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Experimental heat waves induce habitat loss and drive invertebrate relocation to mussel-facilitated patches in the rocky intertidal

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Resumo

As interações positivas, como a facilitação, têm recebido menos atenção do que as interações negativas (por exemplo, predação ou competição) durante muito tempo, apesar de serem relevantes e abundantes no meio marinho. A facilitação é definida quando uma ou mais espécies obtêm algum tipo de benefício e nenhuma das espécies envolvidas é afetada negativamente pela interação. Foram descritos diferentes mecanismos de facilitação envolvendo, por exemplo, a criação de habitat e o estabelecimento de uma superfície de fixação, a melhoria do estresse físico e / ou fisiológico, o fornecimento de refúgios contra a predação e o aumento da oferta de recursos alimentares. Grande parte da investigação sobre facilitação nos últimos anos se refere à Hipótese do Gradiente de Estresse (HGE), que supõe um aumento dos efeitos de facilitação com maiores níveis de estresse ambiental. A maioria dos estudos que apoiam a HGE provêm de comunidades de plantas terrestres e, embora tenham sido feitas algumas investigações no ambiente marinho, ainda são raros os trabalhos experimentais que testam a validade da HGE numa perspetiva de alterações climáticas.

A zona rochosa do entremarés foi descrita como um habitat sentinela, ideal para estudar os impactos das alterações climáticas. As condições ambientais nesse ambiente são frequentemente extremas, sendo que algumas das espécies que o habitam já estão expostas a condições fisiológicas limítrofes. Vários mexilhões mitilídeos actuam como engenheiros ambientais, facilitadores nesse ecossistema, mas já se mostraram vulneráveis às ondas de calor, tendo sido registrados eventos de mortalidade em massa para várias espécies. Prevê-se que essas ondas de calor se tornem mais frequentes e intensas com a progressão das alterações climáticas, ameaçando tanto o facilitador como a comunidade biológica associada, que pode não ter um amortecimento térmico suficiente face às condições futuras.

Este estudo tenta prever se a facilitação nestes bancos de mexilhões está em risco de colapso, mediante a execução de simulações experimentais de ondas de calor *in situ*, aumentando a temperatura intersticial em 5 - 6 °C durante períodos de maré baixa de 2 horas ao longo de 3 dias consecutivos (ou seja, um evento de aquecimento). Após cada um dos três eventos de aquecimento executados, foram obtidas amostras mediante raspagem e os invertebrados associados foram contabilizados e classificados em grandes grupo taxonómicos. Foram calculados três indicadores biológicos gerais (abundância, riqueza e índice de diversidade de Shannon-Wiener) e a estrutura das comunidades foi analisada em condições controle e após o

aquecimento. O estudo foi realizado no Canal de São Sebastião, SP, Brasil, onde predomina um clima subtropical. Na região, os principais detentores de espaço na zona central do entremarés são as cracas do género *Chthalamus*, sobre as quais se estabelecem os mexilhões mitilídeos *Mytilaster solisianus* e *Brachidontes darwinianus*, desenvolvendo uma cobertura secundária homogénea e consituindo bancos bem definidos. A cobertura primária de cracas serve, portanto, de habitat controle, ocupando o mesmo nível vertical no entremarés e sujeita a condições ambientais muito semelhantes às observadas nos bancos de mexilhões.

Relativamente aos três parâmetros biológicos gerais estudados, não mostrou detectados sinais de colapso da facilitação. Os decréscimos de abundância após o aquecimento foram significativamente maiores no habitat controle, em comparação com o habitat facilitado pelos mexilhões, apoiando a HGE e sugerindo uma importância ainda maior desses bancos no futuro. Os declínios na riqueza e diversidade também tenderam a ser maiores no ambiente de cracas, mas essas tendências somente foram significativas em um dos locais de estudo, onde a riqueza e a diversidade eram inicialmente menores. Esse resultado sugere maior vulnerabilidade térmica em comunidades mais simples, consistente com a hipótese de suficiência biológica.

Contrariamente às expectativas, os grupos de invertebrados mais prejudicados com o aquecimento foram os mais resistentes ao estresse térmico, como as larvas de quironomídeos, mas sobretudo os gastrópodes litorinídeos e os ácaros. Apesar do habitat primário desses dois últimos grupos ser a cobertura de cracas, o que sugere aclimatação a condições extremas, as perdas nesse ambiente foram muito maiores do que aquelas sob facilitação, sugerindo que os bancos de mexilhões podem tornar-se o habitat principal também para estes grupos no futuro.

Os efeitos de facilitação (ou seja, as diferenças entre a abundância observada no cobertura de cracas e na cobertura de mexilhões) foram significativamente amplificados pelo aquecimento no caso das larvas de mosquito quironomídeos e dos ácaros. Este facto constituiu uma surpresa, sobretudo para estes últimos, uma vez que não são geralmente descritos como um taxon sensível ao calor. No entanto, o seu pequeno tamanho e o seu comportamento bastante sedentário podem ter impedidos repostas de fuga para microhabitats mais amenos.

Também inesperadamente, foram registadas maiores abundâncias dos dois grupos mais vulneráveis (poliquetas e nemátodos) nas parcelas aquecidas, e não nas parcelas controle. Especialmente no caso dos poliquetas, e considerando que constituem o grupo de maior dimensão corpórea, é possível que o resultado reflita migração de povoamentos de cracas para bancos de mexilhões. Embora as amostras tenham sido colhidas apenas no habitat predominante, as parcelas experimentais, aquecidas ou não, incluiam tanto o habitat focal como o alternativo. No caso das parcelas onde se amostraram os bancos de mexilhões, os números excedentes de poliquetas podem resultar da migração do habitat de cracas, autando como fontes.

Devido à sua maior dimensão e a certos aspectos dos seus hábitos de vida, grupos como os poliquetas e os nemátodos poderão ter algumas vantagens na competição com outros taxa, como os copépodes harpacticoides, que sofreram menores efeitos de facilitação na condição de aquecimento. O declínio de copépodos, uma presa basal nessa rede trófica, é apenas uma mudança na extensa reconfiguração das redes de interação que deve ocorrer com o aumento da frequência e intensidade das ondas de calor.

Apesar de não terem sido identificados sinais de colapso da facilitação, a fisiologia dos mexilhões submetidos a aquecimento artificial será analisada num estudo de acompanhamento para detetar sinais de alerta precoce de aumento da mortalidade. Experiências de aquecimento semelhantes em outras espécies de mitilídeos, para as quais já foram registadas mortalidades em massa relacionadas com ondas de calor (por exemplo, *Mytilus edulis, Mytilus californianus, Perna perna*), também seriam de interesse para esclarecer como a progressão das alterações climáticas poderão impactar essas comunidades de invertebrados no futuro.

Abstract

Despite broad empirical support for increased ecological facilitation upon stress, experimental evidence of the stress-gradient hypothesis (SGH) in a climate change perspective is still scant. Here we address mussel ecosystem engineering in an extreme habitat, where facilitation of several other species could eventually collapse after exposure to heat-wave conditions expected for the near future. Contrary to this prospect and aligned to the SGH, we show that facilitation of invertebrate assemblages may actually exacerbate after *in-situ* experimental heat-wave simulations that raised interstitial temperature by 5-6°C for 2-h low-tide periods over 3 consecutive days (i.e. a heating event). A marked negative impact on overall abundance at the control habitat (barnacle stands, devoid of secondary mussel cover), along with greater resistance at mussel beds, underpinned increased facilitation with stress. Although shore-dependent, the same trend was observed for richness and diversity. Whole-assemblage shifts consisted mostly in substantial declines of the more resistant invertebrates (not more vulnerable, as expected) at the non-facilitated habitat where they originally prevailed. Great losses were detected for chironomid mosquito larvae and, mainly, mites, which are relatively resistant, but more sedentary, hardly capable of escaping to thermal refugia. For those two groups, mussel beds switched from secondary to primary habitat, becoming net facilitators. Surprisingly, the two most vulnerable groups, polychaetes and nematodes, became more abundant after stress, mostly because density increases in the mussel habitat were greater than declines in the control barnacle habitat. These two groups may either crawl or resuspend in the water column, respectively, favoring migration to mussel beds. Owing to their larger size and life habits those groups may also outcompete other invertebrates after stress, such as harpacticoid copepods that became less facilitated after pulse warming. Therefore, heat-waves may not only reduce intertidal habitat for several invertebrate groups, but also trigger extensive rewiring of species interaction networks.

Keywords: habitat amelioration, thermal refugia, climate change, stress-dependent facilitation, positive interactions, foundational species

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List of abbreviations, acronyms and symbols

ASH (Above Substrate Humidity)

AST (Above Substrate Temperature)

AT (Air Temperature)

BC (Barnacle Control)

BH (Barnacle Heated)

BTU (British Thermal Unit)

Ev (Event)

H’ (Shannon-Wiener’s diversity index)

HGE (Hipótese do Gradiente de Estresse)

IPCC (Intergovernmental Panel on Climate Change)

IT (Interstitial Temperature)

MC (Mussel Control)

MH (Mussel Heated)

nMDS (non-metric Multidimensional Scaling)

PC (Principal Component)

PERMANOVA (Permutational Analysis of Variance)

Pl (Plot)

SGH (Stress Gradient Hypothesis)

Sh (Shore)

SIMPER (Similarity Percentages)

SP (São Paulo)

ST (Surface Temperature)

Tr (Treatment)

General introduction

Motivation and relevance

Research on climate change and its impacts has been at the forefront of environmental research in the past two decades, with already some documentation of species' range shifts (Parmesan & Yohe, 2003; Pinsky et al., 2020) and even local extinctions (McLaughlin et al., 2002; Román-Palacios & Wiens, 2020). Responses of whole communities, however, are still rather scarcely represented, especially when attempting to predict climate change effects via an experimental approach and not by solely comparing past observations to the present. Mesocosm experiments are one way to manipulate environmental conditions in situ for an entire community, but they are not feasible for all ecosystems. In the rocky intertidal, conditions are changing almost continuously, creating difficulties for accurate ex situ simulations. In this ecosystem, at least some species have been shown to already live close to their physiological limits (Stillman, 2002; Somero, 2010), especially on tropical rocky shores (Vinagre et al., 2016). making them vulnerable to progressing climate warming. Even though the rate of warming is predicted to be greater in temperate regions than in the tropics (IPCC, 2007; IPCC, 2013), effects of climate change may be detectable earlier in the lower latitudes, as tropical rocky shore inhabitants appear to be less capable of acclimation compared to related temperate species (Vinagre et al., 2016). Tropical mytilid mussel species as critical drivers of highly diverse assemblages have received less attention as their temperate relatives with greater commercial relevance (e.g. *Mytilus edulis*, *Mytilus californianus*), requiring more research to understand the mechanisms playing an important role in their community and its responses to climate change.

Many species in the Mytilidae family enable the establishment and survival of certain associated organisms through diverse mechanisms all falling into the category of facilitation: one or more of the involved species benefit and none are negatively affected by the interaction (Bruno & Bertness, 2001; Stachowicz, 2001). The stress gradient hypothesis (SGH, Bertness & Callaway, 1994) postulates an increase in the effects of facilitation with greater environmental stress but has mostly been supported by studies on terrestrial plant communities (e.g. Cavieres et al., 2002; Forrester et al., 2013). Its testing in the marine environment has been scarce compared to terrestrial ecosystems and more research on the topic is needed (Adams et al., 2022). Some studies have provided support for the SGH in the rocky intertidal (Bogdanski et al., 2024; Kawai & Tokeshi, 2007), but it is unclear whether this will remain the case for more stressful conditions in the future of progressing climate change as well.

By artificially heating the substrate, this experiment simulated heat-waves, which are predicted to increase in frequency and intensity as climate change progresses (IPCC, 2023). Biological parameters of the associated assemblage as well as changes in the abundance of broad taxonomic groups were analysed before and after heating, attempting to fill a gap in the understanding of community responses to climate change effects. Simultaneously, the artificial heating increases environmental stress and provides testing to the SGH in the rocky intertidal for future conditions.

