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Efeito da redução do pH e elevação da temperatura da água do mar sobre a comunidade de meiofauna e associação de Copepoda Harpacticoida

Visnu da Cunha Sarmento

Recife, 2016

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EFEITO DA REDUÇÃO DO pH E ELEVAÇÃO DA TEMPERATURA DA ÁGUA DO MAR SOBRE A COMUNIDADE DE MEIOFAUNA E ASSOCIAÇÃO DE COPEPODA HARPACTICOIDA

Tese apresentada ao Programa de Pós-Graduação em Biologia Animal, da Universidade Federal de Pernambuco (UFPE), como requisito parcial para obtenção do título de Doutor em Biologia Animal.
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"Nós somos feitos da matéria de que são feitos os sonhos;

nossa vida pequenina é cercada pelo sono".

William Shakespeare

RESUMO

Os efeitos do aumento da emissão de gases do efeito estufa, como modificações na temperatura e bioquímica dos oceanos, já podem ser observados e é esperado que se intensifiquem no futuro, causando impactos negativos nos ecossistemas marinhos. O presente estudo teve como principal objetivo investigar os efeitos de diferentes níveis de pH e de temperatura da água do mar sobre as comunidades de meiofauna e de Copepoda Harpacticoida. Para isso foram realizados três experimentos: um experimento para avaliar o efeito de diferentes níveis de pH da água do mar sobre a comunidade de meiofauna do recife de coral do Parque Municipal Marinho do Recife de Fora (Porto Seguro, Bahia); um experimento para avaliar o efeito de diferentes cenários de mudanças climáticas (alterações simultâneas de temperatura e pH da água do mar) sobre a comunidade de meiofauna e de Copepoda Harpacticoida do recife de coral da Praia de Serrambi (Ipojuca, Pernambuco); e um experimento para avaliar o efeito da interação de diferentes níveis de pH e temperatura da água do mar sobre a associação de Copepoda Harpacticoida do costão rochoso da praia de Mounlt Batten (Plymouth, Inglaterra). Foram observadas modificações na estrutura da comunidade de meiofauna e harpacticóides, especialmente quando expostos ao aumento simultâneo de temperatura e acidificação da água do mar. Diferentemente, nos harpacticóides de área temperada coletados em zona de entremaré, os impactos do aumento de temperatura e acidificação foram observados apenas nos tratamentos mais severos. Devido à sensibilidade que os organismos da meiofauna apresentaram aos aumentos de temperatura e acidificação, os resultados apresentados aqui demonstram que o funcionamento trófico dos sistemas bentônicos pode estar seriamente ameaçado pelas mudanças climáticas.

Palavras-chave: meiofauna, copepoda, acidificação, aquecimento, mudança climática.

ABSTRACT

The increase in greenhouse gas emissions has led to unprecedented atmospheric carbon dioxide concentrations. Its impacts have been observed through changes in ocean temperature and biochemistry. However, intensifications of these changes are predicted for the future with serious consequences to marine ecosystems. This study aims to assess the potential effects of different levels of seawater pH and temperatures on meiofauna and harpacticoid copepods communities. For that, three experiments were performed: an experiment was done to assess the effect of different seawater pH on meiofauna community from the coral reefs of Recife de Fora Municipal Marine Park (Porto Seguro, Bahia, Brazil); an experiment was done to evaluate the effect of different climate change scenarios (simultaneous changes in seawater temperature and pH) on meiofauna and harpacticoid communities from the coral reefs of Serrambi beach (Ipojuca, Pernambuco, Brazil); and an experiment was done to evaluate the interactive effects of rising temperature and acidification on harpacticoid community from the rockyshore of Mount Batten beach (Plymouth, UK). Modifications on community structure of meiofauna and Copepod Harpacticoida were observed, especially when organisms were exposed to simultaneous increases in seawater temperature and acidification. Furthermore, harpacticoids from temperate region sampled at the intertidal zone, showed to be affected only at the most severe treatments. Due to the sensibility to ocean warming and acidification that meiofauna organisms exhibited, the results presented here highlights the risk that climate changes poses on the trophic functioning of benthic habitats.

Keywords: meiofauna, copepod, acidification, warming, climate change.

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INTRODUÇÃO GERAL

O sistema climático terrestre é um sistema complexo que consiste da atmosfera, superfície terrestre, neve e gelo, oceanos e outras massas d'água, e dos seres vivos. O clima é normalmente descrito em termos de valores médios e de variação de fatores como temperatura, precipitação e ventos num dado período do tempo, que pode variar desde meses a milhões de anos. O sistema climático evolui ao longo do tempo sob a influência de sua própria dinâmica interna ou devido a mudanças de fatores externos. Uma vez que a radiação solar determina o nosso sistema climático, existem três formas de alterar o equilíbrio dessa radiação na Terra: 1) alterando a entrada de radiação solar (e.g., através de mudanças na orbita da Terra ou no Sol); 2) alterando a fração de radiação solar que é refletida (e.g., através de mudanças na cobertura de nuvens, partículas atmosféricas ou vegetação); e 3) alterando a radiação de ondas longas da Terra que volta para o espaço (e.g., através de mudanças na concentração de gases do efeito estufa) (IPCC 2007). Entretanto, ao longo dos últimos séculos, as atividades humanas se tornaram um componente adicional para o sistema climático terrestre, e nos últimos 50 anos, se tornaram o componente dominante responsável pelo aquecimento global observado (IPCC 2007; Harley et al. 2006; Feely et al. 2004).

As mudanças climáticas originadas das ações antropogênicas são resultantes, primariamente, de mudanças na quantidade dos gases do efeito estufa (principalmente o dióxido de carbono, seguidos de metano, óxido nitroso e halocarbonos) na atmosfera. Esses gases aprisionam parte da energia/calor que re-irradiaria para o espaço, ajudando a aquecer o planeta (Harley et al. 2006; Feely et al. 2004).

O aumento nas emissões dos gases do efeito estufa desde a revolução industrial, movido largamente pelo crescimento econômico e populacional, resultou em

um aumento da concentração de CO_2 atmosférico de cerca de 280 ppm para aproximadamente 400 ppm (Feely et al. 2004; IPCC 2014). A esse aumento nas emissões, é relacionado um aumento de 0,85 [entre 0,65 e 1,06]°C na temperatura global (IPCC 2014).

Nesse processo, os oceanos possuem um papel fundamental obsorvendo grandes quantidades de energia e de CO_2. Aproximadamente, 30% de todo o CO_2 de origem antropogênica emitido foi absorvido pelos oceanos (Sabine et al. 2004). Embora essa captação de CO_2 atenue os efeitos das mudanças climáticas, quando uma grande quantidade de dióxido de carbono de origem antropogênica é dissolvida nos oceanos leva à alterações na bioquímica da água do mar, reduzindo seu pH e causando mudanças no sistema carbonato (Feely et al. 2004, 2009). Quando o CO_2 entra na água do mar, eleva as concentrações de CO_2 dissolvido que, ao combinar com a água forma ácido carbônico (H_2CO_3). Este ácido dissocia-se, em sua maioria, em bicarbonato (HCO_3^-) e íons de hidrogênio. Os íons de hidrogênio produzidos nesse processo diminuem o pH da água do mar. Eles também reagem com íons de carbonato (CO_3^{2-}) que estão presentes na água do mar para forma mais íons de bicarbonato reduzindo a concentração de CO_3^{2-}, que por sua vez reduzem o estado de saturação de minerais de carbonato de cálcio (aragonita e calcita) (Feely et al. 2009). Essas alterações, frequentemente referidas como acidificação dos oceanos, já estão ocorrendo e o pH da água superficial oceânica está 0,1 unidade mais baixo quando comparado aos níveis pré-industriais (Caldeira e Wickett 2003).

Durante a "Conference of the Parties" (COP21), que ocorreu entre os meses de novembro e dezembro de 2015, foram discutidas medidas para manter o aumento na temperatura média global abaixo de 2ºC acima dos níveis pré-industriais até o ano de 2100, e para limitar esse aumento em no máximo 1,5ºC. Essa mobilização global

estabeleceu metas para reduzir ao máximo a emissão de gases do efeito estufa, incentivar o uso de fontes de energia renovável, reduzir ou parar o desmatamento, dentre outras. Apesar de o aumento de 1ºC na temperatura media global ser suficiente para colocar em risco alguns ecossistemas e culturas, o aumento acima de 2ºC geraria consequências muito mais graves para a população humana, como o aumento na intensidade de eventos climáticos extremos (IPCC 2014).

De acordo com o "Intergovernmental Panel on Climate Change" (IPCC), a captação de CO_2 de origem antropogênica pelos oceanos continuará até 2100 considerando todos os cenários de concentração usados nos modelos. Dessa forma, os oceanos irão continuar a aquecer e acidificar. Na atmosfera, o aumento contínuo nas concentrações dos gases do efeito estufa pode alcançar entre 475–1313 ppm no ano de 2100. Consequentemente, é previsto um aumento na temperatura média global entre 2 e 4°C até o final do século 21. Para os oceanos, é previsto uma aumento de 0,6–2ºC nos primeiros 100 m de profundidade (IPCC 2014). Considerando os impactos na bioquímica dos oceanos, estima-se uma diminuição no pH da água superficial oceânica de até 0,4 unidades em 2100 (Caldeira e Wickett 2003; Feely et al. 2004) e de cerca de 0,7 unidades em 2250 (Caldeira e Wickett 2003; Caldeira e Wickett 2005).

É amplamente aceito que as previsões de redução no pH oceânico e do aquecimento global terão efeitos negativos sobre os organismos bentônicos, devido principalmente às alterações fisiológicas e/ou metabólicas que podem desencadear mudanças no crescimento e sobrevivência (Byrne et al. 2010; Findlay et al. 2010; Anthony et al. 2011; Dissanayake e Ishimatsu 2011; Wood et al. 2011). Entretanto, os diferentes grupos taxonômicos e/ou espécies têm apresentado grande divergência nas respostas às mudanças climáticas globais, inclusive ao nível de filo: enquanto os equinodermos, moluscos e cnidários parecem demonstrar grande vulnerabilidade, os

artrópodes e anelídeos parecem ser menos vulneráveis (Orr et al. 2005; Bibby et al. 2007; Widdicombe e Spicer 2008; Hale et al. 2011).

A literatura sobre os impactos das mudanças climáticas nos sistemas e organismos marinhos continua a crescer exponencialmente. Além disso, também é observado um aumento importante no número estudos que abordam os efeitos de múltiplos estressores, que podem ser influenciados pelas mudanças climáticas globais (pCO_2, pH, temperatura, salinidade, oxigênio, poluentes, etc.) sobre os organismos marinhos. Entretanto, apesar desse aumento, a maioria dos estudos realizados com a fauna bentônica ainda é conduzida ao nível de organismos ou espécies individuais (e.g., Findlay et al. 2010; Wood et al. 2011; Calosi et al. 2013; Belivermis et al. 2015; Campanati et al. 2015; Schram et al. 2015; Swiney et al. 2015; Vincent et al. 2015).

Uma das grandes incertezas quanto aos efeitos das mudanças climáticas é como as espécies irão responder dentro de um contexto de comunidades multiespecíficas. É muito provável que uma das consequências mais notáveis das mudanças climáticas surgirá através de alterações nas interações interespecíficas (Gaylord et al. 2015). Acredita-se que a tolerância ou sensibilidade espécie-específica somada às interações biológicas devem representar um papel chave na resposta das comunidades bentônicas marinhas às mudanças climáticas (Widdicombe e Spicer 2008; Widdicombe et al. 2009; Hale et al. 2011). Neste contexto, ainda se sabe pouco sobre como as mudanças climáticas podem afetar os indivíduos e provocar mudanças nas populações, comunidades e ecossistemas.

Quando consideramos os estudos ao nível de comunidades bentônicas de ambientes costeiros, observa-se que a maioria foi conduzida com a macrofauna ou grandes/conspícuos organismos (e.g., Hall-Spencer et al. 2008; Widdicombe et al. 2009; Cigliano et al. 2010; Hale et al. 2011; Kroeker et al. 2011; Fabricius et al. 2011, 2014;

Christen et al. 2013). Entre os estudos com comunidades de meiofauna, a grande maioria foi realizada quase que exclusivamente para investigar os efeitos do armazenamento de CO_2 no fundo do mar (sequestro de carbono), sendo a maioria conduzida na Califórnia central (Barry et al. 2004; Carman et al. 2004; Thistle et al. 2005; Fleeger et al. 2010; Ishida et al. 2013). Os poucos estudos com comunidades de meiofauna de ambientes costeiros/águas rasas foram conduzidos com meiofauna de substrato inconsolidado/sedimento (Kurihara et al. 2007; Dashfield et al. 2008; Widdicombe et al. 2009), e apenas um com meiofauna oriunda de costão rochoso (Meadows et al. 2015).

Experimentos com comunidades naturais podem ser complicados devido à grande variação na estrutura das comunidades em função da heterogeneidade dos habitats (Underwood e Chapman 1996). Particularmente nos ambientes bentônicos as variações das características do substrato influenciam fortemente a estruturação das associações (Snelgrove e Butman 1994; Underwood e Chapman 2006). Dessa forma, uma das dificuldades na avaliação de impactos nas associações é como separar as mudanças causadas pela perturbação antropogênica daquelas que decorrem de variações naturais (Bishop 2005). Para minimizar esse efeito, trabalhos recentes que utilizam comunidades para avaliar impactos de origem antrópica, como as alterações climáticas, tem feito uso de Unidades Artificiais de Substrato (UAS) (Hale et al. 2011; Meadows et al. 2015). O uso das UASs vem sendo recomendado como solução para esses problemas uma vez que permite a amostragem de comunidades padronizadas (Mirto e Danovaro 2004; Bishop 2005; Gobin e Warwick 2006) e tem se demonstrado um método efetivo na representação das comunidades naturais (Mirto e Danovaro 2004; Gwyther e Fairweather 2005; De Troch et al. 2005).

A comunidade meiofaunística, separada da macrofauna e microbiota em função do seu tamanho, é representada por metazoários bentônicos bem definidos biologicamente que ficam retidos entre os intervalos de malha de 0,044 ou 0,062 mm e 0,5 ou 1 mm. Essa comunidade é mais diversa em número de Filos do que qualquer outro componente da biota marinha possuindo representantes de quase todos os Filos de metazoários (Giere 2009). Estes organismos possuem grandes abundâncias (até 10^6 indivíduos/m^2), distribuição ubíqua e rápido tempo de geração. Essas características conferem à meiofauna vantagens quando comparada com a macrofauna no que diz respeito à coleta em campo e manutenção de experimentos em laboratório (Kennedy e Jacoby 1999). Os animais da meiofauna são suficientemente pequenos e numerosos para permitir que amostradores de volumes reduzidos coletem quantidades de fauna estatisticamente adequadas. Portanto, não se faz necessário o uso de amostragens destrutivas que poderiam comprometer a viabilidade das populações naturais. Além disso, o ciclo de vida inteiro de muitos animais da meiofauna pode ser fechado em poucas semanas (Giere 2009). Dessa forma, a meiofauna pode produzir várias gerações em alguns meses, o que pode possibilitar a detecção de impactos em vários parâmetros mais rapidamente do que em organismos da macrofauna (Kennedy e Jacoby 1999; Giere 2009).

Dentre os grupos da meiofauna, Harpacticoida (juntamente com Nematoda) é um dos táxons mais abundantes (Coull et al. 1983; Hall e Bell 1993; Giere, 2009) e apresenta altos valores de diversidade (Hicks 1977). Harpacticoida é uma das nove Ordens da Subclasse Copepoda, sendo constituída por pequenos crustáceos que variam de 0,2 a 2,5 mm de comprimento (Huys e Boxshall 1991). Esta ordem possui aproximadamente 6.000 espécies distribuídas em 645 gêneros e 59 famílias (Wells 2007; Giere 2009; Ahyong et al. 2011). Juntamente com outros animais da meiofauna,

os Copepoda Harpacticoida desempenham um papel importante no fluxo de energia dos ecossistemas bentônicos servindo de alimento para a macrofauna, peixes e outros organismos (Coull 1988; Danovaro et al. 2007; Giere 2009).

Associada à importância econômica e ecológica das comunidades bentônicas e ao risco que as mudanças climáticas globais podem determinar quanto à modificação dessas comunidades, somam-se a escassez de informações consolidadas sobre seus efeitos na meiofauna e na comunidade de Copepoda Harpacticoida como justificativas para o desenvolvimento deste trabalho. Esta tese teve como principal objetivo investigar através de experimentos em laboratório os efeitos de diferentes níveis de pH e de temperatura da água do mar, que podem ser determinados pelas mudanças climáticas globais, sobre a comunidade de meiofauna e de Copepoda Harpacticoida. Especificamente, foram avaliados o efeito da acidificação da água do mar sobre a estrutura da comunidade de meiofauna dos recifes de coral do Parque Nacional Marinho do Recife de Fora (Porto Seguro, Bahia), o efeito de diferentes cenários de mudanças climáticas sobre a estrutura da comunidade de meiofauna e de Copepoda Harpacticoida dos recifes da praia de Serrambi (Ipojuca, Pernambuco) e o efeito da interação de diferentes níveis de pH e temperatura da água do mar sobre a estrutura da associação de Copepoda Harpacticoida de costão rochoso da praia de Mount Batten (Plymouth, UK).

Os resultados deste estudo servirão como a primeira base de dados para avaliar o impacto do aquecimento e acidez da água do mar sobre a comunidade da meiofauna em recifes de coral. Além disso, os dados apresentados aqui possibilitarão gerar uma discussão sobre a sensibilidade desses organismos oriundos de ambientes tropicais e temperados. Estas informações poderão subsidiar medidas de mitigação e de gerenciamento desses ecossistemas no âmbito dos planos de enfrentamento dos efeitos das mudanças climáticas globais tanto no Brasil quanto em outras regiões do mundo.

Esta tese é composta por uma introdução geral, quatro capítulos independentes e conclusão geral. Todos os quatro capítulos foram redigidos em inglês e cada um será destinado a publicações separadas. As referências bibliográficas citadas nos quatro capítulos são apresentadas no final da tese em uma única seção. Referências eventuais aos capítulos serão realizadas como Sarmento (2016a, b, c ou d) para evitar repetições metodológicas e quando for necessária a citação de dados de capítulos anteriores.

CAPÍTULO 1

Effects of seawater acidification on a coral reef meiofauna community

1. Introduction

Approximately 30% of all anthropogenic CO_2 emitted has been absorbed by the ocean surface (Sabine et al. 2004). Ocean uptake of CO_2 will help to moderate future climate change, but when carbon dioxide dissolves in the ocean it lowers the pH and causes changes in the ocean's $CaCO_3$ system (Feely et al. 2004, 2009). These changes, often referred to as 'ocean acidification', are already occurring and are expected to intensify in the future. Surface ocean pH is already 0.1 units lower than pre-industrial levels (Caldeira and Wickett 2003). However, estimates of future atmospheric and oceanic CO_2 concentrations based on the Intergovernmental Panel on Climate Change emission scenarios of human activities suggest that by the end of this century, CO_2 levels could reach from 500 to 1400 ppm, and even exceed 1900 ppm by around the year 2300 (Caldeira and Wickett 2003; IPCC 2013). The corresponding decrease in pH would be about 0.3 to 0.5 pH units in surface waters relative to pre-industrial levels by the year 2100 (Caldeira and Wickett 2003; Feely et al. 2009; IPCC 2013) and about 0.8 units by 2300 (Caldeira and Wickett 2003, 2005).

Ocean acidification poses risks to all marine ecosystems, but coral reefs are widely recognized as the ecosystem that is most threatened by ocean acidification (Hoegh–Guldberg et al. 2007; Kleypas and Yates 2009; Fabricius et al. 2011; IPCC 2014; van Hooidonk et al. 2014). Coral reefs constitute about one-sixth of the world's coastline and are the most biologically diverse habitats in the oceans. They also have an important role in shoreline protection and support a complex food web (Birkeland 1997;

Roberts et al. 2002; Castro and Huber 2010; Gutiérrez et al. 2011). These features indicate that coral reefs provide essential ecosystem services and also provide direct and indirect economic benefits related to fisheries and ecotourism (Wilkinson 1996; Maida and Ferreira 1997; White et al. 2000; Hoegh–Guldberg et al. 2007).

Our present understanding of the impacts of ocean acidification on coral reef ecosystems is almost entirely limited to single-species studies of highly calcifying organisms, particularly those that are critical to the formation of habitats (e.g., coral species) or their maintenance (e.g., grazing echinoderms; e.g., Jokiel et al. 2008; Kleypas and Yates 2009; Morita et al. 2009; Byrne et al. 2013; De'ath et al. 2013; Uthicke et al. 2014). On the other hand, studies at the community level are still incipient. Fabricius et al. (2011, 2014) investigated the consequences of exposure to high CO_2 on coral-reef associated macroorganism communities around three shallow volcanic CO_2 seeps in Papua New Guinea. However, to our knowledge, no investigation on coral-reef meiofauna communities has been conducted to date.

On coral reefs, a major source of primary productivity is derived from the phytal. This environment is often the dominant microhabitat on coral reefs, where algal turfs can cover 30–80% of the total surface area (Maida and Ferreira 1997; Fabricius and De'ath 2001; Wismer et al. 2009; Hoey and Bellwood 2010). Meiofauna is one of the assemblages inhabiting this reef phytal environment. These organisms are likely to be more abundant than macrofauna by at least an order of magnitude (Gibbons and Griffiths 1986) and include representatives from almost all metazoan phyla. Meiofauna densities of up to 10^6 individuals m^{-2} of turf coverage are not uncommon, and some phytal environments are considered "hot spots of meiofaunal production", reaching levels of around 10 g C m^{-2} y^{-1} (Giere 2009). Meiofauna organisms are a biologically and ecologically distinct group of metazoans, operationally defined by their small size

(Giere 2009). The metazoan meiofauna is a key component of the coastal benthos, contributing significantly to energy transfer to higher trophic levels (Danovaro et al. 2007; Kramer et al. 2013). Furthermore, members of the meiofauna community can be a useful tool for studies of human impacts (Kennedy and Jacoby 1999; Giere 2009).

Benthic community variations are strongly influenced by the type of substrate (Snelgrove and Butman 1994; Underwood and Chapman 2006). Furthermore, the wide variability in community structure and diversity caused by habitat heterogeneity can often complicate experiments on natural communities (Underwood and Chapman 1996), and hampers efforts to separate the changes caused by anthropogenic disturbance from those arising from natural variations (Bishop 2005). Artificial substrate units (ASUs) have been used to overcome these problems, allowing the collection of a standardized community (Mirto and Danovaro 2004; Bishop 2005; Gobin and Warwick 2006), and have proved to be an effective method to represent natural communities (Mirto and Danovaro 2004; De Troch et al. 2005). ASUs have been widely applied in recent studies to assess the effects of climate change on benthic communities (Cigliano et al. 2010; Hale et al. 2011; Christen et al. 2013).

Together with the risk that ocean acidification will modify benthic communities, we must consider the great uncertainty about how the impacts of ocean acidification will affect the lower trophic levels, such as the meiofauna. The present study tested the hypothesis that exposure to different levels of seawater acidification that could be caused by global climate change will lead to strong modifications of the phytal meiofauna community from a coral reef, in a mesocosm experiment.

2. Materials and methods

Artificial substrate units (ASUs) colonized by coral reef phytal meiofauna were used in order to collect a standardized and diverse community. Artificial turf (synthetic grass consisting of polyethylene strips 10 mm in height) was used as the ASU, as it mimics the turf algae that cover coral reefs (Kelaher 2003; Matias et al. 2007). Thirty-six ASUs (6 x 6 cm each) were tied up between two nylon ropes what give an appearance of a “belt”. Five belts were set up (six ASUs per belt, distant from each other by 5 cm). Then, each belt was attached on the side of the reef formation called Recife de Fora (S 16° 24' 37.3", W 38° 59' 02.2"). Recife de Fora is located about 9 km off of Porto Seguro city, on the southern coast of Bahia, Brazil. This reef formation is part of the Recife de Fora Municipal Marine Park created in 1998 (Leão and Dominguez 2000). All ASUs were placed in the same location, approximately 4 m deep in a sheltered area (Enseada do Morão), and were therefore exposed to similar conditions (temperature, sunlight, wave exposure; Fig. 1). The ASUs were left in the field for 30 days to allow colonization by a suitable meiofauna community (Mirto and Danovaro 2004; De Troch et al. 2005). Upon collection, each ASU was placed in a small plastic container and then transported for one hour to the mesocosm facility located at Arraial d’Ajuda (Santos et al. 2014; Fig. 1).

