Aquatic Invasions (2013) Volume 8, Issue 3: 261–270
doi: http://dx.doi.org/10.3391/ai.2013.8.3.02

Research Article

Marine invasions on a subtropical island: fouling studies and new records in a recent marina on Madeira Island (Eastern Atlantic Ocean)

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Received: 4 April 2013 / Accepted: 17 June 2013 / Published online: 17 July 2013

Handling editor: John Mark Hanson

Abstract

In recent years, several marine non-indigenous species (NIS) lists have been produced for many European countries but little is known about the diversity and distribution of fouling NIS in Portugal (mainland and islands). We conducted a six-year survey of a marina located on the south coast of Madeira island, Portugal to assess NIS diversity on the island, constituting the first NIS inventory for the archipelago. We found 16 NIS, of which 9 are new records. Both species richness and abundance changed during the course of colonization whether total, NIS, or native diversity were considered. The number of native species decreased with colonization while the number of NIS significantly increased. More importantly, we demonstrated that the number of NIS detections in the marina was correlated with increasing ship traffic over the years.

Key words: biofouling; invasive species; Madeira; marinas; marine invasions; new records; non-indigenous species (NIS)

Introduction

Biological invasions of marine non-indigenous species (NIS) are frequent in coastal communities, and the rate of documented invasions has significantly increased in the last two decades (Ruiz et al. 2009). The greatest proportion of recent marine invasions has been facilitated through the transport of species in ballast water and as hull fouling (Ruiz et al. 2000); the latter vector conveying numerous “fouling” organisms around the world, including sponges, hydroids, tube worms, barnacles, mussels, bryozoans, sea squirts, and algae. In fact, every hard artificial substrate in the marine realm (e.g. ship hulls, piers, pontoons, pilings, seawalls and buoys) is subject to biofouling, i.e., the accumulation of sessile microorganisms and macroorganisms with time (Wahl 1997; Railkin 2004; Dürr and Thomason 2010). The species composition of any fouling community can change considerably over space and time (Railkin 2004; Canning-Clode et al. 2010; Dürr and Thomason 2010). Biofouling assemblages represent an ideal study system for ecologists, due to their amenability for experimentation, their ease of access (without requiring low tides or vessel use), and due to the organisms' proclivity to settle on artificial substrates and their subsequent fast growth (Canning-Clode and Wahl 2010).

In the last decade, lists of marine non-indigenous species have been produced for several European countries such as the UK, Belgium, Denmark, Germany, France, Greece, and Italy (Goulletquer et al. 2002; Occhipinti Ambrogi 2002; Jensen and Knudsen 2005; Pancucci-Papadopoulou et al. 2005; Kerckhof et al. 2007; Minchin et al. 2013). The only NIS inventory for Portuguese waters is for the Azorean archipelago (Cardigos et al. 2006). In addition, although a few new records have been reported for the island of Madeira (e.g. Wirtz 1998; Wirtz and Canning-Clode 2009), little is known about the diversity

