



Winners and losers: prevalence of non-indigenous species under simulated marine heatwaves and high propagule pressure

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ABSTRACT: Global warming is increasing the frequency, duration and intensity of extreme events such as marine heat waves (MHWs). The effects of MHWs include a variety of negative environmental impacts. Extreme weather events can interact with other environmental stressors such as invasion by marine non-indigenous species (NIS). The aim of this study was to (1) compare the responses of fouling assemblages recruited within a harbour (highly invaded) with the responses of those recruited in natural habitats (not invaded) to simulated MHWs of different temperatures and durations, and (2) evaluate the legacy effects of those MHWs on the invasibility of both types of assemblage by deploying them in a marina environment (high NIS propagule pressure). Experimental assemblages were sampled after 5 or 10 d of exposure to 1 of 3 different temperature conditions to examine the effects of varying MHW conditions. Later those assemblages were deployed inside a marina facility to test the invasibility of heat-stressed assemblages. The results revealed that higher temperatures and longer MHWs had an overall negative impact on both native and non-indigenous assemblages. Shorter MHWs had greater effects on assemblages dominated by NIS, while longer MHWs affected native species more. Increasing MHW duration promoted increased variability in the resulting invasive assemblages. Winner and loser species and homogenisation could potentially alter the legacy effects of MHWs on the pattern of NIS recruitment. This study highlights the importance of interactions between environmental stressors to the conservation of coastal communities, crucial ecosystems on oceanic islands.

KEY WORDS: Climate change · Environmental stressors · Biological invasions · Madeira island · Mesocosms

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1. INTRODUCTION

Several anthropogenic activities are causing environmental changes with impacts on global biodiversity and ecosystems, climate change and biological invasions being examples of those threats (Thuiller

2007, Costello et al. 2010). Climate change (a wide term), includes several ocean environmental modifications, such as temperature changes, ocean acidification, sea level rise and variation in ocean stratification, upwellings, currents and weather patterns (Costello et al. 2010, IPCC 2019). Climate change's

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most immediate biological effects are related to species distribution shifts and consequent structural changes in communities (Hampe & Petit 2005). Temperature differences induce modifications in species distribution limits through the displacement of temperate by tropical species (Vergés et al. 2014). The increase in the proportion of warm-water species in temperate or subtropical regions is referred to as tropicalisation (Bianchi & Morri 2003). This phenomenon has been observed globally across several marine areas (Jansen et al. 2007, Sorte et al. 2010a, Canning-Clode et al. 2011, Canning-Clode & Carlton 2017).

Discrete weather events associated with global warming trends are increasing in frequency (IPCC 2013, Coumou et al. 2013), namely floods and droughts (Fabricius et al. 2014, Salvador et al. 2020), storms (Dayton & Tegner 1984, De'ath et al. 2012), cold waves (Canning-Clode et al. 2011, Firth et al. 2011) and heat waves (Garrahou et al. 2009, Wernberg et al. 2013).

In this context, the global average frequency and duration of marine heat waves (MHWs), defined as discrete periods of anomalously high ocean temperatures (Hobday et al. 2016), will rise under continuous global warming (Oliver et al. 2018). The increased severity of MHWs can have consequences for global biodiversity and the structure and functioning of marine ecosystems at a global scale (Oliver et al. 2018, Filbee-Dexter et al. 2020, Smale 2020). Such deleterious effects have corresponding impacts at the community level (Thomson et al. 2015), through the loss of critical foundation species (e.g. seagrass meadows or kelp forests; Wernberg et al. 2016). Moreover, at regional and local scales, MHW events can cause economic losses through impacts on fisheries and aquaculture (Smale et al. 2019).

Parallel with climate change, in the last 2 decades there has been a large rise in biological invasions, with an increase in the number of species (plants and animals) introduced into ecosystems far from their native range (Hewitt 2003, Costello et al. 2010). These introductions are also leading to changes in marine and estuarine communities, altering populations and ecosystem processes (Ruiz et al. 1997) and are recognised as major drivers of global environmental change (Vitousek et al. 1997). Several human-assisted vectors can transport non-indigenous species (NIS) from their native range to other locations (e.g. shipping, aquaculture, live bait, the aquarium trade; Chainho et al. 2015). In the marine system, shipping has been identified as the major pathway for NIS introductions, with ballast water and vessel biofouling from cargo shipping as main vectors of transfer (i.e. accumulation of sessile organisms; Ruiz

et al. 2000, Bailey et al. 2020, Castro et al. 2020). Artificial substrates within harbours and marinas have been identified as key habitats for NIS and are now the focus of numerous monitoring and assessment programmes (Epstein & Smale 2018, Ferrario et al. 2020).

The awareness and study of marine invasions in Madeira is very recent, with only a limited number of NIS documented during the 1990s (e.g. Wirtz 1995). Expanded knowledge and rising numbers of NIS prompted the creation of a dedicated research group in the archipelago, with several ongoing monitoring surveys and experimental studies focused mostly on fouling communities (e.g. Canning-Clode et al. 2013, Gestoso et al. 2019, Ferrario et al. 2020). Consequently, several new NIS have recently been detected and inventoried in the Madeira archipelago (e.g. Canning-Clode et al. 2013, Ramalhosa et al. 2014, 2017, 2019, Souto et al. 2018). These findings have provided vital baseline knowledge to promote further studies in invasion ecology, using fouling communities as model systems for the development of experimental studies to uncover the mechanisms and biotic interactions underlying the invasion process (e.g. Gestoso et al. 2017, 2018, Ferrario et al. 2020). Although some experimental work has been performed in Madeira regarding the tolerance of sessile fouling assemblages to chemical and physical disturbance (Ramalhosa et al. 2019, Ferrario et al. 2020) or biotic resistance and interactions with invaders (Gestoso et al. 2017, Parretti et al. 2021), little is known about the behaviour of NIS in this semi-tropical archipelago under current and future scenarios of climate change. The present work is, therefore, essential to provide answers about current invasions and climate change, especially in an insular ecosystem context.

Every hard substrate in the marine system, including artificial habitats (e.g. piers, pontoons, pilings, seawalls and buoys) is subjected to the process of biofouling over time (Wahl 1997, Dürr & Thomason 2010, Ferrario et al. 2020). Hull fouling transports numerous encrusting sessile organisms (sponges, hydrozoans, polychaetes, crustaceans, bivalves, bryozoans, tunicates and algae) across the globe (Canning-Clode 2015). Sessile fouling assemblages in natural habitats are likely to be dominated by native species, in contrast to the greater abundance of NIS in assemblages recruited in artificial habitats (Ferrario et al. 2020). After colonisation, these communities are potentially vulnerable to environmental changes associated with climate change because they comprise mainly sessile organisms (e.g. sponges,

bryozoans), which are restricted in their ability to directly escape unfavourable conditions (Przeslawski et al. 2008). These sessile assemblages have been extensively used for macroecological studies: their fast growth and ease of settlement on artificial substrates means they represent opportune study systems for ecologists and are therefore often used to investigate ecological models (e.g. Svensson et al. 2007, Canning-Clode et al. 2009, Marraffini et al. 2017, Cacabelos et al. 2020).

Moreover, a few studies suggest that NIS have higher fitness (e.g. higher thermic tolerance) under climate change conditions (Sorte et al. 2010b, Duarte et al. 2016), but some particularities regarding the variability in resilience of biological communities are still poorly understood, under recent and evolving events such as MHWs (Filbee-Dexter et al. 2020).

About 60% of climate-related research publications in the marine realm are focused on independent effects of temperature (Harley et al. 2006). Understanding of the additive, synergistic and antagonistic effects of multiple stressors is limited (see e.g. Vaz-Pinto et al. 2013, Gestoso et al. 2016), although these interactions represent key issues in the conservation and management of marine coastal communities (Côté et al. 2016). One particular aspect that stills needs special attention is the concept of invasibility, i.e. the susceptibility of a system to invasion by a new species (Lonsdale 1999, Richardson et al. 2011), especially in a global change context (e.g. Aronson et al. 2007, Vaz-Pinto et al. 2013). Some authors have reported enhanced invasion success of NIS under climate change conditions, due to their greater competitive ability and survival rate (Byers et al. 2002, Sorte et al. 2010b). For example, in experiments with 2 species of mussels, Gestoso et al. (2016) showed that the invasive species was more resilient to environmental stressors than the native species, although individuals' responses were modulated by the composition of the population. Invasibility is particularly relevant for insular species that are usually more prone to invasions, due to their lack of competitors and vulnerability to subsequent impacts (Stachowicz et al. 2002).

In this context, the current study was designed to (1) compare the responses of fouling assemblages recruited within a harbour (defined here as a highly invaded environment) versus fouling assemblages recruited in natural habitats (defined here as a low invasion environment) to a simulated gradient of MHWs of different temperatures and durations, and (2) evaluate the legacy effects of those MHWs on the invasibility of both sessile assemblages (invaded and native) by deploying them in a harbour habitat (i.e.

exposed to high NIS propagule pressure). It is hypothesised that NIS assemblages will possess higher tolerance and be more resistant to more extreme MHWs (longer and warmer). Moreover, it is predicted that after being subjected to MHWs, the native assemblages will be more predisposed to invasions than NIS assemblages.