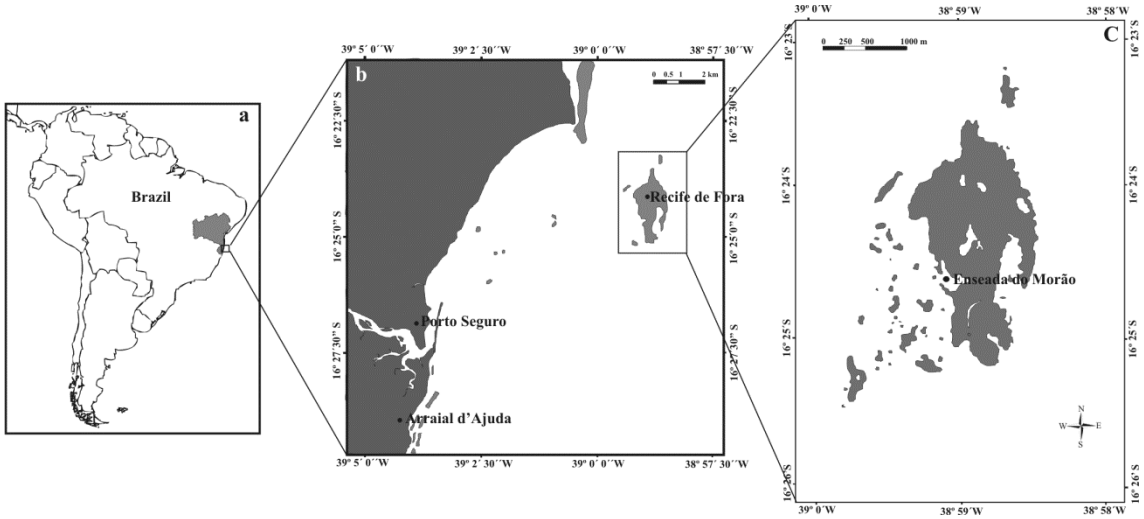

Fig. 1 a) Brazil coastline showing the state of Bahia, b) Porto Seguro and Arraial d'Ajuda, and c) Recife de Fora Municipal Marine Park

Four pH treatments were established with four replicates each. The control was the local/ambient seawater without manipulation, and the decreased pH levels used were 0.3, 0.6, and 0.9 units below the ambient seawater, corresponding to the three levels of acidification. These levels of decrease in the ambient seawater pH are based on predictions of ocean chemistry changes by the years 2100 and 2300, made by a range of models of atmospheric CO_2 emissions (Caldeira and Wickett 2003, 2005; IPCC 2013).

The experimental setup consisted of 16 tanks that were continuously supplied with seawater at a rate of 8.33 L min^{-1}. In this system, seawater is captured 500 m from the shore at an adjacent fringe reef and is pumped to four cisterns of 5,000 L each. In each cistern, the seawater received one of the four pH treatments. CO_2 gas was bubbled through the natural seawater in each cistern, lowering the pH level. Once the pH had fallen to the required level, the supply of CO_2 was stopped. The control and the CO_2-enriched seawater from each cistern supplied four tanks. The acidification process was controlled by a computerized system, Reef Angel© Controller, coupled to pH electrodes (Gehaka 09RBCN). Reef Angel© Controller is an open-source aquarium controller that

allows the level of acidification to follow environmental variations of the pH of seawater collected in the field. Thus, daily and/or seasonal variations are replicated in the tanks. The mesocosm tanks received only natural sunlight and therefore followed natural day/night cycles. To mimic the amount of incident light on the reefs, the tanks were covered with a 70% shade screen, which is equivalent to the mean parameters measured in situ at 2.5 m depth on the Recife de Fora reef (about 250 µmol photons m^{-2} s^{-1}).

At the mesocosm facility, four ASUs were randomly selected and preserved in 4% formalin. These ASUs were used to characterize the community structure before the start of the exposure. The 32 remaining ASUs were randomly allocated to the four treatments. Two ASUs were placed in each of the 16 tanks. No food was supplied. The ASUs remained in the tanks for three days before the exposure started on October 30, 2012. From each tank, one ASU was collected after 15 d (14 November 2012) and another after 30 d (29 November 2012). Each sample was preserved in 4% formalin. During the exposure period, the pH and temperature of the water from each cistern were monitored daily, every 15 min by the Reef Angel© Controller. Measurements of salinity (Instrutemp ITREF 10 optical refractometer), nutrients (Hach DR 890 colorimeter with the reagents NitraVer X and PhosVer 3 for nitrate and phosphate, respectively), and light intensity (LI-COR, LI 250A Light Meter, LI-193 Underwater Spherical Quantum Sensor) were taken weekly. Temperature data from the strongest level of acidification (reduction by 0.9 from the seawater control pH) are not presented because the sensor malfunctioned. Precipitation data were obtained from the National Institute of Meteorology, Brazil (INMET). Nine samples for total alkalinity (TA) of the seawater supplying the mesocosm system were analyzed only in February 2015. An alkalinity titrator (AS-ALK2, Apollo SciTech Inc., Bogart, GA, USA) was used following

Dickson et al. (2007), and certified reference materials were obtained from the laboratory of A. G. Dickson, Scripps Institution of Oceanography. Because of the open mesocosm system, which continuously supplied all treatments, it is expected that the TA of the seawater will not change when bubbled with CO_2 gas (Dickson et al. 2007; Riebesell et al. 2010). The pCO_2s, Ω_{ca} and Ω_{ar} for each treatment were calculated from the TA and mean pH using CO2calc version 1.2.9 (Robbins et al. 2010).

In the laboratory, the fauna was extracted by manual elutriation with filtered water through geological sieves. Samples were sieved through a 300-μm mesh, and a 45-μm mesh was used to retain the meiobenthic organisms. The fraction remaining on the 45-μm mesh was extracted six times with colloidal silica (diluted with distilled water to a final density of 1.18 g cm^{-3}) flotation. The meiofauna retained were analyzed under a Leica EZ4 stereomicroscope to evaluate the densities of the major groups.

2.1 Statistical analysis

Permutational multivariate analyses of variance (PERMANOVA; Anderson 2001; McArdle and Anderson 2001) based on Bray-Curtis dissimilarities on meiofauna log (x+1) transformed data, were used to evaluate the impact of seawater acidification (factor pH) on the structure of communities, considering the two exposure periods, 15 and 30 d (factor Time). For all analyses, 9,999 random permutations were used. Pair-wise *a posteriori* comparisons (the multivariate version of the t statistic) were made when the interaction between factors was significant. A similarity percentage (SIMPER) analysis was applied to determine which groups were responsible for the dissimilarities among the pH treatments for the samples collected after 15 and 30 d. Multi-dimensional scaling (MDS) was used to represent the Bray-Curtis matrix graphically in a two-axis space. The relationship between the density of the major meiofauna groups and the four

pH levels was assessed by linear replicated regression analyses, separately for samples collected after 15 and 30 d of exposure.

PERMANOVA, SIMPER, and MDS were applied using the software Primer® 6 with add-on PERMANOVA+ (Plymouth Routines in Multivariate Ecological Researches). The linear regression analyses were performed using the software BioEstat 5.0. The level of significance was set at $P<0.05$ for all analyses. Confidence intervals of 95% (CI) were used to express the variation of the calculated means. Parametric statistical analysis followed Zar (1996).

3. Results

3.1 Experimental conditions

Figure 2 illustrates the pH levels monitored during the course of the experiment. Nominal pH treatments were successfully maintained throughout the 30-d exposure period (Table 1). The mean (± Confidence Interval, CI) daily total rainfall for the Porto Seguro region during November 2012 was 7.49 mm (±4.27 CI). However, precipitation was lower (4.84 mm, ± 6.26 CI) in the first half of November than in the second (10.14 mm, ± 5.73 CI). The mean temperature was also lower in the first half of the month (24.65ºC, 24.45ºC, 24.62ºC) than in the second (25.7ºC, 25.5ºC, 25.65ºC for pH treatments 8.1, 7.8 and 7.5 respectively). This pattern was also observed for pH. On average, pH levels were lower in the first half of the month (8.05, 7.70, 7.48 and 7.19) than in the second (8.14, 7.77, 7.54 and 7.23). Total alkalinity of the seawater supplying the mesocosm system was 2379.14 (± 2.85 CI) µmol kg^{-1}. The pCO_2 values for the treatments were 351.8 (pH 8.1), 939.0 (pH 7.8), 1683.4 (pH 7.5), and 3494.3 µatm (pH

7.2). The Ω_{ca} and Ω_{ar} values were 5.89 and 3.89 (pH 8.1), 2.98 and 1.96 (pH 7.8), 1.86 and 1.23 (pH 7.5), and 0.97 and 0.64 (pH 7.2).

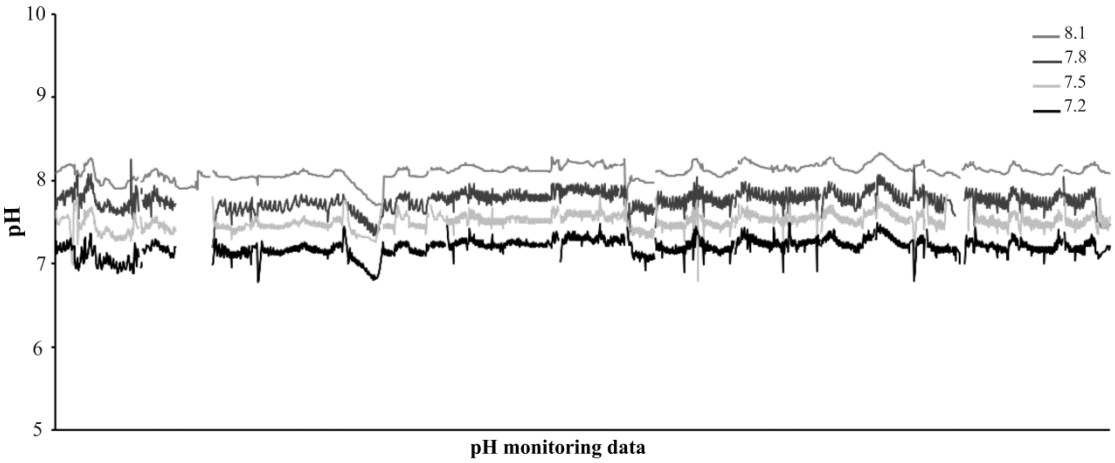

Fig. 2 Levels of pH in the tanks monitored during the 30-day exposure period

Table 1 Seawater physical and chemical conditions maintained in the tanks during the 30-day exposure period. Values: mean ±95% CI

	pH			
	8.1	**7.8**	**7.5**	**7.2**
pH	8.10 0.004	7.74 0.008	7.51 0.006	7.21 0.007
Temperature (ºC)	25.19 0.03	25.00 0.03	25.16 0.03	- -
Salinity	35 0.39	35 0.41	35 0.37	35 0.34
Light (μmol photons m^{-2} s^{-1})	284.97 41.34	296.37 41.51	285.46 33.91	276.98 39.77
Nitrate (mg l^{-1})	0.98 0.20	0.98 0.15	0.88 0.05	0.85 0.06
Phosphate (mg l^{-1})	0.06 0.01	0.06 0.01	0.06 0.01	0.06 0.01

3.2 The mesocosm effects

PERMANOVA comparisons among field and control samples collected after 15 and 30 days showed significant differences in the meiofauna community structure ($F_{(2;9)}$=4.03, p<0.01). A posteriori comparisons showed significant differences between field and 15-days control samples (t=1.89, p=0.04), between 15 and 30 days control samples (t=2.36, p=0.01), but not between field and 30-days control samples (t=1.81, p=0.06).

SIMPER analyses showed that field and 15-days control samples had an average dissimilarity of 32.56%. The groups that most contributed to this dissimilarity were Harpacticoida (43.2%), Nematoda (16.9%), Polychaeta (15.9%), Chironomidae larvae (8.4%) and Nauplii (7.6%). Fifteen and 30-days control samples had an average dissimilarity of 35.8% and Harpacticoida (30.4%), Chironomidae larvae (21%), Polychaeta (17%), Nematoda (16.6%) and Nauplii (7.9%) were the groups that most contributed to the observed dissimilarity.

3.3 Effect of acidification on community

A total of 20,371 meiofaunal organisms were counted. Meiofauna was composed of Copepoda harpacticoids (38.21%), Polychaeta (21.45%), Nematoda (15.86%), Chironomidae larvae (13.56%), Harpacticoid nauplii (3.48%), Ostracoda (2.72%), Turbellaria (2.68%), Tardigrada (1.43%), with Acari, Gastrotricha, and Oligochaeta (<1%).

The MDS analysis representing the similarity matrix of meiofauna samples from the four pH treatments at the two sampling times (Fig. 3) showed a clear pattern of differentiation between samples collected after 15 and 30 d. However, for the 15-d

samples there was no pattern of difference among the different pHs. On the other hand, after 30 d of exposure, there was a clear separation of both the control (pH 8.1) and pH 7.8 samples from the pH 7.5 and 7.2 samples. The PERMANOVA results confirmed the pattern shown in MDS and detected significant differences in the structure of the meiofauna community between samples collected after 15 and 30 d (factor time), among the four levels of pH (factor pH), and also for the interaction between the two factors (Table 2).

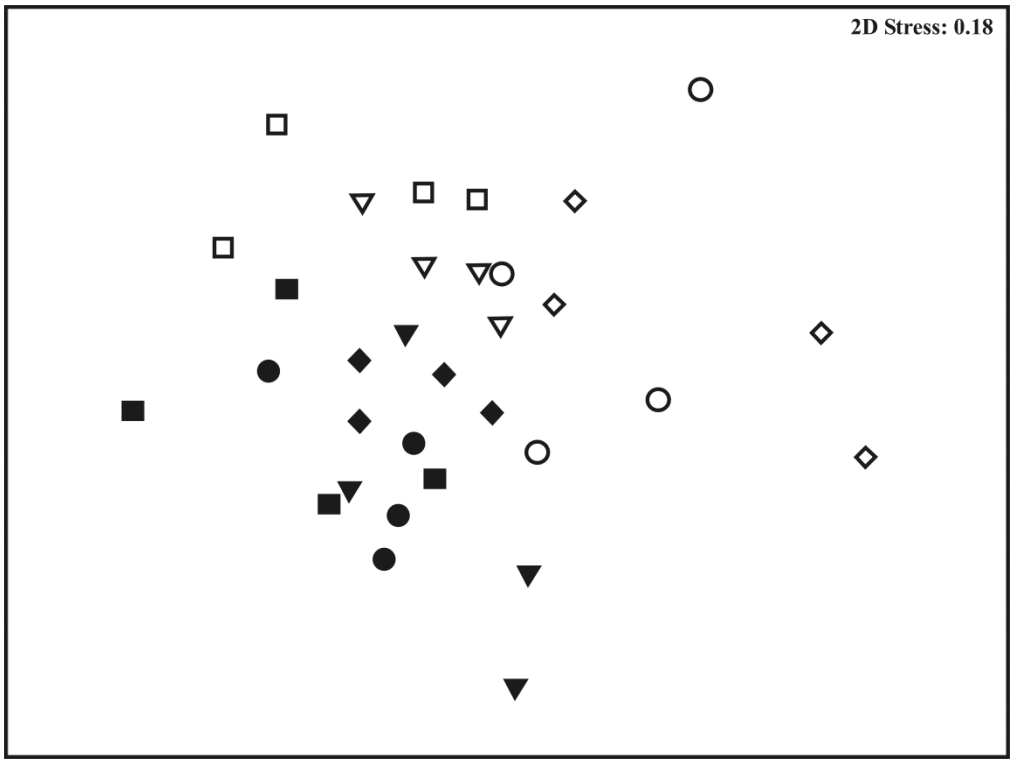

Fig. 3 Non-metric multi-dimensional scaling ordination plots for the Bray-Curtis similarity for the meiofauna community structure. ■ 8.1, ▼ 7.8, ♦ 7.5, ● 7.2. Closed symbols represent samples collected after 15 days, and open symbols after 30 days

Table 2 PERMANOVA results for the meiofauna communities exposed to different pHs and collected after 15 and 30 days. Significant values are highlighted in bold

Source	df	MS	F	P
Time (Ti)	1	566.04	**8.67**	<0.001
pH	3	126.23	**1.93**	0.020
Ti x pH	3	119.99	**1.84**	0.027
Residual	24	65.28		

Pairwise tests for samples collected after 15 d of exposure did not detect significant differences in the meiofauna community structure among the control and the acidification levels ($p>0.42$ for all) or among the three levels of acidification ($p>0.25$ for all). However, samples collected after 30 d of exposure showed a clear pattern of response to seawater acidification. Pairwise tests detected significant differences between the control and the pH 7.5 samples ($t=1.99$, $p=0.029$), and between the control and the pH 7.2 samples ($t=1.85$, $p=0.028$). No significant difference was detected between the control and pH 7.8 ($t=1.20$, $p=0.18$) or among the three levels of acidification ($p>0.06$ for all).

SIMPER analyses showed that the dissimilarities among treatments were greater after 30 d, especially for comparisons between 8.1 and 7.5, and between 8.1 and 7.2; Nauplii was the group that contributed most to these dissimilarities (Table 3).

Table 3 Percent Contribution (Contrib. %) of meiofauna groups to average dissimilarity (Diss.) between different pHs for samples collected after 15 and 30 days. (Chiron. larvae - Chironomidae larvae)

Day 15					
8.1 vs. 7.8		*8.1 vs. 7.5*		*8.1 vs. 7.2*	
Diss.= 11.53	*Contrib.%*	*Diss.= 10.53*	*Contrib.%*	*Diss.= 11.33*	*Contrib.%*
Nauplii	*23.49*	*Tardigrada*	*22.77*	*Tardigrada*	*18.50*
Tardigrada	*19.70*	*Turbellaria*	*18.49*	*Acari*	*14.19*
Acari	*10.89*	*Nauplii*	*12.29*	*Nauplii*	*13.38*
Ostracoda	*9.53*	*Acari*	*11.89*	*Ostracoda*	*12.93*
Turbellaria	*8.98*	*Ostracoda*	*9.38*	*Turbellaria*	*10.09*
Harpacticoida	*7.58*	*Chiron. larvae*	*8.66*	*Chiron. larvae*	*8.41*
Nematoda	*5.99*	*Nematoda*	*6.64*	*Nematoda*	*6.08*
Polychaeta	*5.60*			*Oligocheta*	*5.65*
				Harpacticoida	*4.79*
Day 30					
8.1 vs. 7.8		*8.1 vs. 7.5*		*8.1 vs. 7.2*	
Diss.= 10.51	*Contrib.%*	*Diss.= 16.36*	*Contrib.%*	*Diss.= 14.70*	*Contrib.%*
Turbellaria	*14.02*	*Nauplii*	*21.58*	*Nauplii*	*16.53*
Ostracoda	*13.54*	*Ostracoda*	*12.66*	*Tardigrada*	*12.99*
Tardigrada	*13.30*	*Gastroticha*	*12.34*	*Ostracoda*	*10.70*
Polychaeta	*10.96*	*Nematoda*	*10.14*	*Turbellaria*	*10.39*
Nematoda	*10.09*	*Polychaeta*	*9.48*	*Polychaeta*	*10.07*
Acari	*9.45*	*Tardigrada*	*7.65*	*Nematoda*	*9.47*
Nauplii	*7.72*	*Acari*	*6.74*	*Acari*	*8.58*
Chiron. larvae	*7.71*	*Turbellaria*	*5.96*	*Gastroticha*	*8.21*
Oligocheta	*7.49*	*Chiron. larvae*	*4.59*	*Harpacticoida*	*5.10*

Fluctuations in the density of total meiofauna and of dominant groups were observed between the field and control samples. However, at the end of the experiment, the density of total meiofauna and of the dominant groups increased in the control tanks (Fig. 4).

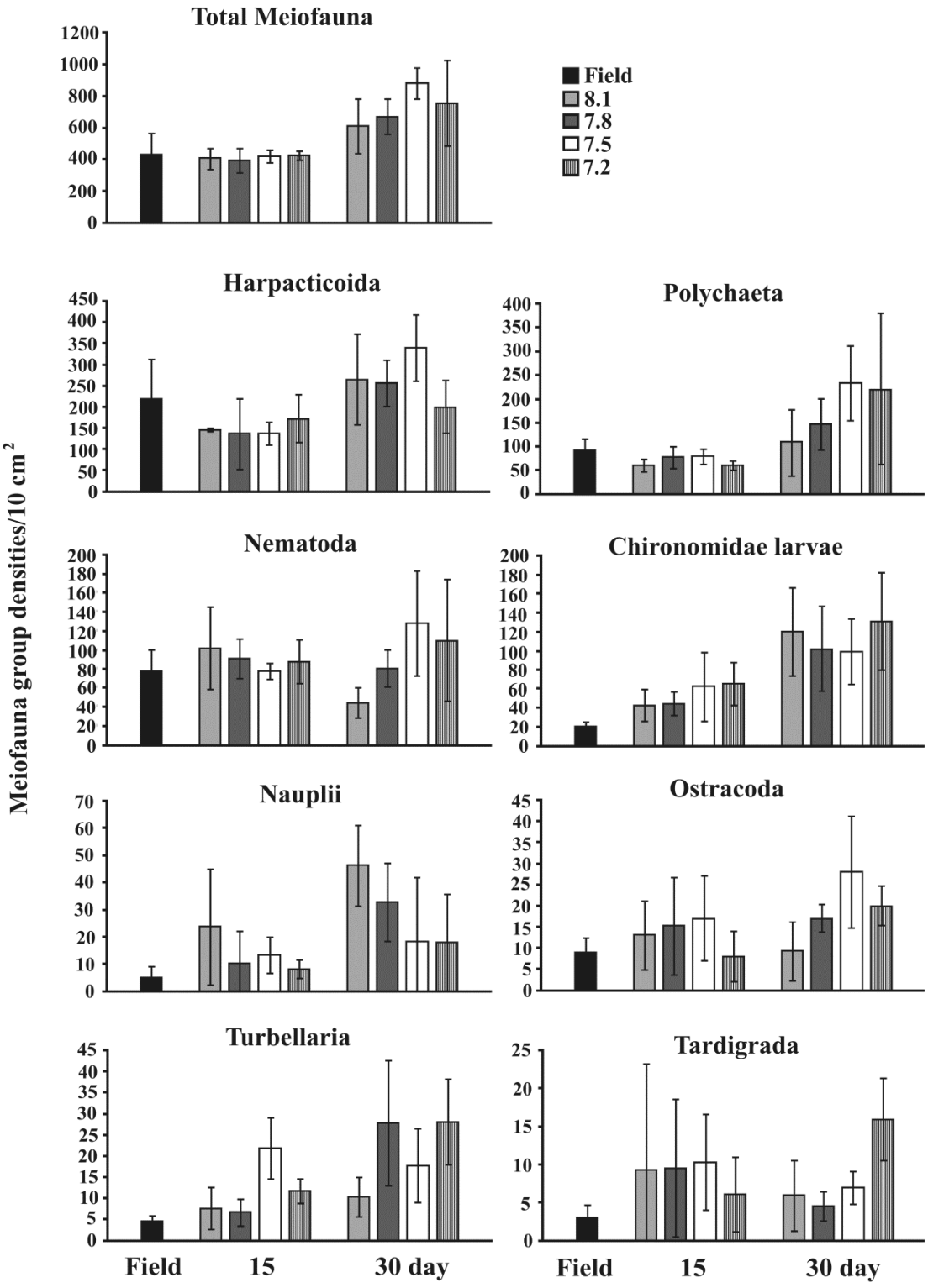

Fig. 4 Mean density (± 95% confidence intervals) of the main groups of meiofauna at different pH levels (8.1, 7.8, 7.5, and 7.2) and sampling times (15 and 30 d)

The results of the linear regression analyses indicated that many groups showed significant relationships between density and the different levels of acidification (Table 4). For samples collected after 15 d, only Turbellaria showed a positive relationship to acidification. On the other hand, for samples collected after 30 d, Nematoda, Ostracoda, Turbellaria, and Tardigrada showed a positive relationship between their densities and acidification. Harpacticoid nauplii was the only group that showed a negative relationship between density and acidification after 30 d of exposure (Fig. 4). However, adult harpacticoid density did not show a significant relationship to the different pHs.

Table 4 Linear replicated regression analyses results for the major groups of meiofauna (data transformation), degrees of freedom= 1;14. Significant values in bold. (untransf. – untransformed data; Chiron. larvae – Chironomidae larvae)

Group	***Day 15***		***Day 30***	
	F	***P***	***F***	***P***
Harpacticoida (untransf.)	*0.556*	*0.526*	*0.285*	*0.607*
Polychaeta (fourth root)	*0.013*	*0.909*	*4.114*	*0.059*
Nematoda (untransf.)	*0.875*	*0.632*	***6.002***	*0.027*
Chiron. larvae (untransf.)	*2.974*	*0.104*	*0.104*	*0.749*
Nauplii (untransf.)	*2.405*	*0.140*	***6.470***	*0.022*
Ostracoda (fourth root)	*0.688*	*0.574*	***5.210***	*0.037*
Turbellaria ($ln_{(X+1)}$)	***4.538***	*0.049*	***4.603***	*0.048*
Tardigrada (untransf.)	*0.205*	*0.661*	***10.133***	*0.007*
Total meiofauna (untransf.)	*0.497*	*0.502*	*2.469*	*0.136*

4. Discussion

In the present study, the acidification system was able to maintain the levels of decrease in the pH treatments following the natural variation of the 'control' seawater. Thus, the meiofauna community was exposed to seawater that showed daily and/or seasonal environmental variations of its parameters, with only the level of acidity varying among treatments due to the amount of CO_2 injected. Furthermore, the total density of meiofauna and their dominant groups in the control conditions increased at the end of the experiment. These results indicate that the conditions in the tanks were suitable for maintaining natural communities with the purpose of conducting studies on anthropogenic impacts such as ocean acidification.

