Aliens in Paradise. Boat density and exotic coastal mollusks in Moorea Island (French Polynesia)

Alba Ardura ^{a, b, *}, Serge Planes ^{b, c}, Eva Garcia-Vazquez ^a

^a Department of Functional Biology, University of Oviedo, C/ Julian Claveria s/n, 33006 Oviedo, Spain

^b USR 3278-CRIOBE-CNRS-EPHE, Laboratoire d'excellence "CORAIL", Université de Perpignan-CBETM, 58 Rue Paul Alduy, 66860 Perpignan CEDEX, France

^c Laboratoire d'Excellence "Corail", Centre de Recherche Insulaire et Observatoire de l'Environnement (CRIOBE), BP 1013, 98 729 Papetoi, Moorea, French Polynesia

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ABSTRACT

Pacific islands are particularly vulnerable to the effects of invasive species. After habitat destruction or modification, invasive species are responsible for more biological extinctions than any other cause. Further, the rate of extinction of native species has been higher on islands than anywhere else in the world. Invasive species have also degraded native ecosystems. In order to detect exotic intertidal mollusk species, an extensive sampling around Moorea Island, a more or less unspoiled island surrounded by a rich coral reef habitat, has been developed considering that sampled points have different characteristics in wave exposure, algae coverage, type of substrate, distance to ports, distance to freshwater, distance sewage and boat traffic. Samples were DNA barcoded for unequivocal species assignment.

The presence of five NIS among 26 species seems an important signal of introduction of alien biota in Moorea Island coast. However they were represented by a total of 38 individuals among 1487 mollusks (2.55%). While the distance to relatively big ports influenced directly species richness, the intensity of maritime traffic measured as boat density near sampling points was significantly associated with the frequency of exotic species. Other environmental factors did not show significant correlation with the frequency of exotics, suggesting that in an environment without big discontinuities, with little habitat modification, local boat traffic is the most influential factor in the spread of exotic species. This could be mitigated relatively easily by reducing boat density in local zones of ecological interest.

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1. Introduction

Coral reefs are complex and fragile ecosystems extremely sensitive to environmental changes and are currently endangered by a variety of problems, from climate change to acidification to pollution (e.g. Wilson et al., 2006; Hoegh-Guldberg et al., 2007). Introduction of alien species can be one of those factors because it is a well recognized threat for marine ecosystems (reviewed by Molnar et al., 2008), but the impacts of non-indigenous species on coral reefs are not well known (Coles and Eldredge, 2002).

There are different gateways for introduction of exotic species into a habitat. Fouling of maritime vessels is likely the most important (e.g. Gollasch, 2002; Godwin, 2003; Ruiz et al., 2009).

* Corresponding author. Université de Perpignan-CBETM, 58 Rue Paul Alduy, 66860 Perpignan CEDEX, France.

E-mail addresses: alarguti@hotmail.com (A. Ardura), planes@univ-perp.fr (S. Planes), egv@uniovi.es (E. Garcia-Vazquez).

International shipping is indeed important for transferring marine species, but intraregional transport has also been reported to be a major factor of alien species dissemination (Wasson et al., 2001). Aquaculture is other way of introduction of exotic species (Naylor et al., 2001), although its particular impact on coral reefs has not been studied yet.

Nearshore coral reefs are of high conservation value, and, as usual in interconnected ecosystems, they are heavily influenced by their proximity to the land, where most human activities are developed. Mollusks and sessile benthic communities from nearshore reefs are particularly sensitive by human impacts associated with land (e.g. Smith et al., 2008). Intertidal areas are the interface between terrestrial environments and nearshore reefs, and their integrity is therefore crucial for the conservation of the latter. Intertidal species, by their capacity of surviving both submerged and covered, are highly adaptable. Some of them can be carried by maritime transport very far from the native habitat, spreading to exotic geographic settings where they can become invasive. The

intertidal zone could therefore be considered a gateway for the introduction of aliens or not indigenous species (NIS) into near-shore coral reefs, NIS using intermediate steps to reach new habitats (e.g. Apte et al., 2000).