1. **Rocky shore intertidal**

1.1. Intertidal definition and substrates

The intertidal stretches from just above the water level at low tide to the highest line of the shore inundated at high tide. By frequently being accessible to marine and terrestrial species, it acts as a connector between the two habitats (Suchanek, 1985; Ning et al., 2019). For instance, shorebirds can feed on marine invertebrates on and inside the substrate, allowing resources generated in the sea to reach terrestrial populations. Moreover, small fish and juveniles of bigger pelagic or migratory species may consume insect larvae and other intertidal invertebrates, creating a carbon flux into marine ecosystems further away from the coast. This intertidal shoreline consists of either a soft bottom or a consolidated bottom. The first can form extensive sand- and mudflats in lagoons and estuaries, while the latter often includes steeper rocky cliffs. On those rocks, environmental conditions vary greatly within short distances (Helmuth et al., 2006; Jurgens & Gaylord, 2018), creating distinct zonation patterns, and leading to the rocky intertidal sometimes being described as an ideal natural laboratory (Benedetti-Cecchi & Trussel, 2014). These environmental conditions can be extreme and harsh, including aerial exposure for up to several hours per day in some regions, leading to potentially extreme heat- (McAfee et al., 2022) and desiccation stress (Benedetti-Cecchi & Trussel, 2014). Additionally, the rocky coast does not enable burrowing activities as easily as in sandy or muddy terrain, meaning sessile and mobile organisms are at risk of being dislodged and washed away (Denny et al., 1985; Bagur et al., 2016). Certain sessile inhabitants can mitigate those strains by increasing the three-dimensional complexity of the substrate, leading to more protected interstitial spaces (Tokeshi & Romero, 1995).

1.2. Intertidal mytilid mussels and their community

Common sessile inhabitants of the rocky intertidal are mytilid mussels, which are known to be a vital part of a rich community on and between the individual bivalves (Seed, 1996; Koivisto, 2011). Depending on the species, they can form multilayered beds of over 20 cm thickness that consist of the mussels themselves, the assemblage of associated species, and amassed detritus (e.g. shell and rock debris, broken-off sea urchin spines, etc.) at the bottom and between the byssal threads of the bivalves (Suchanek, 1985). Greater thickness, age, interstitial space, and structural complexity of the bed have been linked to reduced recovery times after disturbances (Suchanek, 1985), as well as increased diversity (Kanter, 1977; Suchanek, 1979), abundance, and richness (Suchanek, 1985) of the associated invertebrate community. Among other factors, this is likely to be a result of an increase in moisture retention and the quantity of trapped sediment (Kanter, 1977; Koivisto, 2011), as well as a decrease in the impact of wave action and temperature (Bertness & Grosholz, 1985; Jurgens & Gaylord, 2018), therefore relieving the associated species from extreme stress (Jurgens et al., 2022). These associated species can be divided into three categories: sessile epibiota attached to the bivalve shells (e.g. algae, barnacles, or bryozoans), free-living organisms (e.g. nematodes or copepods), and infaunal species (e.g. polychaetes). The latter are particularly dependent on the presence and quantity of accumulated detritus and sediment between the byssal threads at the bottom of the mussel bed. In some regions such as the Pacific coast of South America soft bottom intertidal environments such as lagoons and mud flats are rather scarce (Tokeshi, 1995). Mussel beds cannot replace these ecosystems but are nevertheless an important habitat for these infaunal species that would not establish a population on a rocky shore devoid of mytilid mussels (Suchanek, 1985; Tokeshi & Romero, 1995; Borthagaray & Carranza, 2007; Bagur et al., 2016). In conclusion, it can be stated that intertidal mussel beds provide their associated community with numerous benefits, in more diverse mechanisms than other sessile organisms creating marine benthic habitats (Bruno & Bertness, 2001): by generating habitation and settlement space (Lohse, 1993; Seed, 1996) as well as a refuge from predators (Suchanek, 1985), by reducing physical (Tokeshi & Romero, 1995) and physiological stress (Suchanek, 1985). Lastly, intertidal mussel beds increase the supply or retention of propagules as well as enhance food supply (Bertness, 1984), although these six beneficial mechanisms may not occur in all environments dominated by mytilid mussels around the globe.

1.3. Rocky shores of the São Paulo state, Brazil

Mussel beds at the rocky shore in the state of São Paulo mostly consist of two different species: *Mytilaster solisianus* and *Brachidontes darwinianus* (Eston et al., 1986; Bogdanski et al., 2024), which are both much smaller in comparison to mytilids in other regions. Their relatively limited distribution, from Mexico to Uruguay for *M. solisianus* and from Southeastern Brazil to Patagonia for *B. darwinianus* (Klappenbach, 1965; Rios et al., 1994), and rather small commercial importance (Carranza et al., 2009) may be reasons for the scarcity of research on them compared to other mussels (e.g. *Mytilus edulis*). They form intermixed monolayered beds and are dominant space occupiers at the mid-intertidal zone along the coast of São Paulo state (Petersen et al., 1986). However, a direct settlement on bare rock does not occur, instead they require a more rugose attachment substrate made up of the barnacle *Chthalamus spp.*, and often smother those as bivalve density increases (Eston et al., 1986). These sessile assemblages provide important attachment sites and protected interstitial space for smaller invertebrates, even more so as foliose algae are only abundant from the upper subtidal and downwards (Petersen et al., 1986). The intertidal of São Paulo State is not subjected to aerial exposure for as long as in other regions of the world, with a maximum tidal range of 1.3 m and a semidiurnal tide cycle. Belonging to the subtropical Southeastern Brazil Ecoregion (Spalding et al., 2007), average air and sea-surface temperatures historically measured 25 – 27 °C and 24 – 25 °C, respectively, in the summer months (APAMLN, 2020).

1.4. Heat-waves and their impact on mussel beds and the rocky intertidal

Mussel beds in the rocky intertidal are subjected to multiple threats, one of them being the effects of climate change. One of these effects is the increasing frequency and intensity of heat waves (IPCC, 2023), defined as when the 90th percentile of maximum temperature records of the respective region is surpassed for three or more consecutive days (Perkins & Alexander, 2013). Heat-waves have already caused mass mortalities in several mytilid mussel species of the rocky intertidal, including *Mytilus edulis* (Tsuchiya, 1983), *Mytilus californianus* (Harley, 2008), *Mytilus spp.* (Raymond et al., 2022), and *Perna perna* (Galil et al., 2022). Large-scale mussel losses have certainly caused whole-reef negative impacts.

Rocky shores have generally been described as a good sentinel habitat for climate change (Jurgens et al., 2022), as at least some of the inhabiting species are already living close to their physiological limits (Stillman, 2002; Somero, 2010). This is especially the case for tropical species, which additionally seem to possess a lower acclimation capacity than related temperate species (Vinagre et al., 2016). Warming effects may therefore be more severe and detectable earlier on tropical rocky shores than in temperate regions, even though warming rates are predicted to be greater in higher latitudes (IPCC, 2007; IPCC, 2013).

One of these effects may be the reduction or even loss of habitat for intertidal species on the rocky shore. Their local distributional range has often been postulated to be determined by biological stress (e.g. predation and/or competition) on the lower and by physical stress (e.g. heat and/or desiccation) on the upper end (Stachowicz, 2001). As environmental gradients are often steep in the rocky intertidal (Helmuth et al., 2006; Jurgens & Gaylord, 2018), many species only occur in a very narrow vertical band (Stachowicz, 2001), creating the typical zonation patterns seen in many rocky shore ecosystems. This range may be further restrained with progressing climate change, particularly the upper zone, which may become inhabitable for most species owing to exceedingly high desiccation stress. When predators and competitors setting the lower limit of the species' range are not affected to the same extent, the area on the shore where the species can survive and reproduce successfully may decrease or even disappear.

2. **Facilitation**

There are several biological interactions relevant to the understanding of a community. They can generally be divided into negative interactions, such as predation or competition, and positive interactions, for instance facilitation. The former have historically received significantly more attention than the latter (Stachowicz, 2001; Bertness et al., 2019) although facilitation is reportedly common and important in marine ecosystems (Bruno & Bertness, 2001; Jurgens & Gaylord, 2018). As an attempt to decrease this gap in research, this work will focus on the mechanisms and implications of facilitation.

2.1. General definition

Generally, facilitation is defined as an interaction between organisms that gives an advantage to one or more and no detriment to any of the affected species (Bruno & Bertness, 2001; Stachowicz, 2001). Different processes may be a part of the interaction, such as the establishment of a certain habitat in the first place and providing different types of organisms with an attachment surface (Lohse, 1993; Seed, 1996) as well as a refuge from predation (Suchanek, 1979). Moreover, facilitators may ameliorate physical (Tokeshi & Romero, 1995) and/or physiological stresses (Suchanek, 1985) and increase food supply to surrounding inhabitants

(Bertness, 1984), and many ecosystems characterized by facilitative interactions include multiple of these mechanisms (Bruno & Bertness, 2001). In the marine environment, these facilitating species are often sessile organisms creating a three-dimensional habitat on top of a previously less complex surface, therefore establishing a variety of microhabitats (Gosselin & Chia, 1995). Examples in the subtidal include meadow seagrasses, giant kelps, and reef-building corals, that present a sheltered habitat for countless fish and invertebrate species, many of which have adapted to living in these specific biomes. In the rocky intertidal, common facilitators encompass several species of algae, barnacles, and bivalves, especially important for ameliorating abiotic stresses threatening the survival of associated species in the absence of a facilitator (Jurgens et al., 2022).

2.2. Distinction from and overlap with other concepts: Mutualism, Ecosystem engineering, Foundation species, Keystone species

Facilitation overlaps with several other positive biological interactions, so to avoid confusion these concepts will be defined and distinguished from facilitation. Mutualism is denoted as both involved species benefitting in some way (Stachowicz, 2001), whereas regarding facilitation, the facilitator can remain unaffected. Therefore, all mutualisms are included in the definition of facilitation, but not all facilitative interactions are also mutualisms. Ecosystem engineering on the other hand is a particular type of facilitation: The engineering species creates new habitats or at least physically modifies the existing one (Jones et al., 1994), which increases the abundance and survival of associated species (Seed & Suchanek, 1992). Similarly, foundation species are also facilitators: they determine a major part of the community structure (Dayton, 1972) often by creating a three-dimensional, structural base of the habitat, which can additionally serve as protection from predators or harsh physical stresses. The occurrence of foundation species increases diversity (Suchanek, 1979), as in many cases, certain species can only establish a population in an environment where a foundation species is present, such as mytilid mussels in the rocky intertidal (Suchanek, 1985; Tokeshi & Romero, 1995; Borthagaray & Carranza, 2007). Lastly, the overlap of keystone species and facilitation is more complicated than the previously described intersections. A keystone species was originally described as a native species high in the food web, which greatly impacts the community structure through its activity (Paine, 1969). However, the term has since been used for many different species, which can be grouped into five categories: keystone predator, -prey, -plant, -link, and -modifier (Mills & Doak, 1993). The removal of the latter category would result in a deficit of structures influencing the kind of habitat, as well as the loss of species that depend on those structures. Following this logic, some facilitators would in theory fit into this definition, although sessile invertebrates as discussed here in this work are not usually referred to as keystone species in scientific literature.

2.3. Facilitation theories (SGH, fundamental niche, intermediate disturbance)

Research on facilitation has been focusing on several different aspects, in recent years mainly the stress-gradient hypothesis (SGH; Bertness & Callaway, 1994). It proposes an increase in facilitation effects with higher environmental stress, but research and support for this hypothesis have mostly come from terrestrial plant communities (e.g. Greenlee & Callaway, 1996; Cavieres et al., 2002). A meta-analysis pictured this imbalance by consisting of over three times the number of terrestrial (n = 1313) compared to aquatic (n = 349) studies, but it regarded the SGH well supported in bacterial, plant, and terrestrial research (Adams et al., 2022). Several animal studies found at least partial support for the SGH (Daleo & Iribarne, 2009; Fugère et al., 2012; Barrio et al., 2013; Dangles et al., 2013; Beaudrot et al., 2020), however, as the hypothesis is not widely known among animal researchers, it is often not mentioned by name in papers whose results actually back it up. This hinders progress on the relevance and generality of the SGH and more research on ecological interactions along stress gradients outside of plant-plant interactions is required (Adams et al., 2022).

