season and *Perna* during the dry and high salinity season to augment mariculturists income (Rice 2016; Rice et al. 2016).

There is also a need to investigate the population genetics of the newly established *Mytella* to determine its provenance. Rice and colleagues (2016) suggest that the genetic lineage of the Lingayen Gulf *Mytella* is from the Caribbean coast of South America based on the sequences of the ftmt COI gene. If the Manila Bay population is the source of the Lingayen Gulf populations, then the provenance of the two populations will be the same. However, there is a possibility that the Lingayen Gulf population may represent a separate introduction considering that the gulf hosts the port of Sual, which is an international port.

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