Mollusks are good model species for studying the role of maritime transport in shaping nearshore coral reefs diversity. They constitute an important part of coral reef communities (e.g. Augustin et al., 1999; Zuschin et al., 2001), and can also be dangerous elements contributing to degrading coral ecosystems (Hutchings, 1996). Mollusks are easily transported via ships. Sessile adults can attach to hulls and pelagic larvae can be contained in the ballast water, so they can be transported in different life stages increasing their likelihood to settle into new habitats. Some mollusks are paradigmatic examples of colonizers via ships. This is the case of various gastropoda and bivalvia carried by vessels to Hawaii, of European origin and now evolved to an American invader (Godwin, 2003), and many other species (Molnar et al., 2008).

On the other hand, alien species are particularly dangerous for island ecosystems, where they impact in an extent not well known yet. A paradigmatic example is the invasion of the Moorea Island (French Polynesia) by foreign land snails (e.g. Reaser et al., 2007), which endangered native flora and fauna. Measures of biological control such as the introduction of a predator were taken to eradicate or at least stop the invasion (Civeyrel and Simberloff, 1996), although the efficacy of those measures has been questioned. This island, which possesses an extremely valued lagoon and coral reef around almost all its perimeter, will be the geographical setting of the present project.

In this study we will determine different diversity indices of intertidal mollusk assemblages from Moorea, sampled at different distances from ports and ship harbors around the island. Association of diversity indices with the distance to ports, as well as with boat traffic, will be examined taken also into account other factors like the type of substrate and proximity to freshwater and sewage sources, if any. The objective is to explore the impact of maritime transport on mollusk diversity in intertidal areas connected with nearshore coral reefs, using Moorea as an example. This study will also allow to detecting exotic mollusks because the target gene will be the COI employed in Barcoding projects, which is extremely robust for species identification, and the taxonomic identification was confirmed with a second marker, 16S rRNA.

2. Material and methods

2.1. Study area and sampling

Moorea Island is situated 17°32' South and 149°50' West, 25 km distant from Tahiti in the Society archipelago in French Polynesia. It is a high island, entirely surrounded by a coral reef rim, cut by eleven passes. Like most of the high islands, the Moorea coral reef complex is divided into four "ensembles" or units: outer slope, barrier reef, lagoon and backreef channel and fringing reef (Battistini et al., 1975). Each unit includes zones and biotopes characterized by coral reefs communities (Faure, 1985).

Samples were taken from 16 points (Table 1) located in different ecosystems within the coral reefs that surround the island (Richard, 1985): from water channels in Papetoai, Maharepa and Tema'e; from deep lagoon in Vai'are, Farehau, and Afareaitu; from fringing reef in Tiahura, Maatea, Atiha, Vai'anae, Haapiti, Tiki and Hauru, and finally from bay environment in Opunohu, Paopao and Entre-Deux-Baies. Eight of the sampling points (Hauru, Tiahura, Entre-Deux-Baies –also named Entre2Baies, Maharepa, Tema'e, Farehau, Maatea and Haapiti) were chosen within each of the eight marine protected areas present around the island (Walker, 2003).

Sampling was carried out picking (at random within species)

mollusk specimens from the intertidal range (upper to lower), which is quite short in Moorea (maximum tidal range of 0.40 m) between the 26 August and the 10 September 2011 (Fig. 1). A total of 100 samples were obtained from each site (Table 1). An effort was made for obtaining representative samples, proportional to the abundance of each species. The environmental characteristics considered are reported in Table 1. For the distance to ports, there is only one big port in the island located in Vai'are, where both cargos and passenger ferries arrive. The rest of ports are local fishing and docking ports, with adjacent marinas in most cases. As a proxy of local maritime traffic intensity, the number of boats (fishing and/or recreational) of any size visible from each sampling location was counted for half an hour in each of three different days (one weekend and two working days). For each sampling point distance to freshwater (watercourse mouth) and sewage discharge, degree of substrate artificiality (from natural to totally artificial), algae coverage and exposure to open waves was recorded. A Likert scale was employed for quantifying each factor whenever possible for reasons of data normalization and statistical treatment. We used a 1–5 scale from the minimum to the maximum expression of each factor in the ecosystems sampled in the island.