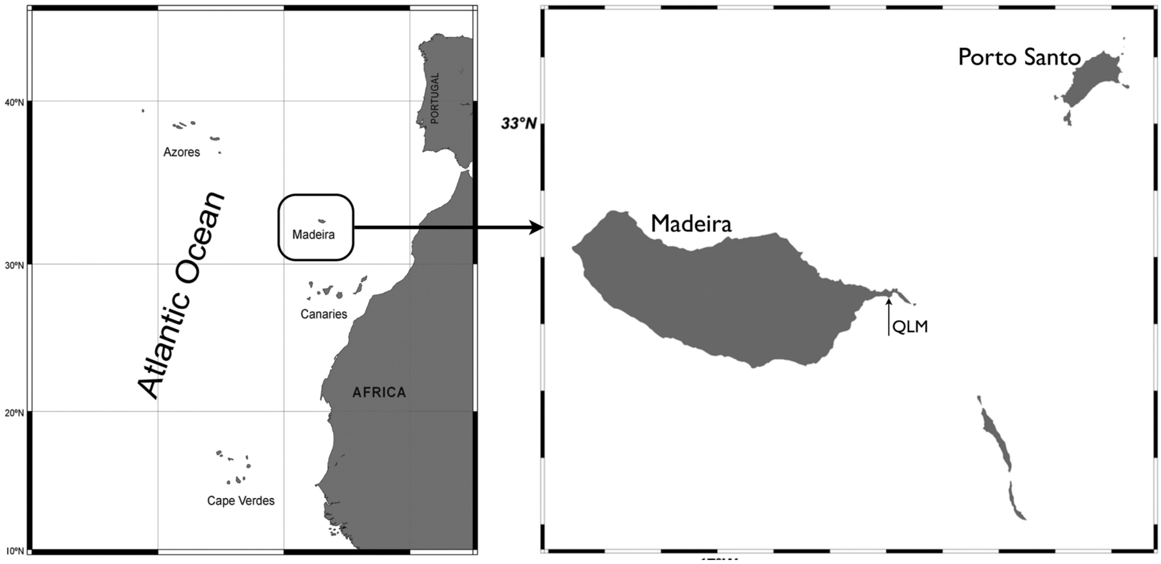

Figure 1. Location of the Quinta do Lorde Marina (QLM) on the southeast coast of Madeira, Portugal.

and distribution of fouling NIS on the island archipelago. Consequently, we conducted a six-year survey of a recently constructed marina located on the southeast coast of Madeira Island to contribute to a first inventory of marine NIS in Madeira. We deployed settling plates and examined diversity and abundance at different stages of the colonization process of the recruiting fouling assemblages. We also examined vessel traffic in the marina in recent years and hypothesized that i) species richness would change with time, regardless of what category was considered (i.e. total, NIS, native or cryptogenic richness); ii) species composition would change in time; and iii) and NIS numbers were positively correlated to vessels traffic in the marina over time.

Methods

Sampling

This study was conducted at Quinta do Lorde Marina (QLM), located at the southeast part of Madeira Island (32º44.5'N, 016º42.8'W; Figure 1). QLM was established in 2002 and is mainly used as a re-fuel and rest stop for yachts sailing between Europe and the Caribbean. In March 2006, 60 polyvinylchloride (PVC) plates (15×15×0.3 cm), vertically attached in 6 PVC rings (60 cm diameter, 25 cm height), were hung from a buoy at approximately 0.5m depth and exposed for colonization (see Canning-Clode et al. 2009 for design details). For the purpose of this analysis, 10 plates were randomly chosen for sampling at different colonization ages: 3 months (T3, July 2006), 6 months (T6, October 2006), 12 months (T12, April 2007), 24 months (T24, April 2008), 44 months (T44, January 2010), 50 months (T50, July 2010) and 74 months (T74, July 2012).

At each sampling event, settling plates were detached, retrieved from the field, and photographed. Plates were then brought back into the field within 3 hours of each sampling event. Species richness and abundance at plate level were determined by recording the number of species identified from the photographs using the image analysis software CPCe (Kohler and Gill 2006). Voucher specimens of each species were further submitted, as necessary, to expert taxonomists for verification or identification. Algae and sessile macroinvertebrates were identified to the lowest possible taxonomic group based on existing literature or taxonomic experts and assigned to one of four categories: native, NIS, cryptogenic (i.e., unspecified origin, that is, unknown whether native or introduced) or unresolved (based on an inability to identify to species level).

Each photographic image was sub-divided into a 3 × 3 grid of 9 cells, with 11 random points per cell resulting in 99 points analyzed per picture.

Table 1. List of the 16 non-indigenous species (NIS) found in Quinta do Lorde Marina (QLM) from 2006 to 2012. For a detailed full species list and abundances over time see Appendix 1.