When the concept of facilitation became the subject of progressively more studies, efforts were made to integrate it into earlier, seemingly contradictory ideas. Ecological niche theory (Grinnell, 1917; Hutchinson, 1991) as one of these concepts is made up of the fundamental niche as the theoretical bounds of environmental conditions, in which a species is able to survive with no regard to any negative interspecific interactions. The realized niche on the other hand is defined as the actual spatial distribution of a species, taking into account all physical and biological parameters. Commonly it is taught that the realized niche must always be smaller than the fundamental niche, but facilitation seems to oppose this (Higashi, 1993; Bruno et al., 2003): For instance, on the rocky shore, some species occur outside their fundamental niche in the presence of a facilitator (Silliman et al., 2011; McAfee et al., 2016), which ameliorates abiotic and biotic factors for neighbouring inhabitants. Neighbouring species were also thought to result in some sort of negative influence to one another, based on the assumption that no two species can coexist in the same niche without competitive exclusion of one of them. With marine facilitators often belonging to different trophic levels than their associated community, they are less likely to compete for resources than for example the species involved in terrestrial plant-plant interactions (Bulleri, 2009).

Related to competition in habitats including a facilitator is the apparent paradox of facilitation in the scope of the intermediate disturbance hypothesis (Connell, 1978; Petraitis et al., 1989): Species diversity should be highest at intermediate levels of disturbance, as competitive exclusion is thought to increase with decreasing disturbance. However, it is suspected that even in the presence of facilitators stress levels are not lowered sufficiently to truly enable competitive exclusion (Bruno & Bertness, 2001), as species diversity has been shown to increase with facilitation in many studies (e.g. Suchanek, 1979; Tokeshi & Romero, 1995; Prado & Castilla, 2006; Bogdanski et al., 2024). This is thought to be possible because many marine facilitators enhance spatial complexity (Robinson et al., 2007), a feature correlated with higher species diversity (Kanter, 1977; Seed & Suchanek, 1992) and thought to reduce competition by adding to the available habitat and settlement space (Bruno & Bertness, 2001). Competitive exclusion is therefore thought to be more relevant to primary space holders, and not as much as initially thought to their associated community (Bruno et al., 2003). For secondary space holders of smaller size than their facilitators, this notion is backed by studies showing a steady increase in diversity through the course of succession rather than a parabolic arc (Johnston & Odum, 1956; McKindsey & Bourget, 2001).

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Experimental heat waves induce habitat loss and drive invertebrate relocation to mussel-facilitated patches in the rocky intertidal

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Abstract

Despite broad empirical support for increased ecological facilitation upon stress, experimental evidence of the stress-gradient hypothesis (SGH) in a climate change perspective is still scant. Here we address mussel ecosystem engineering in an extreme habitat, where facilitation of several other species could eventually collapse after exposure to heat-wave conditions expected for the near future. Contrary to this prospect and aligned to the SGH, we show that facilitation of invertebrate assemblages may actually exacerbate after *in-situ* experimental heat-wave simulations that raised interstitial temperature by 5-6°C for 2-h low-tide periods over 3 consecutive days (i.e. a heating event). A marked negative impact on overall abundance at the control habitat (barnacle stands, devoid of secondary mussel cover), along with greater resistance at mussel beds, underpinned increased facilitation with stress. Although shore-dependent, the same trend was observed for richness and diversity. Whole-assemblage shifts consisted mostly in substantial declines of the more resistant invertebrates (not more vulnerable, as expected) at the non-facilitated habitat where they originally prevailed. Great losses were detected for chironomid mosquito larvae and, mainly, mites, which are relatively resistant, but more sedentary, hardly capable of escaping to thermal refugia. For those two groups, mussel beds switched from secondary to primary habitat, becoming net facilitators. Surprisingly, the two most vulnerable groups, polychaetes and nematodes, became more abundant after stress, mostly because density increases in the mussel habitat were greater than declines in the control barnacle habitat. These two groups may either crawl or resuspend in the water column, respectively, favoring migration to mussel beds. Owing to their larger size and life habits those groups may also outcompete other invertebrates after stress, such as harpacticoid copepods that became less facilitated after pulse warming. Therefore, heat-waves may not only reduce intertidal habitat for several invertebrate groups, but also trigger extensive rewiring of species interaction networks.

1. **Introduction**

Compared to negative species interactions, such as predation and competition, advances on the ecology of positive interactions, including facilitation, has lagged behind (Stachowicz, 2001; Bertness et al., 2019). Facilitation is defined as an interaction allowing advantage to one or more species and detriment to none (Bruno & Bertness, 2001; Stachowicz, 2001), and is ubiquitous in the marine environment (Bruno & Bertness, 2001; Jurgens & Gaylord, 2018). Foundation species (Dayton, 1972), or ecosystem engineers (Jones et al. 1994), are a particular set of facilitators that allow the survival and establishment of many other species through the amelioration of environmental conditions (Jones et al., 1997; Romero et al., 2015), or by creating refuge from predators or disturbance (Bruno & Bertness, 2001; Angelini et al., 2011). Theory predicts that ecological facilitation becomes more important with the increase of environmental stress [i.e. the 'stress gradient hypothesis' (SGH); Bertness & Callaway, 1994; Crain & Bertness, 2006)] and empirical work has been overall supportive, although strongly biased towards plant studies involving only a few species (with prevailing interactions shifting from competition to facilitation, e.g. Maestre et al., 2009). Much less attention has been paid to stress-dependent effects of foundation species on animal communities, limited to the work on a few specific systems, such as social weavers' nests on tress (Lowney and Thomson, 2021, 2022), beech cupules on leaf litter (Melguizo-Ruiz, 2016), oyster (McAfee et al., 2022) and mussel beds on the rocky intertidal (Bogdanski et al., 2024), which provided somewhat conflicting evidence. Additionally, predictions of facilitation effects in present-day stress gradients may not hold for more disruptive change in a climate-change scenario, when the frequency and intensity of heat-waves (i.e. temperature above the 90th percentile over three or more consecutive days; Perkins & Alexander, 2013) are both expected to increase (IPCC, 2023).

Insights from extreme habitats are especially relevant as they may be used as early warnings of more extensive change. The intertidal zone of marine rocky shores is one such habitat, because aerial exposure during low-tide periods may impose severe heat- (Lathlean et al., 2014; McAfee et al., 2022) and desiccation stress (Bell, 1995; Benedetti-Cecchi & Trussel, 2014), especially for vulnerable sessile and sedentary organisms that cannot flee to less stressful microhabitats. In fact, several intertidal species are already living at the edge of their physiological limits (Stillman, 2002; Somero, 2010), especially in the tropics, where temperature maxima more often exceed critical thresholds (Morley et al., 2012; Vinagre et al., 2016). This explains why the intertidal distribution of several different species is restricted to more benign biogenic habitats (e.g. Suchanek, 1985; Tokeshi & Romero, 1995; Borthagaray & Carranza, 2007), and why ecosystem engineering is presently paramount for the stability of invertebrate assemblages (McAfee et al., 2022; Bogdanski et al., 2024). Still in accordance with the SGH, the presence of foundation species may become even more critical, as the gap between the average physical conditions found at the facilitated and the non-facilitated habitat, depending on the buffering capacity of the founder, will likely increase. However, the underlying mechanisms assuring facilitation in present-day conditions may not hold in the future. For example, monarch butterflies currently perform better in an invasive tropical milkweed compared to a native temperate one, but, in a warming scenario, butterflies in the tropical weed presented higher mortality, possibly owing to excess production of toxic chemical defenses by the host (Faldyn et al., 2018). Another study evidenced that warming advanced the phenology of both winter moths and pedunculate oak, their plant host, but to a different extent, so that hatching preceded tree bud openings for several days, causing larval starvation and substantial mortality (van Asch et al., 2007). The functioning of biogenic habitats may thus substantially change with climate, eventually in quite unexpected ways.

Intertidal mussel beds are very important ecosystem engineers in the rocky intertidal, supporting a diversified, self-sustained interstitial community (Tokeshi & Romero, 1995; Arribas et al., 2014; Bogdanski et al., 2024), providing nursery habitat for an additional set of species (Tokeshi and Romero 1995, Flores and Negreiros-Fransozo 1999, Lindsey et al. 2006), and constituting foraging grounds for higher-order consumers, such as birds, fish and decapod crustaceans (Goss-Custard & Dit Durell, 1987; Robles, 1987; Boudreau & Hamilton, 2012), thereby linking terrestrial and marine ecosystems. Besides the provision of structured habitat (Lohse, 1993; Seed, 1996), trophic resources and protection against predators (Suchanek, 1979; Tokeshi & Romero, 1995), mussel beds are one of the few foundation habitats capable of mitigating physiological stress (Suchanek, 1979; Seed & Suchanek, 1992; Bruno et al., 2001). Also, the byssal threads attaching mussels to the rock surface trap suspending particles and create a sediment layer that constitute novel habitat for a set of species otherwise absent from the rocky shore (Sebens, 1991). In the SW Atlantic, mid-intertidal mussel beds, formed by *Mytilaster solisianus* and *Brachidontes darwinianus* (A. d'Orbigny, 1842), are often found interspersed amongst a primary cover of barnacles *Chthamalus spp.* (Eston et al., 1986; Bogdanski et al., 2024). They coexist as nested foundation-species assemblages (*sensu* Angelini et al., 2011) because barnacles, first colonizing bare rock, form the primary layer needed for bivalve attachment and the development of a secondary uniform cover that characterize mussel beds (Eston et al., 1986). Based on differences from the single-layered barnacle habitat, a control condition, Bogdanski and colleagues (2024) reported increased mussel facilitation of invertebrate assemblages along the vertical stress gradient imposed by low-tide exposure to air, in accordance with the SGH. In spite of the extensive declines observed at the control barnacle habitat with increasing stress, invertebrate assemblages remained unaltered in the presence of the secondary mussel cover, suggesting that mussels completely buffer any negative effects of present-day thermal stress and desiccation potential. Habitat amelioration apparently stem from the retention of humidity, as observed by Silliman and collaborators (2011), and the provision of shade within the interstitial microclimate. However, this thermal advantage, compared to control conditions (i.e. as found in barnacle cover), may reverse in extremely hot weather (> 30ºC) when interstitial temperature gets actually higher in the presence of mussels (Bogdanski et al. 2024), indicating that mussel facilitation could collapse with the intensification of heat-waves.

Here we test whether mussel facilitation holds after exposure to heat-wave conditions predicted for the coming decades, considering a plausible climate-change scenario. As a follow-up of Bogdanski and colleagues (2024), we now heated experimental plots with either the predominance of barnacle or mussel cover, at two replicate rocky shores and over three manipulation events, and aimed sampling the associated invertebrate fauna, at both warmed and control plots, one month after each event. We then tested whether pulse warmings mimicking heat-waves increased or decreased mussel facilitation. In all cases, we relied on differences, or ratios, of community attributes between the mussel and the barnacle habitat to back any interpretations. Regardless of facilitation, we expected that warming would impact invertebrate assemblages by causing generalized declines of the groups most vulnerable to heat stress and desiccation. Facilitation would then result because such losses would be more extensive in the control than in the facilitated habitat. An eventual collapse of facilitation would indicate that mussel engineering is insufficient for assemblage recovery after episodical overheating.

2. **Methods**

2.1. *Study area and biological system*

The experiment was conducted from November 2022 to March 2023 at two intertidal rocky shores, Guaecá (23.829 S, 45.447 W) and Feiticeira (23.844 S, 45.408 W), located in the São Sebastião Channel, SP, Brazil. The prevailing climate regime is warm-temperate/subtropical, with a marked seasonal variation of temperature and rainfall (higher during the warm season). Study sites are moderately exposed to wave action and submitted to a microtidal regime, with maximum range around 1.3 m, exhibiting a classic zonation pattern. Mussel patches develop at the midlittoral zone (*sensu* Lewis 1964, Stephenson & Stephenson 1972), typically as a secondary layer on top of the lower half of the chthamalid barnacle cover. Patch size varies widely, from very small clusters of a few cm to horizontal band-like extensions covering tens of meters. More commonly, as found at sampling sites, there is a balanced cover of barnacles and mussels, and mussel patches are typically tens of cm long. As such, and depending on life habits and mobility, vagile invertebrates may migrate from one habitat to another. Mussel facilitation, estimated as differences from the barnacle control condition, benefits stress-vulnerable invertebrates the most (especially polychaetes, flatworms, nematodes and harpacticoid copepods), but also restrains two more resistant groups, that are actually more common in the single-layered barnacle habitat [i.e. mites and littorinid snails; Bogdanski et al. (2024)].