The specimens sampled were photographed and visually identified employing taxonomic guides and voucher specimens from the collection of the CRILOBE, in Moorea (French Polynesia). Tissue samples were stored in absolute ethanol until analysis.

2.2. Classification of species as NIS

The species found in this study were classified as native or NIS according to current inventories of Moorea fauna and the native distribution of each species (World Register of Marine Species, www.marinespecies.org; Encyclopedia of Life, available from <http://www.eol.org>, accessed July 2015).

2.3. Genetic analysis

Total DNA was extracted from a small piece of tissue following the standard protocol described by Estoup et al. (1996), employing Chelex[®] resin (Bio-Rad Laboratories). The E.Z.N.A Mollusc DNA kit (IOMEGA, bio-tek) was used for the species with high content of mucopolysaccharides in muscle tissues, following manufacturer's instructions. In both cases, the tubes were stored at 4 °C for immediate DNA analysis, and aliquots were frozen at –20 °C for long-term preservation.

A fragment of partial COI gene was amplified by polymerase chain reaction (PCR), employing the primers described by Geller et al. (2013). The amplification reaction was performed in a total volume of 40 µl, with Promega (Madison, WI) Buffer, 1x, 2.5 mM MgCl₂, 0.25 mM dNTPs, 20 pmol of each primer, approximately 20 ng of template DNA and 1 U of DNA Taq polymerase (Promega), and the following PCR conditions: initial denaturing at 95 °C for 5 min, 35 cycles of denaturing at 95 °C for 1 min, annealing at 48 °C for 1 min, extension at 72 °C for 1 min and final extension at 72 °C for 5 min.

Additional sequencing of the mitochondrial 16S rRNA gene with the primers described by Palumbi (1996) was carried out for some samples in order to confirm the taxonomic identification with a second marker. The amplification reaction was performed in a total volume of 40 µl with the same conditions described above for the COI gene and the following PCR conditions: initial denaturing at 95 °C for 5 min, 30 cycles of denaturing at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 2 min and final extension at 72 °C for 7 min.

PCR products were visualized in 2% agarose gels with 3 µl of 10 mg/ml ethidium bromide. Sequencing was performed with the

Table 1
Sampling sites characteristics. GPS coordinates; distance to the nearest port (m); intensity of maritime traffic as boat density in a 1–5 Likert scale from least to most intense (Traffic); distance in meters to the nearest urban discharge (Sewage), distance to river/stream in m (Freshwater); algae coverage; substrate type in a 1–5 Likert scale from least to most artificial (Substrate); exposure to wave action in a 1–5 Likert scale from least to most exposed (Waves).

	Latitude	Longitude	Ports	Traffic	Sewage	Freshwater	Algae	Substrate	Waves
Papetoai	1729545	14952346	300	3	100	50	40%	2	3
Tiki	1731887	14954304	6000	3	20	1000	10%	5	2
Atiha	1735561	14949505	200	1	1000	800	10%	1	5
Hauru	1730385	14954936	3000	2	500	1000	10%	5	2
Haapiti	1733197	14952832	3000	1	500	200	10%	1	3
Maharepa	1728752	14947410	200	3	100	20	10%	4	4
Temae	1729837	14945415	300	3	100	1000	10%	5	4
Tiahura	1729459	14953154	500	2	50	1000	30%	3	3
Maatea	1734464	14947611	300	2	20	1000	30%	5	5
Pao-Pao	1730402	14949342	200	4	30	100	10%	5	4
Entre 2 Baies	1729129	14949865	2000	4	200	1000	50%	4	3
Faarehau	1732688	14946705	2000	1	1000	100	20%	3	3
Vaiané	1735184	14950986	200	3	300	500	30%	5	3
Opunohu	1731076	14951055	200	1	100	300	10%	1	1
Afareitou	1733034	14947411	50	3	30	100	10%	4	5
Vai'are	1731441	14946609	0	5	800	800	10%	5	4

Fig. 1. Map of Moorea Island including the sampling locations (black squares), ports with docking (black ships, the big one correspond to the most important port in Vai'are).