Taxon	Common name	First detection	Area of origin	Possible vector of introduction	Reference
Porifera					
Mycale senegalensis (Lévi, 1952)	–	Jan 2010	Senegal	Hull fouling	New record
Paraleucilla magna (Klautau, Monteiro & Borojevic, 2004)	–	Oct 2006	Brazilian coast	Hull fouling	New record
Cnidaria					
Aiptasia diaphana (Rapp, 1829)	Yellow Aiptasia	Apr 2008	Mediterranean	Hull fouling	P. Wirtz, Univ. do Algarve, Portugal
Bryozoa					
Bugula dentate (Lamouroux, 1816)	Dentate moss animal	Apr 2007	Indo-Pacific	Hull fouling	Norman 1909
Bugula stolonifera (Ryland, 1960)	–	Jan 2010	Eastern and western Atlantic; Mediterranean	Hull fouling	New record
Parasmittina protecta (Thornely, 1905)	–	Jan 2010	Red Sea	Hull fouling	New record
Schizoporella pungens (Canu & Bassler, 1928)	–	Jan 2010	Gulf of Mexico	Hull fouling	New record
Scrupocellaria bertholetti (Audouin, 1826)	–	Oct 2006	Gulf of Mexico	Hull fouling	New record
Watersipora subtorquata (d'Orbigny, 1852)	–	Jul 2006	Indo-West Pacific	Hull fouling	New record
Zoobotryon verticillatum (Delle Chiaje, 1822)	Spaghetti bryozoan	Jan 2010	Western Atlantic and the Caribbean	Hull fouling	Wirtz and Canning-Clode 2009
Crustacea					
Balanus trigonus (Darwin, 1854)	Triangle barnacle	Apr 2007	Cosmopolitan; Tropical and warm temperate seas	Hull fouling and/or rafting	Carlton 2011
Chordata					
Botryllus schlosseri (Pallas, 1766)	Star tunicate	Oct 2006	Cosmopolitan; unknown	Hull fouling; ballast water	Canning-Clode et al. 2008
Clavelina lepadiformis (Müller, 1776)	Light bulb tunicate	Apr 2007	Norway to the Atlantic coast of Spain; Mediterranean	Hull fouling; ballast water	Wirtz 1998
Distaplia corolla (Monniot F., 1974)	Button tunicate	Jan 2010	Caribbean	Hull fouling	New record
Microcosmus squamiger (Michaelsen, 1927)	–	Oct 2006	Australia	Hull fouling	Turon et al. 2007
Styela canopus (Savigny, 1816)	Rough sea squirt	Oct 2006	Red Sea	Hull fouling	New record

This stratified random sampling method attributes points to each image region (Kohler and Gill 2006). In addition, each plate was examined using a dissecting microscope for better resolution. During this procedure, fouling assemblages were always submerged in seawater and protected from direct sun exposure. These analyses permitted us to place species into one of three bins in terms of spatial cover: ≤ 1% mean cover; < 10% mean cover, and > 10% mean cover (Appendix 1).

Finally, since QLM only opened in 2002, we were able to compare the number of NIS relative to vessel traffic in the marina since its establishment. All arrivals at QLM since 2003 and every vessel type arriving at this marina (max. length ~ 55 m; min. length ~ 4 meters) were included in this analysis. Data from the neighboring island of Porto Santo included every vessel type arriving at this marina (max. length ~ 55 m; min. length ~ 2 meters) between 2006 and 2012. Vessel traffic in QLM and Porto Santo was provided by Quinta do Lorde Marina administration (Sítio da Piedade, 9200 - 044 Caniçal, Madeira Island).

Table 2. Effects of colonization age on diversity of fouling communities after a 6 year fouling survey conducted at QLM. Results of the linear and polynomial regression analyses are shown for species richness and percent cover.