2.2. *Experimental design*

Twelve experimental plots, at least 3 m apart, were established roughly at the same tidal height in each site, centered at the mid vertical level of the mussel cover (or a nearby reference for barnacle plots) to minimize unwanted effects along the natural vertical stress gradient (see Bogdanski et al. 2024). Six of those plots were delimited over ‘barnacle habitat’ (habitat ‘B’, where a single-layered barnacle cover prevailed) and the other six over mussel cover (habitat ‘M’, where an additional mussel layer covered most of the plot). Within each cover type, three random replicate plots were submitted to experimental heating (manipulation ‘H’) and the other three were left untouched and used as controls (‘C’). Replicate plots for each combination of habitat type and thermal manipulation (‘BH’, ‘BC’, ‘MH’, ‘MC’) were properly interspersed along shorelines (60-70m) to avoid pseudoreplication. The warming potential of our setup and its deployment in the field is given as a supplementary figure (Fig. S1). Heating was achieved by placing infrared burners (Jackwal Mod. 2850; www.jackwal.com.br), delivering 11,300 BTU, 1 m above the rock surface, making possible a relatively homogeneous heating over a radius of 0.5 m (Fig. S1a) and thus the delimitation of ca. 0.79 m^2 experimental plots. Burners were fueled by 12 l cylinder containers filled with liquefied petroleum gas and attached to tripods at the target height (Fig. S1b).

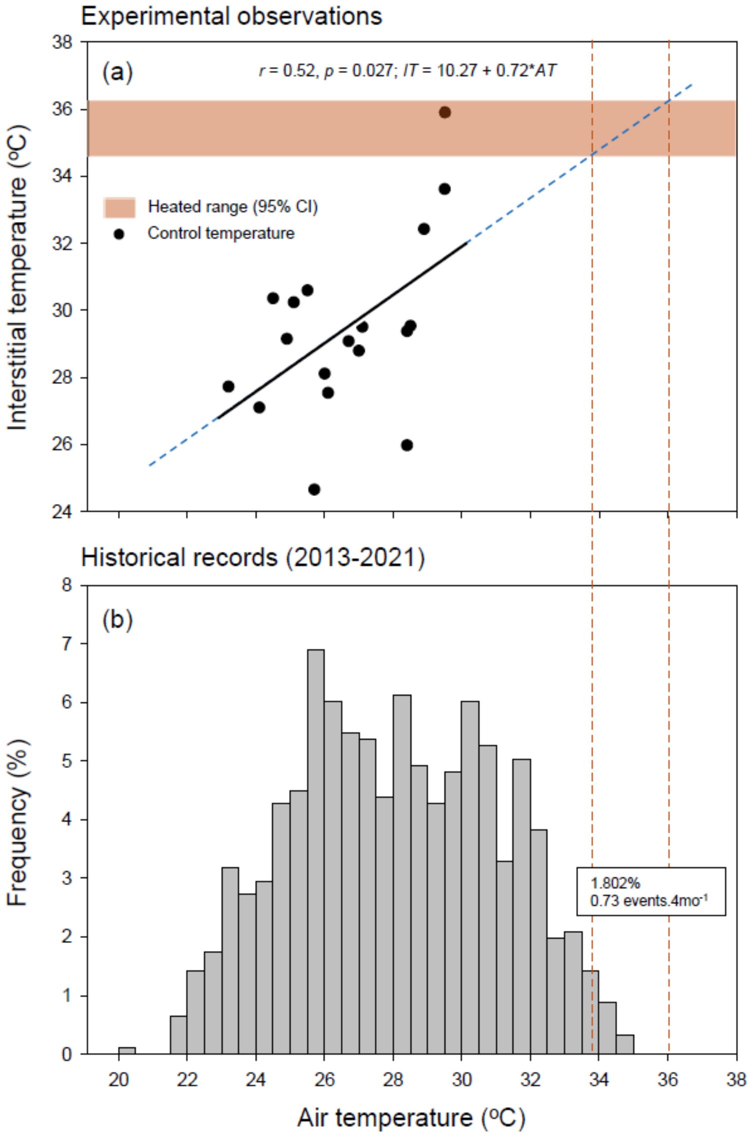

Fig. 1.

Correspondence of heat-wave simulations to historical temperature data. (a) Correlation between air and interstitial temperature at control plots (barnacle and mussel habitats combined) to predict the air temperature range needed to warm up plots to the observed heated interval (95 % CI). Data correspond to daily average values. Air temperature between 33.79 and 36.05 °C would be needed to raise interstitial temperature to the experimental heated range (34.60 – 36.22 °C). Note that this relationship should be interpreted with caution, as predictions extend beyond the range of empirical observations. (b) Frequency distribution of 3-d moving averages of historical temperature data (8 yr from 2013-2021), obtained from the weather station installed at the Centre for Marine Biology of the University of São Paulo, 2-3 km distant from sampling sites. These data consisted of daily temperature averages calculated from readings at 10' intervals from 08:00 am to 12:00 pm, from November 1 to February 28/29 (encompassing the time over low-tides and the season sampled in this study). All possible 3-d periods, used for frequency counts, depict the population of baseline conditions to contextualize 3-d experimental manipulations (e.g. 'events'). Overall, less than 2 % of all possible 3-d periods currently fall in the experimental heat-wave conditions, which translates to an average of 0.73 heat-waves during the warmest season (November to February), as covered in this study. Yearly estimates vary from 0.00 (2017-18, 2019-20) to 2.32 (2016-17).

Severe heat-waves were simulated on repeated spring-tide periods (hereafter 'events') by deploying heaters for 2 h, during diurnal low-tides, for three consecutive days. We aimed three replicate events at Guecá and Feiticeira along subsequent spring-tide periods (i.e. allowing a lunar-month period between heating events at the same site) but weather conditions prevented fieldwork at some dates and adjustments were needed. Time between events was actually 27 and 42 d for Guecá, and 32 and 44 d for Feiticeira. Invertebrate assemblages were sampled 24-30 d and 26-31 d after stress events at Guecá and Feiticeira, respectively (Fig. S2).

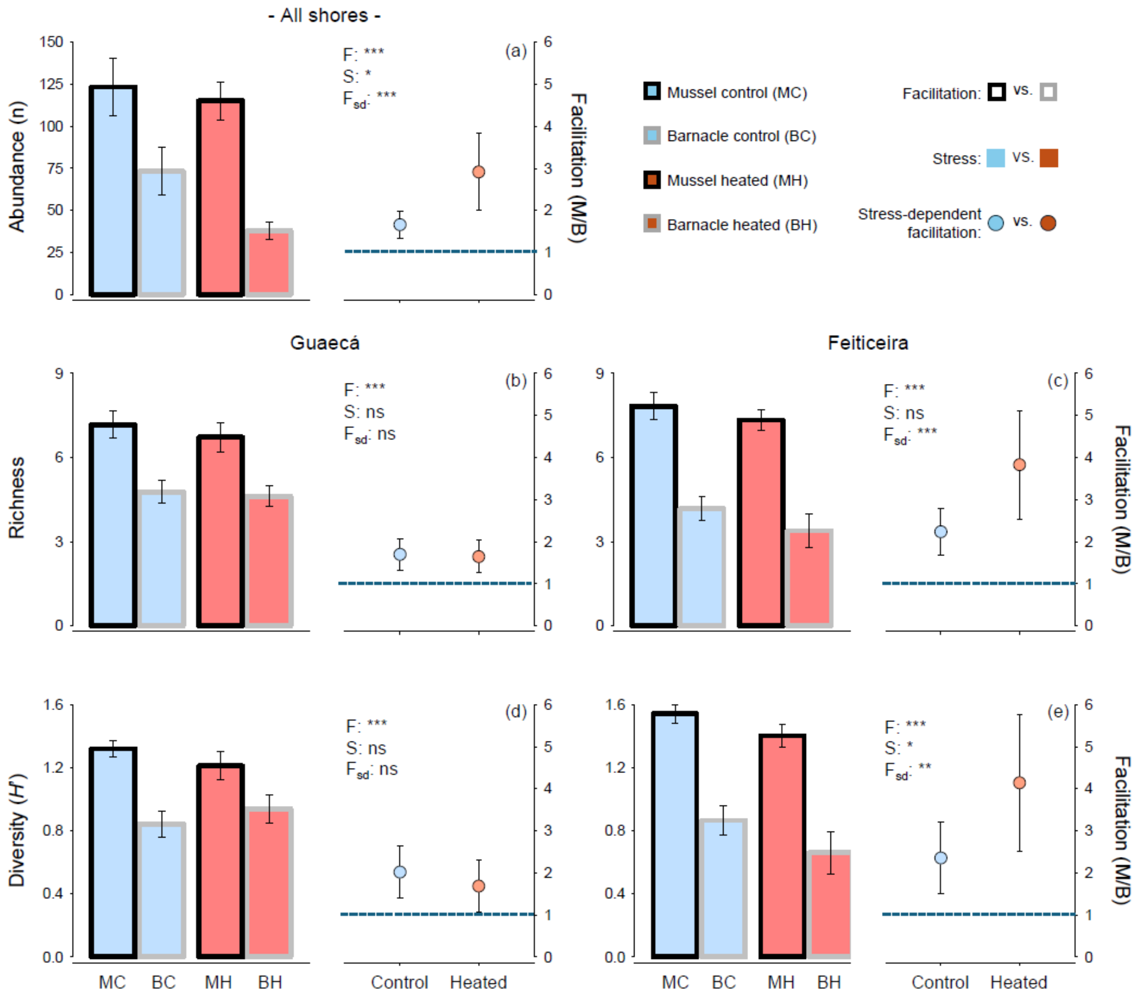

Fig. 2. Overall abundance (a) and shore-specific richness (b-c) and Shannon-Wiener's diversity (d-e) of invertebrate assemblages at the single-layered (barnacle: B) and the double-layered (barnacles plus mussels: M) intertidal habitat, at both control (C) and heated (H) plots. Left panels stand for average values (± 1SE) for all four combinations of factors habitat and thermal manipulation. Comparisons between blue and red bars address heat-stress effects, and comparisons between black and grey-bordered bars address facilitation effects of mussels. Right panels show the relative mussel facilitation, as M/B ratios, at the two thermal conditions. Whiskers represent 95 % confidence intervals and horizontal dashed lines the facilitation threshold. F: Facilitation, S: Stress, F_{sd}: Stress-dependent facilitation. ns: non-significant, *: $p < 0.05$, **: $p < 0.01$, *** $p < 0.001$).

2.3. *Temperature and humidity measurements*

During the 2 h experimental sessions, three different temperature parameters and one humidity measure were recorded at each plot. Five replicate interstitial temperature (IT) readings, using a Fluke 52 II thermometer coupled to a k-type probe (80 PK-26), and whole-plot surface infrared images measuring the surface temperature (ST), using a FLIR C5 camera, were taken two times, 1.5 h apart. Two replicate readings of temperature and humidity, 1 cm above the substrate (AST, ASH, respectively), were taken once in the middle of the session using a Vaisala HM42 probe. Air temperature at chest height (AT) was also recorded during each session every ½ h. Summary statistics of all those parameters, pooled across sites and manipulation events, are shown in Table 1. The interstitial habitat occupied by the target invertebrate assemblages was heated, on average, 5.6 and 6.2 °C above control conditions for the barnacle and the mussel habitat, respectively. This corresponded to much lower temperature increases just above the surface (2.5 – 2.8 °C), but much higher increments just on top of barnacle (7.8 °C) and mussel (8.2 °C) cover. Infrared heating also increased the potential for desiccation stress at both habitats. However, declines in humidity were more substantial at the mussel (5.3 %) compared to the barnacle (3.9 %) habitat (Table 1). A linear relationship of air to interstitial temperature, from control plots (mussels and barnacles combined) and considering average values over each period at the two sampling sites, was used to predict the air temperature needed to elevate interstitial temperature to the range observed for the heated treatment (Fig. 1a). Air temperate would have to exceed 33.79 °C; a condition currently observed in 1.8 % of all possible 3-d periods (considering only readings from 08:00 am to 12:00 pm, and from November to February, following the tidal periods and the season sampled in this study), based on data retrieved from the weather station at the Centre for Marine Biology of the University of São Paulo (available at http://cebimar.usp.br/), only 2-3 km away from sampling sites (Fig. 1b). This translates to an average of 0.73 'heat-wave events' each season (ranging from 0.00 to 2.32 between 2013 and 2021). However, considering heat-wave projections for the coming decades, three events, as imposed in this study, may become common (Perkins-Kirkpatrick & Lewis, 2020).

Table 1. Overall changes of temperature and humidity delivered by infrared burners, pooled across sites and manipulation events. Numbers are average values, followed by respective standard deviations within brackets.