When left in the field, ASUs are colonized by a wide spectrum of organisms (e.g., bacteria, microphytobenthos, meiofauna, and vagile macrofauna). An entire ecosystem, albeit on a small scale, is taken and maintained in the mesocosm system. No interference with the organisms, such as food, maintenance, or handling, was required. Despite the small scale of ASUs (36 cm^2), they constitute a meaningful sampling universe in terms of statistical power to detect biologically important effects on meiofauna due to the small size and high abundance of these animals.

Studies on the effects of acidification on multispecies benthic communities have received increasing attention recently. However, most studies of shallow benthic communities have focused on macrofauna or large and conspicuous organisms (Hall-Spencer et al. 2008; Widdicombe et al. 2009; Cigliano et al. 2010; Hale et al. 2011; Kroeker et al. 2011; Fabricius et al. 2011, 2014; Christen et al. 2013). Studies with meiofauna communities have focused almost exclusively on the effects of direct injection of CO_2 into the deep-sea floor (carbon sequestration), with most of them conducted off central California (Carman et al. 2004; Barry et al. 2004; Thistle et al.

2005; Fleeger et al. 2010; Ishida et al. 2013). All of the very few studies on shallow-water meiofauna communities were from sediment/unconsolidated substrate (Kurihara et al. 2007; Dashfield et al. 2008; Widdicombe et al. 2009) and none from coral reef environments.

Studies on meiofauna communities from shallow areas indicate that they tolerate ocean acidification. In a 56-day microcosm experiment with a meiobenthic community from sediment, Kurihara et al. (2007) found no significant impact in the abundance of meiofauna in response to elevated CO_2 concentrations (pH 7.4). Widdicombe et al. (2009) found that exposure to acidified seawater significantly altered the community structure and reduced diversity for nematode assemblages in a mesocosm experiment. However, the largest differences were observed for pH 5.6 after 20 weeks, and the sediment type (mud or sand) played an important role in the differentiation of the nematode community structure. In a mesocosm experiment, Dashfield et al. (2008) found that the presence of a burrowing urchin was a key factor determining the response of the nematode community to the impact of ocean acidification (pH 7.5), and suggested that any nematode mortality is unlikely to be directly due to differences in pH.

In the present study, acidified seawater caused major changes in the structure of the meiofauna community. These changes were the result of divergent biological responses to acidification. We found that among the numerically dominant meiofaunal taxa, the densities of Harpacticoida and Polychaeta did not show significant differences due to pH after 15 or 30 days. On the other hand, Nematoda, Ostracoda, Turbellaria, and Tardigrada exhibited their highest densities in low-pH treatments (especially at 7.5), while only the harpacticoid nauplii were strongly negatively affected by low pH.

Ocean acidification has been shown to have drastic effects on macrobenthic organisms (e.g., Dupont et al. 2010; Findlay et al. 2010; Byrne et al. 2013; Fabricius et

al. 2014). However, the divergent patterns of response exhibited by meiofauna in this study are not uncommon. It appears that nematodes are likely to be able to withstand short-term exposure to even severe seawater acidification (Wieser et al. 1974; Takeuchi et al. 1997; Ishida et al. 2005; Kurihara et al. 2007; Dashifield et al. 2008; Widdicombe et al. 2009) and also to increase their densities under low-pH conditions (Hale et al. 2011). Similarly, the abundance of polychaetes appears not to be greatly affected by low pH (Hale et al. 2011; Kroeker et al. 2011; Calosi et al. 2013; Christen et al. 2013; Fabricius et al. 2014), and some species even became more abundant at the lowest pH investigated (Cigliano et al. 2010; Hale et al. 2011).

Investigations on the impact of any stressor should consider that other factors/drivers may be involved in the response exhibited by multi-species assemblages (individual performance, species interactions, food supply, and so on; Gaylord et al. 2015). Experiments with macrofaunal species have demonstrated that these organisms are highly sensitive to ocean acidification, with negative impacts on their survival, calcification, growth, reproduction, metabolic rates, and physiology (Bibby et al. 2007; Byrne et al. 2013; Ceballos-Osuna et al. 2013; Cumbo et al. 2013; De'ath et al. 2013; Dupont and Thorndyke 2009; Ellis et al. 2009; Sung et al. 2014). Considering that many macrofaunal organisms are meiofauna predators or competitors, the negative impacts on macrofauna could generate a top-down, indirect positive effect on meiofauna due to the release or reduction of ecological pressures (Cigliano et al. 2010; Hale et al. 2011; Kroeker et al. 2011).

Another indirect impact on meiofauna may occur through a bottom-up effect of ocean acidification on microphytobenthos/primary producer communities. Ocean acidification can alter net primary production due to species-specific sensitivities to increased CO_2 that change the structure of macroalgae (Porzio et al. 2011), diatoms

(Johnson et al. 2013), bacteria (Webster et al. 2013), and biofilm communities (Witt et al. 2011). These modifications are followed by increases in primary production (Hargrave et al. 2009), diatom abundance and biomass (Johnson et al. 2013), biofilm production (Lidbury et al. 2012), and the abundance of bacteria and nanobenthos (Ishida et al. 2005, 2013). Kroeker et al. (2011) suggested that certain indirect effects of low pH could drive the tolerance response of some animals. They suggested that, because of the association between small crustaceans and algal turfs and canopies, the increased abundance of small crustaceans in extreme low-pH zones could be caused by the increased availability of habitat and food. The results presented by Hargrave et al. (2009) show how acidification can have a positive bottom-up effect on primary production and on benthic invertebrate consumers. The potential for ocean acidification to influence bottom-up and top-down processes (Gaylord et al. 2015) concords with the increases in density observed for many meiofaunal groups in this study.

Changes in primary producer communities in response to ocean acidification may have even more subtle consequences for the maintenance and development of benthic populations that depend on them. For instance, there is a consensus that harpacticoid species are able to develop and reproduce while feeding on different diatoms, but that some algal species are more suitable; so although the copepods can survive, the ingestion of some diatoms or bacteria can drastically impact their development and reproductive success (Araújo-Castro and Souza-Santos 2005; Wyckmans et al. 2007; Dahl et al. 2009). Furthermore, indirect effects of ocean acidification can be expected for consumers because of changes in the nutritional quality of their prey (Rossoll et al. 2012). Thus, a ‘bloom’ of some specific diatom or bacteria that benefited from a low-pH environment could serve as food, although could not sustain the full population development of many harpacticoid species for longer

periods. These patterns of response are in accordance with the divergent results found for the total densities of harpacticoids and their nauplii.

The absence of a response of polychaetes and harpacticoids could be the consequence of compensatory response, where reductions in the density of sensitive species are compensated by the opportunistic behavior of others. Opportunistic behavior has been documented for harpacticoid species under stress situations in a coral reef environment. In an assessment of the impact of phytal trampling, Sarmento and Santos (2012) showed that together with the reduction of several more-susceptible species, *Amphiascopsis cinctus* benefited from trampling, which resulted in a lack of differences in the total harpacticoid density.

The apparent higher tolerance observed for the benthic meiofauna in the present study may be related to physiological features of these animals. In contrast to the great vulnerability to high CO_2 of calcifying organisms, marine invertebrate species that do not calcify in the larval stage or have poorly calcified exoskeletons (e.g., copepods, amphipods, barnacles) appear to be resilient to near-future levels of pH/pCO_2 (Kurihara et al. 2004; Ishida et al. 2005; Mayor et al. 2007; Kurihara 2008; Kurihara and Ishimatsu 2008; Dupont and Thorndyke 2009; Dupont et al. 2010; Findlay et al. 2010; Byrne 2012). Thus, it is probable that due to the poorly calcified cuticle of representatives of meiofauna such as the dominant crustaceans Copepoda Harpacticoida, but also Kinorhyncha, Tardigrada, and Nematoda (Ruppert et al. 2004; Giere 2009), the meiofauna community could withstand the effects of ocean acidification at the level tested in the present study.

Considering the direct impacts of ocean acidification on benthic organisms, tolerance to CO_2 has been found to differ between life stages (e.g., larva and adult; (Kurihara 2008; Dupont et al. 2010; Hendriks and Duarte 2010). Adults exposed to

hypercapnia could suffer physiological stress without showing high mortality rates. Such effects are expected to affect long-term growth and reproduction and may thus be harmful at population and species levels (Pörtner et al. 2004). In the present study, although acidification had no significant impact on harpacticoid density, their larval stages were negatively affected. The densities of nauplii were reduced, on average, by 29.2, 60.5, and 61.1% in pH 7.8, 7.5, and 7.2, respectively, compared to the control after 30 days. Some studies with different copepod species (calanoids and harpacticoids) found that adult survival, body size, and growth were not affected by increased seawater acidity (Kurihara et al. 2004; Mayor et al. 2007; Kurihara and Ishimatsu 2008; Pascal et al. 2010), but others found large decreases in egg and naupliar production (Kurihara et al. 2004; Mayor et al. 2007; Fitzer et al. 2012, 2013). These sublethal effects of seawater acidification are in accordance with our results for the absence of response of total harpacticoid density and with the observed decreases in numbers of nauplii.

Some studies have exposed benthic communities to pH reductions >1 unit. In these studies, sharp decreases in density are not surprising, especially for calcifying animals, since such large changes in pH greatly exceed the range of natural environmental variability (Wieser et al. 1974; Widdicombe et al. 2009; Hale et al. 2011; Christen et al. 2013). Although most components of the meiobenthos are not lethally affected by elevated CO_2, it is highly possible that increases in CO_2 will have sublethal effects on reproduction, metabolism, and growth rate (Kurihara et al. 2004; Li and Gao 2012; Fitzer et al. 2012, 2013). The results presented also provide evidence of a negative effect on recruits (larval stages) of Harpacticoida, which may have serious consequences for the long-term population dynamics. It is very likely that the pattern of response shown by the major groups of meiofauna was due to changes in their species composition. Experiments with single meiofauna organisms at a lower taxonomic level

are needed to more closely evaluate the impacts of increased CO_2 and to provide a basis for evaluation of different sensitivities among meiofaunal representatives.

Recent studies have revealed that benthic carnivorous fish are an abundant and important trophic link between a highly nutritious food source (harpacticoid copepods) and higher trophic levels (Kramer et al. 2012, 2013). Berkström et al. (2012) also showed that juveniles of many wrasses are highly dependent on a single food item (harpacticoid copepods), and warned of the potential risk to higher trophic levels if degradation of reefs extends to this resource (meiofauna). This evidence illustrates the important role of phytal environments, together with their associated meiofaunal organisms (especially the diatom-harpacticoid-fish link), in the trophic structure and functioning of a coral reef ecosystem (Berkström et al. 2012; Kramer et al. 2012). It also highlights the fragility of this ecosystem if ocean acidification has major impacts on the meiofauna food base. Thus, our results help to demonstrate that the trophic functioning of coral reefs is seriously threatened by ocean acidification.

CAPÍTULO 2

The impact of predicted climate change scenarios on a coral reef meiofauna community

1. Introduction

Anthropogenic greenhouse gas emissions have increased since the pre-industrial era. Atmospheric CO_2 concentrations have risen from 280 to 380 ppm since the start of the industrial revolution (Feely et al. 2004). Their effects have been detected throughout the climate system and are extremely likely to have been the dominant cause of the observed warming (IPCC 2014)

Together, elevated CO_2 and the resultant increases in global mean temperature will result in a cascade of physical and chemical changes in marine systems (Fig. 1).

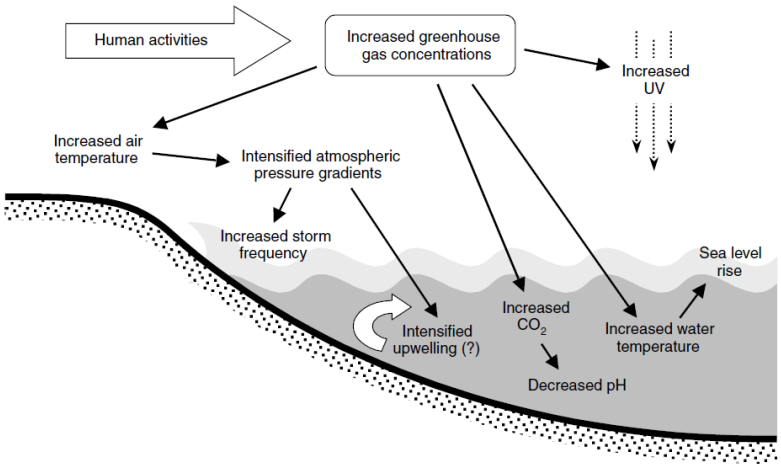

Fig. 1 Abiotic changes associated with global climate changes (from Harley et al. 2006)

These warming trends are expected to accelerate in the current century (IPCC 2001), with implications for several additional abiotic variables. For example, as a result of the thermal expansion of the oceans and freshwater input from ice-melt an increase in sea level is expected; changes in the atmospheric pressure gradient, and thus in the wind, may lead to enhanced upwelling; changes in atmospheric circulation might also increase the storm frequency and precipitation patterns that can affect coastal salinity, turbidity, and inputs of terrestrial-derived nutrients and pollutants (Harley et al 2006; IPCC 2001).

Among all of the expected impacts of the global climate changes in coastal areas, the two major consequences that are already observed are a rise in global average temperature of 0.85ºC, i.e., global warming, and a decrease in the average global surface ocean pH of 0.1 units (a 26% increase in acidity), i.e., the process know as ocean acidification (IPCC 2014). These two processes are connected, and directly and simultaneously correlated with the increase amount of atmospheric CO_2.

Therefore, a continuous and simultaneous increase in seawater temperature and acidification are expected (IPCC 2014). In the atmosphere, the continuous increase of greenhouse gas (GHG) can reach between 475–1313 ppm by the year 2100. Consequently, it is predicted an increase in the global mean temperature between 2 and 4°C by the end of this century. Considering the impacts in the ocean chemistry, is expected a decrease in the superficial seawater pH up to 0.4 units by 2100 (Caldeira and Wickett 2003; Feely et al. 2004) and about 0.7 units by 2250 (Caldeira and Wickett 2003; Caldeira and Wickett 2005).

Anthropogenic GHG emissions are mainly driven by population size, economic activity, lifestyle, energy use, land use patterns, technology and climate policy. The Representative Concentration Pathways (RCPs), which are used for making projections

based on these factors, describe four different 21st century pathways of GHG emissions and atmospheric concentrations, air pollutant emissions and land use (IPCC 2014). The RCPs scenarios (IPCC 2014) together with a range of models of atmospheric CO_2 emissions (Caldeira and Wickett 2003, 2005) are thought very useful to plan more realistic experiments on the effect of ocean acidification and warming on benthic communities.

In the coral reefs, meiofauna is one of the assemblages inhabiting the phytal environment. These organisms are likely to be more abundant than macrofauna by at least an order of magnitude (Gibbons and Griffiths 1986) and include representatives from almost all metazoan phyla. Meiofauna densities of up to 10^6 individuals m^{-2} of turf coverage are not uncommon, and some phytal environments are considered 'hot spots of meiofauna production', reaching levels of around 10 gC m^{-2} yr^{-1} (Giere 2009). Meiofauna organisms are a biologically and ecologically distinct group of metazoans, operationally defined by their small size (Giere 2009). Furthermore, members of the meiofauna community can be a useful tool for studies of human impacts (Kennedy and Jacoby 1999; Giere 2009). Meiofauna is a key component of the trophic web on coastal benthos, contributing significantly to energy transfer to higher trophic levels (Danovaro et al. 2007; Kramer et al. 2013). However, up to now, no studies on the impact of the predicted climate change scenarios have been carried out on meiofauna from coral reef environments.

Due the highly patchy pattern of distribution, studies with meiofauna can be difficult and present challenges to separate the changes caused by anthropogenic disturbance from those arising from natural variations (Bishop 2005). The use of artificial substrate units (ASUs) has been proposed as an alternative to overcome such

problems to an extent by allowing the collection of a standardized community in hard substrates areas as coral reef (Meadows et al. 2015; Sarmento et al. 2016a).

The present study evaluated the response of coral reef phytal meiofauna community to four different climate change scenarios built on the simultaneous modifications in temperature and pH that will accompany the predicted increases in CO_2 emission scenarios discussed in the IPCC reports.

2. Materials and methods

Samples used in this experiment were obtained by artificial substrate units (ASUs) colonized by coral reef phytal meiofauna in order to collect a standardized and diverse community. Artificial turf (synthetic grass, consisting of polyethylene strips 10 mm in height) was used as the ASU (Fig. 2), as it mimics the turf algae that cover coral reefs (Kelaher 2003; Matias et al. 2007). On 8 September, eighty–one ASUs (9 x 5 cm each) were tied up between two nylon ropes what give an appearance of a "belt" (ASUs were distant from each other by 5 cm). Eight "belts" were set up. Then, each "belt" was attached on the side of the reef formation located in Serrambi Beach (Ipojuca, Pernambuco, Northeastern Brazil) (Fig. 3). Serrambi beach (S 8°33' W 35°00') is located in Ipojuca city, about 70 km south of Recife, on the southern coast of Pernambuco, Brazil. The beach is protected by coral reef formations (about 4.5 km long and maximum width reaching 1km), and presents good conservation conditions (CPRH 2013; Jales et al. 2012; Pereira and Accioly 1998).

Fig. 2 Artificial substrate units (ASUs) attached on the side of the reef formation located in Serrambi Beach (Ipojuca, PE)

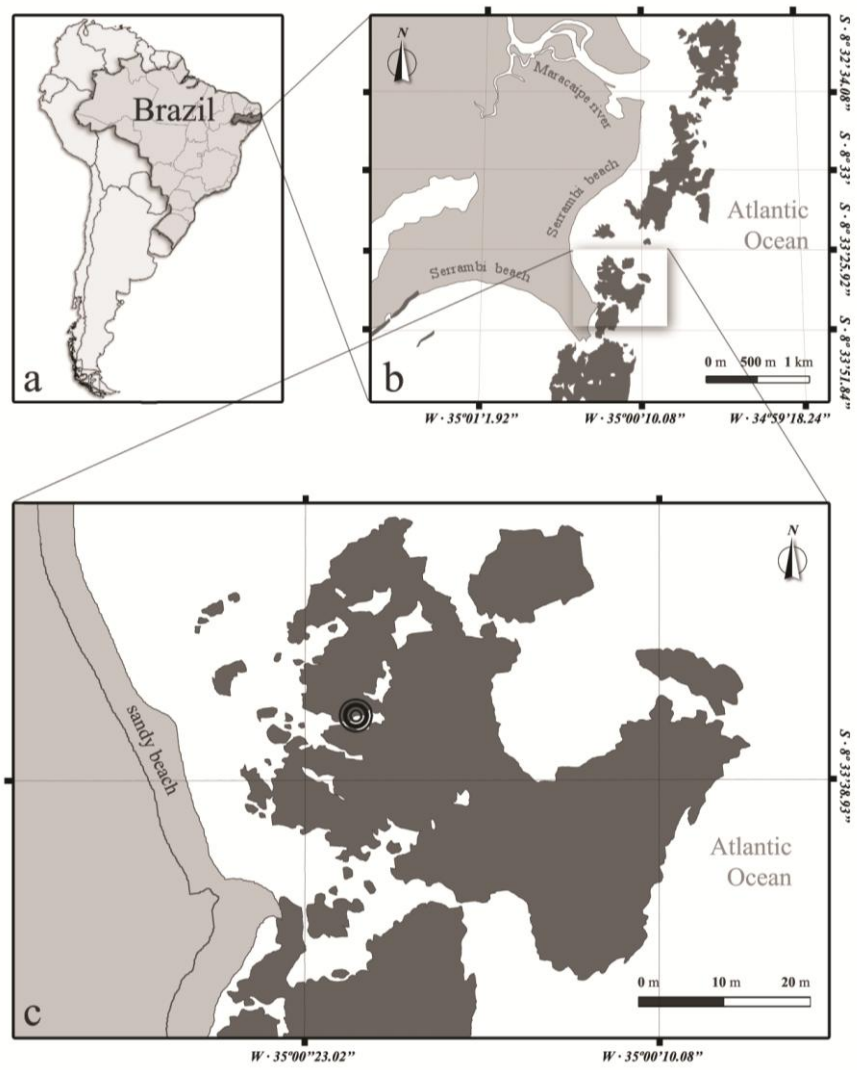

Fig. 3 a) Brazil coastline showing the state of Pernambuco, b) Serrambi Beach and c) the coral reef formations showing the ASUs colonization place

All ASUs were placed between 0.5 and 1 m deep in low tide in a sheltered area (S 8º 33' 35.49'' W 35º 00' 20.99''), where all ASUs would be exposed to similar conditions (temperature, sunlight, wave exposure) (Fig. 3 c). The ASUs were left in the field for 8 weeks to allow colonization by a suitable meiofauna community (Mirto and Danovaro 2004; De Troch et al. 2005). Upon collection on 7 November 2013, each ASU was placed in a small plastic container and then transported during 1:30h to the Population Dynamics Laboratory at Federal University of Pernambuco (Recife, PE).

Seawater physical and chemical measurements (temperature, pH, dissolved oxygen, salinity and total alkalinity) for Serrambi Beach were collected, in situ, during July and October 2013.

Four climate change scenarios were established (each scenario characterized by a specific level of pH and temperature), with nine replicates each. The control was the local/ambient seawater without manipulation, Scenario I was characterized by an increase in seawater temperature by 0.6ºC and a pH drop by 0.1 units, Scenario II was characterized by an increase in seawater temperature by 2ºC and a pH drop by 0.3 units, and Scenario III was characterized by an increase in seawater temperature by 3ºC and a pH drop by 0.7 units.

These levels of changes in the ambient seawater temperature and pH are based on predictions of ocean chemistry changes by the years 2100 and 2300 (Caldeira and Wickett 2003, 2005; IPCC 2013) and on the scenarios or Representative Concentration Pathways (RCP) defined by the scientific community in the IPCC report (IPCC 2013). These RCPs include one mitigation scenario, two stabilization scenarios, and one scenario with very high greenhouse gas emissions. The RCPs were thus used as a guide to set experimental treatments, as they represent a range of 21st century climate policies.

The experimental setup consisted of four tanks (one for each treatment). Each tank received nine small aquariums (17 x 11 x 13 cm). In each tank, aquariums were maintained in water bath. Temperature was regulated by heaters with thermostat (Resun Sunlike-100) and circulation pumps. For each scenario, pH level was attained through direct injection of CO_2 into the seawater of each aquarium separately (CO_2 divisor Aquamazon T3), using small ceramic CO_2 diffusers (Ista I 562). All aquariums were also

continually bubbled with air (Boyu ACQ 001). Sun/daylight simulation lights (Boyu, Red and Blue sunlight tube) were used to keep a 15:09h photoperiod (Fig. 4).

Fig. 4 a) Microcosm system illustrating aquariums with ASUs in water bath, and b) detail of ASUs and CO_2 diffuser

At the microcosm facility, 9 ASUs were randomly selected and preserved in 4% formalin. These ASUs were used to characterize the community structure before the

start of the exposure. The 72 remaining ASUs were randomly allocated to the 4 scenarios. Two ASUs were placed in each of the 36 small aquariums. No food was supplied. Exposure started on 8 November. From each aquarium, one ASU was collected after 15 days (22 November) and another after 29 days (6 December). Each sample was preserved in 4% formalin. During the exposure period, the pH and temperature (Hanna HI 991003) of the water from each aquarium were monitored daily. Measurements of salinity (Atto Instruments REF 201 Refractometer) and O_2 (Instrutherm MO 910) were taken in alternate days.

Three seawater samples for chemical analysis were taken and preserved before exposure started: for total alkalinity (350mL poisoned with 100µL of supersaturated mercury chloride solution) and salinity (100 mL, ambient temperature). Another batch of seawater samples (n=3) were also collected during meiofauna sampling, after 15 and 29 days of exposure respectively. The total alkalinity (TA) was determined by potentiometric titration with dilute H_2SO_4, described in Rounds (2012) with precision of 17 µmol Kg^{-1} and accuracy of 3.5%. Salinity was measured according to the Mohr-Knudsen method described by Strickland and Parsons (1972). The pCO_2s, Ω_{ca} and Ωar for each treatment were calculated from the TA, mean pH, temperature and salinity using CO2Sys Excel Macro (Pierrot et al. 2006) and the carbonic acid dissociation constants of Mehrback et al. (1973) refited by Dickson and Millero (1987) and the dissociation constant of the bisulfate ion described at Dickson (1990).

In the laboratory, the fauna was extracted by manual elutriation with filtered water through geological sieves. Samples were sieved through a 500-µm mesh, and a 45-µm mesh was used to retain the meiobenthic organisms. The meiofauna retained were analyzed under a Leica EZ4 stereomicroscope to evaluate the densities of the major groups.