		Linear		Polynomial	
		R^2	p	R^2	p
Richness	Total	0.03	0.1833	0.46	< 0.001*
	NIS	0.34	< 0.001*	0.50	< 0.001*
	Native	0.08	0.0175*	0.29	< 0.001*
	Cryptogenic	0.33	< 0.001*	0.43	< 0.001*
Cover	Total	0.16	0.0005*	0.34	< 0.001*
	NIS	0.55	< 0.001*	0.58	< 0.001*
	Native	0.01	0.5530	0.25	< 0.001*
	Cryptogenic	0.15	< 0.001*	0.17	0.0017*

Data analysis

For each category of diversity (i.e., total, NIS, native and cryptogenic), linear and quadratic models were used to test for relationships between diversity of fouling assemblages and colonization age. If both linear and quadratic models were significant, the best fit was accepted based on its R^2. For the purpose of this analysis, species classified as unresolved were pooled into the cryptogenic category.

In addition, percent cover data were used for multivariate analyses of similarity (ANOSIM) to evaluate effects of colonization age on species composition. Non-metric multidimensional scaling (MDS) was applied to visualize similarity between compared groups. We then used the SIMPER routine to evaluate the contribution of each taxon to average dissimilarities between groups. The more significant taxa causing these dissimilarities were identified (Clarke and Warwick 1994). Multivariate analysis was performed in the R platform (R Core Team 2013) with the 'vegan' package (Oksanen et al. 2012).

Finally, the relationship between ship traffic at QLM over the years and number of accumulated NIS was tested with linear regression. All statistical analysis was performed in the R platform (R Core Team 2013).

Results

Over the course of six years, 49 taxa were recorded in Quinta do Lorde Marina, of which 16 were categorized as native (33%), 16 as NIS (33%) and 8 as cryptogenic (16%) (NIS shown in Table 1. Full species list and abundances in Appendix 1). Nine of the species classified as NIS were new records for the Madeira Archipelago.

The most frequent taxonomic groups amongst NIS were bryozoans (44%) and ascidians (31%), while other taxonomic groups contributed a smaller number of NIS, such as sponges and barnacles (13% and 6%, respectively). NIS diversity was higher after 44 and 50 months with 13 and 10 species, respectively. In addition, the sponge *Mycale senegalensis* and the ascidian *Distaplia corolla* were both detected after month 44 and have shown the highest abundances thereafter (Table 1 and Appendix 1). As well, certain species here categorized as cryptogenic will probably turn out to be NIS in future surveys once their identification is resolved (e.g. *Haliclona cf. indistincta, Celleporaria cf inaudita, Didemnum cf. perlucidum, Distaplia cf. bermudensis,* see Appendix 1).

Species richness and abundance of fouling communities colonizing settling plates at QLM changed significantly over the course of the study (Table 2, Figure 2). Quadric models of the relationship between diversity and colonization fit better than linear models (Table 2). Total species richness changed significantly over the course of colonization reaching maximum species richness at 12 months, followed by an apparent climax community until month 50, and dropping at month 74 (Figure 2A). In contrast, NIS diversity increased with time (Figure 2B) while native and cryptogenic richness displayed a near linear decrease after 24 months (Figure 2C-D). Species abundances showed very similar patterns to the ones of species richness (Figure 2E-H). All species produced maximum cover after one year of colonization, when communities reached an apparent equilibrium (Figure 2E). As with species richness, NIS cover significantly increased over the course of colonization (Figure 2F). Native species cover showed a similar pattern to the one