	Barnacle habitat			Mussel habitat		
	Control	Heated	Difference	Control	Heated	Difference
Temperature (°C)						
1-cm above surface (AST)	27.7 (3.0)	30.2 (3.3)	+2.5	27.5 (2.8)	30.3 (3.0)	+2.8
Surface (ST)	27.5 (5.9)	36.5 (7.8)	+9.0	27.3 (5.4)	35.5 (6.3)	+8.2
Interstitial (IT)	28.9 (3.2)	34.5 (4.5)	+5.6	29.1 (3.6)	35.3 (4.4)	+6.2
Air at chest height (AT)	26.4 (2.3)					
Humidity (%)						
1-cm above surface (ASH)	77.3 (9.4)	73.4 (11.5)	-3.9	78.2 (8.3)	72.9 (10.5)	-5.3

2.4. *Sampling procedure*

In all sampling dates, two circular samples with a diameter of 6.0 cm (28.3 cm^2) were taken in each plot, 15-50 cm apart, aiming the target habitat (either barnacle or mussel cover). The sample area was first delimited using a hole saw coupled to a battery-powered drill, and then all biological cover was detached from the rock using metal scrapers, brushed onto thin flexible plastic discs (anti-drip wine pouring pieces), and transferred to a container filled with 70 % ethanol. All 144 samples were processed in the laboratory as in Bogdanski et al. (2024). Briefly, sample contents were washed in running saltwater on a 300 µm sieve, freed from any fine sediments and debris (mostly mussel and barnacle shells), and placed in a gridded Petri dish under a dissecting stereomicroscope (16 X) for observation (identification and counting of mobile invertebrates, sorted into 23 broad taxonomic groups).

2.5. *Statistical analyses*

2.5.1. *Univariate response variables* – All analyses of abundance, richness, and the Shannon-Wiener's diversity index [*H*', calculated with the vegan package in R (R Core Team 2021)] stemmed from an omnibus linear mixed model examining 'treatment' effects, consisting of the combined outcomes of habitat type and stress manipulation, pooled in different ways to address specific statistical hypotheses. 'Treatment' (Tr), a fixed factor with four levels (BH, BC, MH, MC), was examined after three specific manipulation ‘Events’ (Ev; fixed, with levels Event 1, 2 and 3) at two different ‘shores’ (Sh; random, with levels Guaecá and Feiticeira), testing the spatial consistency of any treatment effects at a spatial scale of unit km. ‘Plot’ (Pl; three levels, Plot 1, 2, 3 and 4), also a random factor, nested in the triple interaction Sh*Ev*Tr, tested very small scale variation, within shores, that could potentially overwhelm the effects of any manipulations. All analyses were balanced, with replication at the plot level ($n = 2$). Richness and diversity raw data were homoscedastic (Cochran’s C, $p > 0.05$), but abundance data needed to be square-root transformed to meet the assumption ($C = 0.1445$, $p > 0.05$). The GMAV5 software (Underwood & Chapman 1997) was used to run this general model.

We limited specific planned comparisons to the cases where a treatment main effect, or its interaction with factors ‘event’ (indicating either one-off or cumulative effects) and ‘shore’ (suggesting spatial-dependent responses), were significant. Because the omnibus procedure was mainly used to obtain proper error terms for contrasts, a higher critical type-I error (0.10) was initially used (Ruxton & Beauchamp 2008). Three planned comparisons involving treatment levels directly addressed specific hypotheses: First, we tested for overall mussel facilitation [Comparison 1 - $H_a : \mu_{(BC+BH)/2} < \mu_{(MC+MH)/2}$], then for overall heating effects [Comparison 2 - $H_o : \mu_{(BC+MC)/2} > \mu_{(BH+MH)/2}$], and finally we tested whether facilitation, as mussel-to-barnacle-ratios (M/B), changed after heating [Comparison 3 - $H_a : (\mu_{(MH/BH)} / \mu_{(MC/BC)}) \neq 1$], in this latter case a two-tailed approach since we considered either the collapse or the enhancement of facilitation (i.e. supportive of the SGH). For the three comparisons, all possible pairs of observations (B*+B*, M*+M*, B*+M*, M*/B*) were used to compute those average values and respective standard deviations, keeping however sample size (n) as 36 and 18 to compute test statistics for cases of significant main effects and second-order interactions, respectively (see ‘Results’). For Comparison 3, mean square error estimates from the omnibus procedure could not be used, and separate t-tests were conducted instead, correcting for heteroscedasticity when needed.

2.5.2. *Multivariate analyses of community structure* – An analogous model to the one described above, with the same random and fixed factors, and respective levels, was run as the omnibus procedure but using permutational analysis of variance (PERMANOVA) instead (Anderson 2001, McArdle and Anderson 2001). Data were first 4th root transformed, as taxonomic groups most sensible to heat stress may not be the most abundant (Bogdanski et al. 2024) and then a resemblance matrix was built using Bray Curtis similarity. Variance terms were estimated with type III sums of squares, and *p*-values were obtained through unrestricted 9,999 permutations.

Two-dimensional nMDS plots indicating the position of samples of the four treatments levels (BC, BH, MC, MH) were produced to illustrate cases of significant comparisons. Similarity percentage analyses (SIMPER) identified the invertebrates responsible for most dissimilarity between contrasting groups. For comparisons 1 and 2 we specifically set facilitation (BC, BH vs. MC, MH) and heat stress (BC, MC vs. BH, MH) planned contrasts. When significant, similarity percentage analyses (SIMPER) identified the invertebrates responsible for most dissimilarity between groups (relative contribution > 8 %). For comparison 3, testing whether facilitation changed under thermal manipulation, we used PC1 and PC2 scores of nMDS plots to calculate all possible distances between mussel and barnacle samples for the control (d_c) and the heated (d_h) condition. Again, this was tested through a two-tailed test [$H_a : \mu_{(dc)} \neq \mu_{(dh)}$] using the degrees of freedom corresponding to the number of independent observations. For all groups identified in SIMPER analyses, count differences between control (MC – BC) and heated (MH – BH) samples were transformed into *z*-scores for a more direct assessment of how thermal manipulation affected their facilitation. nMDS plots and SIMPER analyses were carried out in R (R Core Team (2021). PERMANOVA analyses were done in Primer 6 (v.6.1.13) & PERMANOVA (v.1.0.3).

3. **Results and Discussion**

3.1. *Overall numbers: facilitation of stress-vulnerable species and unexpected losers under heat stress*

A total of 12,586 individuals were sampled over the study, comprising 16 major invertebrate groups, 12 of them (listed in Table 2) in sufficient numbers allowing overall comparisons between habitats and thermal manipulation treatments. Just as observed in a preceding study (Bogdanski et al. 2024), the mussel habitat hosts a more abundant and diversified invertebrate fauna compared to the barnacle habitat, and the extent of mussel facilitation appeared to proxy the vulnerability of each invertebrate group to desiccation stress. Nematodes, polychaetes, harpacticoid copepods, amphipods and flatworms were the most facilitated, while the larvae of ptychopterid insects, littorinid snails and mites, more resistant to desiccation (Sømme & Block 1984, Sokolova and Pörtner 2001), were actually restrained in the mussel habitat. Leathery sea slugs and ribbon worms were only found in mussel-facilitated plots (Table 2). Experimental heating caused an overall 22.1 % decline of invertebrate abundance (Table 2, Fig. 2a), but contrary to expectations losses were not positively correlated to stress vulnerability. Four out of the six most abundant groups declined with thermal manipulations, and three of them, littorinid snails (-36.2 %), chironomid larvae (-8.4 %) and mites (-67.4 %; the second most impacted group) are in fact the most resistant invertebrates. This suggests that very common species presently thriving in challenging conditions may be among the most affected in the future, as niche space in the rocky intertidal will probably be reduced with the increasing intensity and frequency of heat waves. Harpacticoid copepods (-55.0 %), still within the most abundant groups, along with flatworms (-32.2 %) and leathery sea slugs (-95.0 %), comprised the less resistant groups negatively affected by heating. Unexpectedly, other vulnerable invertebrates tended to be more abundant in heated than in control plots (*i.e.* nematodes, polychaetes, isopods and amphipods), suggesting that migration between habitat patches and competition may ultimately contribute to the structure of warmed assemblages.

Table 2. Overall number of individuals within main invertebrate groups, sorted by habitat (barnacle vs. mussel) and thermal manipulation (control vs. heated) and pooled across sites and manipulation events.

		Habitat			Manipulation		
	All plots	Barnacle	Mussel	Change (%)	Control	Heated	Change (%)
Nematodes (Nematoda)	3448	96	3352	3391.7	1622	1826	12.6
Mites (Arthropoda: Arachnida)	2532	1791	741	-58.6	1909	623	-67.4
Chironomid larvae (Arthropoda: Insecta)	1803	826	977	18.3	941	862	-8.4
Polychaetes (Annelida: Polychaeta)	1680	68	1612	2270.6	790	890	12.7
Harpacticoids (Crustacea: Copepoda)	1012	55	957	1640.0	698	314	-55.0
Littorinids (Mollusca: Gastropoda)	1009	817	192	-76.5	616	393	-36.2
Isopods (Crustacea: Isopoda)	626	255	371	45.5	287	339	18.1
Amphipods (Crustacea: Amphipoda)	307	33	274	730.3	116	191	64.7
Ptychopterid larvae (Arthropoda: Insecta)	66	51	15	-70.6	26	40	53.8
Flatworms (Platyhelminthes)	52	7	45	542.9	31	21	-32.3
Leathery sea slugs (Mollusca: Gastropoda: Onchidella)	21	0	21	-	20	1	-95.0
Ribbon worms (Nemertea)	20	0	20	-	10	10	0.0
Others	10	2	8	300.0	10	0	-100.0
Total	12586	4001	8585	114.6	7076	5510	-22.1

*Others: other gastropods, tanaids, scaphopods, echinoids, non-identified individuals

3.2. *Losses on general community attributes in the absence of facilitation*

Overall mussel facilitation was pervasive across sites and throughout the experiment, but the general effects of thermal stress, and stress-dependent facilitation, apparently hinge on the relative vulnerability of the single-layered non-facilitated barnacle habitat. More specifically, results suggest that below certain threshold levels of assemblage attributes, invertebrate communities may rapidly decline after short-term thermal stress. Invertebrate abundance revealed overarching treatment effects (habitat and thermal manipulation combined; $F_{(3,3)} = 12.08$, $p = 0.035$; Table 3), but outcomes on richness and diversity were shore dependent (respectively; $F_{(3,48)} = 2.29$, $p = 0.090$; $F_{(3,48)} = 4.53$, $p = 0.007$; Table 3). Regardless of shore, and despite of marked spatial variation at the two scales tested (shore and plot effects; $p \leq 0.001$, Table 3), abundance was higher on the mussel habitat compared to the barnacle habitat ($t_{(142)} = 6.15$, $p < 0.0001$; Fig. 2a), supporting mussel facilitation, and higher on control compared to heated plots ($t_{(142)} = 1.87$, $p < 0.032$; Fig. 2a), indicating general effects of thermal stress, in accordance with the overall numbers shown in Table 2 (i.e. abundance increasing 114.6 % through facilitation and declining 22.1 % after heating). Based on abundance ratios between the mussel and the barnacle habitat (M/B), stress-dependent facilitation on overall invertebrate abundance, consistent to the stress-gradient hypothesis (SGH), is supported. The M/B ratio for heated plots was 75 % higher than for control plots (2.91 vs. 1.66; $t_{(44)} = 3.84$, $p < 0.001$), mostly owing to a much-reduced abundance in the barnacle habitat after heating (Fig. 2a). At Guaecá and Feiticeira mussel facilitation also increased richness (respectively $t_{(70)} = 5.03$, $t_{(70)} = 7.92$; $p < 0.0001$) and diversity (respectively $t_{(70)} = 4.68$, $t_{(70)} = 7.55$; $p < 0.0001$) but the extent of faunal declines in the non-facilitated habitat after heating apparently explains shore-dependent effects on those two community properties (Fig. 2 b-e). Low average richness levels, around four invertebrate groups per sample, combined with *H*' diversity indices nearing 0.80, as typically observed at the barnacle habitat in Feiticeira, are seemingly insufficient to stand the level of thermal stress introduced in the system, explaining the observed declines in richness (although with marginal statistical support; $t_{(70)} = 1.34$, $p = 0.092$; Fig. 2c) and diversity ($t_{(70)} = 1.83$, $p = 0.035$; Fig. 2e). As an outcome, stress-dependent facilitation consistent to the SGH is supported for the two parameters at this shore (respectively; $t_{(23)} = 3.39$, $t_{(23)} = 3.31$, $p < 0.01$), where M/B ratios were on average 72-76 % higher after heating (Fig. 2c,e). Conversely, effects of thermal stress (respectively for richness and diversity; $t_{(70)} = 0.68$, $t_{(70)} = 0.08$; $p > 0.05$) and stress-dependent facilitation (respectively; $t_{(34)} = 0.20$, $t_{(70)} = 0.74$, $p > 0.05$; Fig. 2b,d), were not held at Guaecá. Notably, treatment effects on those general univariate attributes remained similar along the succession of thermal manipulation events (i.e. non-significant shore*treatment interactions in Table 3), suggesting that eventual chronic or cumulative outcomes would be very subtle at most.