2. MATERIALS AND METHODS

This experimental study took place in 3 stages (Fig. 1): Phase 1 — deployment of experimental structures inside and outside a marina for the recruitment of different assemblages (native and non-native); Phase 2 — conducting mesocosm experiments using the recruited assemblages (Phase 1), exposing them to MHWs of different temperatures and durations; Phase 3 — placing the native and non-native assemblages previously exposed to MHWs inside the marina (high NIS propagule pressure) for a field test of invasibility.

2.1. Phase 1 — Study area and assemblage recruitment

This study was conducted in Quinta do Lorde Marina in the southeast part of Madeira Island (Portugal; 32° 44.5' N, 16° 42.8' W; Fig. 1). Following the methodology applied by Ferrario et al. (2020), 60 polyvinylchloride (PVC) plates (14 × 14 × 0.3 cm) were installed at 5 m depth; 30 plates inside the marina and 30 plates along the natural rocky shore outside it (Fig. 1; see also Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m668p021_supp.pdf). The marina is a high NIS propagule pressure area and is known to host higher numbers of NIS than the surrounding natural areas (Canning-Clode et al. 2013, Ramalhosa et al. 2019, Ferrario et al. 2020); therefore, the inside and outside locations were selected to recruit assemblages dominated by NIS and native species, respectively. After 6 mo submerged (from 5 August 2018 to 5 February 2019), all experimental units were retrieved and brought back to the laboratory for sampling as detailed below and in Fig. S1.

Dissolved oxygen, pH, salinity and water temperature were measured once per week inside the marina and in the outside area using a multiparameter probe (YSI Professional Plus) to detect any anomalies during the experiment (recruitment stage and mesocosm experiment).

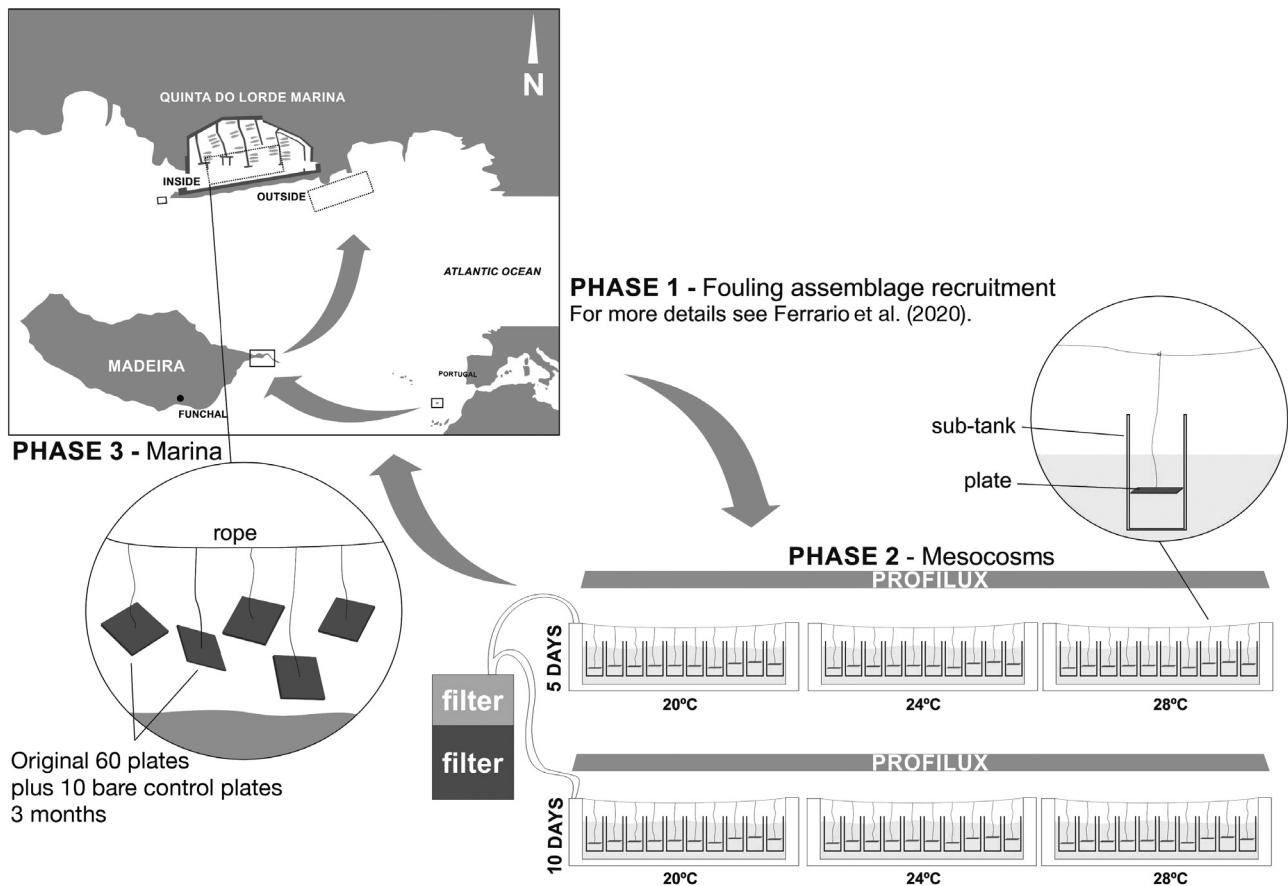


Fig. 1. Map of Madeira archipelago showing the location of Quinta do Lorde Marina, where the present study was carried out, and a diagram showing the different phases of the experimental layout (Phase 1—Assemblage recruitment; Phase 2—Mesocosm experiment (simulated marine heat waves, MHWs); Phase 3—Field test of invasibility: MHW legacy effects)

2.2. Phase 2—Mesocosm experiment

An experiment was designed to examine the response of the 2 fouling assemblages (i.e. non-indigenous vs. native assemblages) to 2 types of MHW that differed in duration (short: 5 d vs. long: 10 d) and temperature (ambient: 20°C, moderate: 24°C and extreme: 28°C). The duration of the MHWs was based on Hobday et al. (2016), where the shortest MHW was 5 d. For the present study, it was assumed that 10 d would represent a long MHW, although we recognise that in nature MHWs may last much longer (max. duration observed by Hobday et al. 2016 = 95 d). The selected temperatures were aligned with the predictions of the IPCC (2013) and followed the definition of MHW proposed by Hobday et al. 2016, i.e. seawater temperatures exceeding a seasonally-varying threshold (usually the 90th percentile) for at least 5 consecutive days. The chosen temperatures were based on findings by Schäfer et al. (2019) and unpublished data from the MARE (Marine and Environmental Re-

search Centre)-Madeira databases, recording seawater warming anomalies of ~4°C for Madeira. Accordingly, moderate MHW temperature was established as 24°C, i.e. 4°C above the ambient temperature of 20°C registered at the time of the experiment, and extreme MHW was established as 28°C to simulate a maximum warming peak during summer, i.e. 4°C above the ~24°C maximum summer sea surface temperature in Madeira (Martins et al. 2007).

This experiment was conducted inside the mesocosm system in the laboratory facilities of the Madeira research unit of MARE, located at Quinta do Lorde Marina. For the experimental setup, 6 mesocosm tanks (350 l each) were used to simulate the 3 different temperature regimes of 20, 24 and 28°C. The 2 tanks per each temperature regime were assigned to different MHW durations: short (5 d) vs. long (10 d) (Fig. 1). Seawater (filtered: 10 µm) was pumped into the system in a continuous flow of about 30 ml s⁻¹, resulting in complete water turnover every 4 h. The use of filtered seawater excluded the possibility of

new recruits, so that hypotheses were tested on only the original fouling assemblages. The 60 PVC plates from inside and outside the marina were distributed randomly in groups of 10 (5 plates from inside and 5 from outside) per mesocosm tank. Those tanks were used as water baths in which the PVC plates were held independently inside 10 l sub-tanks (Fig. S2).

After seawater catchment and filtration, and before filling the mesocosm tanks, seawater temperature was increased using three 200 l pre-heating tanks with aquarium heaters (Schego 600). Each mesocosm tank was equipped with an aquarium controller (Profilux 4, 5.1-D-PAB powerbars; GHL Advanced Technology) that automatically regulated seawater temperature. Additionally, constant aeration systems were placed in each sub-tank and artificial light was provided by 4 LED bars (eco+ LED bar REEF 11000K, 80 cm) mounted above each mesocosm tank, with a photoperiod of 12:12 h (light:dark). The experiment lasted 13 d and was divided into 2 sampling times corresponding to the end of short and long duration MHWs. After the retrieved PVC plates were sampled (see details in Section 2.4), they were placed in the mesocosm system. After an acclimation period of 3 d at ambient temperature (20°C), the temperature regime was established (20, 24 and 28°C), at a rate of 1°C h⁻¹, representing a heat peak (Hobday et al. 2016) lasting 5 and 10 d for the short and long MHW durations respectively (from February 6 to 18, 2019; Fig. S2). At the end of each phase, the fouling assemblages were sampled (see details in Section 2.4.)