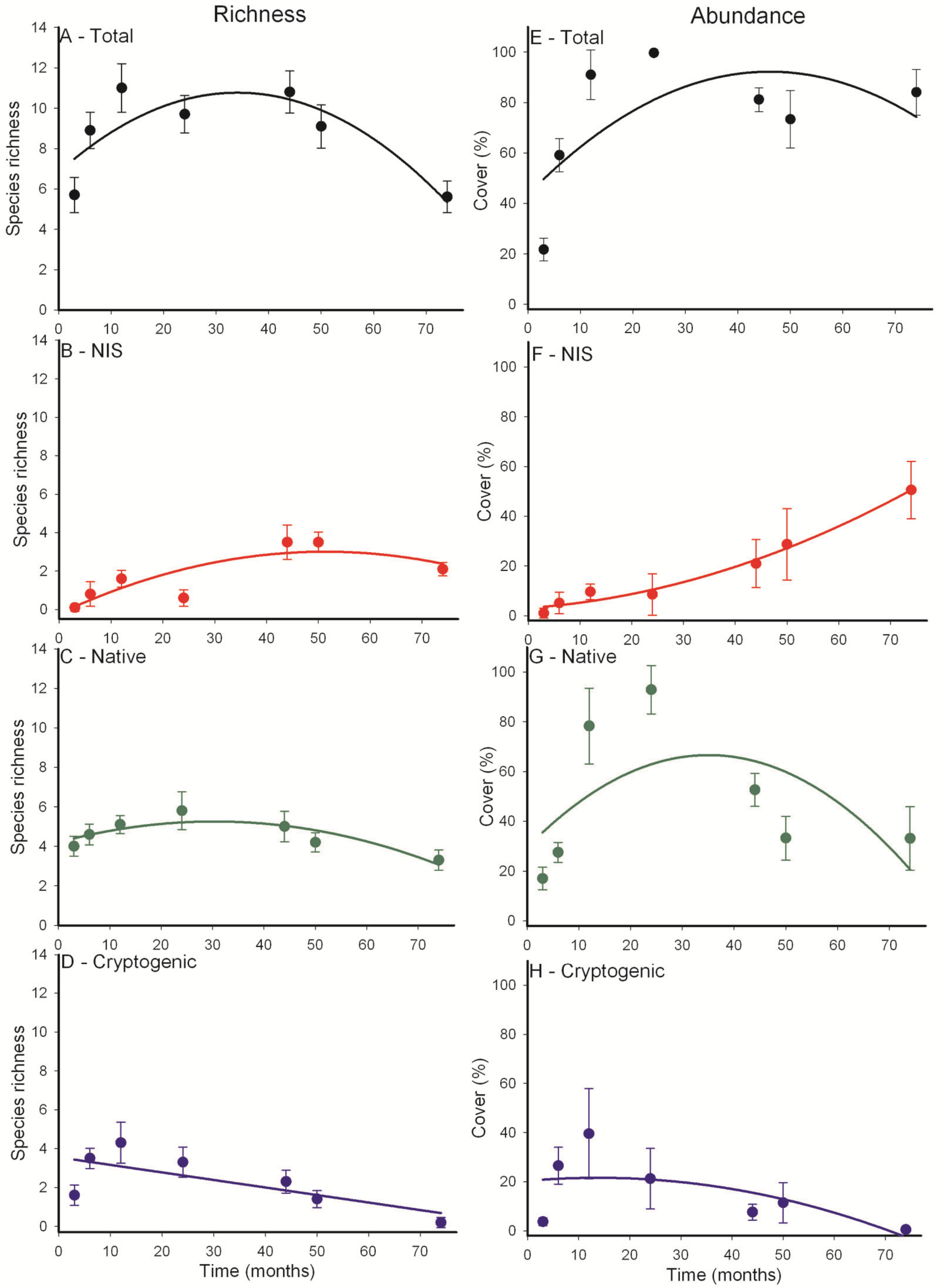

Figure 2. Relationship between species richness and colonization (panels A–D) and relationship between species abundances and colonization (panels E–H) at Quinta do Lorde Marina after 74 months of colonization. Means and 95% confidence intervals are indicated (n = 10).