DNA sequencing service Macrogen Europe (The Netherlands).

2.4. Sequence edition and phylogenetic analysis

Sequences were visualized and edited employing the BioEdit Sequence Alignment Editor software (Hall, 1999) and aligned with the ClustalW application (Thompson et al., 1994) included in BioEdit. The sequences obtained were compared with international databases employing the program BLAST within NCBI (<http://www.ncbi.nlm.nih.gov/>) and BOLD system, in the case of COI sequences, (<http://www.boldsystems.org/>) for identifying the species.

The phylogenetic analysis was performed using MEGA 6 (Tamura et al., 2013). This software was used to construct the phylogenetic tree based on the aligned sequences, using the Neighbor-Joining methodology, since is the standard method of

phylogenetic inference in DNA barcoding studies (Hebert et al., 2003); its use in DNA barcoding studies is in part due to its strong track record in being able to rapidly analyze large species assemblages (Kumar and Gadadkar, 2000). The phylogenetic tree was constructed with the Tamura-Nei model (Tamura and Nei, 1993) and uniform rates, based on the software jModelTest (Posada, 2008), which determine the best suited model of sequences evolution. Robutness of the NJ tree topology was assessed using 1000 bootstrap replicates.

2.5. Statistical analysis

Diversity indices were calculated employing the PRIMER 6 software. The indices analyzed were the Simpson index that takes into account the number of species in the habitat and their relative

abundance, and the total phylogenetic diversity (PD) to know the phylogenetic structure of each sampling point.

The environmental variation of the study sites was analyzed employing a principal components analysis (PCA) with the software PAST (Hammer et al., 2001) with correlation option and 0.7 Jolliffe's cut-off (the threshold value of significant component, as estimated by the software). The contribution of the site characteristics to the total variance was estimated, as well as the correlation between sampling site characteristics and diversity indices. We have used non-parametric statistics (Kendall's Tau correlation tests) given the non-parametric nature of many data (traffic intensity, wave exposure, algae coverage and type of substrate). When correlation values > 0.8 were obtained between two parameters, one of those was eliminated from the PCA to avoid autocorrelation biases. Having thus established an independent (non-correlated) environmental dataset, the diversity indices (Phylogenetic diversity and Simpson index) were used as dependent variables in two independent Generalized Linear Models, with AIC as a guide for best fit model, to test directly for the contribution of the different environmental variables. In addition the diversity indices and the frequency of exotics were used as dependent variables in a multivariate multiple linear analysis to test for the relative contribution of each environmental variable to frequency of exotics and to each diversity indices with another test.

A linkage tree analysis was performed using the LINKTREE routine included in PRIMER 6 software, which creates binary splits of species based on maximizing the rank similarity statistics, R. At each split, the routine provides a measure of the separation between the two groups based on the entire ranked similarity matrix, such that splits further down the y-axis (smaller %B values) have smaller overall differences between groups.

3. Results

DNA sequences of COI 594 nucleotide long were retrieved from 1487 samples of molluscs. The haplotypes found were submitted to GenBank database, where they are available with the accession numbers (Supplementary Table 1). From BLAST analysis they were unequivocally assigned to 26 different species (Supplementary Table 1) with E-values $<< 0.0001$ in all cases. The sequences exhibited a strong phylogenetic signal that allowed separating species by order in different clusters (Fig. 2). The 16S rDNA sequences obtained confirmed species assignment from COI, as expected. They are also available in the GenBank (Supplementary Table 1).

The number of species, species richness and phylogenetic diversity varied greatly between sampling locations (Table 2). The extremes were Tiki, with only one species, and Vai'are with 12 species. Five non indigenous species (NIS) were found from six out of the 16 sampling locations, ranging between 1.1% and 15.6% individuals for Faarehau and PaoPao respectively. Three of the five NIS (*Drupa albolabris* from Philippines, *Littoraria glabrata* from the Indian Ocean, *Thais tissoti* from the Indo-west Pacific) were from other areas of the Pacific Ocean. One species, *Nerita tessellata*, is native to the West Atlantic Ocean. Finally, *Saccostrea cucullata* or hooded oyster, present at high frequency in PaoPao, is recognized as an invasive species (e.g. Ruesink et al., 2005) native to the Indian Ocean.