During the experiment, to detect any deviance in physical–chemical parameters, the dissolved oxygen, pH, salinity and water temperature were assessed twice per day in 3 randomly selected sub-tanks per mesocosm tank using a multiparameter probe (YSI Professional Plus).

2.3. Phase 3 – Field test of invasibility: MHW legacy effects

After the mesocosm experimental manipulation, all PVC plates were translocated to inside the marina area (i.e. high NIS propagule pressure) to evaluate the invasibility of previously heat-stressed assemblages. For that, the 60 PVC plates with fouling assemblages (from Phase 2) and an additional 10 bare PVC plates used as controls were deployed randomly facing downwards using a rope attached to the marina pontoons at 1 m depth (Figs. 1 & S3). After 3 mo, all PVC plates were re-sampled. To avoid over-estimation of invasibility in the field test due to the

presence of NIS recruited in Phase 1, only NIS detected in Phase 3 on control plates were considered for this analysis.

2.4. Sampling procedure

All fouling species were identified under a stereomicroscope (Leica S8APO) using taxonomic keys in the existing literature (e.g. Hayward & Ryland 1995, Souto et al. 2015, 2018), or by consulting taxonomic experts. Their biogeographic status was assigned (native, cryptogenic, NIS or unresolved) based on known databases or the scientific literature (e.g. Canning-Clode et al. 2013, Chainho et al. 2015, Gestoso et al. 2017, Ramalhosa et al. 2019, Ferrario et al. 2020). All plates were photographed using an Olympus Stylus TG-5 camera and images were subsequently analysed with CPCe software (Coral Point Count with Excel extensions; Kohler & Gill 2006) to assess the abundance (in percentage cover) of the fouling species by considering 99 random points on each plate. This stratified random sampling method divides the photograph into a 3 × 3 grid of 9 cells, with 11 random points per cell, resulting in 99 points analysed per image, with points distributed equally to each image region (Kohler & Gill 2006).

In Phase 1, only the abundance and assemblage structure of the fouling assemblages were examined. For Phase 2, the sampling method was similar; however, the health status of each species was assessed using the semi-quantitative index developed by Ferrario et al. (2020). Accordingly, and to evaluate the effects of MHWs on the fouling assemblages, for each species the stress effect index (SEI) was calculated as follows:

$$SEI = \frac{(\% \text{ cover before MHW}) - (\% \text{ cover after MHW})}{(\% \text{ cover before MHW})} \quad (1)$$

Sampling for Phase 3 was similar to Phase 1. It was first performed for the PVC plates originally subjected to short MHWs (along with 5 control PVC plates), then 5 d later for those subjected to long MHWs (plus the 5 remaining control PVC plates). This way all plates were subjected to the same 3 mo period of NIS recruitment.

2.5. Data analysis

To be conservative, cryptogenic, native and unresolved species were included in the same category for all analyses (Marchini et al. 2015) as native spe-

cies. For Phase 1, to detect variation in the structure and composition of fouling assemblages (native and non-indigenous) a 1-way permutational multivariate analysis of variance (PERMANOVA; Anderson 2005) was performed to verify differences between the recruited fouling assemblages according to their origin, with location as a unique factor (2 levels: inside and outside, fixed, $n = 30$). The PERMANOVA was conducted with unrestricted permutations of the raw data. A principal coordinate ordination (PCO; Clarke et al. 2006) was used to visualise variation in the assemblages (% cover). When significant differences were detected for the analysed factor, a SIMPER (similarity percentages) procedure was conducted to assess which species contributed most to the similarity or dissimilarity within and between the different levels of that factor, respectively (cut-off level 90%; only species with a percentage contribution of $\geq 2\%$ were reported; Clarke 1993).

In Phase 2, several attributes of the fouling assemblages were calculated for each species based on SEI: species richness loss, total cover loss (%), and Shannon-Wiener diversity index loss. Uni- and multivariate PERMANOVA analyses were performed separately for each of the 3 computed attributes and fouling assemblages, respectively. The design employed for these analyses included 3 orthogonal factors: location (2 levels: inside and outside, fixed), MHW (2 levels: short and long, fixed) and temperature (3 levels: 20, 24 and 28°C, fixed) ($n = 5$). PCO was used to visualise variations in species percentage cover (%) across assemblages. SIMPER analysis was carried out to detect which taxa contributed most to similarity within and dissimilarity among significant factors for the analysis of the assemblages as a whole. The PERMANOVA main test and pairwise tests were conducted with unrestricted permutations of the raw data.

To investigate the legacy effects of MHWs on the invasibility of fouling assemblages (Phase 3), similar uni- and multivariate PERMANOVA, PCO and SIMPER analyses were performed, using the same design (with control plates as an additional location level) and attributes (in this case number of NIS species recruited, total cover of NIS recruited (%) and Shannon-Wiener index considering only NIS organisms).

All multivariate tests were conducted on square root transformed data (except for Phase 2 when the fourth root transformation was used), using Bray-Curtis similarity as the resemblance measure, while for univariate analyses the Euclidean distance was used as a resemblance measure (Clarke et al. 2006).

Prior to multivariate analysis, PERMDISP was used to check the heterogeneity of data dispersion to determine differences in multivariate group dispersion. The software PRIMER v6 with the PERMANOVA+ add-on was used for all data analyses (Clarke & Gorley 2006, Anderson et al. 2008).

3. RESULTS

3.1. Phase 1 — Assemblage recruitment

After 6 mo of colonisation, the 60 PVC plates collected a total of 76 taxonomic entities: 47 identified to the species level and 29 in higher taxonomic groups. Outside the marina, 60 different taxa were observed and inside the marina, 53 taxa were observed, with 11 and 13 classified as NIS, respectively (Table 1). The main taxonomic groups inside the marina were bryozoans (28%) and ascidians (21%), while bryozoans (32%) and cnidarians (17%) dominated the outside assemblage (Table 1).

Multivariate analysis of the structure of the 2 fouling assemblages showed significant differences between the 2 locations (PERMANOVA: pseudo- $F_{1,59} = 31.945$, $P(\text{perm}) = 0.001$, PERMDISP- $P(\text{perm}) = 0.761$). The exploration of the PCO ordination indicated that the first 2 axes explained almost 50% of the total variation in assemblage structure, with PCO1 accumulating 39% of the total variation and indicating a distinct pattern for the assemblages recruited inside the marina vs. those recruited outside (Fig. 2A). The overlapped vectors showed that fouling assemblages recruited inside the marina had a higher abundance of NIS. On the other hand, the outside assemblage had a higher contribution of native and cryptogenic species (Fig. 2A). The SIMPER analysis indicated an average dissimilarity of 73.16% between locations, and the species *Spirobranchus triqueter* (Linnaeus, 1758), *Parasmittina alba* Ramalho, Muricy & Taylor, 2011, *Celleporaria inaudita* Tilbrook, Hayward & Gordon, 2001 and *Cribrilaria radiata* (Moll, 1803) were the taxa that contributed most to these differences (21.83% accumulated average dissimilarity; Table S1). Specifically, *P. alba* (20.21%) (NIS), bare space (18.23%) and *C. inaudita* (11.00%) (NIS) contributed to most of the similarity inside the marina, whereas for the outside habitat the sessile assemblage was dominated by *S. triqueter* (15.64%), bare space (11.20%) and *P. alba* (10.41%) (NIS; Table S1). In contrast, seawater parameters were rather similar inside and outside the marina (Table S2).

Table 1. List of species recorded on PVC plates in Phase 1 (inside and outside the marina of Quinta do Lorde), including frequency of occurrence (% of plates) and biogeographic status (native [N], non-indigenous species [NIS], cryptogenic [C] or unresolved [U] due to inability to identify to species level), with the respective reference for that status determination