2.1 Statistical analysis

To evaluate changes on the natural communities due to the experiment, a Permutational multivariate analyses of variance (PERMANOVA) (Anderson 2001; McArdle and Anderson 2001) based on Bray-Curtis dissimilarities on meiofauna log (x+1) transformed data was used to detect if significant differences existed in the structure of the meiofauna assemblages among field (the 9 ASUs preserved immediately after collection from the field) and control samples collected after 15 and 29 days (factor Time). Similarity percentage (SIMPER) analysis was applied to determine which groups were responsible for the dissimilarities among field and control samples collected after 15 and 29 days. Multi-dimensional scaling (MDS) was used to represent the Bray-Curtis matrix graphically in a two-axis space.

PERMANOVA analyses based on Bray-Curtis dissimilarities on meiofauna log (x+1) transformed data, were also used to evaluate the impact of different climate change scenarios (factor Scenario) on the structure of communities, considering the two exposure periods, 15 and 29 days (factor Time). For all analyses, 9,999 random permutations were used. Pair-wise *a posteriori* comparisons (the multivariate version of the *t* statistic) were made when the interaction between factors was significant. A similarity percentage (SIMPER) analysis was applied to determine which groups were responsible for the dissimilarities among scenarios for the samples collected after 15 and 29 days. Multi-dimensional scaling (MDS) was used to represent the Bray-Curtis matrix graphically in a two-axis space.

Two-way analysis of variance (ANOVA) was used to examine the effects of the different scenarios (factor Scenario) on total meiofauna density and on the densities of the meiofauna major groups, considering the two exposure periods, 15 and 29 days (factor Time).

PERMANOVA, SIMPER and MDS were applied using the software Primer® 6 with add-on PERMANOVA+ (Plymouth Routines in Multivariate Ecological Researches). The two-way ANOVAs were calculated using the software STATISTICA 12. Only groups with density >1% were included in analysis. The level of significance was set at $P<0.05$ for all analyses. Confidence intervals of 95% (CI) were used to express the variation of the calculated means. Parametric statistical analysis followed Zar (1996).

3. Results

3.1 Seawater physical and chemical characterization of Serrambi Beach

Two expeditions were made to characterize Serrambi seawater physical and chemical conditions (Table 1). However, chemical analysis of dissolved oxygen, salinity, pH and total alkalinity were done only for samples collected in the first expedition. Data on carbonate chemistry were calculated from the data collected in 10 July using the average pH, temperature and chemical salinity: pCO_2= 362.077 µatm, Ωca = 5.8 and Ωar = 3.842.

Table 1 Seawater physical and chemical conditions characterization of Serrambi Beach

10 July 2013								
Time	T (ºC)	pH (*in situ*)	Sal (*in situ*)	DO mg/l (*in situ*)	DO (Chem)	Sal (Chem)	pH (Chem)	TA (µmol/kg^{-1})
9:00	26.7	7.92	36	-	5.55	33.6	8.15	2402
10:00	27.2	7.94	36	-	5.67	33.5	8.15	2102
11:00	27.4	8.1	36.5	-	5.92	33.7	8.29	2402
12:00	27.9	8.12	36	-	5.61	33.5	8.33	2302
13:00	27.9	8.16	36	-	5.67	33.7	8.34	2302
14:00	28.0	8.24	36	-	5.38	33.5	8.33	2302
14 October 2013								
Time	T (ºC)	pH (*in situ*)	Sal (*in situ*)	DO mg/l (*in situ*)	DO (Chem)	Sal (Chem)	pH (Chem)	TA (µmol/kg^{-1})
8:05	28.8	-	37	6.9	-	-	-	-
9:00	28.5	7.99	37	5.2	-	-	-	-
10:00	29.1	7.98	37	6.7	-	-	-	-
11:00	28.7	8.03	37	-	-	-	-	-
12:00	29.2	7.97	37	6.2	-	-	-	-

T = temperature, pH = pH measured, Sal = salinity, DO = dissolved oxygen, TA = total alkalinity, Chem = data obtained from chemical technique,

3.2 Experimental conditions

Nominal pH treatments were satisfactory attained and maintained throughout the 29-day exposure period (Table 2).

Table 2 Seawater physical and chemical conditions maintained in the aquariums during the entire exposure period. Values: mean ± SD

07 November									
	Temp ºC	Temp ºC	pH	pH	Sal $_{(refrat.)}$	Sal $_{(refrat.)}$	Sal $_{(chem.)}$ (mean day)	DO mg/l	DO mg/l
Initial	27.5	-	8.00	-	37	-	39.2 (0.18)	6.3	-
22 November									
	Temp ºC (mean day)	Temp ºC (mean 14 d)	pH (mean day)	pH (mean 14 d)	Sal $_{(refrat.)}$ (mean day)	Sal $_{(refrat.)}$ (mean 14 d)	Sal $_{(chem.)}$ (mean day)	DO mg/l (mean day)	DO mg/l (mean 14 d)
Control	27.12 (0.04)	26.93 (0.03)	8.02 (0.02)	8.01 (0.01)	38.1 (0.33)	38.14 (0.36)	39.2 (0.84)	7.38 (0.04)	7.52 (0.11)
Scenario I	27.66 (0.02)	27.63 (0.03)	7.97 (0.02)	7.93 (0.03)	38.7 (1.00)	38.28 (0.28)	38.3 (0.13)	7.33 (0.06)	7.43 (0.15)
Scenario II	29.28 (0.04)	29.22 (0.03)	7.74 (0.07)	7.75 (0.07)	38.3 (0.44)	38.06 (0.19)	39.1 (0.78)	7.03 (0.05)	7.12 (0.11)
Scenario III	30.44 (0.05)	30.34 (0.03)	7.31 (0.12)	7.38 (0.11)	38.7 (0.56)	38.10 (0.38)	39.2 (0.43)	6.9 (0.1)	6.95 (0.07)
06 December									
	Temp ºC (mean day)	Temp ºC (mean 29 d)	pH (mean day)	pH (mean 29 d)	Sal $_{(refrat.)}$ (mean day)	Sal $_{(refrat.)}$ (mean 29 d)	Sal $_{(chem.)}$ (mean day)	DO mg/l (mean day)	DO mg/l (mean 29 d)
Control	27.01 (0.04)	26.98 (0.46)	7.91 (0.01)	7.97 (0.05)	37.4 (0.42)	37.94 (0.59)	36.0 (0.35)	7.83 (0.05)	7.60 (0.14)
Scenario I	27.74 (0.05)	27.67 (0.13)	7.79 (0.07)	7.88 (0.06)	37.6 (0.65)	37.97 (0.53)	36.6 (0.14)	7.59 (0.03)	7.46 (0.14)
Scenario II	29.22 (0.04)	29.22 (0.05)	7.69 (0.08)	7.71 (0.05)	38.58 (1.36)	37.88 (0.58)	37.1 (0.33)	7.31 (0.06)	7.21 (0.14)
Scenario III	30.43 (0.04)	30.39 (0.13)	7.28 (0.08)	7.34 (0.08)	39.38 (0.25)	38.08 (0.70)	38.3 (0.36)	7.09 (0.03)	7.02 (0.10)

Temp = temperature, pH = pH measured, Sal $_{(refrat.)}$ = salinity data obtained with refractometer, Sal $_{(chem.)}$ = salinity data taken from chemical technique, DO = dissolved oxygen. Mean day = average value collected in sampling moment, Mean 14 d = Mean value calculated from the 14 days daily data, Mean 29 d = Mean value calculated from the 29 days daily data.

Carbonate chemistry data (pCO_2, Ω_{ca} and Ω_{ar}) presented in table 3 were calculated from average (14 and 29 days daily data) pH, temperature and chemical salinity.

Table 3 Treatments seawater carbonate chemistry. Values: mean ±SD

07 November				
	TA (µmol/Kg^{-1})	pCO_2	Ω_{ca}	Ω_{ar}
Initial	2453.76 (62.24)	464.341	5.612	3.744
22 November				
Control	1725.44 (51.89)	311.246	3.867	2.575
Scenario I	2012.80 (96.27)	462.633	4.022	2.683
Scenario II	2389.76 (53.94)	899.856	3.630	2.436
Scenario III	3913.60 (173.45)	3839.622	2.926	1.971
06 December				
Control	1806.72 (50.03)	375.967	3.720	2.471
Scenario I	2031.36 (124.98)	543.124	3.666	2.442
Scenario II	2267.52 (42.46)	957.849	3.131	2.098
Scenario III	3905.92 (35.63)	4239.560	2.662	1.793

TA = total alkalinity, pCO_2 = partial pressure of carbon dioxide calculated Ω_{ca} – calcite saturation state calculated (where 1 is saturation), Ω_{ar} - aragonite saturation state calculated**.**

3.3 Comparison between field and control samples

The MDS analysis represented the similarity matrix of meiofauna samples of field and control samples (Fig. 5). MDS showed a pattern of differentiation between field and control samples collected after 15 and 29 days.

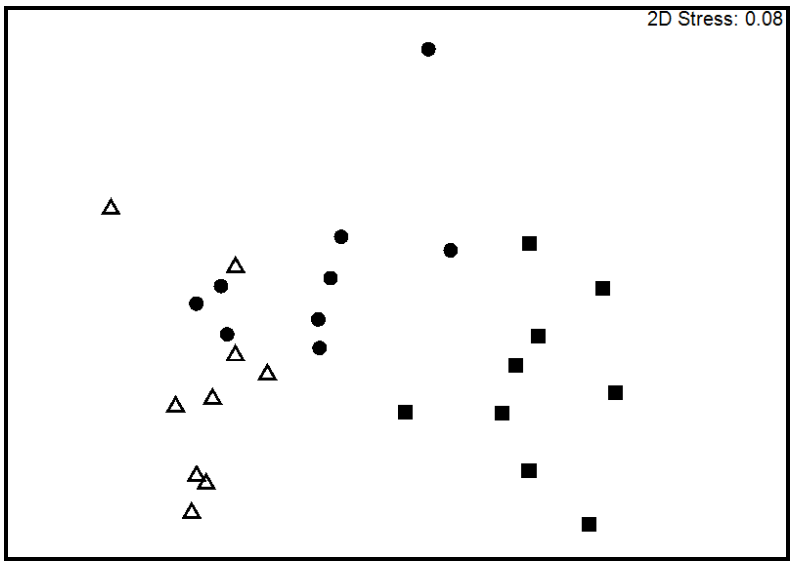

Fig. 5 Non-metric multi-dimensional scaling ordination plots for the Bray–Curtis similarity for the meiofauna community structure. Δ (triangle) Field, ● (closed circle) Control 15 days, ■ (closed squares) Control 29 days

PERMANOVA comparisons among field and control samples collected after 15 and 29 days showed significant differences in the meiofauna community structure ($F_{(2;24)}$=16.67, $p<0.001$). A posteriori comparisons showed significant differences between field and 15-days control samples ($t= 2.71$, $p<0.01$), between field and 29-days control samples ($t=5.43$, $p<0.001$), and between 15 and 29-days control samples ($t=3.75$, $p<0.001$).

SIMPER analyses showed that the dissimilarities among field and control samples were greater after 29d; Ostracoda was the group that contributed most to these dissimilarities (Table 4).

Table 4 Percent contribution (Contrib. %) of meiofauna groups to average dissimilarity (Diss.) among field and control samples collected after 15 and 29 d

Field *vs* Control 15		Field *vs* Control 29 d		Control 15 *vs* Control 29 d	
Diss.= 10.94	Contrib.%	Diss.= 18.07	Contrib.%	Diss.= 14.73	Contrib.%
Ostracoda	27.44	Ostracoda	38.40	Ostracoda	29.68
Turbellaria	16.20	Turbellaria	21.82	Nematoda	18.98
Harpacticoida	15.49	Nematoda	11.58	Turbellaria	16.77
Polychaeta	15.37	Polychaeta	10.94	Harpacticoida	12.25
Nematoda	12.93	Nauplius	9.05	Nauplius	11.43
Nauplius	12.57			Polychaeta	10.90

3.4 Effect of scenarios on meiofauna community

A total of 45,263 meiofaunal organisms were counted. Meiofauna was composed of 25 taxonomic groups, of which Copepoda Harpacticoida (44.64%) and their Nauplii (15.10%), Polychaeta (19.82%), Nematoda (11.45%), Ostracoda (3.41%) and Turbellaria (1.73%) accounted for 96.15% of total. The dominant group in the field samples was Copepoda Harpacticoida (46.60%), followed by Polychaeta (20.75%), Nauplii (15.10%), Ostracoda (4.99%), Nematoda (3.49%) and Turbellaria (2.79%). For samples collected after 15 days, the dominance pattern was similar: Copepoda Harpacticoida (45.71%), followed by Polychaeta (22.20%), Nauplii (13.23%), Nematoda (9.53%), Ostracoda (4.73%) and Turbellaria (1.77%). For the samples collected after 29 days, it was observed a shift in the dominance pattern: Copepoda

Harpacticoida (41.69%), followed now by Nematoda (19.93%), Nauplii (17.78%), Polychaeta (15.74%) and Dinophillidea (3.25%).

The MDS analysis representing the similarity matrix of meiofauna samples from the field and the four treatments/scenarios at the two sampling times showed a clear pattern of differentiation between samples collected after 15 and 29 days and among scenarios (Fig. 6).

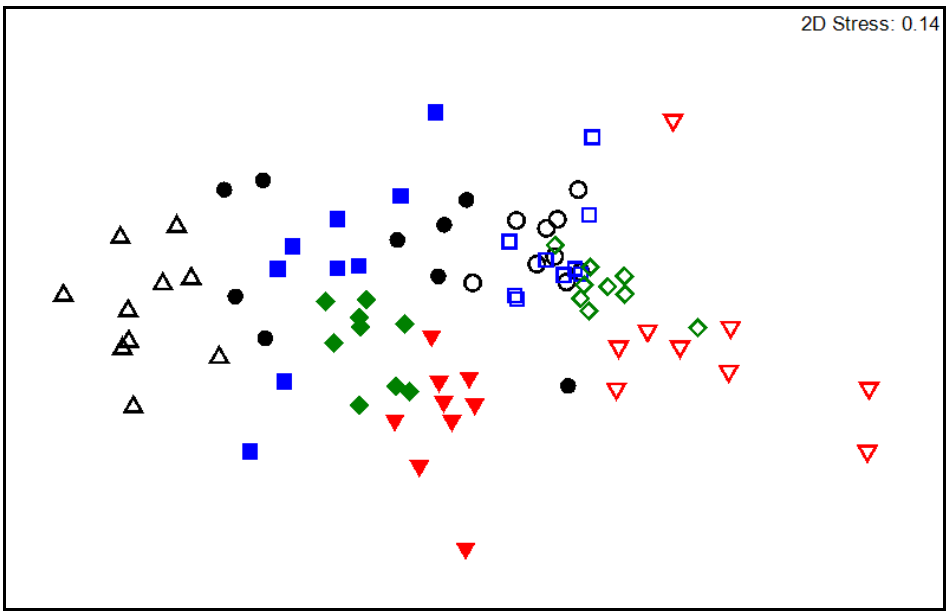

Fig. 6 Non-metric multi-dimensional scaling ordination plots for the Bray–Curtis similarity for the meiofauna community structure. Δ (open triangle) Field, ● (circle) Control, ■ (square) scenario I, ♦ (diamond) scenario II, ▼ (inverted triangle) scenario III. Closed symbols represent samples collected after 15 d, and open symbols after 29 d

The PERMANOVA results confirmed the pattern shown in MDS, and detected significant differences in the structure of the meiofauna community between samples collected after 15 and 29 days (factor Time), among the four scenarios (factor Scenario), and also for the interaction between the two factors (Table 5).

Table 5 PERMANOVA results for the meiofauna communities exposed to different climate change scenarios and collected after 15 and 29 days. Significant values are highlighted in bold

Source	*df*	MS	*F*	*P*
Time (Ti)	1	3494.2	**63.45**	<0.001
Scenario (Sc)	3	2651.4	**16.05**	<0.001
Ti x Sc	3	373.69	**2.26**	0.03
Residual	64	3524.5		

Significant values are highlighted in bold

Pairwise tests for samples collected after 15 days of exposure did not detect significant differences in the meiofauna community structure only for comparisons between control and scenario I, and scenario II and scenario II. However, samples collected after 29 days of exposure showed significant differences among control and all scenario samples and among the scenarios (Table 6).

Table 6 Pair-wise *a posteriori* comparisons among climate change scenarios for samples collected after 15 and 30 d

Comparisons	Day 15		Day 29	
	t	*P*	*t*	*P*
Control *vs* Scenario I	1.43	0.128	**1.59**	0.043
Control *vs* Scenario II	**2.82**	<0.01	**2.60**	<0.01
Control *vs* Scenario III	**3.16**	<0.01	**4.30**	<0.01
Scenario I *vs* Scenario II	1.02	0.334	**1.68**	0.014
Scenario I *vs* Scenario III	**2.76**	<0.01	**3.99**	<0.01
Scenario II *vs* Scenario III	**3.80**	<0.01	**2.86**	<0.01

Significant values are highlighted in bold

SIMPER analyses showed that the dissimilarities were greater among control and scenario III, especially after 29 d. Nauplii was the group that contributed most to these dissimilarities (Table 7).

Table 7 Percent contribution (Contrib. %) of meiofauna groups to average dissimilarity (Diss.) among different climate change scenarios for samples collected after 15 and 29 d

Day 15					
Control *vs* Scenario I		Control *vs* Scenario II		Control *vs* Scenario III	
Diss.= 10.40	Contrib.%	Diss.= 10.64	Contrib.%	Diss.= 12.69	Contrib.%
Ostracoda	21.10	Nematoda	26.26	Nauplius	30.94
Nematoda	20.26	Ostracoda	23.77	Harpacticoida	19.36
Turbellaria	19.13	Polychaeta	16.42	Nematoda	15.87
Harpacticoida	16.05	Harpacticoida	15.00	Ostracoda	14.40
Polychaeta	15.33	Nauplius	9.79	Polychaeta	10.75
Day 29					
Control *vs* Scenario I		Control *vs* Scenario II		Control *vs* Scenario III	
Diss.= 9.36	Contrib.%	Diss.= 11.66	Contrib.%	Diss.= 21.66	Contrib.%
Nauplius	24.63	Nauplius	29.95	Nauplius	39.26
Ostracoda	20.92	Harpacticoida	21.18	Harpacticoida	23.84
Turbellaria	15.68	Ostracoda	14.36	Polychaeta	12.6
Polychaeta	13.60	Polychaeta	12.19	Turbellaria	9.78
Harpacticoida	13.18	Nematoda	11.82	Ostracoda	7.86
Nematoda	11.98	Turbellaria	10.5		

Differences in the density of total meiofauna and of the dominant groups were observed between the field and control samples. The groups of meiofauna presented different sensibilities/responses to the different climate change scenarios. Harpacticoida and their Nauplii showed to be more sensitive to climate change scenarios, especially after 29 days of exposition. On the other hand, Nematoda increased their density under the climate change scenario conditions (Fig. 7).

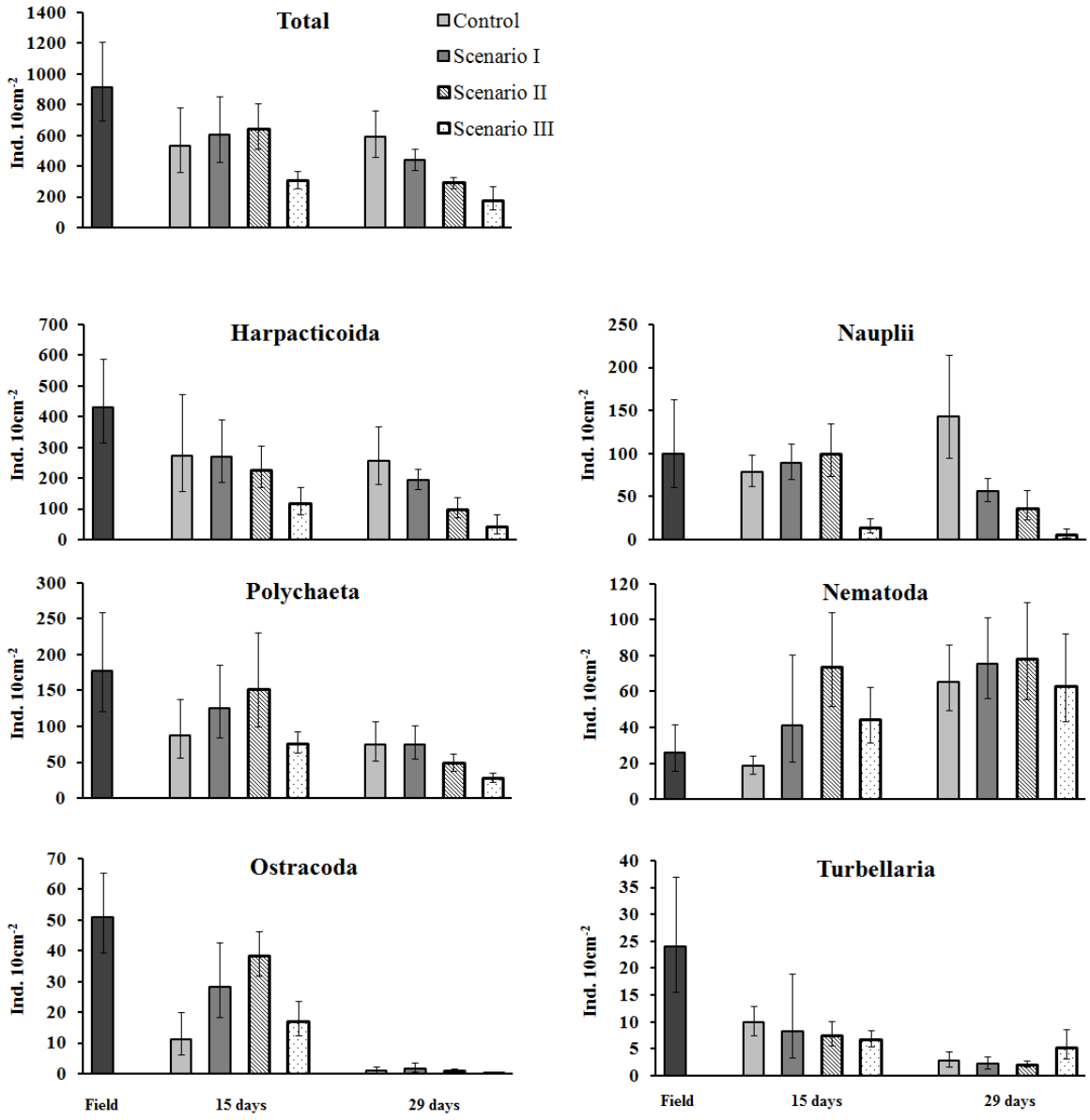

Fig.7 Mean density (± 95% confidence intervals) of the main groups of meiofauna at different scenarios (Control, Scenario I, II and III) and sampling times (15 and 29 days)

The two-way Anova results indicated that Polychaeta, Nauplii, Nematoda, Ostracoda and total meiofauna densities showed significant interaction between the factors Time and Scenario. Harpacticoida was sensitive to both factors, but not for the interaction. Turbellaria was sensitive only to the factor Time (Table 8).

Table 8 Two-way ANOVA results for meiofauna major groups densities in response to different climate change scenarios and sampling times (15 and 29 d). Significant ($p<0.05$) *F* values in bold

Source	*df*	*MS*	*F*	*p*	*MS*	*F*	*p*
		Harpacticoida			Polychaeta		
Time (Ti)	1	5.68	**14.19**	<0.01	8.55	**33.45**	<0.01
Scenario (Sc)	3	6.26	**15.65**	<0.01	1.91	**7.48**	<0.01
Ti x Sc	3	0.91	2.27	0.089	0.88	**3.46**	0.021
Residual	64	0.40			0.26		
		Nauplii			Nematoda		
Time (Ti)	1	2.88	**7.08**	0.01	5.54	**16.70**	<0.01
Scenario (Sc)	3	19.52	**47.9**	<0.01	1.74	**5.24**	<0.01
Ti x Sc	3	2.21	**5.42**	<0.01	1.09	**3.29**	0.026
Residual	64	0.408			0.331		
		Ostracoda			Turbellaria		
Time (Ti)	1	109.38	**291.1**	<0.01	12.57	**35.68**	<0.01
Scenario (Sc)	3	1.97	**5.25**	<0.01	0.36	1.01	0.39
Ti x Sc	3	1.12	**2.99**	0.037	0.80	2.27	0.09
Residual	64	0.376			0.352		
		Total meiofauna					
Time (Ti)	1	2.67	**14.60**	<0.01			
Scenario (Sc)	3	2.77	**15.19**	<0.01			
Ti x Sc	3	0.63	**3.48**	0.02			
Residual	64	0.183					

The results of the *a posteriori* test (Fisher LSD) indicated that Harpacticoida was negatively affected in Scenario II ($p<0.01$) and III ($p<0.01$) when compared to Control samples.