Table 3. Summary results of the general linear model testing the combined effects of habitat [mussel (M) vs. barnacle (B) cover] and thermal stress [control (C) vs. heated (H)], *i.e.* ‘treatment effects’, over sequential experimental ‘events’, on univariate attributes of invertebrate assemblages (abundance, richness and diversity). The random factor ‘shore’ tests for spatial consistency (unit km) of any invertebrate responses to fixed-factor main effects, or their interaction. Significant p-values ($p < 0.10$) for the terms relevant to planned contrasts are shown in bold. Follow-up specific comparisons (α = 0.5) involving all four treatments (MC, MH, BC, BH) are explained in the main text.

		Abundance			Richness			Diversity		
Source	d.f.	MS	F	p	MS	F	p	MS	F	p
Shore: Sh	1	168.7	13.39	0.001	0.6	0.17	0.684	0.07	0.67	0.417
Event: Ev	2	160.9	5.73	0.149	75.1	7.06	0.124	1.86	8.44	0.106
Treatment: Tr	3	185.3	12.08	**0.035**	111.8	14.57	0.027	3.55	7.47	0.066
Sh*Ev	2	28.1	2.23	0.119	10.6	3.18	0.051	0.22	2.09	0.134
Sh*Tr	3	15.3	1.22	0.314	7.7	2.29	**0.090**	0.47	4.53	**0.007**
Ev*Tr	6	18.4	1.09	0.460	3.6	0.67	0.677	0.40	2.34	0.162
Sh*Ev*Tr	6	16.9	1.34	0.257	5.3	1.60	0.169	0.17	1.63	0.160
Plot: Pl (Sh*Ev*Tr)	48	12.6	2.45	0.000	3.3	1.70	0.020	0.10	1.27	0.176
Res.	72	5.1			2.0			0.08		
Transform			sqrt (x+1)			none			none	
Cochran’s C			0.1445 (ns)			0.0883 (ns)			0.1074 (ns)	

Higher facilitation upon stress, consistent with the SGH, seemed to be primarily determined by increased stress mitigation through mussel ecosystem engineering, but responses observed for richness and diversity, combined, suggest a secondary effect of the state of non-facilitated assemblages prior to thermal manipulation. For instance, a typical low-richness sample from the barnacle habitat, equaling four, would probably contain mites, midge larvae, littorinid snails and isopods (based on the overall numbers of Table 2). Mites would strongly rely on midge larvae (either as prey or as parasitic hosts; Martin & Stur 2006, Pozojević et al. 2019), while the other three groups on more basal resources (chiefly biofilms and detritus; Norton et al. 1990, Hillebrand 2009, Prato et al. 2012). A richer sample of five groups would likely include nematodes, which could be an alternative food resource for mite populations (as in terrestrial systems, e.g. Rueda-Ramírez et al. 2023), potentially reducing their chance to collapse upon warming. This additional interaction, as many others emerging from adding a simple invertebrate group, could ultimately promote assemblage resistance to thermal stress, as predicted by the biological insurance hypothesis, which advocates enhanced community stability through fulfilment of niche space and functional redundancy (Naeem & Li 1997, Yachi & Loreau 1999, Loreau et al. 2021). Still, a much better appraisal of the strength and direction of the biological interactions among the invertebrate groups focused on this study is needed to properly predict the impact of declines, or local extinctions, on assemblage networks.

3.3. *Stress-dependent facilitation of main invertebrate groups*

Despite of very marked differences between shores (Table 4), along with uncontrolled dynamics that led to overall drift and increasing variation among samples over time (Fig. 3a,b,d,e,g,h), we detected context-dependent effects of habitat and thermal manipulation on the structure of invertebrate assemblages. Besides a spatial component (‘shore*treatment’; $F_{(3,48)} = 1.96$, $p = 0.012$), as observed for diversity and richness, there was also a temporal component modulating treatment outcomes (‘event*treatment’; $F_{(6,48)} = 2.16$, $p = 0.031$; Table 4), both shown in the green-shaded plots of Figure 3 (c,f,i,j,k). Pairwise planned contrasts within shores (21.6 < Pseudo-$F_{(1,70)}$ < 21.9, $p < 0.001$) and events (12.7 < Pseudo-$F_{(1,46)}$ < 21.2, $p \leq 0.001$) indicate, again, overwhelming habitat effects (largely as mussel facilitation), but less general responses to thermal stress, restricted to Feiticeira (Pseudo-$F_{(1,70)} = 2.83$, $p = 0.014$) and, of marginal statistical support, after Event 1 (Pseudo-$F_{(1,46)} = 1.93$, $p = 0.087$; Fig. 3). Consistent to the SGH, and considering the two-factor interactions depicted in the marginal shaded plots of Figure 3, average distances between mussel and barnacle samples were always higher for heated compared to control plots, although statistical support was limited to Feiticeira ($t_{(34)} = 2.49$, $p = 0.009$) and for samples obtained after Event 2 ($t_{(22)} = 3.64$, $p < 0.001$). Eight invertebrate groups repeatedly explained most dissimilarity between groups: nematodes, polychaetes, harpacticoid copepods, amphipods, isopods, midge larvae, mites and littorinid snails (see SIMPER results in Supplementary Tables S1-S4). Those can be roughly separated into three categories: groups that tend to be mussel-facilitated and winners after warming (++; nematodes, polychaetes, isopods and amphipods), mussel-facilitated and losers after warming (+-; chironomid larvae and harpacticoid copepods), and mussel-restrained and losers after warming (--; mites and littorinid snails; Table 2, Tables S1-S3).

Table 4. Summary results of permutational analyses of variance (PERMANOVA) testing responses of invertebrate assemblage structure to combinations of habitat (mussel vs. barnacle cover) and thermal stress (control vs. heated), i.e. ‘treatment effects’, over sequential experimental ‘events’. The random factor ‘shore’ tests for spatial consistency (unit km) of any invertebrate responses to fixed-factor main effects, or their interaction. Significant *p*-values for the terms relevant to planned comparisons are shown in bold.

		Assemblage structure		
Source	d.f.	MS	Pseudo-*F*	*p*
Shore: Sh	1	8782	4.84	<0.001
Event: Ev	2	15299	3.99	0.132
Treatment: Tr	3	24079	6.76	0.066
Sh*Ev	2	3836	2.11	0.020
Sh*Tr	3	3563	1.96	**0.012**
Ev*Tr	6	2645	2.16	**0.031**
Sh*Ev*Tr	6	1226	0.68	0.926
Plot: Pl(Sh*Ev*Tr)	48	1814	2.15	< 0.001
Res.	72	844		

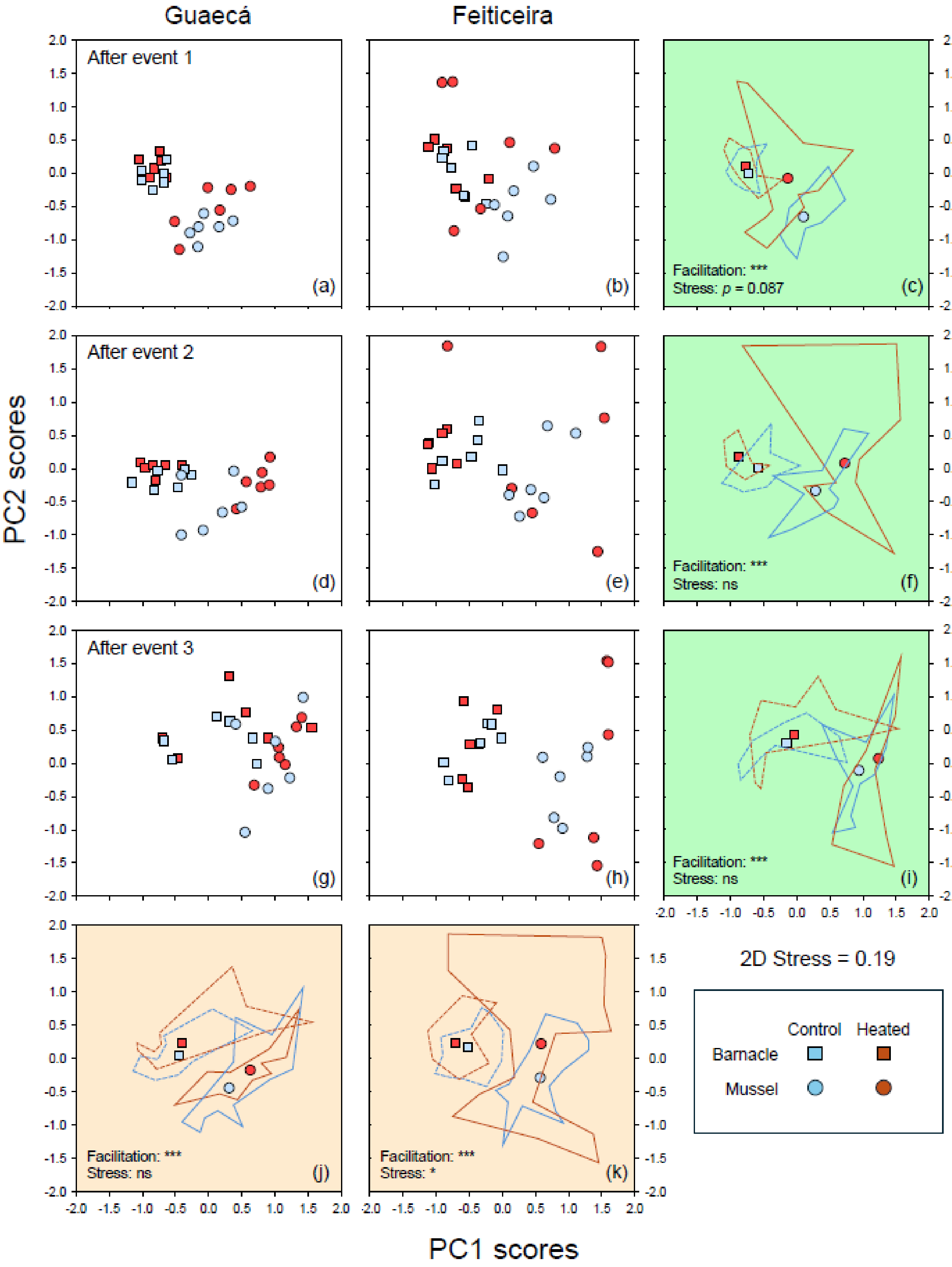

Fig. 3. Non-metric multi-dimensional scaling plots showing effects of heat stress on invertebrate assemblage structure at barnacle and mussel-covered habitats. White plots (a,b,d,e,g,h) show sample clustering for each shore and manipulation event, separately. Plots representing significant two-order interactions are shaded in green [treatment*event; (c, f, i)] or salmon [treatment*shore; (j, k)]. For these two-order interaction plots, symbols and lines represent respectively sample centroids and spread for each combination of factor levels (Mussel control: MC, Barnacle control: BC, Mussel heated: MH, Barnacle heated: BH). Results of planned contrasts testing for facilitation (MC-MH vs BC-BH) and stress (MH-BH vs MC-BC) are shown in the lower left corner of plots (ns: non-significant, *: $p < 0.05$, *** $p < 0.001$). In all plots, scores of principal components 1 and 2 were calculated jointly for the whole dataset allowing direct comparisons.