| Taxon | Status | Frequency of occurrence (%) | | Reference |
|---|--------|-----------------------------|---------|--|
| | | Inside | Outside | |
| Chlorophyta | | | | |
| Chlorophyta n.i. | U | 3.33 | 3.33 | |
| <i>Valonia</i> sp. C. Agardh, 1823 | U | 6.67 | | |
| Ochrophyta | | | | |
| <i>Colpomenia</i> sp. (Endlicher) Derbès & Solier, 1851 | U | | 13.33 | |
| <i>Dictyota</i> sp. J.V. Lamouroux, 1809 | U | | 66.67 | |
| <i>Lobophora</i> sp. J. Agardh, 1894 | U | 36.67 | 66.67 | |
| <i>Sargassum</i> sp. C. Agardh, 1820 | U | | 3.33 | |
| Rhodophyta | | | | |
| <i>Jania</i> sp. J.V. Lamouroux, 1812 | U | 3.33 | 20.00 | |
| <i>Lithophyllum incrustans</i> Philippi, 1837 | N | 63.33 | 93.33 | Canning-Clode et al. (2013) |
| <i>Polysiphonia</i> sp. Greville, 1823 | U | 66.67 | 10.00 | |
| Foraminifera | | | | |
| <i>Miniacina miniacea</i> (Pallas, 1766) | C | | 26.67 | George (2014) |
| Porifera | | | | |
| <i>Clathrina clathrus</i> (Schmidt, 1864) | N | | 36.67 | Ramalhos et al. (2019) |
| <i>Mycale (Carmia) senegalensis</i> Lévi, 1952 | NIS | 43.33 | 20.00 | Canning-Clode et al. (2013), Ramalhos et al. (2019) |
| <i>Mycale</i> sp. morphotype 1 Gray, 1867 | U | 6.67 | 53.33 | |
| <i>Mycale</i> sp. morphotype 2 Gray, 1867 | U | 23.33 | 30.00 | |
| <i>Paraleucilla magna</i> Klautau, Monteiro & Borojevic, 2004 | NIS | 20.00 | 50.00 | Canning-Clode et al. (2013) |
| Sponge morphotype 1 | U | 56.67 | 10.00 | |
| Sponge morphotype, 2 | U | 10.00 | 6.67 | |
| <i>Sycon</i> sp. Risso, 1827 | U | 53.33 | 50.00 | |
| Cnidarians | | | | |
| <i>Aglaophenia pluma</i> (Linnaeus, 1758) | C | 3.33 | 10.00 | Wirtz (2007) |
| <i>Clytia hemisphaerica</i> (Linnaeus, 1767) | C | | 13.33 | Wirtz (2007) |
| <i>Ectopleura crocea</i> (Agassiz, 1862) | NIS | | 10.00 | Chainho et al. (2015) |
| <i>Ectopleura</i> sp. L. Agassiz, 1862 | U | 3.33 | 53.33 | |
| <i>Exaiptasia diaphana</i> (Rapp, 1829) | NIS | 56.67 | 13.33 | Canning-Clode et al. (2013) |
| <i>Kirchenpaueria halecioides</i> (Alder, 1859) | C | 6.67 | 6.67 | Wirtz (2007), Ramalhos et al. (2019) |
| <i>Obelia</i> sp. morphotype 1 Péron & Lesueur, 1810 | U | 3.33 | 6.67 | |
| <i>Obelia</i> sp. morphotype 2 Péron & Lesueur, 1810 | U | 6.67 | | |
| <i>Pennaria disticha</i> Goldfuss, 1820 | C | | 6.67 | Wirtz (2007) |
| <i>Sertularella</i> sp. morphotype 1 Gray, 1848 | | | 23.33 | |
| <i>Sertularella</i> sp. morphotype 2 Gray, 1848 | | | 53.33 | |
| Entoprocta | | | | |
| <i>Barentsia discreta</i> (Busk, 1886) | C | 3.33 | 6.67 | Ramalhos et al. (2019) |
| Echinoderms | | | | |
| <i>Arbacia lixula</i> (Linnaeus, 1758) | N | 3.33 | | Canning-Clode & Carlton (2017) |
| Annelida | | | | |
| <i>Branchiomma bairdi</i> (McIntosh, 1885) | NIS | 6.67 | | Ramalhos et al. (2014) |
| <i>Sabellidae</i> sp. Latreille, 1825 | U | 10.00 | 3.33 | |
| <i>Salmacina dysteri</i> (Huxley, 1855) | N | 53.33 | 70.00 | Ramalhos et al. (2019) |
| <i>Spirobranchus triqueter</i> (Linnaeus, 1758) | N | 86.67 | 100.00 | Ramalhos et al. (2019) |
| <i>Spirorbis</i> sp. Daudin, 1800 | U | 96.67 | | |
| Bryozoans | | | | |
| <i>Aetea</i> sp. Lamouroux, 1812 | U | 6.67 | 46.67 | |
| <i>Beania maxilladentata</i> Ramalho, Muricy & Taylor, 2010 | NIS | | 46.67 | Souto et al. (2015) |
| Bryozoan morphotype 1 | U | | 3.33 | |
| Bryozoan morphotype, 2 | U | | 6.67 | |
| <i>Bugulina simplex</i> (Hincks, 1886) | NIS | 40.00 | | Ramalhos et al. (2017) |
| <i>Celleporaria inaudita</i> Tilbrook, Hayward & Gordon, 2001 | NIS | 86.67 | 3.33 | Souto et al. (2018), Canning-Clode et al. (2013) |
| <i>Cradoscrupocellaria bertholletii</i> (Audouin, 1826) | NIS | 53.33 | | |

(continued on next page)

Table 1 (continued)

| Taxon | Status | Frequency of occurrence (%) | | Reference |
|--|--------|-----------------------------|---------|--|
| | | Inside | Outside | |
| <i>Cribrilaria radiata</i> (Moll, 1803) | C | | 93.33 | Waters (1899), Ferrario et al. (2020) |
| <i>Crisia denticulata</i> (Lamarck, 1816) | C | 6.67 | | Gestoso et al. (2017) |
| <i>Crisia</i> sp. Lamouroux, 1812 | U | 30.00 | 30.00 | |
| <i>Favosipora purpurea</i> Souto, Kaufmann & Canning-Clode, 2015 | N | 3.33 | 93.33 | Souto et al. (2015) |
| <i>Hemicyclopora multispinata</i> (Busk, 1861) | C | | 13.33 | Norman (1909) |
| <i>Hippoporella maderensis</i> Souto, Reverter-Gil & Ostrovsky, 2014 | C | | 3.33 | Souto et al. (2014) |
| <i>Mollia</i> sp. Lamouroux, 1816 | U | 3.33 | 3.33 | |
| <i>Nolella gigantea</i> (Busk, 1856) | C | 30.00 | | Ramalhos et al. (2019) |
| <i>Parasmittina alba</i> Ramalho, Muricy & Taylor, 2011 | NIS | 90.00 | 83.33 | Souto et al. (2018) |
| <i>Parasmittina</i> sp. morphotype 1 Osburn, 1952 | U | | 13.33 | |
| <i>Parasmittina</i> sp. morphotype 2 Osburn, 1952 | U | 3.33 | | |
| <i>Reptadeonella violacea</i> (Johnston, 1847) | C | | 6.67 | Norman (1909) |
| <i>Schizoporella errata</i> (Waters, 1878) | C | 6.67 | 6.67 | Ramalhos et al. (2019) |
| <i>Schizoporella pungens</i> Canu & Bassler, 1928 | NIS | 3.33 | 63.33 | Canning-Clode et al. (2013) |
| <i>Schizoporella unicornis</i> (Johnston in Wood, 1844) | C | | 60.00 | Norman 1909), Souto et al. (2015) |
| <i>Scrupocaberea maderensis</i> (Busk, 1860) | N | | 73.33 | Norman 1909), Souto et al. (2015) |
| <i>Tubulipora</i> sp. Lamarck, 1816 | U | 3.33 | 30.00 | |
| Molluscs | | | | |
| Vermetidae Rafinesque, 1815 | U | 40.00 | 53.33 | |
| Cirripedia | | | | |
| <i>Balanus trigonus</i> Darwin, 1854 | NIS | | 23.33 | Pilsbry (1916), Chainho et al. (2015) |
| Chordata | | | | |
| <i>Ascidia</i> sp. Linnaeus, 1767 | U | 3.33 | 3.33 | |
| <i>Asciidiella aspersa</i> (Müller, 1776) | NIS | 10.00 | | Ramalhos et al. (2021) |
| <i>Botrylloides leachii</i> (Savigny, 1816) | C | 3.33 | | Canning-Clode et al. (2013) |
| <i>Botrylloides niger</i> Herdman, 1886 | NIS | 70.00 | 20.00 | Gestoso et al. (2017), Ramalhos et al. (2019) |
| <i>Botryllus schlosseri</i> (Pallas, 1766) | C | 3.33 | | Canning-Clode et al. (2008) |
| <i>Diplosoma listerianum</i> (Milne Edwards, 1841) | C | 16.67 | 20.00 | Ramalhos et al. (2019) |
| <i>Distaplia corolla</i> Monniot F., 1974 | NIS | 63.33 | 6.67 | Wirtz (2007), Canning-Clode et al. (2013) |
| <i>Ecteinascidia</i> sp. Herdman, 1880 | U | 6.67 | | |
| <i>Eudistoma angolanum</i> (Michaelsen, 1914) | N | | 3.33 | Ramalhos et al. (2021) |
| <i>Microcosmus</i> sp. Heller, 1877 | U | 13.33 | 3.33 | |
| <i>Perophora listeri</i> Wiegman, 1835 | C | 33.33 | | Ramalhos et al. (2019) |
| <i>Perophora</i> sp. Wiegmann, 1835 | U | 3.33 | 6.67 | |
| <i>Symplegma brakenhielmi</i> (Michaelsen, 1904) | NIS | 3.33 | | Gestoso et al. (2017) |
| <i>Trididemnum cereum</i> (Giard, 1872) | N | | 20.00 | Ramalhos et al. (2019) |