Several variables were highly significantly correlated, principally diversity indices, with r -values > 0.8 , and one from each pair of correlated variables of the same nature was removed from further analyses to prevent internal autocorrelations and biased results (Table 3). We retained Simpson's ecological diversity index, and the phylogenetic diversity (PD) as phylogenetic index. The principal component analysis (Table 4) revealed that two components

contributed $> 50\%$ total variance. The main factors in these components were the two diversity indices, distance to port, maritime traffic, distance to freshwater and the degree of substrate artificiality.

The plot graph of the two principal components (Fig. 3) showed NIS (Exotics) in the same quadrant of maritime traffic (indicated by boat density), and the diversity indices inversely associated with the distance to ports.

The least influential factor taking into account its coefficient in the two PC was exposure to waves that was therefore removed from further analyses.

In a dichotomy tree (Fig. 4) organized grouping communities by the most influential variables, Vai'are was alone in a branch clearly separated from the rest due to the high influence of the big port and traffic, also for being the most diverse community at phylogenetic level. The spatial clustering of the sixteen communities analyzed was similar to that retrieved from PCA (Fig. 3) except for the position of Entre-2-Baies, which clustered with Tiki and Hauru in the tree whereas was closer to Pao-Pao in the PCA.

The generalized linear model constructed from the most influential variables entering in PC1 was significant ($p < 0.05$) for traffic with significant contribution to frequency of exotics ($y = 13.696x + 2.2148$, $G = 5.568$) and to phylogenetic diversity ($y = 0.00434x + 1.1473$, $G = 7.477$), and for algae coverage with significant contribution to phylogenetic and Simpson's diversity indices ($y = 0.036x + 6.989$ with $G = 516.4$, and $y = 26.085 + 5.6653$ with $G = 529.16$, respectively).

Multivariate multiple linear analysis (Table 5) showed that maritime traffic was the only significant factor correlated with phylogenetic diversity (Table 5a), and that the distance to ports was significantly and negatively associated with Simpson's index of ecological diversity (Table 5b), confirming PCA results.

Considering the situation of samples on different ecosystems around the island, significant differences for the average number of species were found between locations placed on the fringing reef and the rest of locations, placed in deeper waters (bays, channels in contact with the open ocean, deep lagoon), with mean species per site of 3 (95% confidence interval 1.8177–4.1823) and 6 (95% confidence interval between 3.8259 and 8.1741) respectively and $t = 2.708$ with $P = 0.016$ for samples with equal variance ($F = 4$ with $P = 0.084$ for different variance, not significant). Not significant differences were found between samples inside and outside of Marine Protected Areas (mean 4.25 and 5.13 respectively, $t = 0.623$ with $P = 0.543$ for difference between means, not significant). For NIS and taken globally, within marine protected areas only four NIS individuals were found (0.55%), in contrast with 34 NIS in the eight locations out of MPA (4.49%). The difference between MPA and non-MPA for NIS frequency, as a whole, was statistically significant (Chi-square of contingency = 22.4, 1 degree of freedom, $p < 0.001$).

4. Discussion

The results presented in this study demonstrate significant differences in diversity of mollusk communities in Moorea Island. Diversity values are significantly associated to maritime traffic and negatively correlated with the distance to ports. Moreover, the frequency of NIS (exotic species) was correlated with boat density. Taken together these data are indicators of a strong influence of shipping and, in general, maritime navigation, on the intertidal molluscan community. These significant differences detected from the very narrow intertidal of this small island may emphasize the role of this interphase area as habitat of interest for marine biodiversity and invasion studies (e.g. Apte et al., 2000; Smith et al., 2008). The results also suggest a progressive decline of marine mollusk biodiversity in the zone. As an example, mollusk inventory

Fig. 2. Neighbor-joining tree reconstructed from the COI sequences obtained from Moorea Island molluscs. Bootstrapping is marked on the branches (in percent). Numbers 1 to 8 correspond to Sorbeoconcha, Patellogastropoda, Vetigastropoda, Neritimorpha, Cephalaspidea, Pulmonata, Ostreoida and Pteriomorphia orders respectively. Outgroup: *Octopus vulgaris*, GenBank Accession Number: KC789330.