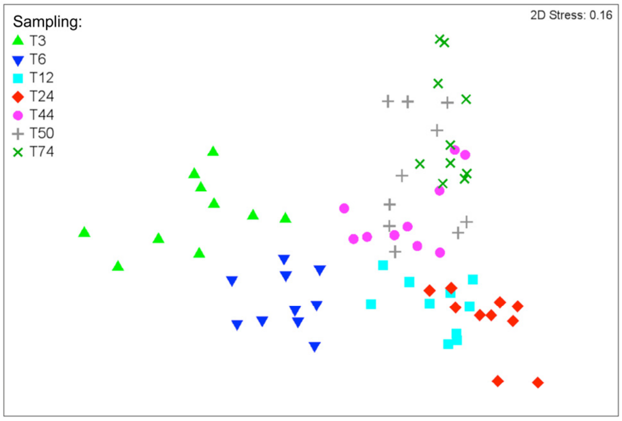

Figure 3. Multidimensional scaling (MDS) plot of the fouling communities colonizing PVC plates at different stages of the colonization process (T3 – 3 month old communities; T6 – 6 months; T12 – 12 months; T24 – 24 months; T44 – 44 months; T50 – 50 months; T74 – 74 months).

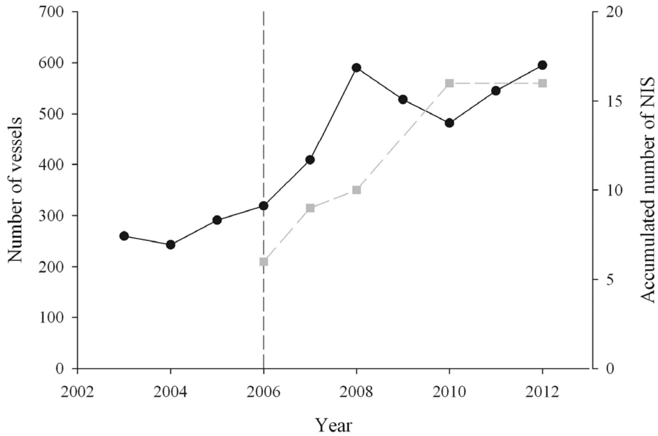

Figure 4. Number of vessels arriving at Quinta do Lorde Marina since its construction and accumulated number of NIS detected during the present study. Black line and black circles for number of vessel arrivals. Gray line and gray squares for accumulative number of NIS. Dashed line indicates the beginning of our survey.

displayed by total species cover with maximum cover after 24 months of colonization (Figure 2G). Finally, percent cover of species included in the cryptogenic category significantly decreased with time (Figure 2H).

Multivariate analysis showed clear differences in community composition between all colonization ages (ANOSIM: global R = 0.779, $p < 0.01$). Differences between young (T3) and older communities was very pronounced (Figure 3; ANOSIM: R = 0.99, p <0.01 for T3–T74; R = 0.94, p <0.01 for T3–T50). In contrast, communities at intermediate and late stages of colonization showed little difference in species composition (ANOSIM: R = 0.17, p <0.05 for T44–T50; R = 0.26, p <0.05 for T50–T74).

Finally, according to SIMPER routines, the invasive ascidian *Distaplia corolla* was essential in differentiating early colonization communities from late assemblages (Table 3). Accordingly, average abundances of *Distaplia corolla* increased over the colonization process and had a 20% positive contribution to dissimilarities between T3 and T50 communities and over 45% positive contribution to dissimilarities between T3 and T74 assemblages (Table 3).

The number of vessels arriving at Quinta do Lorde Marina increased after we commenced our work in 2006, from pre-2006 levels of 300 vessels or less, to vessel arrivals of more than 400 (and up to 600) per year (black line, Figure 4).

Similarly, we detected a significant positive relationship between the accumulative number of vessel arrivals and cumulative number of NIS during the survey period ($P > 0.05$; $R^2 = 0.76$).

Approximately 50% of vessels arriving every year at Quinta do Lorde Marina come from the neighboring island of Porto Santo. Other significant ports of origin were: Portugal mainland (6%); Canary Islands (6%); Mediterranean (5%);

Table 3. Results from the SIMPER routine performed with multivariate data from Quinta do Lorde Marina to identify which taxa contributed more (>10%) to observed changes in community composition between colonization ages. Contribution (%) and direction of change (+ positive; - negative) are indicated.