3.2. Phase 2—Mesocosm experiment

Multivariate analyses indicated that different fouling assemblages (inside and outside) were affected differently by the duration of MHWs (Fig. 2B). The PCO multivariate analysis showed some point dispersion, indicative of differences in the pattern of responses to the duration of the heat stress (Fig. 2B). The overlapped vectors indicated that shorter MHW had greater effects on the assemblages dominated by NIS (Fig. 2B). In contrast, a greater proportion of native/cryptogenic species was lost during longer MHWs (i.e. those assemblages recruited outside the marina). In addition, PERMANOVA analysis con-

firmed, to some extent, the trends visualised in the PCO analysis, showing significant effects for MHW and location, but not for temperature (Table S3). SIMPER analysis showed that shorter MHWs (average similarity of 39.71% for the inside and 40.96% for the outside) had more impact on NIS species such as *P. alba* (9.32%), *Botrylloides niger* Herdman, 1886 (9.20%) or *C. inaudita* (7.89%) than on native species, resulting in greater NIS loss in inside assemblages (Table 2). In natural habitats (i.e. outside the marina), apart from the native/cryptogenic species *S. triqueter* (15.40%) and *Spirobis* sp. Daudin, 1800 (15.35%), most of the species affected by shorter MHWs were also NIS, representing almost 50% of

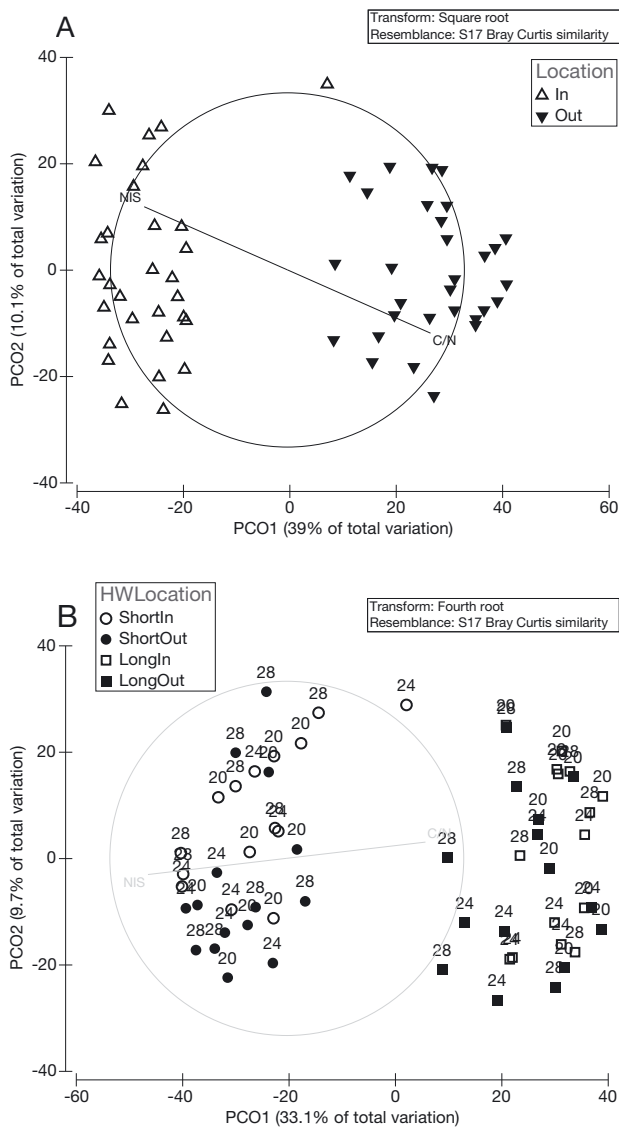


Fig. 2. (A) Phase 1: principal coordinate ordinations (PCOs) showing the variance in the structure of the fouling assemblages recorded on the fouling plates inside (in) the Quinta do Lorde Marina and in the outside natural area (out). (B) Phase 2: PCO showing the variance in the structure of the fouling assemblages between outside and inside after being subjected to simulated MHWs (short: 5 d long: 10 d) and different temperatures (20, 24 or 28°C). Vectors represent the abundance of NIS vs. cryptogenic/native species (C/N)

losses (Table 2). In contrast, longer MHWs (average similarity of 40.96% for the inside and 40.50% for the outside) resulted in a higher percentage of loss of native species: for example, *Scrupocaberea madeirensis* (Busk, 1860) (12.52%), *S. triqueter* (9.99%) and *Favosipora purpurea* Souto, Kaufmann & Canning-Clode, 2015 (7.63%) in inside assemblages or *P. radiata* (13.69%) and *S. triqueter* (11.17%) in outside assemblages (Table 2).

Univariate analysis revealed significant effects of MHW duration on the number of affected species (species loss PERMANOVA: pseudo- $F = 13.674$, $P(\text{perm}) = 0.001$) although no other interactions between temperature or location were detected (Table S4). The mean number of affected species increased with duration of MHWs (Fig. 3A, Table S4). Fouling assemblages from inside the marina showed greater numbers of affected species, on average, than those from outside (Fig. 3A). Although not significant, a trend was also detected in temperature, with higher temperatures impacting a greater number of species, which was more evident in the inside NIS-dominated assemblages (Fig. 3A). No significant effects were detected for the mean total cover loss (%) (Fig. 3B, Table S4). Interestingly, inside assemblages showed higher losses at increased temperatures, but only during long MHWs, this effect being greatest at 24°C (Fig. 3B, Table S4). This trend was also observed in diversity loss after the impacts of MHWs (Fig. 3C), although in this case it was not significant (Table S4).

Average measurements of the physical-chemical parameters of tanks were similar (Table S2), as was the variability in temperature in all tanks (Tables S2 & S5).

3.3. Phase 3 — Field test of invasibility: MHW legacy effects

The control plates recruited a total of 9 NIS, including 2 species not found during the initial recruitment: *Bugula neritina* (Linnaeus, 1758) and *Watersipora subtorquata* (d’Orbigny, 1852) (Table 3). Overall, at the end of the exposure period inside the marina, the main taxonomic groups present were bryozoans (44.4%), cnidarians (22.2%), ascidians (22.2%) and cirripedia (11.1%; Table 3). After being subjected to high NIS propagule pressure, the abundance of newly recruited NIS (i.e. only those recorded on the control plates) displayed distinct patterns across the fouling assemblages previously exposed to MHW treatments, as indicated by the PCO diagram (Fig. 4). However, the PERMANOVA analysis showed a significant effect only for the factor location (PERMANOVA: pseudo- $F_{1,68} = 8.982$, $P(\text{perm}) = 0.001$, PERMDISP- $P(\text{perm}) = 0.498$; Table S6). Percentage similarity increased from 43.68% for the control plates to 44.73% in the outside assemblages and 51.75% in the inside assemblages. For the control plates, bare space (52.97%) and *Cradosrupocellaria bertholletii* (Audouin, 1826) (24.79%) contributed almost 78% of the cumulative percent-

Table 2. Phase 2: SIMPER analysis showing similarities in loss of each taxon after PVC plates colonized inside or outside the Quinta do Lorde Marina were subjected to 2 different durations of simulated marine heat waves (MHWs). NIS species shown in **bold**