Results for significant interactions between factors indicated that for samples collected after 15 days, the density of Polychaeta significantly increased in Scenario II ($p=0.03$) in comparison to Controls, while no differences were observed between Control and Scenario I ($p=0.15$) and Scenario III ($p=0.56$). After 29 days of exposition, no differences were observed among Control and Scenarios I ($p=0.99$) and II ($p=0.08$). However, the density of Polychaeta significantly decreased in Scenario III ($p<0.01$) in

comparison to Control. No differences were observed between Control samples collected after 15 and 29 d ($p=0.52$).

After 15 days, the density of harpacticoid's nauplii significantly decreased in Scenario III ($p<0.001$) when compared to Control. However, after 29 days, nauplii's density significantly decreased in Scenario I ($p=0.003$), II ($p<0.001$) and III ($p<0.001$) when compared to Control. No differences were observed between Control samples collected after 15 and 29 d ($p=0.052$).

Nematoda density significantly increased in Scenario I ($p=0.006$), II ($p<0.01$) and III ($p=0.003$) when compared to Controls after 15 days. After 29 days, no differences were observed among Control and Scenarios ($p>0.5$ for all comparisons). Nematoda density in Control samples collected after 29 days was significantly higher than in Control samples collected after 15 days ($p<0.001$).

Ostracoda density significantly increased in Scenario I ($p=0.004$) and II ($p<0.01$) when compared to Controls after 15 days but no differences were observed among Control and Scenarios ($p>0.12$ for all comparisons). Nematoda density in Control samples collected after 29 days was significantly lower than in Control samples collected after 15 days ($p<0.001$).

After 15 days, the total density of meiofauna significantly decreased only in Scenario III ($p<0.01$) when compared to Control. However, after 29 days, the total density of meiofauna decreased in Scenario II ($p<0.01$) and III ($p<0.001$) when compared to Control. No differences were observed between Control samples collected after 15 and 29 d ($p=0.061$).

4. Discussion

The microcosm system set-up for this study was able to attain and maintain the different climate change scenarios with low variation within treatments. The choice of scenarios approach, based in the Representative Concentration Pathways (RCP) defined by the scientific community in the IPCC report (IPCC 2013) showed to be a more realistic guide to set the experimental treatments, as it represents a range of 21st century climate policies. Moreover, the use of colonized artificial substrates in the present study (despite the small scale of ASUs, 45 cm^2), enabled the settlement of a meaningful meiofauna sampling universe, considering both density and diversity, for assessment of anthropogenic impacts associated to climate change (Meadows et al. 2015; Sarmento et al. 2016a).

Modifications in meiofauna community structure among field and control samples kept in laboratory were observed. However, despite the significant differences, dissimilarities values among field and control samples were quite low and mainly due to decreases in Ostracoda and Turbellaria densities, both being not dominant groups in the present study. These changes are resultant of artificially imposed stress once the organisms were removed from their natural surroundings. This experimental stress is very common in laboratory experiment (Riebesell et al. 2007), besides, modification over the time in control samples can be observed, and are expected, even in field experiments (Sarmento et al. 2013). The modification observed in the present study was considered low and not relevant to interfere in the evaluation of the impact of the climate change scenarios.

In the present study, it was observed a clear pattern of impact of the different treatments in the meiofauna community structure from controls toward the most severe scenario and over time. After only fifteen days of exposure, the community showed to

be sensitive to scenarios II and III. However, at the end of 29 days, meiofauna community showed to be even more susceptible and all scenarios were different from controls.

Modifications in community structure were the result of the different patterns of response of the numerically dominant meiofaunal taxa to scenarios. Harpacticoid copepods were negatively affected with decreases in scenario II and III after 15 and 29 days. However, harpacticoid nauplii were strongly negatively affected in all three scenarios. Polychaeta showed to be a more resistant group. After 15 days, Polychaeta presents an increase in their density in scenario II and was negatively affected only after 29 days in the most severe scenario. On the other hand, Nematoda exhibited its highest densities in all the warming and low pH scenarios.

Divergent biological responses of meiofauna taxa have been observed in previous climate change studies. In a mesocosm experiment with phytal meiofauna from coral reefs, Sarmento et al. (2016a) found that Harpacticoida and Polychaeta did not show significant differences due to pH after 15 or 30 days. On the other hand, Nematoda, Ostracoda, Turbellaria, and Tardigrada exhibited their highest densities in low-pH treatments (especially at 7.5), while only the harpacticoid nauplii have been strongly negatively affected by low pH. In other studies on meiofauna communities from shallow areas it has been found that they tolerate ocean acidification. In a 56-day microcosm experiment with a meiobenthic community from sediment, Kurihara et al. (2007) found no significant impact in the abundance of meiofauna in response to elevated CO_2 concentrations (pH 7.4). Widdicombe et al. (2009) found that exposure to acidified seawater significantly altered the community structure and reduced diversity for nematode assemblages in a mesocosm experiment. However, the largest differences were observed for pH 5.6 after 20 weeks, and the sediment type (mud or sand) played

an important role in the differentiation of the nematode community structure. In a mesocosm experiment, Dashfield et al. (2008) found that the presence of a burrowing urchin was a key factor determining the response of the nematode community to the impact of ocean acidification (pH 7.5), and suggested that any nematode mortality is unlikely to be directly due to differences in pH.

Considering the majority of studies, meiofauna organisms appear to show some resistance to ocean acidification. This apparent higher tolerance observed for the benthic meiofauna may be related to physiological features of these animals. In contrast to the great vulnerability to high CO_2 of calcifying organisms, marine invertebrate species that do not calcify in the larval stage or have poorly calcified exoskeletons (e.g., copepods, amphipods and barnacles) appear to be resilient to near-future levels of pH/pCO_2 (Kurihara et al. 2004; Ishida et al. 2005; Mayor et al. 2007; Kurihara 2008; Kurihara and Ishimatsu 2008; Dupont and Thorndyke 2009; Dupont et al. 2010; Findlay et al. 2010; Byrne 2012). Thus, it is probable that due to the poorly calcified cuticle of representatives of meiofauna such as the dominant crustaceans Copepoda Harpacticoida, but also Kinorhyncha, Tardigrada, and Nematoda (Ruppert et al. 2004; Giere 2009), the meiofauna community could withstand the effects of ocean acidification at some level.

Ocean acidification is projected to impact all areas of the ocean, from the deep sea to coastal areas as coral reefs and estuaries (Orr et al. 2005; Feely et al. 2009), with potentially important impacts on marine life (Doney et al. 2009). Moreover, the increasing levels of atmospheric CO_2 are concurrently driving ocean warming (Meehl et al. 2007). Ocean acidification has been shown to have drastic effects on a broad range of macrobenthic marine organisms decreasing survival, calcification, growth, development and abundance (e.g., Dupont et al. 2010; Findlay et al. 2010; Byrne et al.

2013; Fabricius et al. 2014). However, a trend to enhance sensitivity when organisms are concurrently exposed to elevated seawater temperature is observed (Pörtner 2008; Byrne 2011; Kroeker et al. 2013).

In general, the number of studies on the impact of climate change in meiofauna is increasing slowly (Zeppilli et al. 2015), and up to date, only one had addressed both elevated temperature and acidification on meiofauna community (Meadows et al. 2015). The effects of increase temperature and decrease pH on intertidal, meiofaunal assemblages were investigated by Meadows et al. (2015) using a mesocosm experiment. Artificial Substrate Units containing meiofauna were exposed for 60 days to eight experimental treatments comprising four pH levels: 8.0, 7.7, 7.3 and 6.7, crossed with two temperatures: 12 °C and 16 °C. Meiofauna and Nematoda community structure was significantly affected by pH (especially by the pH 6.7) and temperature. The response of meiofauna organisms to ocean acidification varied with temperature. Copepodites were affected with significant difference between pH 7.7 and 8.0 at 16 °C and between 12 and 16 °C at pH 8.0 and 7.3. Copepod abundance was affected by pH and temperature showing a drastic decline at pH 6.7. Moreover, average copepod relative abundance values were greater at 16 °C compared to 12 °C for all pH treatments lower than pH 8.0. A positive response in nematode abundance was observed in response to lowered pH at the 12 and 16 °C temperatures, but the effects of temperature are only evident at pH 6.7, whereby nematode abundance is substantially higher at 12 °C. However, estimated nematode species diversity, species evenness, and the maturity index, were substantially lower at 16 °C.

Differently from ocean acidification studies, where meiofauna appear to be resistant in some extent, meiofauna have respond to seawater warming impact. Recent studies have shown that freshwater meiofauna showed important changes across

thermal gradient (O'Gorman et al. 2012) and that, in subtropical Nematoda communities, an increase of temperature negatively affects abundance, biomass and species richness (Gingold et al. 2013).

The large majority of studies that consider the impact of both warming and/or acidification were conducted on macrobenthic organisms (Pörter 2008; Byrne 2011; Byrne and Przeslawski 2013; Kroeker et al. 2013). In general, it is observed that increasing temperature has a stimulatory effect on development, but beyond the optimum temperature, further warming can denature proteins and is implicated in mass mortality, increased disease, physiological limitation in oxygen delivery and increased costs of metabolism. CO_2 elicits acidosis not only in the water, but also in tissues and body fluids. Compensatory accumulation of bicarbonate, acid-base parameters (pH, bicarbonate and CO_2 levels) and ion levels could reach new steady-state values, with specific, long-term effects on metabolic functions (Pörtner 2008; Byrne et al. 2011).

Since temperature is fundamental to biological processes, thought, it is likely to have a direct impact on physiological responses to ocean acidification. The combined effects of concurrent warming and acidification can have complex interactive effects with synergistic effects (increased stress greater than the sum of the effects of individual stressors) or antagonistic effects (decreased stress) on biological processes (Folt et al. 1999; Hale et al. 2011; Byrne and Przeslawski 2013; Kroeker et al. 2014; Wood et al. 2011; Meadows et al. 2015).

In fact, it appears that some meiofaunal groups of meiofauna, regardless to show no sensibility to ocean acidification, become negatively affected when exposed to simultaneous decrease in pH and increase in seawater temperature. Polychaetes appears not to be greatly affected by low pH (Hale et al. 2011; Kroeker et al. 2011; Calosi et al. 2013; Christen et al. 2013; Fabricius et al. 2014; Sarmento et al. 2016a), and some

species even became more abundant at the lowest pH investigated (Cigliano et al. 2010; Hale et al. 2011). However, in the present study, when polychaetes were exposed to simultaneous decrease in seawater pH and elevated temperatures, it was observed a negative impact in its densities. Many studies with different copepod species found that adult survival, body size, metabolism and growth were not affected by increased seawater acidity (Kurihara et al. 2004; Mayor et al. 2007; Kurihara and Ishimatsu 2008; Pascal et al. 2010; Pedersen et al. 2014; Isari et al. 2015). But found decreases in egg and naupliar production (Kurihara et al. 2004; Mayor et al. 2007; Fitzer et al. 2012, 2013). Studies that addressed the impact of warming and ocean acidification showed that these organisms can present sublethal effects in response to synergistic effect (Hildebrand et al. 2014; Li et al. 2015). Harpacticoid copepods when exposed to simultaneous seawater warming and acidification had their density negatively affected in scenario II and III. Nematodes have been showed to be able to withstand short-term exposure to even severe seawater acidification (Wieser et al. 1974; Takeuchi et al. 1997; Ishida et al. 2005; Kurihara et al. 2007; Dashfield et al. 2008; Widdicombe et al. 2009) and also to increase their densities under low-pH conditions (Hale et al. 2011).

In the present study, the simultaneous sea water warming and acidification exhibited a synergistic effect on meiofauna taxonomic groups increasing the sensibility. On the other hand, nematodes showed to be the most resistant and opportunistic group with its density even increasing under the scenarios of elevated temperature and low pH. It could be related to antagonistic effects of increasing warming and ocean acidification in addition to the reduction of ecological constraints, such as predation caused by a decrease in macrofaunal abundance Melo et al. (2015, data not published) and competition due decreases in the densities of the other groups.

In the present study, the exposure of meiofauna organisms from coral reefs phytal environment to simultaneous increase of warming and ocean acidification have shown to be more threatening than when exposed to a single stressor (Kurihara et al. 2007; Dashfield et al. 2008; Sarmento et al. 2016a; Widdicombe et al. 2009). Meiofauna have historically been used as an indicator of a wide range of antropogenic impacts (Coull and Chandler 1992) and more recently, have shown to be a very useful tool responding to climate change impact (Meadows et al. 2015; Zeppilli et al. 2015) reflecting changes among different levels of temperature and pH and been sensitive to temporal changes as well. The same pattern of response of the dominant meiofaunal groups, where copepods (and their juveniles) are the most sensitive to changing environmental conditions while nematode are particularly tolerant to stress follow previous studies and highlight the advantages of using meiofauna to evaluate anthropogenic impacts in different ecosystems and levels of stress.

The results displayed by meiofauna community from coral reefs phytal environment (Sarmento et al. 2016a) indicate that this fauna seemed to be more sensitive to ocean acidification when compared to meiofauna from sediment environments (Kurihara et al. 2007; Dashfield et al. 2008; Widdicombe et al. 2009). This could be related to the fact that, under present conditions, CO_2 represents an abiotic factor that remains more or less constant in most of the zones of the sea. However, CO_2 levels will fluctuate where occur volcanic emissions or excessive respiration in confined areas filled with plant and animal life, e.g. in rockpools. It also fluctuates in marine sediments or hypoxic bottom waters as it depends on the oxidation of organic matter, rates of oxygen consumption and anaerobic metabolism of bacteria, meio- and macrofauna in an environment where mixing with the surface water is poor (Pötner et al. 2004).

On the reef flat, the hard substrate is mainly dominated by turf-forming macroalgae (Maida and Ferreira 1997). Macroalgae are among the major contributors to reef primary production, and provide shelter to an extremely diverse fish and invertebrate fauna. Among the groups of meiofauna, copepods are regularly the most abundant group in the phytal environment (Hicks 1977; Coull et al. 1983; Hall and Bell 1993), with high diversities (Hicks 1985). The frequent epibenthic occurrence of harpacticoids makes them a preferred prey for many small, often juvenile demersal fishes, carnivorous crustaceans (shrimps and their larvae) and polychaetes. Derived from their preferred diatom food harpacticoids have high fatty acid contents, thus playing a decisive nutritional role for small fish (Coull 1999; Giere 2009; Berkström et al. 2012; Kramer et al. 2012, 2013).

In the light of the recent studies and the data presented here, it is likely that some species of nematodes can be favored by the occurrence of extreme environmental conditions. Indeed, as environmental conditions change, there is a concern that ecosystems will become more simplified, resulting in homogenization and reduced functional diversity (Kroeker et al. 2013). Nematodes apparent high tolerance to impacts of increasing ocean acidification (even when concurrently with warming), in addition to the selective impact in the biofilm assemblage that could promote an increase in the amount of detritus and in bacteria density in ASUs, would highly benefited generalists and/or detritivores animals (e.g. nematode) in detriment of those that have selective food preferences or are more sensitive to low pH (e.g. copepods). The presence of nematode extreme species can be used as warning signal of global change. Thereby, a shift in meiofauna community dominance from copepods towards the resistant nematodes is a real threaten to the functioning of the trophic web in coral reefs.

Coral reefs are widely recognized as one of the ecosystem that are most threatened by ocean acidification, especially due to its great impact in highly calcifying organisms, particularly those that are critical to the formation of habitats (e.g., coral species) or their maintenance (e.g., grazing echinoderms; e.g., Jokiel et al. 2008; Kleypas and Yates 2009; Morita et al. 2009; Byrne et al. 2013; De'ath et al. 2013; Uthicke et al. 2014). The observed high sensibility of meiofauna to ocean acidification with the concurred warming demonstrates the great risk that climate change poses to benthic lower trophic levels. Therefore, the results presented in this study, helps to highlight the great sensibility of coral reefs environments to the consequences of the global climate change.

CAPÍTULO 3

Synergistic impact of ocean acidification and warming on harpacticoid copepod community from coral reefs

1. Introduction

Human influence on the climate system is clear, and recent anthropogenic emissions of greenhouse gases are the highest in history. Recent climate changes have had widespread impacts on human and natural systems. Anthropogenic greenhouse gas emissions have increased since the pre-industrial era, driven largely by economic and population growth, and are now higher than ever. This has led to atmospheric concentrations of carbon dioxide, methane and nitrous oxide that are unprecedented in at least the last 800,000 years (IPCC 2014). In fact, the observed increase in atmospheric carbon dioxide (CO_2) concentrations does not reveal the full extent of human emissions in that it accounts for ~50% of the CO_2 fraction of the combined fossil-fuel and land-use emissions released by human activity. The rest has been taken up by plants on land and ocean has absorbed about one-quarter of anthropogenic carbon emissions (Sabine et al. 2004; Feely et al. 2009).

Warming and ocean acidification are two of the most prominent anthropogenic changes in the ocean. Both are driven by elevated CO_2 and threaten to have widespread ecological consequences. The magnified greenhouse effect associated with rising atmosphere CO_2 concentrations is predicted to cause a global rise in sea surface temperatures from 0.6–2ºC within this century (IPCC 2013). But global mean surface temperatures by the end of 21st century are likely to attain 2.6ºC to 4.8ºC (IPCC 2014).

The substantial proportion of the CO_2 emitted into the atmosphere that is absorbed by the oceans cause changes in carbonate chemistry and decline in seawater

pH. The pH of ocean surface waters has already decreased by about 0.1 since the industrial era began (Caldeira and Wickett 2003). The predicted increases in CO_2 levels would result in an additional decrease in surface water pH of 0.15 to 0.4 units from pre-industrial, by 2100, which represents an increase in the ocean's hydrogen ion (H^+) concentration by 2.5 times relative to the beginning of the industrial era (Caldeira and Wickett 2003; Feely et al. 2009; IPCC 2014). However, further predictions estimate a pH decreases of up to 0.7 units by 2250 (Caldeira and Wickett 2003, 2005).

In the natural environment marine organisms and ecosystems will often be subjected to elevated CO_2 levels and elevated temperatures and so investigations into the potential synergistic impacts should be addressed. The consequences of climate change impacts on human societies, land and marine ecosystems have attained growing recognition at intergovernmental level (e.g. "Our ocean Conference" and "Conference of the Parties") and an important growing in this research area is observed (Harley et al. 2006; Gattuso and Hansson 2011). Though, the vast majority of those studies compass single stress perturbation experiments specially ocean acidification (e.g. Kroeker et al. 2010; Dupon et al. 2010), and still, most studies are conducted at individual species level (e.g. Cripps et al. 2014; Chan-Gyung et al. 2014; Iglesias-Prieto et al. 2014; Chan et al. 2015; Cross et al. 2015). Recent discussions have alluded that one of the biggest unknown is how species will respond to ocean acidification and warming within the context of their communities (Gaylord et al. 2015). In this context, well-constrained multiple-stress studies conducted at community level are urgently needed.

Future greenhouse gas emissions are the product of very complex dynamic systems, determined by driving forces such as demographic development, socio-economic development and technological change. Scenarios are alternative images of how the future might unfold and they are useful tools for scientific assessments of

climate change impacts and for policymaking, assisting climate change analysis and debates on adaptation and mitigation plans (IPCC 2000). Therefore, scenarios that embrace the current trend of increasing atmospheric CO_2 together with the simultaneous changes in temperature and ocean chemistry are necessary to understand the impacts of climate changes on the response of marine communities.

In Brazil, coral reefs are among the most prominent marine ecosystems of tropical zone. These reefs are distributed along 3,000 km of the northeastern coast, and they include the southernmost coral reef communities in the Atlantic. The Brazilian coral reefs form structures that are significantly different from most of the well-known coral reef models in the world (Leão and Dominguez 2000).

Despite the ecological importance of coral reef ecosystems, and all direct and indirect economic benefits the coral reefs provide to fisheries and ecotourism (White et al. 2000), shallow waters coral reefs are subjected to a variety of local impacts such as overfishing, nitrification, pollution related to agriculture and industry, tourism and engineering interference (Leão and Kikuchi 2005; Davenport and Davenport 2006; Defeo et al. 2009). In addition to these threats, coral reefs are widely recognized as the ecosystem that is most threatened by climate change impacts, specially ocean acidification and warming (Hughes et al. 2003; Hoegh-Guldberg et al. 2007; Kleypas and Yates 2009; Anthony et al. 2011; Fabricius et al. 2011; van Hooidonk et al. 2014).

On coral reefs, a major source of primary productivity is derived from the phytal. Meiofauna is the most abundant assemblage inhabiting this reef phytal environment. Meiofauna organisms are a biologically and ecologically distinct group of metazoans, operationally defined by their small size (Giere 2009). Due to their high abundance and diversity, their widespread distribution, their rapid generation time and fast metabolic rates, meiofaunal organisms are vital contributors to ecosystem function,

including nutrient cycling and the provision of energy to higher trophic levels ((Danovaro et al. 2007; Kramer et al. 2013). Meiofauna are characterized by a high sensitivity to environmental changes due to their short generation time and the lack of pelagic larval dispersion for the dominant meiofaunal groups (Kennedy and Jacoby 1999; Giere 2009).

Among the groups of meiofauna, copepods of the order Harpacticoida are regularly the most abundant group in the phytal environment (Hicks 1977; Coull et al. 1983; Hall and Bell 1993), with high diversities (Hicks 1985; Sarmento and Santos 2012). This taxonomic group is already recognized as valuable for predicting global climate changes since they show very sensitive to changing environmental conditions (Sarmento et al. 2016b; Zeppilli et al. 2015). Moreover, harpacticoids have high fatty-acid contents, derived from their preferred diatom food, and play a decisive nutritional role for small fish, carnivorous crustaceans (shrimp and their larvae), and polychaetes (Coull 1999; Giere 2009). Thus, these metazoans are a key component of the benthic ecosystem, contributing significantly to energy transfer to higher trophic levels (Coull 1988; Danovaro et al. 2007).

To understand how climate change affects harpacticoid community that colonized artificial substrate units from a tropical coral reef environment, the present study experimentally determined the effect of four different scenarios of ocean acidification and warming, which may be determined by global climate change. The following hypothesis was tested: the different scenarios will generate different impacts on harpacticoid community structure, ecological indexes and on the numerically dominant species.

2. Materials and methods

The samples used in this study are from the same experiment described in detail by Sarmento et al. (2016b – chapter 2). However, due to the high number of replicates and since harpacticoid identification is a high time consuming task, only forty-five samples (five replicates per scenario) were randomly selected for analysis.

At the end of 4 weeks exposure, the fauna was extracted from the ASUs by manual elutriation with filtered water through geological sieves. Samples were sieved through a 500-µm mesh, and a 45-µm mesh was used to retain the meiobenthic organisms. Under a Leica EZ4 stereomicroscope, the individuals of copepods were selected from each replicate and placed in Eppendorf tubes with 70% alcohol. The identification of Copepoda Harpacticoida was done under optical microscope (Leica DM 2500) following the taxonomic keys of Lang (1948, 1965), Huys et al. (1996) and Wells (2007) as well as publications with specific descriptions.

2.1 Statistical analysis

To evaluate changes on the natural communities due to the experiment, a Permutational multivariate analyses of variance (PERMANOVA) (Anderson 2001; McArdle and Anderson 2001) based on Bray-Curtis dissimilarities on copepods not transformed data was used to detect if significant differences existed in the structure of the Copepoda assemblages among field (the 9 ASUs preserved immediately after collection from the field) and control samples collected after 15 and 29 days (factor Time). Similarity percentage (SIMPER) analysis was applied to determine which species were responsible for the dissimilarities among field and control samples

collected after 15 and 29 days. Multi-dimensional scaling (MDS) was used to represent the Bray-Curtis matrix graphically in a two-axis space.

PERMANOVA analyses based on Bray-Curtis dissimilarities on copepods not transformed data, were also used to evaluate the impact of different climate change scenarios (factor Scenario) on the structure of communities, considering the two exposure periods, 15 and 29 days (factor Time). For all analyses, 9,999 random permutations were used. Pair-wise *a posteriori* comparisons (the multivariate version of the *t* statistic) were made when the interaction between factors was significant. A similarity percentage (SIMPER) analysis was applied to determine which species were responsible for the dissimilarities among scenarios for the samples collected after 15 and 29 days. Multi-dimensional scaling (MDS) was used to represent the Bray-Curtis matrix graphically in a two-axis space.

The Shannon–Wiener (H′, using $\log_2$), Pielou's evenness (J′) and species richness indices were calculated. The population parameters ratio of malformed animals, density of copepodites, Copepodite and Nauplii ratios, and ovigerous female and female/male ratios were also calculated.

Two-way analysis of variance (ANOVA) was used to examine the effects of the different scenarios (factor Scenario) on the densities of adult cyclopoids and harpacticoid's species (>2% of total), ecological indexes (S, J' and H') and on population parameters (ratios of copepodites, nauplii, female/male, ovigerous females and malformed animals), considering the two exposure periods, 15 and 29 days (factor Time).