Compared colonization ages	Taxa	Status	Contribution
T3, T6	*Ceramium* sp.	U	26.9(+)
T3, T12	*Salmacina dysteri*	N	19.2(+)
	Lithophyllum incrustans	N	16.5(+)
	Lobophora variegate	N	12.0(+)
T3, T24	*Salmacina dysteri*	N	23.2(+)
	Sphacelaria rigidula	N	19.0(+)
	Lobophora variegate	N	15.2(+)
	Lithophyllum incrustans	N	12.3(+)
T3, T50	*Distaplia corolla*	NIS	20.3(+)
	Lithophyllum incrustans	N	17.2(+)
	Bugula dentata	NIS	11.7(+)
	Salmacina dysteri	N	10.7(+)
T3, T74	*Distaplia corolla*	NIS	46.2(+)
	Lobophora variegate	N	11.5(+)
	Lithophyllum incrustans	N	10.8(+)
T12, T24	*Sphacelaria rigidula*	N	19.9(+)
	Salmacina dysteri	N	13.9(+)
	Lithophyllum incrustans	N	10.7(-)
T12, T50	*Salmacina dysteri*	N	14.8(-)
	Distaplia corolla	NIS	13.4(+)
	Lithophyllum incrustans	N	10.8(-)
T12, T74	*Distaplia corolla*	NIS	28.4(+)
	Salmacina dysteri	N	13.4(-)
	Lithophyllum incrustans	N	10.1(-)
T24, T50	*Sphacelaria rigidula*	N	18.5(-)
	Salmacina dysteri	N	17.2(-)
	Lobophora variegate	N	11.1(-)
	Distaplia corolla	NIS	10.6(+)
T24, T74	*Distaplia corolla*	NIS	23.7(+)
	Salmacina dysteri	N	17.4(-)
	Sphacelaria rigidula	N	16.8(-)
T50, T74	*Distaplia corolla*	NIS	36.5(+)
	Lithophyllum incrustans	N	12.6(-)
	Lobophora variegate	N	11.9(+)
	Bugula dentata	NIS	10.8(-)

and Northern Europe (4%) (Figure 5A). Occasionally, vessels from different biogeographic regions arrived, namely from North Africa (2%) and the Caribbean (<1%). In comparison, most boats that arrive in Porto Santo Marina come from Portugal mainland (41%), Northern Europe (9%) and Mediterranean (9%), with a few entries from North Africa (2%) and the Canary Islands (2%) (Figure 5B).

Discussion

Although a few new records of fouling NIS have been reported for Portugal (continental and islands) during the last decade, such as the barnacle *Austrominius modestus* (O'riordan and Ramsay 1999), the ascidian *Styela clava* (Davis and Davis 2005), and the bryozoan *Zoobotryon verticillatum* (Amat and Tempera 2009; Wirtz and Canning-Clode 2009), little was known about the diversity and distribution of fouling NIS in this region. Although the data we present here were restricted to one single marina in the island of Madeira, the present work constitutes the first NIS inventory for the Madeira archipelago. During this study we found 16 NIS, of which 9 are new records to Madeira archipelago. Most of the NIS present in the marina were bryozoans, ascidians and sponges and they were detected after month 44. Indeed, several recent studies conducted in marinas and bays across the globe have found ascidians and bryozoans among the most abundant organisms colonizing settling plates (e.g. Stachowicz et al. 2002; Valdivia et al. 2005; Sugden et al. 2008; Canning-Clode et al. 2011; Crooks et al. 2011). Both species richness and abundance changed during the course of colonization of settling plates in QLM. The relationship between the colonization and total richness (and total abundance to some extent) displayed a unimodal pattern with maximum diversity at middle stages of colonization. Moreover, while the number of native species seemed to decrease with colonization time, the number (and abundance) of NIS increased. These findings seem to be consistent with other studies focusing invasions in fouling assemblages. For example, in a study to assess the effects of diversity on the invasion of sea squirts in a fouling community, Stachowicz et al. (2002) observed a decline in native diversity as the abundance of invaders increased over time.