| Short MHW Taxon | Inside — Average similarity: 39.71 | | | | Outside — Average similarity: 40.96 | | | | | | |
|---|------------------------------------|--------|----------|----------|-------------------------------------|---|----------|----------|-------|-------|-------|
| | Av.Abund | Av.Sim | Sim/SD | Contrib% | Cum.% | Taxon | Cum.% | Contrib% | Cum.% | | |
| <i>Spirorbis</i> sp. | 1.55 | 4.83 | 1.79 | 12.15 | 12.15 | <i>Spirobranchus triqueter</i> | 2.26 | 7.14 | 2.81 | 15.40 | 15.40 |
| <i>Parasmittina alba</i> | 1.37 | 3.70 | 1.04 | 9.32 | 21.47 | <i>Spirorbis</i> sp. | 2.23 | 7.12 | 2.83 | 15.35 | 30.74 |
| <i>Botrylloides niger</i> | 1.53 | 3.65 | 0.76 | 9.20 | 30.67 | <i>Parasmittina alba</i> | 2.13 | 6.91 | 3.10 | 14.89 | 45.64 |
| <i>Celleporaria inaudita</i> | 1.11 | 3.13 | 1.16 | 7.89 | 38.56 | <i>Celleporaria inaudita</i> | 2.21 | 6.82 | 1.80 | 14.70 | 60.34 |
| <i>Polysiphonia</i> sp. | 0.92 | 2.99 | 1.20 | 7.52 | 46.08 | <i>Botrylloides niger</i> | 1.77 | 4.13 | 0.90 | 8.90 | 69.24 |
| <i>Spirobranchus triqueter</i> | 1.25 | 2.92 | 0.94 | 7.35 | 53.43 | <i>Cradosrupocellaria bertholletii</i> | 1.44 | 2.50 | 0.65 | 5.39 | 74.63 |
| <i>Lithophyllum incrustans</i> | 0.86 | 2.30 | 0.99 | 5.78 | 59.21 | <i>Distaplia corolla</i> | 1.26 | 2.28 | 0.66 | 4.93 | 79.56 |
| <i>Distaplia corolla</i> | 0.95 | 2.01 | 0.77 | 5.07 | 64.28 | <i>Exaiptasia diaphana</i> | 0.74 | 1.37 | 0.70 | 2.96 | 82.52 |
| <i>Salmacina dysteri</i> | 0.98 | 1.89 | 0.63 | 4.75 | 69.03 | <i>Lithophyllum incrustans</i> | 0.72 | 1.15 | 0.58 | 2.49 | 85.01 |
| Sponge morphotype 1 | 0.81 | 1.84 | 0.84 | 4.65 | 73.67 | <i>Sycon</i> sp. | 0.78 | 1.15 | 0.56 | 2.48 | 87.48 |
| <i>Mycale</i> sp. morphotype 1 | 0.88 | 1.62 | 0.69 | 4.07 | 77.75 | <i>Bugula simplex</i> | 0.99 | 0.99 | 0.39 | 2.12 | 89.61 |
| <i>Bugula simplex</i> | 1.03 | 1.40 | 0.46 | 3.53 | 81.27 | <i>Polysiphonia</i> sp. | 0.75 | 0.95 | 0.47 | 2.05 | 91.66 |
| Vermetidae | 0.77 | 1.27 | 0.57 | 3.20 | 84.47 | | | | | | |
| <i>Exaiptasia diaphana</i> | 0.82 | 1.17 | 0.56 | 2.94 | 87.42 | | | | | | |
| <i>Cradosrupocellaria bertholletii</i> | 0.69 | 1.02 | 0.47 | 2.58 | 89.99 | | | | | | |
| <i>Sycon</i> sp. | 0.47 | 0.92 | 0.48 | 2.32 | 92.32 | | | | | | |
| Long MHW Taxon | Inside — Average similarity: 40.96 | | | | Outside — Average similarity: 40.50 | | | | | | |
| Av.Abund | Av.Sim | Sim/SD | Contrib% | Cum.% | Taxon | Cum.% | Contrib% | Cum.% | | | |
| <i>Scrupocaberea maderensis</i> | 2.19 | 5.13 | 1.15 | 12.52 | 12.52 | <i>Puellina radiata</i> | 2.12 | 5.55 | 2.26 | 13.69 | 13.69 |
| <i>Spirobranchus triqueter</i> | 1.71 | 4.09 | 2.99 | 9.99 | 22.51 | <i>Spirobranchus triqueter</i> | 1.97 | 4.52 | 1.71 | 11.17 | 24.86 |
| <i>Favosipora purpurea</i> | 1.12 | 3.12 | 1.99 | 7.63 | 30.14 | <i>Parasmittina alba</i> | 1.68 | 3.75 | 1.32 | 9.27 | 34.13 |
| <i>Puellina radiata</i> | 1.49 | 2.89 | 1.44 | 7.06 | 37.20 | <i>Lithophyllum incrustans</i> | 1.24 | 3.59 | 3.02 | 8.87 | 43.00 |
| <i>Dictyota</i> sp. | 1.01 | 2.69 | 1.48 | 6.56 | 43.76 | <i>Scrupocaberea maderensis</i> | 1.94 | 3.57 | 0.84 | 8.82 | 51.81 |
| <i>Parasmittina alba</i> | 1.40 | 2.56 | 1.15 | 6.25 | 50.01 | <i>Favosipora purpurea</i> | 1.28 | 3.23 | 1.81 | 7.97 | 59.78 |
| <i>Lithophyllum incrustans</i> | 0.80 | 2.14 | 1.18 | 5.22 | 55.23 | <i>Schizoporella pungens</i> | 1.49 | 2.71 | 0.94 | 6.69 | 66.47 |
| <i>Mycale</i> sp. morphotype 2 | 1.15 | 2.03 | 0.94 | 4.96 | 60.19 | <i>Sertularella</i> sp. morphotype 1 | 1.43 | 1.81 | 0.54 | 4.46 | 70.94 |
| <i>Salmacina dysteri</i> | 1.02 | 1.74 | 0.96 | 4.26 | 64.45 | <i>Scrupocellaria unicornis</i> | 1.10 | 1.57 | 0.66 | 3.89 | 74.82 |
| <i>Aetea</i> sp. | 1.12 | 1.60 | 0.75 | 3.91 | 68.36 | <i>Salmacina dysteri</i> | 1.07 | 1.29 | 0.65 | 3.18 | 78.00 |
| <i>Scrupocellaria unicornis</i> | 1.20 | 1.51 | 0.65 | 3.68 | 72.04 | <i>Paraleucilla magna</i> | 1.10 | 1.23 | 0.63 | 3.04 | 81.04 |
| Vermetidae | 1.10 | 1.41 | 0.63 | 3.45 | 75.49 | <i>Vermetidae</i> | 0.92 | 0.89 | 0.46 | 2.19 | 83.23 |
| <i>Sycon</i> sp. | 0.93 | 1.30 | 0.67 | 3.19 | 78.67 | <i>Ectopleura</i> sp. | 0.93 | 0.86 | 0.45 | 2.12 | 85.35 |
| <i>Ectopleura</i> sp. | 1.01 | 1.29 | 0.49 | 3.14 | 81.81 | <i>Botrylloides niger</i> | 0.97 | 0.74 | 0.32 | 1.84 | 87.19 |
| <i>Sertularella</i> sp. morphotype, 2 | 1.09 | 1.26 | 0.55 | 3.07 | 84.89 | <i>Beania maxilladentata</i> | 0.73 | 0.73 | 0.38 | 1.81 | 89.00 |
| <i>Schizoporella pungens</i> | 0.85 | 1.07 | 0.57 | 2.61 | 87.50 | <i>Dictyota</i> sp. | 0.70 | 0.72 | 0.47 | 1.77 | 90.77 |
| <i>Clathrina clathrus</i> | 0.91 | 0.92 | 0.36 | 2.23 | 89.73 | | | | | | |
| <i>Paraleucilla magna</i> | 0.77 | 0.61 | 0.36 | 1.48 | 91.21 | | | | | | |

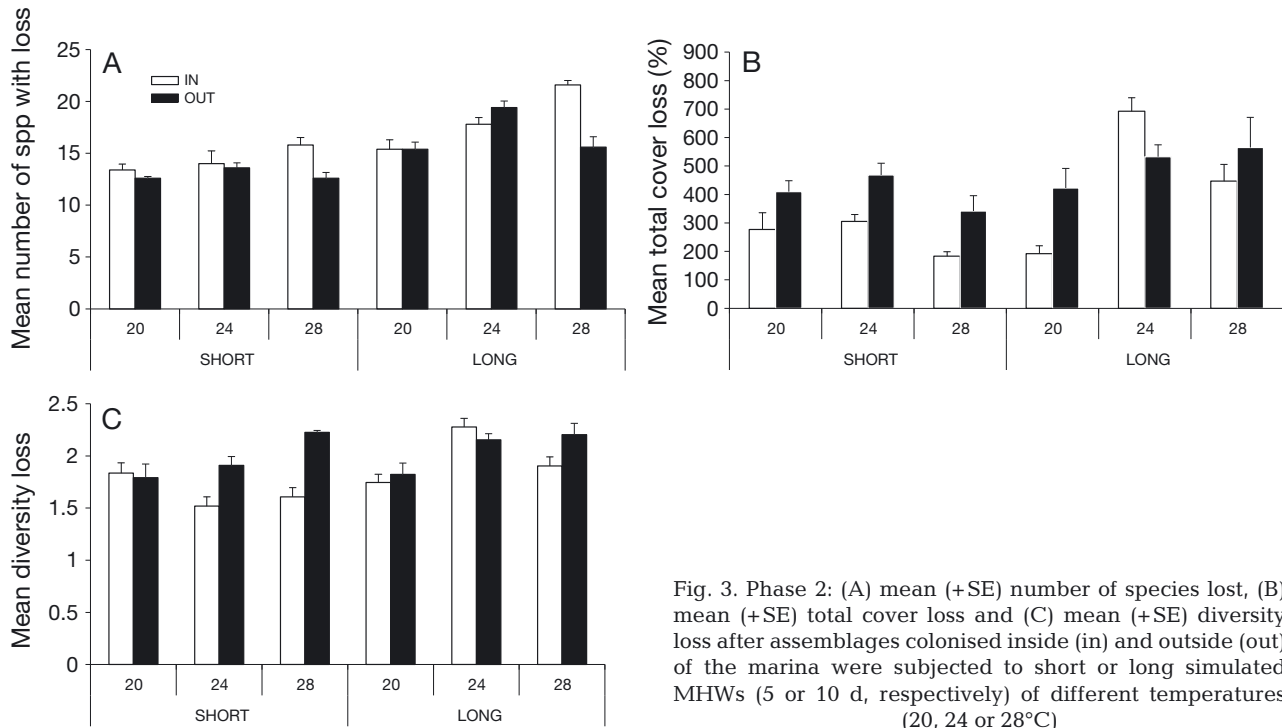


Fig. 3. Phase 2: (A) mean (+SE) number of species lost, (B) mean (+SE) total cover loss and (C) mean (+SE) diversity loss after assemblages colonised inside (in) and outside (out) of the marina were subjected to short or long simulated MHWs (5 or 10 d, respectively) of different temperatures (20, 24 or 28°C)

age (Table S7). In the assemblages originally recruited inside the marina, *C. inaudita* (44.68%) and bare space (16.18%) were the major contributors (Table S7), whereas *Distaplia corolla* Monniot F., 1974 (39.49%) and *Balanus trigonus* Darwin, 1854 (25.55%) were the species present in the greatest abundance in the assemblages recruited in natural habitats (Table S7). In relation to the richness of re-

cruited NIS, the PERMANOVA analysis revealed significant effects of temperature only (Table S8). Overall, assemblages previously subjected to high temperatures recruited a greater number of NIS and, although not significant, there were also a greater number of NIS recruits in the inside assemblages (Fig. 5A). Concerning mean percentage cover of NIS recruits, the analysis detected differences for location