Table 2

Diversity values for mollusk communities found in the considered locations around Moorea Island. Number of species (NSp), species richness (SR), proportion of exotic molluscs (NIS, non-indigenous species), Shannon's and Simpson's diversity indices, taxonomic diversity (Tax div), taxonomic distance (Tax dit), phylogenetic diversity (PD) and average phylogenetic diversity (AvPD).

	NSp	SR (d)	NIS	Shannon	Simpson	Tax div	Tax dist	PD	AvPD
Papetoai	6	1.095	0.0625	1.549	0.7761	56.97	73.41	420	70
Tiki	1	0	0	0	0	0	0	100	100
Atiha	3	0.4402	0	0.478	0.2743	21.94	80	260	86.67
Hauru	2	0.2191	0	0.0579	0.02083	1.667	80	180	90
Haapiti	2	0.2191	0	0.6211	0.4342	34.74	80	180	90
Maharepa	4	0.6635	0	0.7827	0.4871	38.68	76.67	320	80
Temae	3	0.4382	0	0.8408	0.534	16.67	60	200	66.67
Tiahura	5	0.8784	0.0316	1.112	0.5991	47.03	82	380	76
Maatea	3	0.4456	0	0.696	0.4668	10.68	60	200	66.67
Pao-Pao	5	0.8764	0.156	1.068	0.5195	43.29	86	420	84
Entre2Baies	9	1.842	0	1.273	0.6364	50.36	78.33	660	73.33
Faarehau	6	1.117	0.0114	1.146	0.6157	47.36	72	380	63.33
Vaiané	5	0.9294	0.0811	1.387	0.7149	45.78	60	280	56
Opunohu	5	0.8764	0	1.129	0.5934	38.22	68	300	60
Afareitou	4	0.6573	0	1.056	0.6287	42.39	66.67	260	65
Vai'are	12	2.34	0.0636	1.747	0.7248	55.98	70	680	56.67

from Tiahura site on a smaller total surface ($24 \times 5 \text{ m}^2$, 120 m^2 in total compared with 200 m^2 in the present study) from a complete transect of the fringing reef retrieved a total of 70 species in 1971 and only 37 in 1995 (Augustin et al., 1999). Although the sampling methods are not identical because our sampling effort was lower, in the present 2011 study we found five species (13.5% of 1995's species). It is true that our sampling was limited to the intertidal

(<50 cm in the vertical transect) and expected diversity is lower therein than in all the fringing reef; notwithstanding it, such very small number may indicate a continued trend of diversity decline in the island as suggested by Augustin et al. (1999) between 1971 and 1995.

The effect of maritime traffic on diversity here seems to be undeniable. Boats move species around the island, as well as to and

Table 3
Correlations between variable analyzed. P-values in the upper part and r-values below the diagonal of the table.