Overall, results suggest that mussel-facilitation will become more important as heat-wave frequency and intensity increase over the next decades, consistent to the SGH hypothesis and contrary to the notion that mussel foundational effects may collapse. Analyses on stress-dependent facilitation, summarized as *z*-scores spanning the relative facilitation in control and heated conditions (shaded panels in Fig. 4), identified 15 cases of statistical significance ($z \neq 0$, $p < 0.05$), 10 indicating enhanced facilitation and 5 pointing to decreased facilitation after thermal stress. Increased facilitation after warming in mites was markedly consistent, checked for the two sites (Fig. 4j,k) and after all sampling events (Fig. 4c,f,i), and repeatedly observed for littorinid snails (at Feiticeira, Fig. 4k, and after Event 2, Fig. 4f). Those two groups, together with midge larvae at Guaecá (Fig. 4j) responded similarly to warming, basically as pronounced declines in the barnacle habitat (e.g. Fig. 4b,d,g,h), suggesting that populations of those species may shrink in the future owing to reduced niche space in the rocky intertidal. This is especially true for mites and midge larvae, as they are more sedentary and would not seek for microrefugia over the thermal landscape. Mussel beds may thus become paramount for those groups, and not secondary habitat as they are at present. Other cases of group-specific stress-dependent facilitation are more difficult to interpret and likely reflect more complex processes, such as migration. Because experimental plots included remnant areas of the non-focal cover, individual movements between habitat patches may explain the unexpected cases in which polychaetes (Fig. 4b,d,e), and to a lesser extent nematodes (Fig. 4d,e) and amphipods (Fig. 4a,d,g), reached higher densities in mussel beds after heating. Heat stress at the barnacle habitat may trigger an escape response, with nematodes eventually resuspending in the water and actively screening habitat upon descending (e.g. Ullberg & Ólafsson 2003), and larger polychaetes directly crawling (e.g. Negrello et al. 2006; Aberson et al. 2011) to the less stressful mussel cover. Amphipods are chiefly swimming, highly mobile peracarids (Steele 1988) and brood their young (Schram 1986), which makes them capable to rapidly reach and colonize novel habitat (Edgar 1991, Vázquez-Luis et al. 2012). Higher mobility, together with the competitive advantage given by their larger size, would thus explain why polychaetes (Fig. 4f) and amphipods (Fig. 4j) may become more facilitated by mussel engineering with the increasing frequency of warming events. The less common cases of decreased facilitation after warming, as repeatedly observed for harpacticoid copepods (Fig. 4, shaded plots), are still unclear and may reflect indirect species interactions. Harpacticoids are among the most vulnerable invertebrates and their extensive decline with warming (-55.0 %, Table 2), even within mussel beds (Fig. 3), could simply indicate insufficient mitigation of physical stress. Yet, together with nematodes, they constitute main prey for larger invertebrate predators, including chironomid larvae and flatworms (Ptatscheck et al. 2020). Those two predators are overall expected to decline with warming (Table 2), but higher losses of much larger flatworms (-32.3 %) may explain the rise of nematodes (+12.6 %, Table 2), their main prey (Beier et al. 2004, Kreuzinger-Janik et al. 2018). This may cascade to abundance declines of similarly sized harpacticoid copepods, as those two groups may compete for refugia and food (Meadows et al. 2015). Increased competition after heating may also explain reverse trends for larger crustaceans in Guaecá (Fig. 3j), as immigrant amphipods may have displaced isopods from the mussel habitat. It should be pointed out, however, that species interactions within the interstices of barnacle and mussel beds are still largely unknow and the tentative explanations above remain speculation, pending formal testing. Still, the changes observed for assemblage structure at the two different habitats, along with their probable driving mechanisms, strongly suggest extensive ‘rewiring’ of the interactive network (as in Watts & Strogatz 1998, Dupont et al. 2009) connecting the different interstitial invertebrate groups.

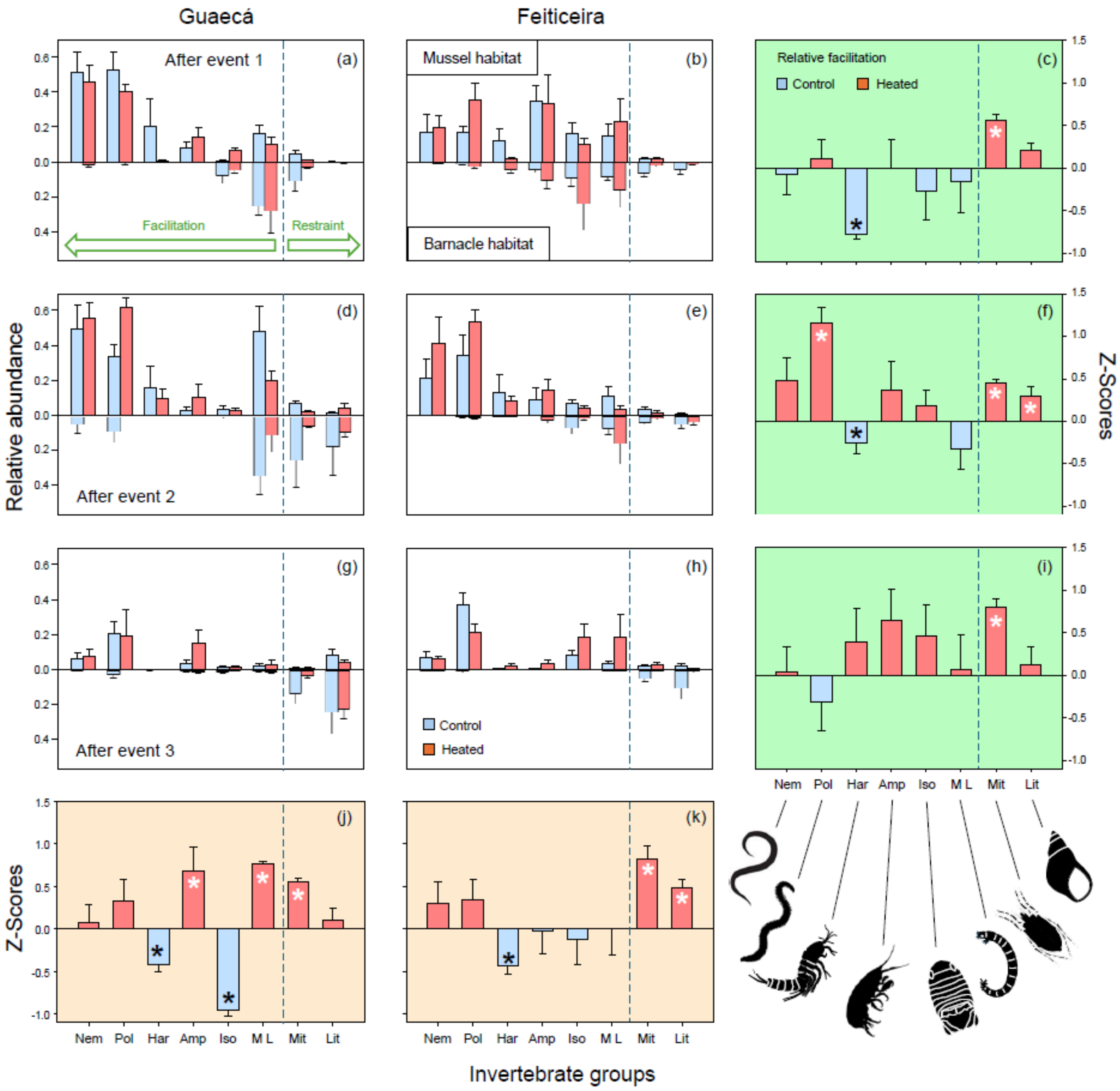

Fig. 4. (a,b,d,e,g,h): Relative abundance (based on x_i/x_{max} data) of key invertebrate groups responsible for whole-assemblage contrasts owing to facilitation (Mussel control - Mussel heated vs Barnacle control – Barnacle heated) and thermal stress (Mussel heated – Barnacle heated vs Mussel control – Barnacle control) at each shore and after each heating event. Invertebrate groups are ordered from the most (left) to the least (right) facilitated and vertical dashed lines separate groups that are overall facilitated and restrained. Bars stand for average values and whiskers for +1SE. Plots shaded in green (c, f, i) and salmon (j, k) show the relative facilitation of invertebrate groups after each thermal manipulation event (shores pooled) and at each shore (events pooled), respectively. Invertebrate groups for which heating tended to increase and decrease facilitation are shown in red and blue, respectively. Whiskers represent +1SE and significant cases ($z \neq 0$, $p < 0.05$) are marked with an asterisk. Nem: Nematodes; Pol: Polychaetes; Har: Harpacticoid copepods; Amp: Amphipods; Iso: Isopods; ML: Midge larvae; Mit: Mites; Lit: Littorinid snails.

4. **Conclusion and final remarks**

This is a follow-up study of Bogdanski and colleagues (2024), who showed increased facilitation of mobile invertebrates along the natural stress gradient delivered by the time they are exposed to air, which increases linearly cross-shore, from the lower to the upper vertical limit of mussel beds. While assemblages in the control barnacle habitat became gradually impoverished along the gradient, the assemblages at the mussel-facilitated habitat remained remarkably unchanged, suggesting complete mitigation of stress. Here, we worked on the very same system and tested whether mussel facilitation will still hold in the near future by setting pulse thermal manipulations in the field as to increase the ambient temperature for the interstitial associated fauna in around 5 °C. The short answer is 'yes', but contrary to expectations, and considering midlittoral invertebrates as a whole (barnacle and mussel habitat combined) climate change may impact thermally-resistant species the most, and indirectly benefit thermally-vulnerable, but more mobile, species that may flee from harsh to amenable spots within the thermal mosaic. Worryingly, the niche space presently occupied by the most resistant species at the barnacle habitat could drastically shrink.

Within the facilitated habitat, interspecific competition, and much likely increased settlement due to intensified chemical cueing released by immigrants, especially polychaetes, may greatly alter species interactions in the mussel-facilitated habitat. How indirect effects cascade down the species interaction network, and what are the effects of successive heat-waves over periods longer than the single season reported here, remain pending questions. Also, the role of rarer invertebrate groups, not selected in SIMPER routines (in spite of using 4^{th} root transformed data in analyses), may be downplayed. The density of flatworms, leathery sea slugs and sea-urchins were overall reduced to a third after heating, and therefore any disproportional consumptive effects (i.e. through keystone predation) may greatly decline.

Finally, our interpretation of results assumes that mussels themselves are resistant to heat-waves, which may not be the case (e.g. White et al. 2023). Events of mussel mass mortality could lead to catastrophic events, as the very diversified fauna they facilitate would be also greatly impacted, with effects surely surpassing the limits of the rocky intertidal, as several passing-by higher-order consumers, such as fish, crabs and birds, actively forage on mussel beds and thereby connect land and open-ocean ecosystems. A next stage of this line of work should include an appraisal on the effects of heat-waves on mussel performance, including physiological metrics, as such responses can be used as early signals of greater damage.