To detect non-random distributions of species (indicator species) between scenarios and sampling times, the Indicator Species Analysis developed by Dufrêne and Legendre (1997) was used. A species is an indicator when it characterizes a group of

sites/samples; it is found mostly in a single group and is present at the majority of the sites belonging to that group (Dufrêne and Legendre 1997). This asymmetrical approach is analyzed on the basis of a priori partition of areas and is based on an indicator value index (IndVal). The IndVal coefficient combines both the species relative abundance (specificity) with its relative frequency of occurrence (fidelity) in a defined group of areas. The statistical significance of the species indicator values was evaluated using a Monte Carlo test (permutation number = 1,000).

PERMANOVA, SIMPER and MDS were applied using the software Primer® 6 with add-on PERMANOVA+ (Plymouth Routines in Multivariate Ecological Researches). The two-way ANOVAs were calculated using the software STATISTICA 12. The IndVal values were calculated using the software PC-ORD 4.0. The level of significance was set at $P<0.05$ for all analyses. Confidence intervals of 95% (CI) were used to express the variation of the calculated means. Parametric statistical analysis followed Zar (1996).

3. Results

3.1 Serrambi seawater characterization

Previous studies (Jales et al. 2012; Monteiro et al. 2012) characterized Serrambi seawater as presenting temperature values varying between 25 to 33 ºC, pH between 8.1 to 8.8, salinity between 28 to 37, dissolved oxygen between 4.15 to 5.42mg/L, ammonium between undetected to 0.39µm L^{-1} or 0.001 to 0.097µg/L, nitrite between undetected to 0.13µm L^{-1} or 0.04 to 0.555µg/L, nitrate between 0.13 to 2.1µm L^{-1} or 0.003 to 0.920µg/L, phosphate between undetected to 0.15µm L^{-1} or 0.001 to 0.075 µg/L and silicate between 1.99 to 13.31µm L^{-1} or 5.172 to 25.767µg/L.

Seawater measurements taken in July and October 2013 showed that seawater temperature varied between 26.7 to 29.2 ºC, pH between 7.92 to 8.24, salinity between 36 to 37, dissolved oxygen between 5.2 to 6.9 mg/L and total alkalinity varies between 2102 to 2402 µmol kg^{-1}. The carbonate system parameters calculated for Serrambi beach seawater were pCO_2 (µatm) = 362.077, Ωca = 5.800 and Ωar = 3.842.

3.2 Experimental Conditions

Nominal pH and temperature for each scenario were satisfactory attained and maintained throughout the 29-day exposure period. Carbonate chemistry data (pCO_2, Ω_{ca} and Ω_{ar}) were calculated from data media (14 and 29 days) of pH, temperature and chemical salinity (Table 1).

Table 1 Seawater physical and chemical conditions maintained in the microcosm during the exposure period (values: mean ± standard deviation)

	Temp (ºC)	pH	Sal $_{(refrat)}$	Sal $_{(chem)}$ (Mean day)	DO (mg/l)	Total alkalinity (µmol kg^{-1})	pCO_2 (µatm)	Ω_{ca}	Ω_{ar}
07 November									
	27.5	8.00	37	39.2	6.3	2453.76 (62.24)	464.341	5.612	3.744
	Temp ºC (Mean 14 d)	pH (Mean 14 d)	Sal $_{(refrat)}$ (Mean 14 d)	Sal $_{(chem)}$ (Mean day)	DO (mg/l) (Mean 14 d)	Total alkalinity (µmol kg^{-1})	pCO_2	Ω_{ca}	Ω_{ar}
22 November									
Control	26.93 (0.03)	8.01 (0.01)	38.14 (0.36)	39.2	7.52 (0.11)	1725.44 (51.89)	311.246	3.867	2.575
Scenario I	27.63 (0.03)	7.93 (0.03)	38.28 (0.28)	38.3	7.43 (0.15)	2012.80 (96.27)	462.633	4.022	2.683
Scenario II	29.22 (0.03)	7.75 (0.07)	38.06 (0.19)	39.1	7.12 (0.11)	2389.76 (53.94)	899.856	3.630	2.436
Scenario III	30.34 (0.03)	7.38 (0.11)	38.10 (0.38)	39.2	6.95 (0.07)	3913.60 (173.45)	3839.622	2.926	1.971
	Temp ºC (Mean 29 d)	pH (Mean 29 d)	Sal $_{(refrat)}$ (Mean 29 d)	Sal $_{(chem)}$ (Mean day)	DO (mg/l) (Mean 29 d)	Total alkalinity (µmol kg^{-1})	pCO_2	Ω_{ca}	Ω_{ar}
06 December									
Control	26.98 (0.46)	7.97 (0.05)	37.94 (0.59)	36.0	7.6 (0.14)	1806.72 (50.03)	375.967	3.720	2.471
Scenario I	27.67 (0.13)	7.88 (0.06)	37.97 (0.53)	36.6	7.46 (0.14)	2031.36 (124.98)	543.124	3.666	2.442
Scenario II	29.22 (0.05)	7.71 (0.05)	37.88 (0.58)	37.1	7.21 (0.14)	2267.52 (42.46)	957.849	3.131	2.098
Scenario III	30.39 (0.13)	7.34 (0.08)	38.08 (0.70)	38.3	7.02 (0.10)	3905.92 (35.63)	4239.560	2.662	1.793

Temp = temperature, pH = pH measured, Sal $_{(refrat)}$ = salinity data taken with refractometer, Sal $_{(chem)}$ = salinity data taken from chemical technique, DO = dissolved oxygen, pCO_2 = partial pressure of carbon dioxide calculated, Ω_{ca} - calcite saturation state calculated (where 1 issaturation), Ω_{ar} - aragonite saturation state calculated. Mean 14 d = Mean value calculated from the 14 days taken data, Mean 29 d = Mean value calculated from the 29 days taken data.

3.3 Comparison between field and control samples

MDS ordination showed that the copepods community brought from field tended to change under laboratory experimental conditions, especially after 29 days (Fig 1).

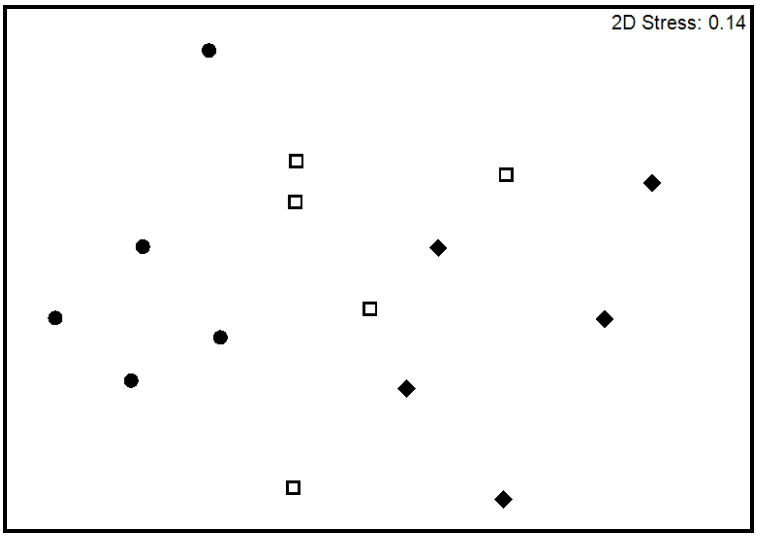

Fig. 1 Non-metric multi-dimensional scaling ordination plots for the Bray–Curtis similarity for the copepods community structure. ● (closed circles) Field, □ (open squares) Control 15 days, ♦ (closed diamond) Control 29 days

PERMANOVA comparisons among field and control samples collected after 15 and 29 days confirmed the pattern showed in MDS and indicated significant differences in the copepod community structure ($F_{(2;12)}$=2.28, p=0.003). A posteriori comparisons showed no significant differences between field and 15-days control samples (t=1.26, p=0.07) or between 15 and 29-days control samples (t=1.16, p=0.23). On the other hand, significant differences were found between field and 29-days control samples (t=1.98, p=0.009).

SIMPER analyses showed that the dissimilarities among field and control samples were greater after 29d; *Tisbe* sp was the species that contributed most to these dissimilarities (Table 2).

Table 2 Percent contribution (Contrib. %) of Cyclopoida and Harpacticoida species to average dissimilarity (Diss.) among field and control samples collected after 15 and 29 d (Cut off for low contributions: 70%)

Field *vs* Control 15 d		Field *vs* Control 29 d		Control 15 *vs* Control 29 d	
Diss.= 67.98	Contrib.%	Diss.= 80.27	Contrib.%	Diss.= 66.09	Contrib.%
Stenhelia sp	12.62	*Tisbe* sp	15.20	*Tisbe* sp	18.61
Mesochra sp	9.17	*Stenhelia* sp	11.79	*Dactylopodamphiascopsis* sp	7.70
Tisbe sp	8.22	*Mesochra* sp	8.36	*Ameira* sp	6.10
Ectinosoma sp1	7.86	*Ectinosoma* sp1	8.35	*Paradactylopodia* sp	5.95
Dactylopodamphiascopsis sp	6.21	*Dactylopusia* sp	5.30	*Ectinosoma* sp1	5.91
Amonardia sp	5.53	*Ameira* sp	4.99	*Dactylopusia* sp	5.66
Paradactylopodia sp	4.92	Cyclopoida	4.53	*Stenhelia* sp	5.49
Ameira sp	4.63	*Amonardia* sp	4.38	Cyclopoida	5.37
Dactylopusia sp	3.99	*Ectinosoma* sp2	3.93	*Amonardia* sp	5.29
Amphiascus (Pacificus) sp	3.64				
Robertsonia sp1	2.89				

3.4 Copepod community structure and species-specific response to climate change scenarios

A total of 2622 copepod individuals was analyzed, 61% of which were harpacticoids identified to the species level, 6.8% were adult cyclopoids, 31.7% were copepodites and 0.6% were broken animals which could not be determined to species. Among the harpacticoids, 14 families, 37 genera and 54 species were recorded (Table 3).

Table 3 List of Copepoda Harpacticoida species collected in the artificial substrate unit from Serrambi beach, northeastern Brazil

Order Harpacticoida Sars, 1903 Suborder Oligoarthra Lang, 1944	
Family Laophontidae T. Scott, 1905	**Family Dactylopusiidae Lang, 1936**
Echinolaophonte sp.	*Dactylopusia* sp.
Laophonte sp.	*Paradactylopodia* sp.
Laophontinae sp.1	*Diarthrodes* sp.
Laophontinae sp.2	**Family Ectinosomatidae Sars, 1903**
Laophontinae sp.3	*Bradyellopsis* sp.1
Paralaophonte sp.	*Bradyellopsis* sp.2
Family Miraciidae Dana, 1846	*Ectinosoma* sp.1
Amonardia sp.	*Ectinosoma* sp.2
Amphiascoides sp.1	*Ectinosoma* sp.3
Amphiascoides sp.2	*Ectinosoma* sp.4
Amphiascopsis sp.	*Halophytophilus* sp.1
Amphiascus (Minutus) sp.	*Halophytophilus* sp.2
Amphiascus (Pacificus) sp.	*Pseudobradya* sp.
Amphiascus (Varians) sp.	*Sigmatidium* sp.
Dactylopodamphiascopsis sp.	**Family Longipediidae Boeck, 1865**
Delavalia sp.	*Longipedia* sp.
Diosaccus sp.	**Family Normanellidae Lang, 1944**
Haloschizopera sp.	*Normanella* sp.
Melima sp.1	**Family Harpacticidae Dana, 1846**
Melima sp.2	*Harpacticus* sp.
Melima sp.3	**Family Pseudotachidiidae Lang, 1936**
Melima sp.4	*Idomene* sp.
Paramphiascella sp.	**Family Peltidiidae Claus, 1860**
Pseudamphiascopsis sp.1	*Eupelte* sp.
Robertgurneya sp.	**Family Tisbidae Stebbing, 1910**
Robertsonia sp.1	*Tisbe* sp.
Robertsonia sp.2	
Stenhelia sp.	**Family Orthopsyllidae Huys, 1990**
Family Ameiridae Boeck, 1865	*Orthopsyllus* sp.
Ameira sp.	**Family Cletodidae T. Scott, 1905**
Sarsameira sp.1	*Cletodes* sp.
Sarsameira sp.2	
Family Canthocamptidae Brady, 1880	Harpacticoida sp.
Mesochra sp.	
Nannomesochra sp.	

Ectinosoma sp1 (21.72%), adult cyclopoid (9.41%), *Tisbe* sp (7.45%), *Stenhelia* sp (5.56%), *Paramphiascella* sp (5.07%), *Ectinosoma* sp2 (4.81%), *Dactylopusia* sp (4.66%), *Ameira* sp (4.56%), *Mesocha* sp (3.86%), *Paradactylopodia* sp (3.74%), *Dactylopodamphiascopsis* sp (3.14%), *Amonardia* sp (3%) and *Amphiascoides* sp1 (2.65%) accounted for 79.63% of total. In the field samples, *Stenhelia* sp (17.67%), *Ectinosoma* sp1 (15.03%), *Mesochra* sp (12.25%), *Dactylopusia* sp (7.14%), *Amonardia* sp (6.11%), *Paradactylopodia* sp (4.93%), *Amphiascus* (Pacificus) sp (4.78), *Ameira* sp (4.46%), *Paramphiascella* sp (3.59%), *Robertsonia* sp1 (2.43%), adult Cyclopoida (2.05%) and *Melima* sp1 (2.03%) accounted for 82.48%.

For samples collected after 15 days, *Ectinosoma* sp1 (19.85%), adult cyclopoid (9.26%), *Paramphiascella* sp (6.45%), *Dactylopusia* sp (6.40%), *Dactylopodamphiascopsis* sp (5.73%), *Tisbe* sp (5.13%), *Paradactylopodia* sp (5.01%), *Ectinosoma* sp2 (4.39%), *Amonardia* sp (3.57%), *Ameira* sp (3.54%), *Robertgurneya* sp (3.37%), *Amphiascoides* sp1 (3%), *Stenhelia* sp (2.86%), *Mesocha* sp (2.84%), and *Paralaophonte* sp (2.15%) accounted for 83.55%.

For the samples collected after 29 days, *Ectinosoma* sp1 (28.13%), *Tisbe* sp (14.19%), adult cyclopoid (13.91%), *Ectinosoma* sp2 (8.17%), *Ameira* sp (5.98%), *Paramphiascella* sp (4.13%), *Amphiascoides* sp1 (2.87%), Haloschizopera sp (2.59%) and *Stenhelia* sp (2.06%), accounted for 82.04%.

MDS ordination analyses indicated marked differences in the structure of copepod community among Control and Scenarios samples. Important differences were also observed between the two sampling moments (Fig. 2).

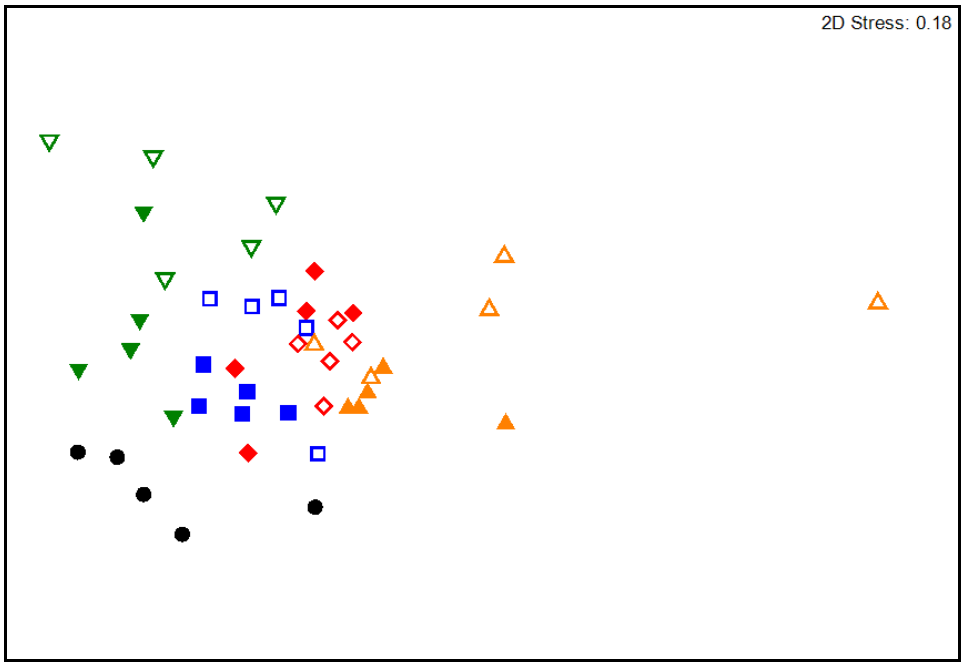

Fig. 2 MDS ordination plots for the Bray–Curtis similarity for Copepod community structure. ● (circle) Field samples, ▼ (inverted triangle) Control, ■ (square) Scenario I, ♦ (diamond) Scenario II, ▲ (triangle) Scenario III. Closed symbols represent samples collected after 15 d, and open symbols after 29 d

The pattern illustrated in the MDS ordination was confirmed by PERMANOVA. Significant differences in the structure of the copepod community were detected among scenarios, between the two sampling moments, and also for the interaction between the two factors (Table 4).

Table 4 PERMANOVA results for the Copepod community exposed to different climate change scenarios and collected after 15 and 29 days. Significant values are highlighted in bold

Source	*df*	MS	*F*	*P*
Time (Ti)	1	5259.3	**3.44**	0.0003
Scenario (Sc)	3	8123.9	**5.31**	0.0001
Ti x Sc	3	2443	**1.59**	0.0237
Residual	32	1530.7		

The response of copepod community structure to scenarios varies according to the sampling moment. After 15 days of exposition, differences between Control and Scenario I, and between Scenario I and Scenario II were not observed. However, after 29 days, differences were observed among all treatments (Table 5).

Table 5 Pair-wise *a posteriori* comparisons among climate change scenarios for samples collected after 15 and 29 d

Comparisons	Day 15		Day 29	
	t	*P*	*t*	*P*
Control *vs* Scenario I	1.32	0.053	**1.93**	0.008
Control *vs* Scenario II	**1.76**	0.008	**2.45**	0.008
Control *vs* Scenario III	**2.20**	0.008	**1.86**	0.007
Scenario I *vs* Scenario II	1.29	0.070	**1.65**	0.024
Scenario I *vs* Scenario III	**2.02**	0.007	**1.99**	0.008
Scenario II *vs* Scenario III	**1.98**	0.009	**1.60**	0.025

SIMPER analyses showed that dissimilarities were greater between Control and Scenarios II and III for both sampling moments. *Tisbe* sp was the species that contributed most to these dissimilarities. The importance of this species was even sharper after 29 days (Table 6).

Table 6 Percent contribution (Contrib. %) of Cyclopoida and Harpacticoida species to average dissimilarity (Diss.) among different climate change scenarios for samples collected after 15 and 29 d (Cut off for low contributions: 70%)

Day 15					
Control *vs* Scenario I		Control *vs* Scenario II		Control *vs* Scenario III	
Diss.= 63.34	Contrib.%	Diss.= 71.79	Contrib.%	Diss.= 74.27	Contrib.%
Tisbe sp	9.13	*Tisbe* sp	10.72	*Tisbe* sp	13.31
Dactylopodamphiascopsis sp	7.77	Cyclopoida	8.60	*Dactylopodamphiascopsis* sp	9.09
Ectinosoma sp1	6.83	*Ectinosoma* sp1	8.53	*Ameira* sp	7.67
Cyclopoida	5.99	*Dactylopodamphiascopsis* sp	7.34	*Paradactylopodia* sp	7.17
Paramphiascella sp	5.92	*Paradactylopodia* sp	5.83	*Stenhelia* sp	6.95
Amonardia sp	5.72	*Ameira* sp	5.78	*Dactylopusia* sp	6.89
Stenhelia sp	5.43	*Stenhelia* sp	5.60	*Amonardia* sp	6.38
Paradactylopodia sp	5.19	*Amonardia* sp	5.09	*Paramphiascella* sp	5.61
Dactylopusia sp	5.17	*Dactylopusia* sp	4.87	*Ectinosoma* sp1	5.23
Ectinosoma sp2	4.62	*Ectinosoma* sp2	3.53		
Robertgurneya sp	4.13	*Robertgurneya* sp	2.76		
Ameira sp	4.04				
Day 29					
Control *vs* Scenario I		Control *vs* Scenario II		Control *vs* Scenario III	
Diss.= 67.91	Contrib.%	Diss.= 71.58	Contrib.%	Diss.= 81.69	Contrib.%
Ectinosoma sp1	22.65	*Tisbe* sp	25.59	*Tisbe* sp	27.16
Tisbe sp	22.06	*Ectinosoma* sp1	18.52	Cyclopoida	9.43
Cyclopoida	8.73	*Ameira* sp	8.69	*Ameira* sp	9.4
Ameira sp	6.75	*Stenhelia* sp	5.58	*Ectinosoma* sp1	8.81
Ectinosoma sp2	5.03	Cyclopoida	5.43	*Stenhelia* sp	6.53
Haloschizopera sp	4.66	*Haloschizopera* sp	5.16	*Haloschizopera* sp	5.52

ANOVA results for species richness, evenness and diversity showed significant differences for the factor Time ($p<0.03$ for all), but not for factor Scenario or for interaction between the two factors (Fig. 3).

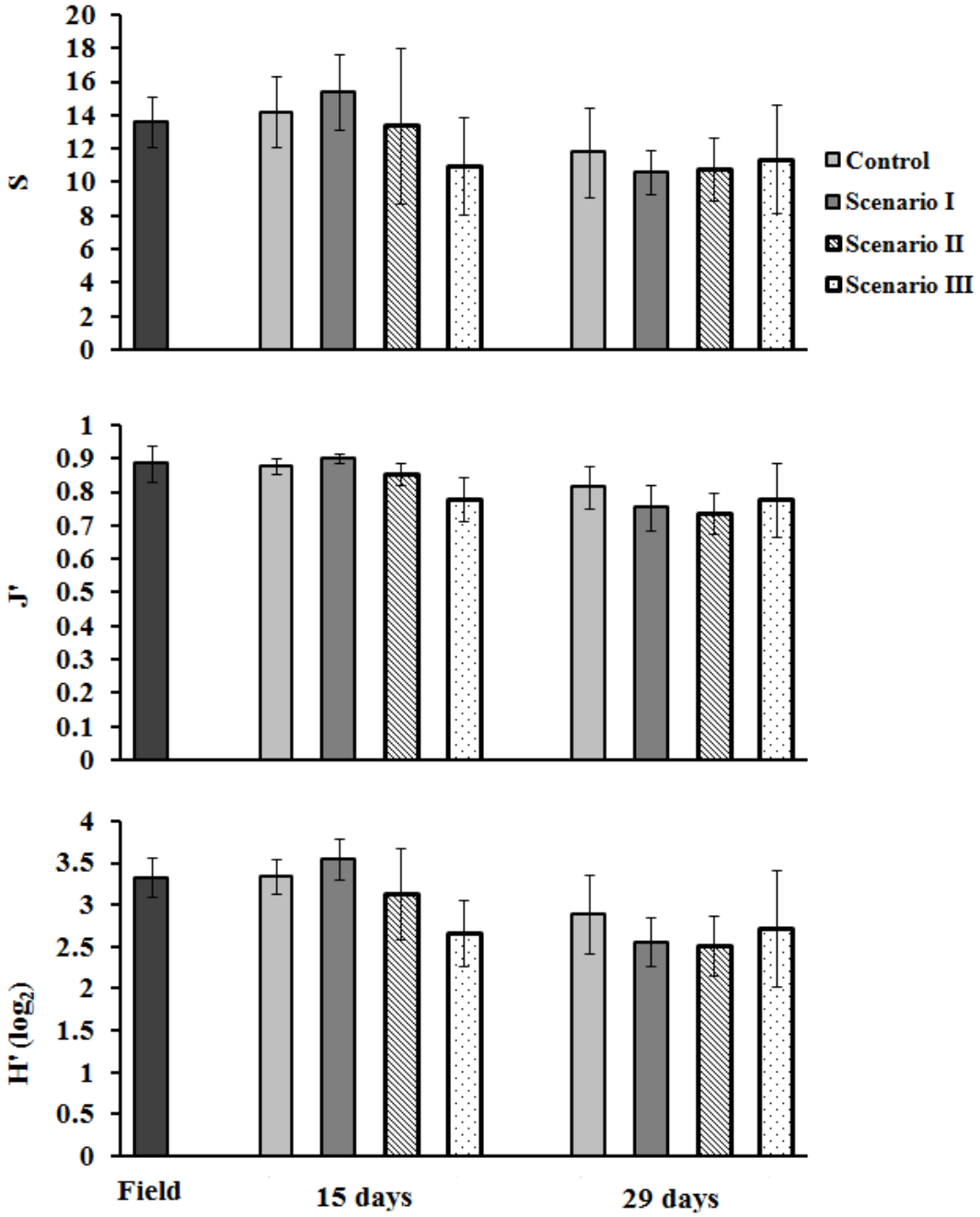

Fig. 3 Species richness (S), Pielou's evenness (J') and Shannon diversity (H'$\log_2$) for copepod community at different climate change scenarios and sampling times (15 and 29 d). Values: mean ±95 % confidence intervals

ANOVA results for some copepod population parameters showed no differences for female/male ratio ($p>0.2$ for all factors). Copepodite ratio showed significant differences for factor Scenario ($F_{(3,32)}= 3.06$; $p=0.04$), but not for interaction between factors nor for factor Time ($p>0.7$ for both). The *a posteriori* Fisher test indicated that copepodite ratio was higher at Scenario III when compared to control ($p=0.025$), Scenario I ($p=0.029$) and Scenario II ($p=0.011$). The same pattern was observed for Nauplii ratio and significant differences were observed only for the factor Scenario ($F_{(3,32)}= 7.02$; $p<0.01$). Nauplii ratio was higher at Scenario III when compared to control ($p<0.001$), Scenario I ($p<0.01$) and Scenario II ($p<0.01$). ANOVA results for the density of copepodites showed significant difference for factor Scenario ($F_{(3,32)}= 9.0$; $p<0.001$) and for interaction between factor Scenario and Time ($F_{(3,32)}= 2.94$; $p=0.048$). The *a posteriori* Fisher test indicated that there was no differences in the density of copepodites between Scenarios and Control samples ($p>0.06$ for all comparisons) for samples collected after 15 days. Although, for samples collected after 29 days, the density of copepodites was lower at Scenario II ($p<0.01$) and III ($p<0.001$) when compared to control, while no differences were found between Scenario I and Control ($p=0.14$). The density of copepodites was higher in Control samples collected after 29 days than after 15 days ($p=0.04$). The percentage of ovigerous female showed no differences for both factors or for interaction between then ($p>0.27$ for all factors). Malformed animals ratio showed significant differences only for factor Scenario ($F_{(3,32)}= 4.96$; $p<0.01$). Fisher test indicated a significant increase of malformed animals in Scenario III when compared to Control ($p<0.001$), Scenario I ($p=0.012$) and Scenario II ($p=0.032$) (Fig.4).