The number of NIS detections in the marina was not independent from vessel traffic; there is a positive relationship between accumulative number of NIS and accumulative vessel traffic. The majority of vessels arriving at QLM came from the neighboring island of Porto Santo; a significant portion of arrivals in both marinas (Quinta do Lorde and Porto Santo) came from Portugal mainland, Mediterranean and Northern Europe. This fact may suggest that most NIS detected in this study were likely secondary or tertiary introductions. That is, Madeira is receiving species introduced earlier to other European ports, and is not (at this time) a site for novel invasions of the European theatre. The resulting introduction of NIS into marinas via secondary introduction has been recently detected in different biogeographic regions (e.g. Johnson et al. 2001; Floerl and Inglis 2005; Ashton et al. 2006; Clarke Murray et al. 2012; Minchin et al. 2013). Therefore, recreational boating may play a key role in the secondary spread of marine NIS (Ashton et al. 2006; Clarke Murray et al. 2011).

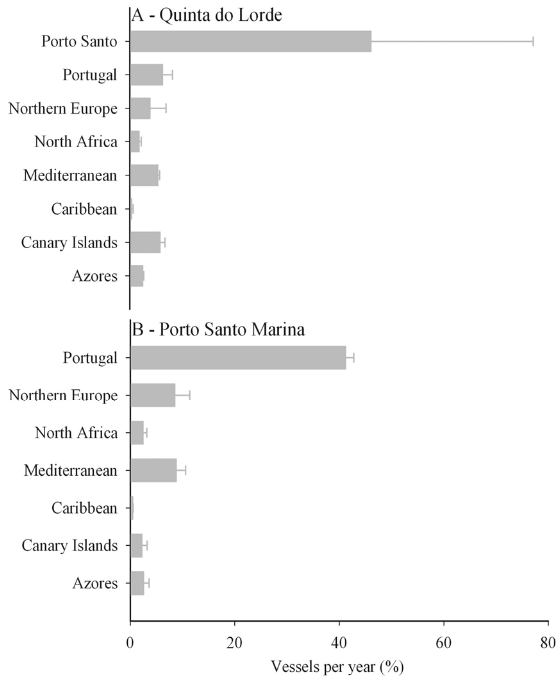

Figure 5. Percentage of yearly vessel arrivals from different biogeographic regions at Quinta do Lorde Marina (A) during 2008 and 2011 and at Porto Santo Marina (B) during the same period. Means and standard deviations are indicated.

Finally, due to budget restrictions we conducted this survey in only one marina. One marina is unlikely to be representative of the full NIS diversity in the archipelago. For this reason, future studies should incorporate other marinas, as well as other habitats, on these islands. We believe additional surveys in other marinas and habitats (e.g. pontoon, dock) would result in other marine NIS detections.

Acknowledgements

We thank Quinta do Lorde Marina for allowing us to perform this survey and particularly Cátia Carvalho for logistic support. We thank Xavier Turon, Rolando Bastida-Zavala, Sara Ferreira, Ana Neto, Konstantinos Tsiamis, Joana Xavier, Francis Kerckhof and Peter Wirtz for species identifications. We thank three anonymous reviewers for helpful criticism and suggestions that significantly improved the early version of this manuscript. João Canning-Clode holds a FCT post-doctoral grant (SFRH/BPD/75775/2011). The service charges for this open access publication have been covered by "LARSyS Associated Laboratory" through FCT/MCE project PEst-OE/EEI/LA0009/2013. This is contribution number 20 from Marine Biology Station of Funchal.

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Supplementary material

The following supplementary material is available for this article:

Appendix 1. List of macroalgae and macroinvertebrates found in Quinta do Lorde Marina (QLM) from 2006 to 2012.

This material is available as part of online article from:
http://www.aquaticinvasions.net/2013/Supplements/AI_2013_CanningClode_etal_Supplement.pdf