Table 3. Phase 3: List of non-indigenous species (NIS) recorded on PVC plates with assemblages of different origins (control, inside and outside) after being subjected for 3 mo to the invasion process inside the marina, showing frequency of NIS occurrence (%), and references for species biogeographic status

| Species | Status | Frequency of occurrence (%) | | | Reference |
|---|--------|-----------------------------|--------|---------|---|
| | | Control | Inside | Outside | |
| Cnidarians | | | | | |
| <i>Ectopleura crocea</i> (Agassiz, 1862) | NIS | 10.00 | 13.33 | 6.90 | Wirtz (1995), Chainho et al. (2015) |
| <i>Exaiptasia diaphana</i> (Rapp, 1829) | NIS | 50.00 | 50.00 | 31.03 | Canning-Clode et al. (2013) |
| Bryozoans | | | | | |
| <i>Bugula neritina</i> (Linnaeus, 1758) | NIS | 10.00 | 6.67 | 3.45 | Norman (1909), Ramalhosa et al. (2017) |
| <i>Celleporaria inaudita</i> Tilbrook, Hayward & Gordon, 2001 | NIS | 10.00 | 86.67 | 13.79 | Ramalhosa et al. (2017) |
| <i>Cradoscrupocellaria bertholletii</i> (Audouin, 1826) | NIS | 60.00 | 43.33 | 20.69 | Canning-Clode et al. (2013) |
| <i>Watersipora subtorquata</i> (d'Orbigny, 1852) | NIS | 20.00 | 13.33 | 6.90 | Canning-Clode et al. (2013) |
| Arthropoda | | | | | |
| <i>Balanus trigonus</i> Darwin, 1854 | NIS | 30.00 | 66.67 | 27.59 | Pilsbry (1916), Chainho et al. (2015) |
| Chordata | | | | | |
| <i>Botrylloides niger</i> Herdman, 1886 | NIS | 10.00 | 33.33 | 10.34 | Gestoso et al. (2017) |
| <i>Distaplia corolla</i> Monniot F., 1974 | NIS | 50.00 | 76.67 | 27.59 | Wirtz (2007), Canning-Clode et al. (2013) |

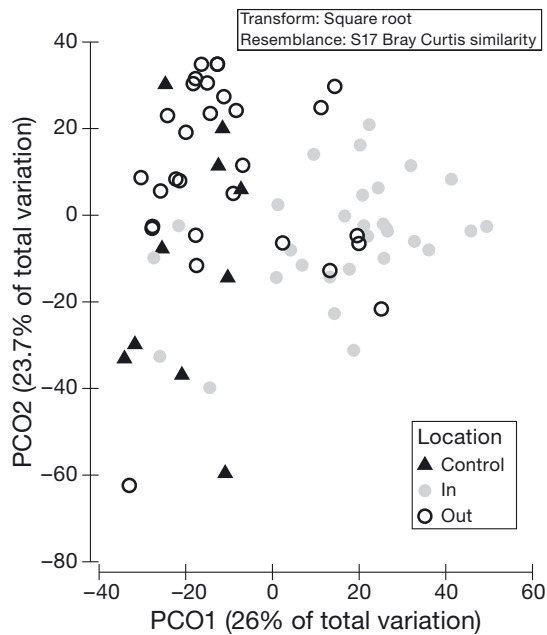
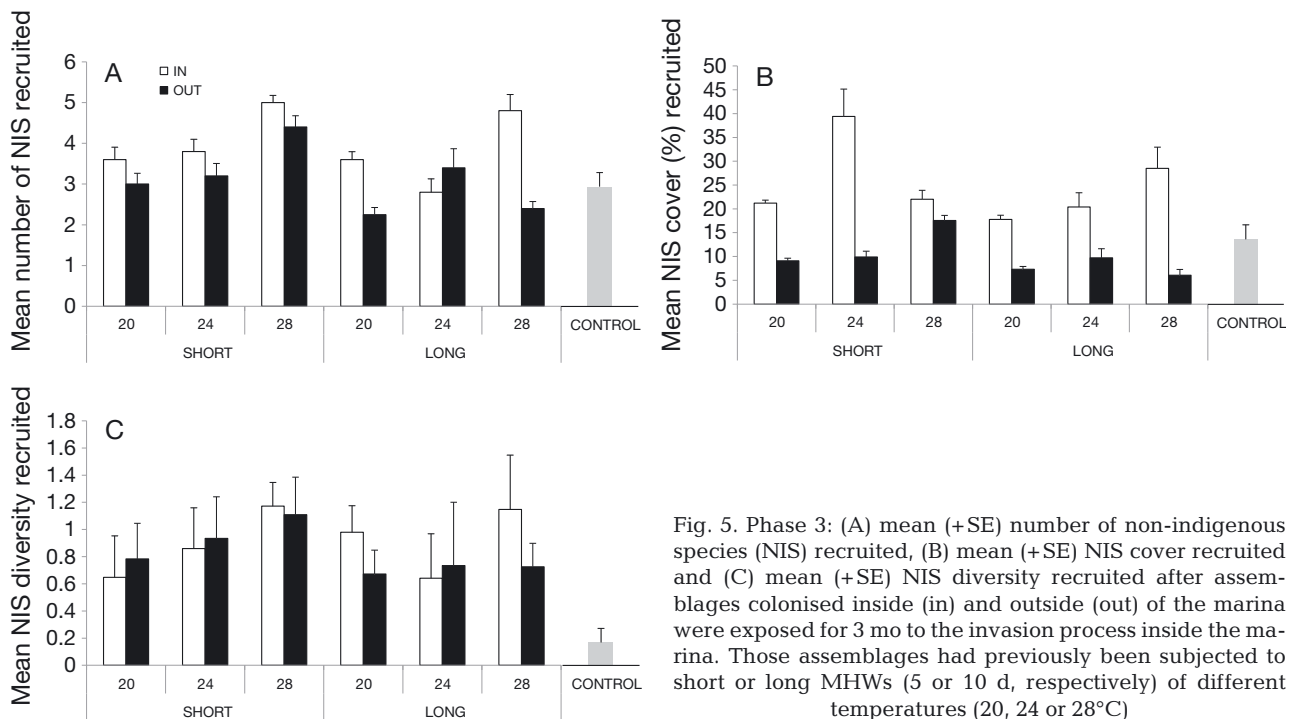


Fig. 4. Phase 3: Principal coordinate ordination (PCO) showing variation in the structure of fouling assemblages from different origins (inside the marina, outside the marina and control plates) after being subjected for 3 mo to the invasion process inside the marina

only (PERMANOVA: pseudo- $F_{1,68} = 24.255$, $P(\text{perm}) = 0.001$), showing greater cover by NIS recruits in the inside assemblages (Fig. 5B, Table S8). The analysis for the diversity index of NIS recruits did not reveal any statistical differences (Fig. 5C, Table S8).



Overall, location was significant in all phases of the study in terms of assemblage structure, as well as the duration of MHWs in Phase 2 (Table 4). In univariate analyses, MHW and temperature were significantly related to species richness in Phases 2 and 3 (loss of species in Phase 2 and NIS recruitment in Phase 3) (Table 4). In relation to total cover of NIS recruitment (Phase 3), only the covariate location was significant (Table 4).

4. DISCUSSION

The present work was designed to compare the responses of fouling assemblages recruited within a marina (highly invaded environment) and in natural habitats (low invasion environment) to a simulated gradient of MHWs of different temperatures and durations, and to investigate the legacy effects that these warming events could have on the invasibility of the assemblages. Longer MHWs had stronger impacts on native assemblages, while shorter MHWs affected NIS-dominated assemblages more. Overall, both assemblages were affected by extreme temperatures and longer MHWs. In terms of invasibility, outside assemblages were similar to the control plates, where resistance to invasion was low or null. Inside assemblages underwent greater NIS recruitment, implying facilitation. The present findings suggest that the final features of an assemblage's structure

Table 4. PERMANOVA results showing differences detected during phases of the study and the significant factors ($p < 0.05$) in each analysis. Studied factors were long and short marine heat waves (MHWs; 5 and 10 d), different experimental temperatures (T; 20, 24 and 28°C) and location (Lo), which refers to the origin of the assemblages (inside or outside the marina area). For Phase 2, all indices measured loss after MHWs. For Phase 3, all indices related to the recruitment of non-indigenous species (NIS) detected on the control plates. N: native species

| | Multivariate analysis Assemblage structure | Univariate analysis | | |
|---------|---|------------------------|-------------------|----------------|
| | | Richness | Total cover | Shannon-Wiener |
| Phase 1 | Lo (inside > NIS) | – | – | – |
| Phase 2 | MHW and Lo (5 d >NIS; 10 d > N) | MHW (10 d > NIS and N) | No diff. | No diff. |
| Phase 3 | Lo (inside > NIS; outside = control) | T (28°C > NIS) | Lo (inside > NIS) | No diff. |

and composition depend on an interplay between resistant and resilient species, classified as winners (mostly NIS), which dominated the assemblages, and less resistant species (mostly native), classified as losers, whose presence was reduced by the former, providing the opportunity for new NIS settlers to dominate that space.