	N	S	SR _(d)	NIS	Algae coverage	Distance to ports	Waves exposure	Type of substrate	Distance to freshwater	Distance to sewage	Boat density	Shannon	Simpson	Tax div	Tax dist	AvPD	PD
N																	
S	0.8234 0.0607																
SR _(d)	0.0104	0.9986															
NIS	0.0412	0.3521	0.34633														
Algae coverage	-0.6225	0.3770	0.4123	0.0788													
Distance to ports	0.1430	-0.6259	-0.63129	-0.31846	-0.29306												
Waves exposure	0.0729	0.0607	0.05253	0.08787	-0.05543	-0.38708											
Type of substrate	-0.0934	0.0936	0.09409	0.26927	0.00802	0.02268	0.19227										
Distance to freshwater	-0.0346	-0.0441	-0.04139	-0.30168	0.16918	0.41588	-0.03360	0.36534									
Distance to sewage	0.1164	0.2465	0.23716	-0.1026	-0.20366	0.10638	0.09795	-0.29492	0.00179								
Boat density	0.1038	0.5776	0.57375	0.5038	0.17356	-0.2956	0.2222	0.67327	0.10783	-0.28578							
Shannon	-0.0617	0.8373	0.8392	0.4807	0.4398	-0.8557	0.1171	-0.055	-0.3391	0.0256	0.4265						
Simpson	-0.1552	0.6908	0.6972	0.3674	0.45332	-0.9245	0.2010	-0.1259	-0.4139	-0.068	0.2893	0.9593					
Tax div	-0.0826	0.7604	0.7662	0.4534	0.39518	-0.7604	0.0615	-0.2541	-0.5324	0.0927	0.3068	0.9104	6.22E-06	0.1108	0.00011	0.00867	
Tax dist	-0.0102	0.3236	0.3270	0.2236	0.13511	-0.4207	0.2327	-0.3339	-0.3038	0.2663	-0.1090	0.3810	0.41415	0.50131	0.02322	0.00053	
AvPD	0.1539	-0.6073	-0.6091	-0.149	-0.2780	0.8064	-0.1322	-0.0800	0.1638	-0.0421	-0.183	-0.797	-0.8170	-0.56284	-0.2027	0.45156	0.09953
PD	0.0092	0.9609	0.9653	0.3825	0.44782	-0.5794	0.0794	0.0510	-0.0535	0.1951	0.5838	0.7721	0.63166	0.76668	0.4264	-0.4348	

Table 4

Results of the Principal Components Analysis for environmental factors of the sampling sites, indicating the variance explained by each component (in percent) and the coefficient of the eigenvector within each component. The three variables with bigger influence in each component are marked in bold.

Variance	Component 1	Component 2
	31.35%	20.20%
Phylogenetic diversity	0.436	-0.023
Simpson Index	0.468	-0.276
Frequency of exotics	0.370	0.075
Distance to port	-0.389	0.286
Boat density	0.387	0.435
Distance to sewage	-0.085	-0.293
Distance to freshwater	-0.145	0.441
Waves exposure	-0.213	-0.029
Algae coverage	0.245	0.057
Type of substrate	0.142	0.603

from neighboring islands, and increase local biodiversity by transfer of individuals and species from other populations. Maritime traffic so contributes to spatial homogenization of biodiversity (e.g. Molnar et al., 2008), especially in islands and archipelagos (see a review in Fitzpatrick, 2007, and references therein) where boats are the most frequent transport. Both ballast water and hull fouling are principal vectors for marine NIS dispersal (Ruiz et al., 2009), so the ports that receive cargos and big ships will be in principle more vulnerable than marinas where ballast water will have smaller or negligible influence. In this study boat density was more influential on biodiversity than the distance to ports, and this applies also to Moorea sampling sites far from the biggest Vai'are port where long-distance cruises and cargos arrive. For example, Entre-Deux-Baies is far from Vai'are and exhibited nine different species in the short intertidal zone. Without denying the influence of long-distance maritime traffic from far last port of call, since biodiversity was greatest in Vai'are, the results of this study suggest that small boat traffic is spreading species around Moorea Island. The frequency of NIS is associated with boat density, but not significantly correlated with the distance to ports. This supports the importance of the often overlooked secondary spread of species promoted from local short-distance maritime traffic (e.g. Wasson et al., 2001; Zabin et al., 2014). The NIS would logically enter first in the big port, and be further transported repeatedly in all directions around the island by local boats. This would contribute to disperse them and homogenize their distribution regardless the distance to the entry point (the big port). Local conditions favorable to invasions would promote expansions of several NIS, for example in Pao-Pao abundant exotic oysters would be favored by relatively sheltered conditions.