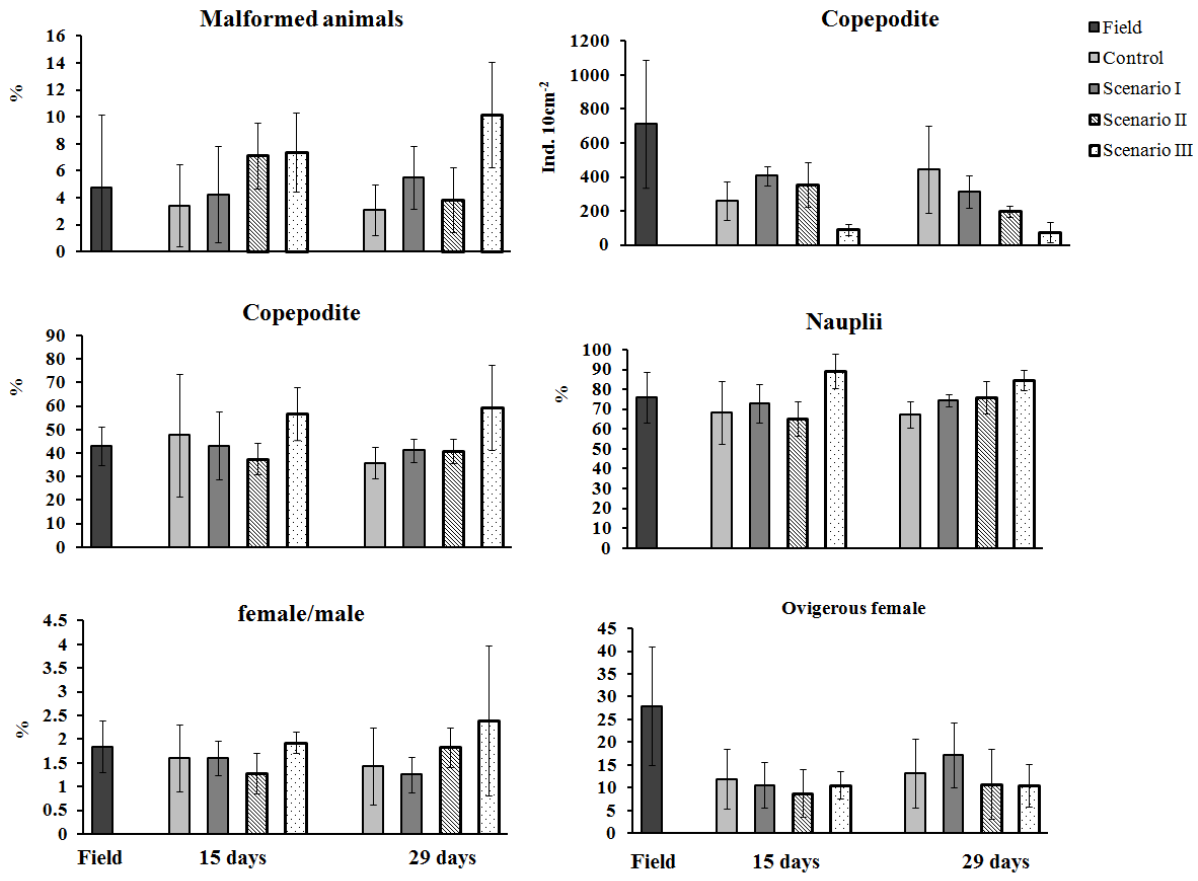

Fig. 4 Mean (±95 % confidence intervals) of malformed animals ratio, density of copepodites, Copepodite and Nauplii ratios, ovigerous female and female/male ratios at different climate change scenarios and sampling times (15 and 29 d)

The different species composing this coral reef copepod community presented different sensibilities/responses to the different levels of seawater warming and acidification (Fig. 5). The two-way ANOVA results (Table 7) indicated that *Ectinosoma* sp2 and *Mesochra* sp densities showed significant interaction between the factors Time and Scenario. Cyclopoida and the species *Ectinosoma* sp1, *Tisbe* sp, *Stenhelia* sp, *Paramphiascella* sp and *Ameira* sp were sensitive to the factor Scenario while *Paradactylopodia* sp and *Amonardia* sp were sensitive only for the factor Time. *Dactylopusia* sp was sensitive to both factors, but not for the interaction.

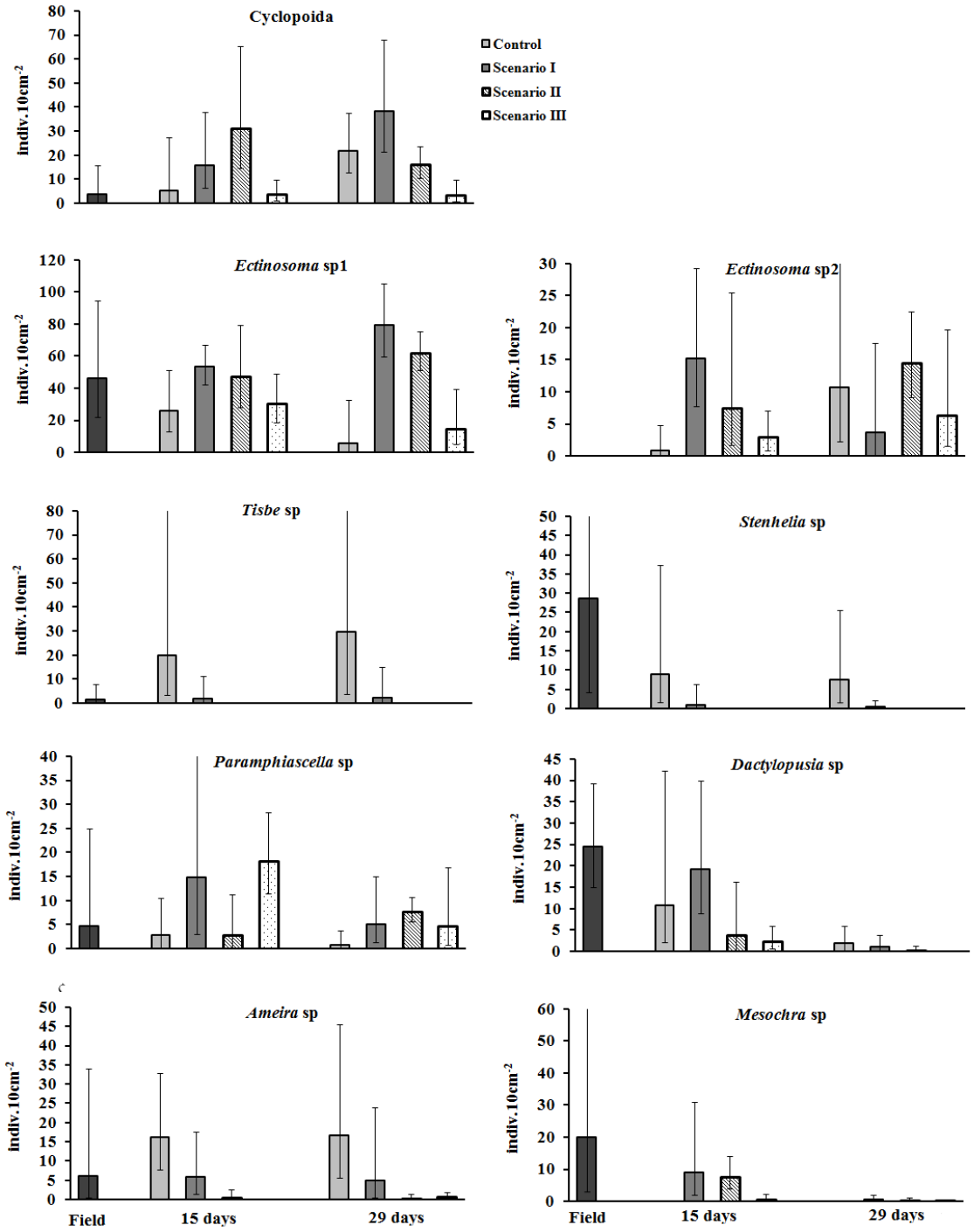

Fig. 5 Mean density (±95 % confidence intervals) of Cyclopoida and of the main Harpacticoida species at different climate change scenarios and sampling times (15 and 29 d)

Table 7 Two-way ANOVA results for Cyclopoida and the main harpacticoid species densities in response to different climate change scenarios and sampling times (15 and 29 d). Significant ($p<0.05$) *F* values in bold

Source	*df*	*MS*	*F*	*p*	*MS*	*F*	*p*
		Cyclopoida			*Ectinosoma* sp1		
Time (Ti)	1	1.31	1.43	0.24	1.22	1.68	0.20
Scenario (Sc)	3	6.33	**6.93**	<0.01	5.61	**7.72**	<0.01
Ti x Sc	3	1.88	2.06	0.13	1.71	2.35	0.09
Residual	32	0.91			0.73		
		Amphiascoides sp1			*Tisbe* sp		
Time (Ti)	1	1.07	0.87	0.36	0.15	0.09	0.76
Scenario (Sc)	3	1.55	1.26	0.30	23.25	**14.21**	<0.01
Ti x Sc	3	1.06	0.86	0.47	0.08	0.05	0.99
Residual	32	1.23			1.64		
		Stenhelia sp			*Paramphiascella* sp		
Time (Ti)	1	0.13	0.14	0.71	3.00	2.31	0.14
Scenario (Sc)	3	11.01	**12.47**	<0.01	4.24	**3.27**	0.03
Ti x Sc	3	0.05	0.06	0.98	2.11	1.62	0.20
Residual	32	0.88			1.30		
		Dactylopusia sp			*Ameira* sp		
Time (Ti)	1	23.85	**23.52**	<0.01	0.03	0.04	0.85
Scenario (Sc)	3	3.85	**3.80**	0.02	15.80	**16.63**	<0.01
Ti x Sc	3	0.65	0.64	0.60	0.22	0.23	0.88
Residual	32	1.01			0.95		
		Ectinosoma sp2			*Paradactylopodia* sp		
Time (Ti)	1	2.11	1.56	0.22	8.73	**5.99**	0.02
Scenario (Sc)	3	1.79	1.32	0.28	1.27	0.87	0.47
Ti x Sc	3	4.10	**3.03**	0.04	1.71	1.17	0.34
Residual	32	1.35			1.46		
		Dactylopodamphiascopsis sp			*Amonardia* sp		
Time (Ti)	1	6.69	3.63	0.07	5.85	**4.50**	0.04
Scenario (Sc)	3	1.45	0.78	0.51	1.22	0.94	0.43
Ti x Sc	3	1.70	0.92	0.44	0.14	0.11	0.95
Residual	32	1.84			1.30		
		Mesochra sp					
Time (Ti)	1	10.82	**21.72**	<0.01			
Scenario (Sc)	3	4.28	**8.58**	<0.01			
Ti x Sc	3	2.58	**5.19**	<0.01			
Residual	32	0.50					

The results of the *a posteriori* test (Fisher LSD) indicated that Cyclopoida density was lower in Scenario III when compared to Control ($p=0.03$), Scenario I ($p<0.001$) and II ($p<0.001$). No differences were observed between Scenario I ($p=0.086$)

nor Scenario II (p=0.13) when compared to control. The density of *Ectinosoma* sp1 significantly increased in Scenario I (p<0.001) and in Scenario II (p<0.001) when compared to Controls. On the other hand, no differences were observed between Control and Scenario III samples (p=0.2). *Tisbe* sp was very sensitive to Scenarios, and was significantly reduced in Scenario I (p=0.001), Scenario II (p<0.001) and Scenario III (p<0.001) in comparison to control samples. *Stenhelia* sp presented a similar pattern and was also significantly reduced in Scenario I (p<0.001), Scenario II (p<0.001) and Scenario III (p<0.001) in comparison to Control. *Ameira* sp also showed great sensibility to Scenarios and was significantly reduced in Scenario I (p=0.03), Scenario II (p<0.001) and Scenario III (p<0.001). On the other hand, the density of *Paramphiascella* sp increased significantly in Scenario I (p=0.01) and Scenario III (p=0.01) in comparison to Control. *Dactylopusia* sp was significantly reduced only in Scenario III (p=0.14) in comparison to control.

Results for significant interactions between factors indicated that for samples collected after 15 days, the density of *Ectinosoma* sp2 significantly increased in Scenario I (p=0.005) and Scenario II (p=0.044) in comparison to Controls, while no differences were observed between Control and Scenario III (p=0.3). Similarly, *Mesochra* sp density increased in Scenario I (p<0.001) and Scenario II (p<0.001), while no differences were observed between Control and Scenario III (p=0.25). After 29 days of exposition, no differences were observed among Control and Scenarios for both species *Ectinosoma* sp2 and *Mesochra* sp (p>0.05 for all comparisons).

3.5 Indicator species

Samples collected after 15 days were represented by four significant indicator (characteristic) species: *Amonardia* sp (IndVal=36.7%; p=0.03), *Amphiascoides* sp2

(IndVal=31.9%; p=0.01), *Dactylopusia* sp (IndVal=72.5%; p=0.002) and *Mesochra* sp (IndVal=51%; p=0.005). The species *Bradyellopsis* sp2 (IndVal=40%; p=0.002) and Laophontinae sp1 (IndVal=29.27%; p=0.037) were significant indicators of samples collected after 29 days. Among the scenarios, only Control and Scenario I were characterized by significant indicator species. *Ameira* sp (IndVal=66.8%; p=0.001), *Robertsonia* sp1 (IndVal=33.3%; p=0.03), *Tisbe* sp (IndVal=69.4%; p=0.001) and *Stenhelia* sp (IndVal=66.2%; p=0.001) were significant indicators of Control samples, while *Ectinosoma* sp1 (IndVal=38.5%; p=0.002) and Cyclopoida (IndVal=39.5%; p=0.04) were significant indicators of Scenario I.

Samples of Scenario I collected after 15 days were represented by *Dactylopusia* sp (IndVal=40.9%; p=0.02). For samples collected after 29 days, *Ameira* sp (IndVal=37.3%; p=0.03) was indicator of Control, *Ectinosoma* sp1 (IndVal=23.1%; p=0.011) was indicator of Scenario I and *Bradyellopsi*s sp2 (IndVal=38.6%; p=0.025) was indicator of Scenario II.

4. Discussion

The differences observed among copepod communities from field and control samples kept in the microcosm were even smaller than those showed by the meiofauna at major taxonomic groups (Sarmento et al. 2016b). Moreover, those differences were due the increases in the density of the species *Tisbe* sp in control samples over time.

The use of artificial substrate units (ASUs) has been used to overcome problems related to variability in community structure and diversity caused by habitat heterogeneity that can, often, complicate experiments on natural communities (Underwood and Chapman 1996), and hamper efforts to separate the changes caused by anthropogenic disturbance from those arising from natural variations (Bishop 2005).

Therefore, ASUs have been widely applied in recent studies to assess the effects of antropogenic impacts such as climate change on benthic communities (Cigliano et al. 2010; Hale et al. 2011; Christen et al. 2013; Sarmento et al. 2016a, 2016b). In addition to allow the collection of a standardized community (Mirto and Danovaro 2004; Bishop 2005; Gobin and Warwick 2006), ASUs have proved to be an effective method to represent natural communities (Mirto and Danovaro 2004; De Troch et al. 2005; Sarmento et al. 2016a). In the present study, a highly diverse community colonized ASUs. The community brought to microcosm system was composed of fifty-four species and a global $H'_{(log2)}$= 4.37 were registered. Thus, this copepod community presents the characteristic high diversity of communities found in natural phytal environments (Sarmento et al. 2012).

In the present study the response of a benthic copepod community from coral reefs to different climate change scenarios were assessed. A general sensibility to simultaneously warming and ocean acidification was observed. Similarly to the results found for meiofauna community, analyzed at major taxonomic groups level from the same experiment (Sarmento et al. 2016b), it was observed a clear separation among control samples and scenarios. After 15 d, copepod community was affected in Scenario I and II and after 29 d, all scenarios were different from control.

Copepods at major taxonomic group showed high sensibility to scenarios (Sarmento et al. 2016b). However, at species level, a complex pattern of response was perceived. The combined ANOVA and Indicator Species Analysis approach allowed the detection of many species that respond to future climate change scenarios, of which, some respond negatively while others positively. The species *Tisbe* sp, *Stenhelia* sp and *Ameira* sp showed to be the most sensitive, exhibiting severe reductions in their densities in Scenarios I, II and III. *Robertsonia* sp2 was representative of Control

samples in general. Cyclopoida and *Dactylopusia* sp were negatively affected in Scenario III, but had their densities increased in Scenario I. In contrast, *Ectinosoma* sp1, *Ectinosoma* sp2 and *Mesochra* sp had their densities improved at Scenario I and II with no differences between Control and Scenario III samples. *Paramphiascella* sp, in turn, had it density improved in Scenario I and III, and *Bradyelopsis* sp was representative of Scenario II after 29d.

The temperature influence in population dynamics of different species of copepods (calanoids and harpacticoids) has been assessed since the 70s both in laboratory as well as in field studies (e.g. Landry 1975; Gaudy and Guerin 1982; Moreira et al. 1985; Matias-Peralta et al. 2005; Santos et al. 1999; Santos et al. 2003; Chertoprud and Azovsky 2006). Data from laboratory experiments showed that, in general, reproductive and population parameters are negatively affected by temperature after reaching an optimum level. For *Tisbe holofhuriae*, it was observed that the larval development time, longevity of adults and offspring reaching adulthood were inversely related to temperature. However, with increasing temperature the number of *T. holothuriae* egg sacs produced increased substantially (Gaudy and Guerin 1982). For *Pseudocalanus minutus* the mean length of adult females was inversely related to temperature (Lock and MacLaren 1970) and for *Nitocra affinis f. californica* development time was shortest at above the determined optimum temperature level (Matias-Peralta et al. 2005). Considering the reproductive development times of *Tigriopus brevicornis*, fastest ovary development was observed in the highest temperature, but the highest number of nauplii produced was in the mid temperature (McAllen and Brennan 2009). For *Pseudodiaptomus pelagicus* results indicate that survival from early nauplii to adult and, the number of nauplii were significantly affected by increasing temperature (Rhyne et al. 2009). Metabolic as well as

reproductive activity of *Itunella muelleri* was reduced under high temperatures (Steinarsdóttir and Ingólfsson 2011). In a study on the reproduction of *Tisbe battagliai* it was found that there was an inverse relationship between female lifespan and temperature, with females reared at highest temperature living approximately twice as less. Increases in temperature and food concentration resulted in a reduction in the time interval between hatching of successive broods, but there was no clear trend of temperature on brood size (Willian and Jones 1999).

Studies on the impact of ocean acidification on copepods have increased since the last decade, but the majority of studies were conducted on planktonic calanoid species. Studies with different copepod species found that when animals are exposed to increased seawater acidity alone, the physiological performance, mating behavior, adult survival, body size and growth were not affected (Kurihara et al. 2004; Mayor et al. 2007; Kurihara and Ishimatsu 2008; Pascal et al. 2010; Fitzer et al. 2012a; Isari et al. 2015). However, large decreases in egg and naupliar production were found (Kurihara et al. 2004; Mayor et al. 2007; Fitzer et al. 2012b, 2013).

Studies that evaluate the simultaneous impact of ocean acidification and warming are scarce, and again mainly focused on planktonic calanoid species. Those studies indicated that, while no clear response to ocean acidification alone is observed, it may synergistically act with ocean warming to impact the copepods. Zervoudaki et al. (2013) found that, while ocean acidification had no discernible effect, the combined effect of low pH and raised temperature on *Acartia clause* significantly decreased egg production rate and hatching success. Moreover, temperature appeared to have a positive effect on respiration and excretion. Acidification had no clear effect on respiration, but a negative effect on excretion was observed. Acidification and warming resulted in an increase of the excretion rate and the increase was higher than that

observed by warming only. Vehmaa et al. (2013) found that acidification did not have any significant direct effects on either oxidative status variables or reproductive output variables on *Acartia bifilosa*. However, acidification together with higher temperature reduced copepod antioxidant capacity. Higher temperature also decreased egg viability, nauplii development, and oxidative status. Li et al. (2015) demonstrate that the harpacticoid *Tigriopus japonicus* responds more sensitively to heat shocks rather than to seawater acidification. And, that high pCO_2 concentration and heat shock did not induce any mortality, though respiration increased before being depressed at high temperature. Hildebrant et al. (2014) found that respiration rates, body mass and mortality in *Calanus glacialis* and *Calanus hyperboreus* did not change in different pCO_2. But when incubated at higher temperatures they were induced to sublthel stress.

Despite the increasing body of literature on the impact of ocean acidification and/or warming on copepods, they are all conducted on single species. The present study exhibit, for the first time, the response of copepod species in the context of a community. Studies conducted on population- and community-level processes suggest that global climatic changes impacts on individual organisms do not necessarily translate directly into changes in distribution and abundance (Harley et al. 2006). Thus, experiments on community level can be more informative than single species ones, revealing complex changes in ecological and biological interactions. A precautionary approach may be required when interpreting predictions from single species studies since many of the most striking consequences of climate change will arise through altered species interactions (e.g. Fabricius et al. 2011; Kroeker et al. 2013; Gaylord et al. 2015).

It has been shown that different species, even those taxonomically close, can display different pattern of response to ocean acidification or warming (Pörter 2008;

Byrne 2011; Wood et al. 2011; Kroeker et al. 2013). For instance, in Pascal et al. (2010), two harpacticoid species from the same family showed different sensibility to ocean acidity. They suggested that this response could be associated to the fact that the two copepod species are associated with different environments and thought, copepods living in environments more prone to hypercapnia, such as mudflats where *Shizopera knabeni* lives, may be less sensitive to future acidification than *Amphiascoides stopus* found on large sand grains beaches. However, in the present study all species came from the same community and were subjected to similar selective pressures regarding seawater physics and chemistry.

Even though ocean acidification is expected to reduce biodiversity (Widdicombe and Spicer 2008), some species may benefit from these new environmental conditions (Dupont and Thorndyke 2009). In the present study, it is likely that some species showed differential specie-specific and time-dependent sensibility (McConville et al. 2013) via physiological pathways that would couple with stress associated to acidity, hypercapnia and/or elevated temperatures. Furthermore, some studies showed evidence of alleviation of ocean acidification effects as a result of transgenerational effects suggesting that copepods may have adaptive potential to withstand the direct long-term effects of even pessimistic future ocean acidification (Pedersen et al. 2014; Thor and Dupont 2015).

In addition to the differences in the direct sensibility to ocean acidification and/or elevated temperature, it is very likely that, together with the reduction of susceptible species, changes in primary producer communities in response to ocean acidification (e.g. Porzio et al. 2011; Johnson et al. 2013; Webster et al. 2013; Witt et al. 2011) and temperature may have consequences for the maintenance and development of benthic copepods that depend on them. It is well know that harpacticoid species are able

to develop and reproduce while feeding on different diatoms, but that some algal species are more suitable; so although the copepods can survive, the ingestion of some diatoms or bacteria can drastically impact their development and reproductive success (Araújo-Castro and Souza-Santos 2005; Wyckmans et al. 2007; Dahl et al. 2009).