4.1. Phase 1 — Assemblage recruitment

Shipping plays an important role in NIS dispersal worldwide (Bailey et al. 2020, Castro et al. 2020). On Madeira, as in other locations, areas inside ports and marinas are known to host the majority of NIS (Canning-Clode et al. 2013, Airoldi et al. 2015, Marchini et al. 2015), while outside these areas native species dominate more diverse communities (Afonso et al. 2020, Ferrario et al. 2020). This premise allowed us to conduct the present study and subsequent experiments. The PVC plates located outside the marina were colonised by more diverse assemblages with a higher abundance of native species, while the assemblages recruited on plates inside the marina were less diverse and mainly dominated by NIS, in accordance with Afonso et al. (2020) and Ferrario et al. (2020). Climate change in all its forms is shaping ecosystems (Costello et al. 2010, IPCC 2019). Recent work on Madeira documented the arrival and establishment of several species — e.g. *Cronius ruber* (Lamarck, 1818); *Millepora alcicornis* Linnaeus, 1758; *Avrainvillea canariensis* A. Gepp & E. S. Gepp, 1911 — due to ongoing temperature increases in the region (Ribeiro et al. 2019, Schäfer et al. 2019).

4.2. Phase 2 — Mesocosm experiment

Discrete events such as MHWs have been suggested as important drivers of massive mortality events of particularly abundant species (Gizzi et al.

2020). Worldwide, MHWs are becoming more frequent, of longer duration and greater intensity (Oliver et al. 2018). The hypothesis tested in this work, that fouling assemblages from natural habitats would be less well adapted to MHWs than those from artificial habitats, was not totally supported. Particularly in terms of assemblage structure, our results indicated that the duration of the simulated MHW had different impacts on assemblages from the 2 locations. Independent of the temperature regime, longer MHWs greatly impacted native/cryptogenic species (i.e. those recruited outside the marina), partially supporting the hypothesis. However, shorter MHWs strongly affected the assemblages dominated by NIS (i.e. those recruited inside the marina). Therefore, MHW duration seems to be an important factor to consider, not only the warming intensity.

A long-lasting MHW (2015–2016) in Tasmania (Australia) had impacts on local ecosystems that varied from new disease outbreaks in aquaculture organisms to the mortality of native species (Oliver et al. 2017). The duration of the MHW is linked statistically to a decrease in ecological health of foundation species, indicating that these events are altering the structure of ecosystems (Smale et al. 2019). In the present case, the winners (mostly NIS) persevering after the MHW (i.e. highly resistant species) are widespread warm-water organisms that thrive in heavily polluted environments, such as *B. niger*, *P. magna* and *Schizoporella pungens* Canu & Bassler, 1928 (Longo et al. 2007, Canning-Clode et al. 2013, Sheets et al. 2016). Additionally, the presence and establishment of those species may also be a consequence of ocean warming, where a poleward movement of numerous species has been observed, with the frequency of climate-mediated invasions linked to warming or cooling periods (Canning-Clode et al. 2011, Canning-Clode & Carlton 2017).

Interestingly, the observed loss in NIS cover (%) under longer MHWs, together with a similar decline (although not significant) in the diversity index,

seemed to indicate a tendency towards homogenisation in the inside assemblages. According to Scheibling & Gagnon (2009), MHWs could facilitate the presence of highly resistant species, e.g. non-indigenous bryozoans, modifying the structure of assemblages by reducing the cover of more vulnerable and/or less resistant species (i.e. losers) and thus creating more homogeneous assemblages.

With some exceptions (e.g. Shannon-Wiener diversity index), all indices indicated that the combined effects of prolonged exposure and extreme temperatures could induce a more evident decline in both inside and outside assemblages. Therefore, in agreement with previous studies (e.g. Garrabou et al. 2009, Smale et al. 2019), an MHW of sufficient extent (duration) and intensity (elevated temperature) could result in widespread mortality even in the most resistant species (Sorte et al. 2010b), giving more adapted species (i.e. winners) the chance to replace and dominate assemblages. The winners could eventually possess features that enhance NIS competitiveness and invasibility, such as well documented plasticity and adaptations. For example, the non-indigenous hydrozoan *Macrorhynchia philippina* Kirchenpauer, 1872 has recently spread in the Canary Islands, colonising a wide variety of habitats due to its ecological plasticity (Espino et al. 2020). Also, non-native seaweeds can adapt to varying levels of resource availability through changes in their morphology and physiology (Serebryakova 2017). Climate change can potentially facilitate the spread of biological invasions, either indirectly by promoting more resistant invasive species and/or directly by damaging strong native species or communities (Scheibling & Gagnon 2009, Sorte et al. 2010b). In recent research, Atkinson et al. (2020) verified that, during MHWs, physiological thresholds of native species may be exceeded, while the performance of invasive species may be enhanced. Consequently, MHWs could change an ecosystem's invasion dynamics, but such interactions are poorly understood.

4.3. Phase 3 — Field test of invasibility: MHW legacy effects

However, it is still not clear what effects MHWs can have on the invasibility of resident communities and consequent alterations in bioinvasion patterns in the context of climate change (Sorte et al. 2010b, Vaz-Pinto et al. 2013, Uyà et al. 2020). In the present study, after exposing PVC plates to high NIS propagule pressure, the native assemblages (i.e. from out-

side the marina) previously maintained under heat-stressed conditions, were akin to the bare control plates, showing no biotic resistance against NIS recruitment. Ecological theory indicates that communities with high species richness offer more biotic resistance against biological invasions by maintaining high levels of predation pressure or increased competition for space and resources (Freestone et al. 2013, Gestoso et al. 2018). Additionally, using PVC plates similar to those used in the present study, Gestoso et al. (2018) recruited communities from a marine protected area (MPA) on Madeira and relocated them to a high-pressure NIS environment to test their resistance to NIS invasion. Their results suggest that communities from the MPA showed lower NIS colonisation success compared to other communities, displaying some degree of biotic resistance against NIS invasion. Therefore, legacy effects from heat stress seemed to reduce the biotic resistance of native communities (i.e. via diversity reduction) and their capacity to hinder NIS recruitment (Elton 1958). Responses to invasion are strongly influenced by interactions between invaders and native species. If native species provide biotic resistance by consuming or competing with an invader, invasion may be slowed, and/or invasive populations may be limited (Berke et al. 2020).

A variety of mechanisms can be involved in determining the outcome of biotic interactions between incoming invasive and native and/or resident species (Mitchell et al. 2006). For example, species identity can be more relevant than the diversity of the community when resisting invasions (Arenas et al. 2006). In this sense, a reduction in the cover of specific native/cryptogenic species, such as the tubeworms *Spirorbis* sp. or *S. triqueter*, could potentially have reduced their ability to resist settlement by incomers (Sorte et al. 2010b). Furthermore, after Phase 3, a greater number of NIS were recruited on plates previously subjected to higher temperatures. Moreover, recruitment of NIS was always greater in NIS-dominated assemblages than in native assemblages, suggesting facilitation versus resistance tendencies, respectively (Uyà et al. 2020). These results could be related to the homogenisation tendency detected after the mesocosm experiment if longer MHWs promoted the dominance of particular species. Hypothetically, after long MHWs, some of the winning species could have had enough time to dominate assemblages, whereas short MHWs only reduced the presence of thermal losers, providing the chance for NIS incomers to occupy these empty spaces. This results of this study suggested that inside assemblages, with fewer species,

provided less biotic resistance to newcomers, although the Shannon-Wiener index for NIS did not reveal any significant results. The origin of the assemblages (location) had an influence on total NIS cover recruitment: inside assemblages had a higher cover recruitment than outside assemblages. However, these findings should be interpreted with caution, as they could be biased because some recruited NIS (even considering only those NIS recruited on the bare control plates) were at least partially present in the sessile assemblages from initial colonisation (Phase 1; i.e. *D. corolla*). In addition, some differences might be explained by temperature-mediated variability in damage to microbial biofilms, key mediators for settlement of macrofoulers (Cacabelos et al. 2020), as some authors report that biofilms affect strongly the efficacy of chemical antimicrofouling defences (Rohde et al. 2008, Wahl et al. 2010). However, since we did not assess the biofilms, such a hypothesis requires further testing.

4.4. Final remarks

In conclusion, this work showed that longer and more intense MWHs have an overall negative impact on native and non-indigenous assemblages. The invasibility of sessile assemblages is affected by heat stress, with high variability of responses in relation to biotic interactions, species resistance/adaptation/plasticity/identity (winners and losers) and homogenisation effects.

Finally, this study contributes to an understanding of the consequences that MHWs can have for the conservation of coastal communities. Management actions should give proper attention to potential interactive impacts that simultaneous global change stressors, here MHWs and biological invasions, can have on the conservation of marine coastal communities, especially in the context of an island ecosystem.

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