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Supplementary Material

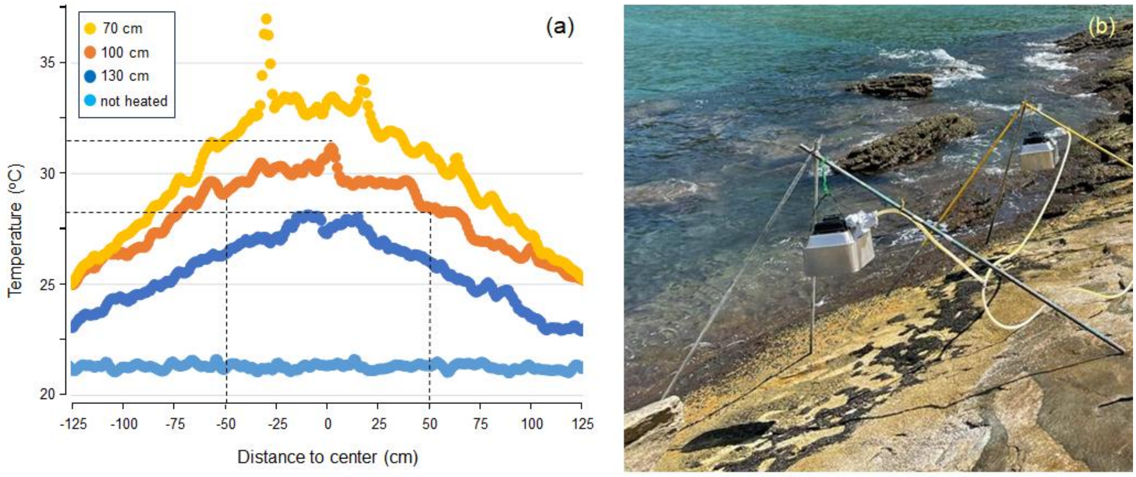
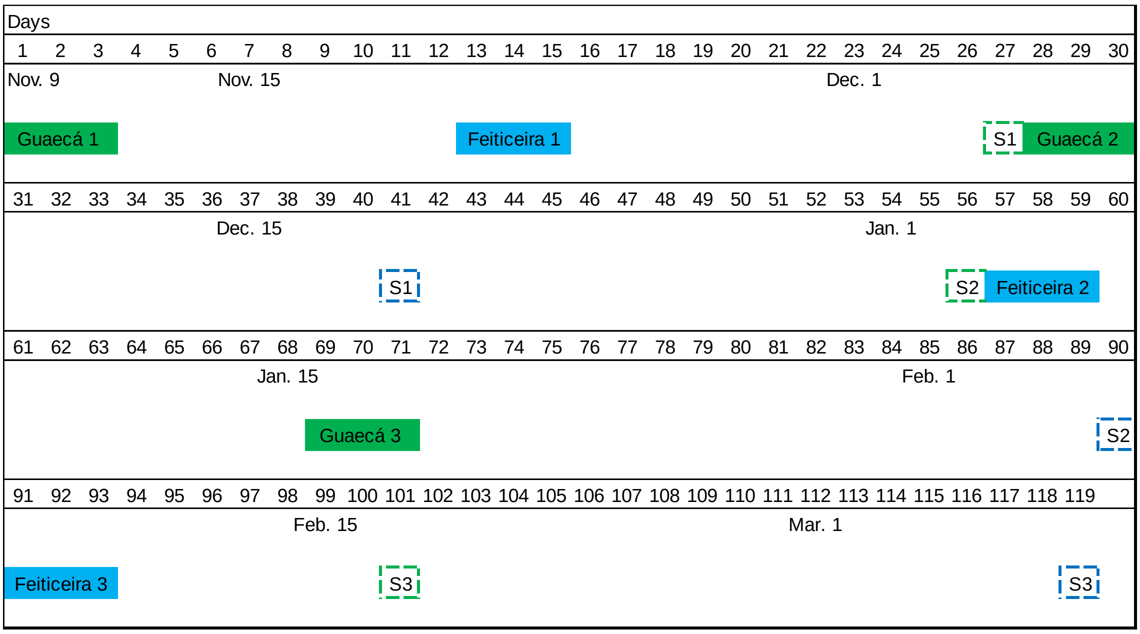


Figure S1. Preliminary work and *in situ* experimental heating in the midlittoral zone at Guaecá, SP, Brazil. (a) Pilot heating trials, on outdoors cemented ground, to determine the height of heaters and the size of experimental plots. The 100 cm height was chosen, as it delivered, in the trial, surface temperatures ranging from 28.2 to 31.4 °C (average: 29.8 °C) over a radius of 0.5 m, exceeding 7.0-10.2 °C (average: 8.6 °C) control temperatures measured 3m away (ca. 21.2 ºC). Surface temperature readings were obtained through infrared thermography (FLIR mod. C5). (b) A set of two heaters (see Methods) deployed on the shore at low-tide.



Days																													
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Guaecá 1												Feiticeira 1														S1	Guaecá 2		
31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
						Dec. 15																	Jan. 1						
										S1															S2	Feiticeira 2			
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
							Jan. 15																	Feb. 1					
								Guaecá 3																					S2
91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	
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Feiticeira 3										S3																	S3		

Fig. S2. Timeline of the field experimental work. Thermal manipulations (1 to 3), reproducing heat waves over three consecutive days, were conducted at two replicate intertidal rocky shores (Guaecá and Feiticeira) in the São Sebastião Channel, SP, Brazil. Manipulations were undertaken during low-tide spring tides, and invertebrate assemblages were sampled (S1-S3) approximately one lunar month ahead (26-28 d).

Table S1. Similarity percentage (SIMPER) analyses detailing interactive ‘event’*‘treatment’ effects, indicated in the omnibus procedure (see Table 3) and suggesting ecological facilitation (mussel vs. barnacle treatments; $p < 0.0001$ in all pairwise comparisons). Invertebrate groups responsible for most dissimilarities between habitats (percent contribution higher than 8) are shaded in gray.

	Habitat			
Groups	Mussel (ind.plot^{-1})$^{0.5}$	Barnacle (ind.plot-1)$^{0.5}$	Contrib. %	Cum. %
After event 1		Average dissimilarity = 62.07		
Nematoda	6.76	0.70	24.51	24.51
Polychaeta	4.58	0.44	17.06	41.57
Chironom. Larvae	3.12	3.34	9.87	51.44
Harpacticoida	2.76	0.55	9.63	61.07
Acari	3.02	3.79	8.52	69.59
Isopoda	2.00	2.27	7.76	77.36
Amphipoda	2.19	0.56	7.72	85.07
Chironom. Pupae	0.46	0.94	3.74	88.82
Littorinidae	0.41	0.77	3.50	92.32
After event 2		Average dissimilarity = 68.02		
Nematoda	7.59	0.83	25.25	25.25
Polychaeta	5.22	0.78	17.96	43.21
Harpacticoida	3.51	0.41	11.08	54.30
Chironom. Larvae	3.34	2.82	10.39	64.68
Acari	3.36	4.19	10.31	74.99
Littorinidae	0.92	2.38	7.93	82.92
Isopoda	1.46	0.76	5.44	88.36
Amphipoda	0.98	0.25	3.58	91.93
After event 3		Average dissimilarity = 70.69		
Polychaeta	3.36	0.23	19.11	19.11
Littorinidae	1.61	3.28	17.67	36.78
Nematoda	2.70	0.21	14.85	51.63
Acari	2.21	3.34	14.55	66.17
Isopoda	1.71	0.13	9.89	76.07
Chironom. Larvae	1.44	0.39	7.28	83.34
Amphipoda	0.72	0.13	6.02	89.37
Chironom. Pupae	0.77	0.13	3.97	93.34

Table S2. Similarity percentage (SIMPER) analyses detailing interactive ‘shore’*‘treatment’ effects, indicated in the omnibus procedure (see Table 3) and suggesting ecological facilitation (mussel vs. barnacle treatments; $p < 0.0001$ in all pairwise comparisons). Invertebrate groups responsible for most dissimilarities between habitats (percent contribution higher than 8) are shaded in gray.

	Habitat			
	Mussel	Barnacle		
Groups	(ind.plot^{-1})$^{0.5}$	(ind.plot-1)$^{0.5}$	Contrib. %	Cum. %
Guaecá	Average dissimilarity = 65.96			
Nematoda	6.78	0.76	23.52	23.52
Polychaeta	4.42	0.50	16.02	39.54
Acari	2.97	5.05	12.92	52.46
Littorinidae	1.36	2.86	11.51	63.97
Chironom. Larvae	3.01	2.78	11.06	75.03
Harpacticoida	2.17	0.12	6.74	81.77
Amphipoda	1.12	0.11	5.01	86.77
Isopoda	1.09	0.77	4.90	91.67
Feiticeira	Average dissimilarity = 72.36			
Polychaeta	4.34	0.36	19.35	19.35
Nematoda	4.61	0.19	18.98	38.33
Isopoda	2.39	1.27	10.04	48.37
Chironom. Larvae	2.27	1.56	9.96	58.33
Acari	2.72	2.57	9.18	67.51
Harpacticoida	2.36	0.47	9.16	76.67
Littorinidae	0.57	1.49	6.82	83.49
Amphipoda	1.54	0.42	6.46	89.95
Chironom. Pupae	0.65	0.27	3.15	93.09

Table S3. Similarity percentage (SIMPER) analyses detailing interactive 'event'*'treatment' and 'shore'*'treatment' effects, indicated in the omnibus procedure (see Table 3) and suggesting thermal stress (heated vs. control treatments; significant pairwise comparisons: Event 1, $p < 0.05$; Feiticeira, $p = 0.087$). Invertebrate groups responsible for most dissimilarities between habitats (percent contribution higher than 8) are shaded in gray.

	Thermal manipulation			
Groups	Heated (ind.plot^{-1})$^{0.5}$	Control (ind.plot-1)$^{0.5}$	Contrib. %	Cum. %
Event 1		Average dissimilarity = 53.86		
Nematoda	3.85	3.61	19.25	19.25
Chironom. Larvae	3.03	3.43	12.79	32.04
Polychaeta	2.68	2.35	12.47	44.51
Acari	2.61	4.20	11.18	55.69
Isopoda	2.46	1.81	10.12	65.81
Harpacticoida	1.24	2.07	9.44	75.25
Amphipoda	1.45	1.30	7.29	82.55
Chironom. Pupae	0.76	0.64	4.61	87.16
Littorinidae	0.51	0.67	4.45	91.60
Feiticeira		Average dissimilarity = 65.05		
Nematoda	2.68	2.12	14.30	14.30
Acari	1.95	3.33	13.94	28.24
Polychaeta	2.48	2.22	13.37	41.60
Chironom. Larvae	1.90	1.93	12.43	54.03
Isopoda	1.85	1.81	10.80	64.83
Littorinidae	0.70	1.36	9.45	74.28
Harpacticoida	1.50	1.33	8.82	83.10
Amphipoda	1.10	0.86	6.27	89.37
Chironom. Pupae	0.50	0.42	3.55	92.92

Table S4. Summary results of SIMPER analyses for significant pairwise comparisons related to facilitation (mussel vs. barnacle treatments) and thermal manipulation (heated vs, control treatments) effects, following significance of the two-order interaction terms ‘event’*‘treatment’ and ‘shore’*‘treatment’ (Table 3). M: Mussel, B: Barnacle, H: Heated, C: Control.

Facilitation (Mussel vs. Barnacle treatments)

Thermal manipulation (Heated vs. Control treatments)

Ranked by mean contribution to dissimilarity (%)

	Events			Shores		
	Event 1	Event 2	Event 3	Guaecá	Feiticeira	Average
Nematoda	24.51	25.25	14.85	23.52	18.98	**21.42**
Polychaeta	17.06	17.96	19.11	16.02	19.35	**17.90**
Acari	8.52	10.31	14.55	12.92	9.18	**11.10**
Chironom. Larvae	9.87	10.39	7.28	11.06	9.96	**9.71**
Littorinidae	3.50	7.93	17.67	11.51	6.82	**9.49**
Isopoda	7.76	5.44	9.89	4.90	10.04	**7.61**
Harpacticoida	9.63	11.08	0.00	6.74	9.16	**7.32**
Amphipoda	7.72	3.58	6.02	5.01	6.46	**5.76**

	Event 1	Guaecá	Average
Nematoda	19.25	14.30	**16.78**
Polychaeta	12.47	13.37	**12.92**
Chironom. Larvae	12.79	12.43	**12.61**
Acari	11.18	13.94	**12.56**
Isopoda	10.12	10.80	**10.46**
Harpacticoida	9.44	8.82	**9.13**
Littorinidae	4.45	9.45	**6.95**
Amphipoda	7.29	6.27	**6.78**

Ranked by mean M/B ratios

	Events			Shores		
	Event 1	Event 2	Event 3	Guaecá	Feiticeira	Average
Nematoda	9.66	9.14	12.86	8.92	24.26	**12.97**
Polychaeta	10.41	6.69	14.61	8.84	12.06	**10.52**
Harpacticoida	5.02	8.56	0.00	18.08	5.02	**7.34**
Amphipoda	3.91	3.92	5.54	10.18	3.67	**5.44**
Isopoda	0.88	1.92	13.15	1.42	1.88	**3.85**
Chironom. Larvae	0.93	1.18	3.69	1.08	1.46	**1.67**
Acari	0.80	0.80	0.66	0.59	1.06	**0.78**
Littorinidae	0.53	0.39	0.49	0.48	0.38	**0.45**

Ranked by mean H/C ratios

	Event 1	Guaecá	Average
Amphipoda	1.12	1.28	**1.20**
Isopoda	1.36	1.02	**1.19**
Nematoda	1.07	1.26	**1.17**
Polychaeta	1.14	1.12	**1.13**
Chironom. Larvae	0.88	0.98	**0.93**
Harpacticoida	0.60	1.13	**0.86**
Littorinidae	0.76	0.51	**0.64**
Acari	0.62	0.59	**0.60**