Another factor that promoted mollusk biodiversity in this study was algae coverage. This is not unexpected, since many mollusks are algae grazers and, in addition, algae offer protection from air predators (birds) and desiccation in low tides, therefore algae coverage is one of the key factors for explaining mollusk diversity in general, and in Moorea Island in particular (e.g. Adjerdoud, 1997).

The presence of five NIS among 26 species (19% species) seems an important signal of introduction of alien biota in Moorea Island coast. However they were represented by a total of 38 individuals among 1487 molluscs (2.55%), in only six locations and mainly out of marine protected areas. Other authors have pointed out that, logically, contaminants (biological and chemical) can surpass freely the limits of MPA if there are no barriers isolating them from the rest of the sea; consequently, MPA would not offer protection against the spread of exotic species (e.g. Allison et al., 1998, and

Fig. 3. Plot of the principal components PC1 and PC2, with the relative contribution of each factor proportional to diagonal length.

Fig. 4. Linktree constructed from the main characteristics of the analyzed communities clustering them based on their values at the most influential variables.

references therein). However, our results suggest that the status of MPA, with relatively fewer human activities than in unprotected spaces, confers at least some protection against NIS introduction. Restriction or moderation of maritime traffic would be therefore recommended in ecologically key areas such as those nearby coral reefs for preventing biological invasions from spread.

Last but not least, this study emphasizes the value of DNA Barcoding for biodiversity inventory. This method is especially applicable for marine mollusks, since there are many reference sequences available for this taxonomic group obtained from many projects and different geographical areas (e.g. Bucklin et al., 2011). In particular for Moorea Island there is an online database available for biodiversity studies derived from Moorea BIOCODE project (<http://www.mooreabiocode.org>), where species-specific DNA

signatures (COI sequence), voucher specimens and tissues are available for some of the species found in this study (<http://biocode.berkeley.edu/>); for example *Nerita argus* and *Smaradigmella calyculata*. Although in our study we have employed the worldwide databases GenBank and BOLD as references, local databases can be especially useful for accessing more detailed information about the species found. This helps to solve uncertainties, especially in cases of polymorphisms and phenotypic plasticity.

5. Conclusions

Although exposed to increasing anthropogenic modifications, Moorea Island still contains a small proportion of non-indigenous mollusks in the intertidal (in individual counts), which reflects a

Table 5

Multivariate multiple linear analysis. Slopes for each environmental factor with their estimated errors, results of t-tests and associated p-values. Factors with significant slopes are marked in bold.

	Slope	Standard error	t	p
(a) With phylogenetic diversity as the dependent variable.				
Constant	−32.251	147.32	−0.2189	0.8330
Simpson Index	184.96	250.01	0.7398	0.4835
Frequency of exotics	31.878	772.57	0.0412	0.9682
Distance to port	−0.0041	0.0251	−0.1641	0.8442
Boat density	108.91	36.884	2.9529	0.0213
Distance to sewage	0.1799	0.0822	2.1883	0.0648
Distance to freshwater	0.0284	0.0912	0.3118	0.7643
Algae covering	3.1642	2.8537	1.1088	0.3042
Type of substrate	−38.41	26.295	−1.4607	0.1875
b) With Simpson Index as the dependent variable				
Constant	0.4342	0.1393	3.1183	0.0168
Simpson Index	0.0004	0.00053	0.7398	0.4835
Frequency of exotics	−0.3069	1.119	−0.2743	0.7918
Distance to port	−6.62 × 10^{−5}	2.68 × 10^{−5}	−2.4718	0.0427
Boat traffic	0.0124	0.0803	0.1546	0.8815
Distance to sewage	1.8 × 10 ^{−5}	0.00015	−0.1163	0.9107
Distance to freshwater	0.0002	0.00011	−1.6142	0.1505
Algae covering	0.0057	0.0040	1.4251	0.1972
Type of substrate	−0.0015	0.0437	−0.0344	0.9735

good conservation status. Maritime traffic seems to be the most influential factor on the introduction of exotic molluscs in the island coast. Reducing local boat traffic in areas of ecological interest is recommended for mitigating or preventing exotics spread.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.marenvres.2015.08.007>.

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