Therefore, we can suggest that species like *Ectinosoma* sp1, *Ectinosoma* sp2 and *Mesochra* sp, display some resistance to ocean acidification and warming in addition to food flexibility. These species could be benefited since the ecological pressures as competition were ameliorated or suppressed due to damage of those species that had been negatively affected. Those finds highlight the importance of studies conducted at community level.

Indicator taxa can be evaluated for groups of samples defined by environmental parameters or intensity of degradation. Since Dufrêne and Legendre (1997) introduced this new and flexible asymmetrical approach to identify indicator species, the IndVal method has been widely used in ecological studies (Punti et al. 2009; Kubosova et al. 2010). Despite the ecological advantages of this method for conservation studies of marine environments, as highlighted by Mouillot et al. (2002), this approach has only been applied recently to evaluate human impact on marine meiofauna (Sarmento and Santos 2012). In the present study, the IndVal approach allowed the detection of indicator species for Scenarios and Time samples as a whole, or for only one specific Scenario collected after 15 or 29d, even when these species were rather scarce. Thereby, IndVal showed to be an important tool to assist future studies of environmental stress assessments as climate change.

No significant differences were observed for the ecological indexes species richness, evenness and diversity. It is likely that the period of exposure wasn't enough to generate modifications in these ecological parameters among scenarios. Besides, the

antagonist patterns of response of the dominant species could act as a compensatory mechanism.

Copepods as major taxonomic groups showed to be very sensitive to scenarios: the densities of copepods were reduced, on average, by 24.9, 61.63, and 83.85 % in Scenario I, II and III, respectively, compared with the control after 29 d (Sarmento et al. 2016b). Copepodite density showed similar pattern of sensibility to scenarios, with decreases, on average, by 29, 55.35, and 83.07 % in Scenario I, II and III, respectively, compared with the control after 29 d. However, nauplii stage showed even higher sensibility to scenarios, with reductions, on average, by 60.44, 74.41, and 95.83 % in Scenario I, II and III, respectively, compared with the control after 29 d (Sarmento et al. 2016b). These results are in accordance with previous studies that showed that the different developmental stages can be affected differently to factors such temperature, where nauplii stage is predicted to suffer higher mortality as compared to copepodite stages or adults (Santos et al. 2003; Rhyne et al. 2009).

The positive increase of malformed adult animals with the increase level of warming and ocean acidification is presented for the first time for copepods. This kind of approach has been conducted only in large representatives of macrobenthic species at early development stages. For those animals, an increase in abnormal development in larval and juveniles stages of some coral, molluscs and echinoderms has been observed with increases in ocean acidification and warming (Byrne 2011). Since the time elapsed between nauplii and the last copepodite phase can be very short for most of harpacticoid species (Giere 2009), the evaluation of abnormality at these stages would be a very difficult task, and for studies at community level almost impossible. Though, to assess the presence of malformed appendices in adults in the microscope do not increase the time spent during identification. The analysis of this parameter suggested that, species

that apparently can couple with the stress associated to ocean acidification and warming and entering the adult stage are not free from the sublethal symptoms that could have even worse consequences for population in long periods of exposition.

In this study, the response of harpacticoid species to ocean acidification and warming impact is presented for the first time in the community context, highlighting the importance of biological interactions. Our results, together with those presented in the recent literature, emphasize that mobile crustaceans such as copepods might not be as tolerant to acidification as previously suggested (Whiteley 2011; Kroeker et al. 2013), once that, in combination with increasing temperatures they present high sensibility to ocean acidification.

CAPÍTULO 4

Effects of elevated co_2 and temperature on an intertidal harpacticoid copepod community

1. Introduction

Increasing atmospheric carbon dioxide (CO_2) is altering the levels of co-occurring stressors, resulting in increasing sea surface temperatures and pCO_2, as well as decreasing the oceans' pH and its level of saturation of carbonate minerals (Doney et al. 2009a; Feely et al. 2009). Since the beginning of the industrial revolution in the mid-eighteenth century, the release of carbon dioxide from humankind's combined industrial and agricultural activities has resulted in an increase by nearly 40% in atmospheric CO_2 concentrations from approximately 280 to 387 parts per million (ppm) (Feely et al. 2004, 2009). Earth's atmospheric present levels of CO_2 are higher than anytime in at least the last 800,000 years (Lüthi et al. 2008), and it is expected to continue to rise at an accelerating rate, leading to significant temperature increases in the atmosphere and the surface ocean in the coming decades (Feely et al. 2009). Globally averaged combined land and ocean surface temperature data show a warming of 0.85 [0.65 to 1.06] °C over the period 1880 to 2012.

The oceans cover 70% of the earth's surface. Due to their large volume and the ability of seawater to buffer CO_2, oceans have absorbed nearly a third of anthropogenic carbon added to the atmosphere what attenuated its effects (Sabine et al. 2004). Though, since the beginning of the industrial era, oceanic uptake of CO_2 has resulted in changes in seawater carbonate chemistry, the process known as 'ocean acidification' and the pH of ocean surface water has decreased by 0.1 units, corresponding to a 26% increase in acidity (IPCC 2014).

The rise in greenhouse gas atmospheric concentrations is predicted to continue, with estimates for the year 2100 ranging from 475 to 1313 ppm. As a result of this, the global ocean will continue to warm during the 21st century. Best estimates of ocean warming in the top one hundred meters are about 0.6 °C to 2.0 °C by the end of the 21st century (IPCC 2013). However, an additional warming of global mean surface temperatures is forecasted to attain 2.6ºC to 4.8ºC by the end of 21st century (IPCC 2014). As a consequence of ocean CO_2 intake, an additional drop in ocean pH of 0.3 units by 2100 (Caldeira and Wickett 2003; Feely et al. 2004) and 0.7 units by 2250 is predicted (Caldeira and Wickett 2003).

Political, social and environmental pressures to reduce CO_2 emissions have led several governments to seek new options of mitigation actions for stabilization of atmospheric greenhouse gas CO_2. Due to this high storage capacity, the ocean at first appeared to be a suitable place for the disposal of CO_2, via diffusive entry or via industrial scale of carbon capture and storage (CCS). The term CCS represents a number of methods by which anthropogenically-generated CO_2 is collected at source and released into mesopelagic waters, seafloor depressions or geological sub-surface formations (IPCC 2005). While it is assumed that storage sites would be selected to minimize the potential for leakage, subsurface storage leaks are possible over time (Hawkins 2004). Despite this, little is known about long-term issues which may arise from underground storage of CO_2.

Temperature and pH are among the most important environmental factors controlling the distribution, physiological performance, morphology and behavior of marine invertebrates (Pörtner et al. 2004, 2008; Widdicombe and Spicer 2008; Doney et al. 2009b; Feng et al. 2009). Climate change is thus causing alterations to marine

ecosystems with impacts that are evident from polar to tropical regions (Harley et al. 2006; Hoegh-Guldberg et al. 2007; IPCC 2014).

Organisms naturally occur in multi-specific assemblages and the response of any individual to its environment will be influenced by its interaction with abiotic factors as well as by interactions with other species of the community (Menge and Sutherland 1987). In response to the growing concern of how climate changes can impact marine ecosystems and human societies, studies on natural multi-specific assemblages conducted under multifactorial experiments should be encouraged in order to assess the potential impacts of stressor associated to the global climate changes.

Environmental stressors can have simple additive effects (both significant, but no significant interaction) or have complex interactive effects where they have synergistic (increased stress) or antagonistic (decreased stress) effects on biological processes (Folt et al. 1999). Despite the well-known controlling influence of temperature on metabolism and development, the interactive effects of ocean warming and CO_2- driven acidification on organisms at community level are still sparse and require use of factorial experimental designs.

Copepods are very small aquatic crustaceans that, in terms of their size, abundance and diversity can be regarded as the "insects of the seas". Copepods have successfully colonized all salinity and temperature regimes, as well as an immense vertical range. They are the most important primary consumers in marine plankton communities, as such, form the base of virtually all pelagic food chain. In the benthic environment, harpacticoid copepods are very important in terms of abundance and diversity. Due to their high nutritional value, harpacticoids are the predominant meiofaunal element in the diet of many fishes of both ecological and economic importance (Huys and Boxshall 1991).

The present study comes from a mesocosm experiment performed by Hale et al. (2011) who investigated the combined effects of elevated CO_2 and temperature on macrofauna recruited from the intertidal zone using Artificial Substrate Units (ASUs). Here, we assessed the potential interactive impact of different levels of elevated CO_2 and temperature on harpacticoid copepods community. The null hypothesis tested was that exposure to low pH hypercapnia and elevated temperature will have no significant effect on the community structure and diversity of a benthic harpacticoid community.

2. Materials and methods

The meiofauna samples used in this study are from a mesocosm experiment carried out at Plymouth Marine Laboratory in 2009 (Hale et al. 2011), where intertidal benthic communities were exposed to elevated temperature crossed with different levels of reduction in the pH of seawater. The colonization of artificial substrate units and mesocosm experimental set-up was described in detail by Hale et al. (2011) and Meadows et al. (2015), and is summarized here.

2.1 Material collection

Fifty Artificial Substrate Units (ASU, each one made from 4 nylon mesh pan scourers tied together, 9 cm ø , 2.5 cm thick) were attached to a sheltered area of a rocky shore at Mount Batten, Plymouth, UK (50°35′67″N, 4°12′77″W) (Fig. 1A). They were attached between 0.6 m and 1 m above lowest chart datum (LCD), during the spring low tide on the 14 January, in an area where all ASUs would be exposed to similar conditions (temperature, sunlight, wave exposure, elevation). They were left for a period of twelve weeks to allow colonization and collected on 8 April 2009. The ASUs were retrieved and transported in plastic bags to the mesocosm facility at the

Plymouth Marine Laboratory (PML) 1h after collection. Once at PML, five ASUs were randomly selected and preserved in 10% formaldehyde solution. These ASUs were used to determine the community structure and diversity present at the start of the exposure period.

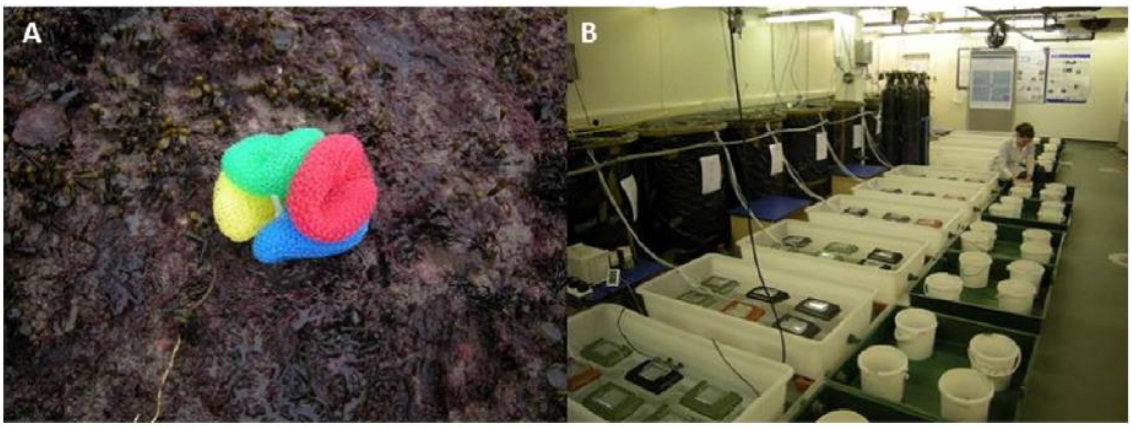

Fig. 1 (A) one of the Artificial Substrate Units (ASUs) attached to rocks in the intertidal; (B) image of the mesocosm experiment setup (figure from Meadows et al. 2015)

2.2 Mesocosm experiment

Forty of the remaining ASUs were each placed in separate food grade plastic buckets (vol. 6 L) containing ambient pH and temperature natural seawater (Fig. 1B e Fig. 2). Each bucket was randomly allocated to one of eight treatments (four pH levels crossed with two temperature levels), with five replicates for each combination. Control pH was 8.0 (the ambient seawater pH measured at the fauna collection site), and the decreased pH levels used were 0.3 units below ambient (the predicted drop in ocean pH by 2100), 0.7 units below ambient (the predicted drop in pH by 2250; Gitay et al. 2002; Caldeira and Wickett 2003) and a pH of 6.7 (mimicking a continuous point source leakage of CO_2 storage, Blackford et al. 2009). The mesocosm was held at a control

temperature of 12 °C (the ambient temperature measured at the fauna collection site) and the elevated temperature treatment was 4 °C above the control. Artificial manipulation of temperature was achieved by placing the treatment buckets in water baths containing heaters.

Fig. 2 Image of the experiment setup conducted in the mesocosm seawater acidification facility housed at the Plymouth Marine Laboratory (figure from Riebesell et al. 2010)

Seawater acidification was achieved by bubbling with 100% CO_2 gas following the methods described by Widdicombe and Needham (2007) and the experimental setup is shown in Fig. 3. Each 6 L bucket was continuously supplied with seawater and oxygen was bubbled through the water held within the buckets to assist with maintenance of the correct pH and to increase water mixing and oxygen levels. Each bucket was fed 1.68 ml of shellfish feed once a week to approximate the nutrient levels available to a community located at Mount Batten. The natural light regime was

approximated using daylight simulation lights within the mesocosm with an average 8-h photoperiod per day. No tidal cycle was applied to the buckets.

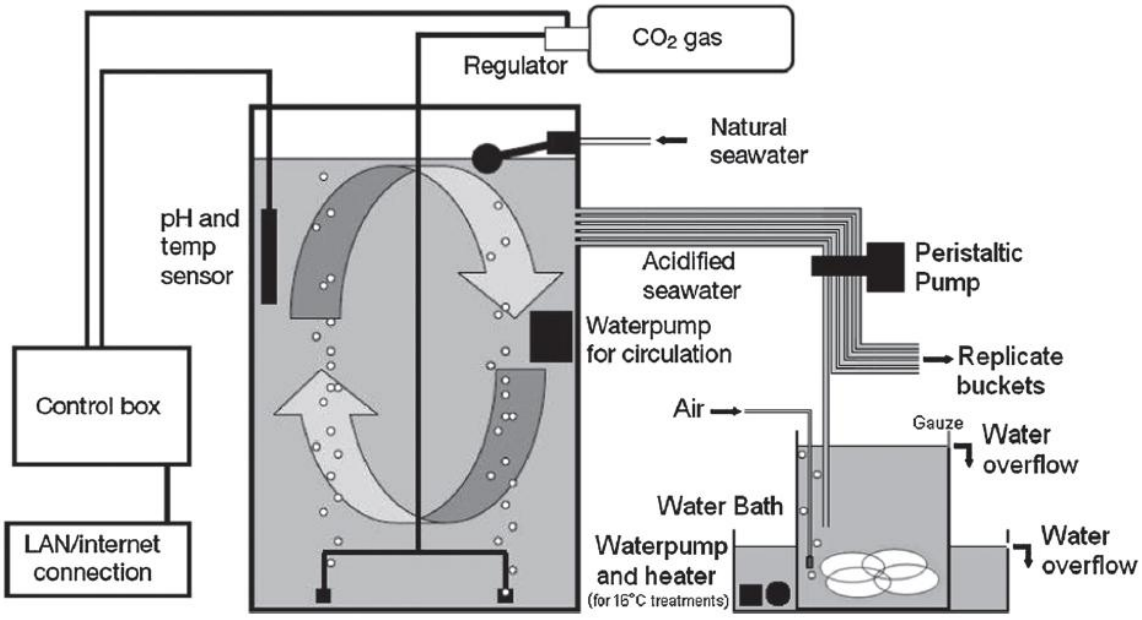

Fig. 3 Schematic diagram of the experimental setup used during the mesocosm experiment (diagram from Hale et al. 2009)

2.3 Monitoring of experimental conditions

The water within each of the eight reservoir tanks and 40 buckets was monitored bi-weekly using a combined pH and temperature probe and a salinity probe. Water samples were taken to obtain total CO_2 values using a DIC analyser calibrated with 2 g L^{-1} CO_2 standard reagent. From these data other carbonate parameters were calculated using the CO2SYS program (Pierrot et al. 2006). The system maintained the nominated pH and temperature treatments throughout the experimental period with little variation (Table 1) and was therefore considered a suitable method for the artificial manipulation of seawater pH and temperature. Carbonate concentration declined with pH. The control pH 8.0 treatments were oversaturated with respect to both calcite and

aragonite within the reservoir tanks and the 6 L buckets. Within the acidified treatments those held at pH 7.7 and below were undersaturated with respect to aragonite and those held at pH 7.3 and below were undersaturated with respect to calcite.

Table 1 Seawater chemistry within a) buckets and b) reservoir tanks during the experimental exposure period (Hale et al. 2011). (Sal – salinity, TCO_2 – total water carbon dioxide concentration, TA – total alkalinity, $_pCO_2$ – partial pressure of carbon dioxide, Ω_{Ca} – calcite saturation state, Ω_{Ar} – argonite saturation state, HCO_3- – bicarbonate concentration, CO_3^{2-} – carbonate concentration). Values: mean, ±SD, 95% CI

	Nominal pH	Temp (°C)	pH	Sal	TCO_2	TA	pCO_2 (µatm)	Ω_{Ca}	Ω_{Ar}	HCO_3-	CO_3^{2-}
a)											
12°C	8	11.78	7.86	34.88	1358.80	1956.61	729.23	1.59	1.01	1784.99	66.59
		0.35	0.09	0.19	314.04	293.36	160.4	0.3	0.19	275.4	12.5
		0.07	0.02	0.05	72.54	74.23	40.59	0.08	0.05	69.68	3.16
	7.7	11.93	7.66	34.89	2084.49	2155.52	1295.53	1.17	0.75	2031.97	49.03
		0.35	0.08	0.24	331.50	303.58	244.51	0.24	0.15	286.88	10.09
		0.07	0.01	0.06	76.57	76.81	61.87	0.06	0.04	72.59	2.55
	7.3	11.66	7.35	34.94	2181.94	2098.44	2729.23	0.55	0.35	2039.57	23.22
		0.41	0.07	0.13	227.68	228.21	499.36	0.11	0.07	222.75	4.5
		0.09	0.01	0.03	52.59	57.74	126.35	0.03	0.02	56.36	1.14
	6.7	11.53	6.81	34.82	2409.95	1942.01	2268.73	0.16	0.1	1925.14	6.6
		0.38	0.23	0.14	313.33	221.04	3127.42	0.11	0.07	220.99	4.56
		0.08	0.04	0.04	72.37	55.93	791.33	0.03	0.02	55.92	1.15
16°C	8	16.04	7.85	35.31	1915.97	1984.25	822.18	1.91	1.23	1779.26	80.3
		0.40	0.13	0.26	216.12	226.77	743.4	0.38	0.27	204.34	15.83
		0.08	0.02	0.07	49.92	57.38	188.1	0.1	0.06	51.7	4.01
	7.7	16.01	7.61	35.13	2046.30	2072.27	1422.98	1.21	0.78	1943.53	50.94
		0.63	0.15	0.21	241.83	246.16	388.9	0.29	0.19	233.63	12.18
		0.13	0.03	0.05	55.86	62.29	98.4	0.07	0.05	59.12	3.08
	7.3	15.76	7.37	35.06	2105.32	2051.74	2611.66	0.67	0.43	1980.85	28.01
		0.31	0.1	0.15	254.15	232.74	547.02	0.15	0.1	225.48	6.43
		0.07	0.02	0.04	58.70	58.89	138.41	0.04	0.02	57.05	1.63
	6.7	15.48	6.66	34.99	2423.61	1957.02	3010.36	0.15	0.1	1940.63	6.5
		1.52	0.19	0.18	284.04	25.08	4141.2	0.08	0.05	212.54	3.41
		0.32	0.04	0.04	65.60	54.42	1047.85	0.02	0.01	53.78	0.86
b)											
	8	14.08	7.89	34.95	1930	2018.97	680.76	1.93	1.24	1811.93	81.15
		0.44	0.14	0.10	273.13	307.01	156.68	0.31	0.2	287.81	12.89
		0.19	0.06	0.05	138.22	166.89	85.17	0.17	0.11	156.45	7.01
		15.56	7.98	34.84	1860	1970.58	527.15	2.38	1.53	1715.41	99.82
		0.41	0.14	0.13	257.68	290.3	96.68	0.39	0.25	231.67	16.49
		0.18	0.06	0.07	130.40	157.81	52.56	0.21	0.14	142.08	8.97
	7.7	15.46	7.68	34.82	2086.67	2116.32	1211.65	1.38	0.89	1970.45	57.92
		0.43	0.19	0.12	206.13	225.18	243.27	0.23	0.15	216.55	9.6
		0.19	0.08	0.06	104.31	122.41	132.24	0.12	0.08	117.71	5.22
		14.33	7.63	34.75	2033.33	2084.55	1308.57	1.21	0.77	1957.12	50.53

	0.56	0.18	0.15	287.90	323.71	230.09	0.37	0.23	297.01	15.36
	0.24	0.08	0.08	145.70	175.97	125.07	0.2	0.13	161.46	8.35
7.3	15.39	7.26	34.86	2156.67	2066.85	3279.5	0.54	0.35	2010.12	22.48
	0.55	0.19	0.13	130.21	141.82	584.35	0.12	0.08	135.28	5.21
	0.24	0.08	0.07	65.90	77.09	317.65	0.07	0.04	73.54	2.83
	14.26	7.35	34.82	2106.67	2043.55	2905.94	0.55	0.35	1984.88	23.14
	0.43	0.2	0.14	264.49	286.94	484.55	0.12	0.08	278.31	5.16
	0.19	0.08	0.07	133.85	155.98	263.4	0.07	0.04	151.29	2.8
6.7	13.86	6.33	34.76	2686.67	1770.66	243.69	0.05	0.03	1765.47	2.16
	0.44	0.14	0.14	311.7	247	42.29	0.02	0.01	245.84	0.64
	0.19	0.06	0.08	157.74	134.27	22.99	0.01	0.01	133.64	0.35
	15.3	6.34	34.76	2693.33	1763.27	238.83	0.06	0.04	1757.66	2.31
	0.7	0.2	0.16	358.17	220.64	41.23	0.02	0.01	219.47	0.63
	0.3	0.08	0.09	181.26	119.94	22.41	0.01	0.01	119.31	0.34

The experiment ran for 60 days. At the end of the mesocosm experiment, the ASUs were placed into the 1-litre pot and the pots filled with diluted 10% formalin solution. The fauna were separated from the pan scourers in a fume hood by washing the scourers with water, then the material was passed through two sieves (0.5 mm and 63 μm) to separate the macrofauna fraction from the meiofauna fraction (Somerfield et al. 2007).

Due to the high number of meiofauna organisms, thirty-six samples (four replicates per treatment + 4 initial samples) were randomly selected for analysis. Under a stereo microscope, the first sixty individuals of copepods were selected from each replicate, placed in Eppendorf tubes and preserved in 75% Industrial Methylated Spirit (IMS). The identification of Copepoda Harpacticoida was done under optical microscope following the taxonomic keys of Lang (1948, 1965), Huys et al. (1996) and Wells (2007) as well as publications with specific descriptions.

2.4 Statistical analysis

To represent graphically the changes on the natural communities due to the experiment, the multi-dimensional scaling (MDS) based on Bray-Curtis dissimilarities on relative abundance of copepods communities data was done. Similarity percentage (SIMPER) analysis was applied to determine which species were responsible for the dissimilarities among field and control samples collected at the end of experiment.

Permutational multivariate analyses of variance (PERMANOVA) (Anderson 2001; McArdle and Anderson 2001) based on Bray-Curtis dissimilarities on copepods abundance $\log_{(x+1)}$ transformed data was used to evaluate the impact of different temperatures (factor Temperature) and pH levels (factor pH) on the structure of communities. For all analyses, 9,999 random permutations were used. Pair-wise a posteriori comparisons (the multivariate version of the t statistic) were made for significant differences. A similarity percentage (SIMPER) analysis was applied to determine which species were responsible for the dissimilarities among pH and temperatures. The Shannon–Wiener (H′, using $\log_2$), Pielou's evenness (J′) and species richness indices were calculated. The population parameters malformed animal ratio, Copepodite ratio, ovigerous female and female/male ratios were also calculated.

Two-way analysis of variance (ANOVA) was used to examine the effects of the different pH and temperatures on the densities of harpacticoid's species (>2% of total), ecological indexes (S, J' and H') and on population parameters (ratios of copepodites, female/male, ovigerous females and malformed animals).

PERMANOVA, SIMPER and MDS were applied using the software Primer® 6 with add-on PERMANOVA+ (Plymouth Routines in Multivariate Ecological Researches). The two-way ANOVAs were calculated using the software STATISTICA 12. The IndVal values were calculated using the software PC-ORD 4.0. The level of

significance was set at $P<0.05$ for all analyses. Confidence intervals of 95% (CI) were used to express the variation of the calculated means. Parametric statistical analysis followed Zar (1996).

3. Results

3.1 Comparison between field and control samples

MDS ordination showed that the copepod community brought from field was modified under laboratory conditions during the 60 days experiment (Fig 4).

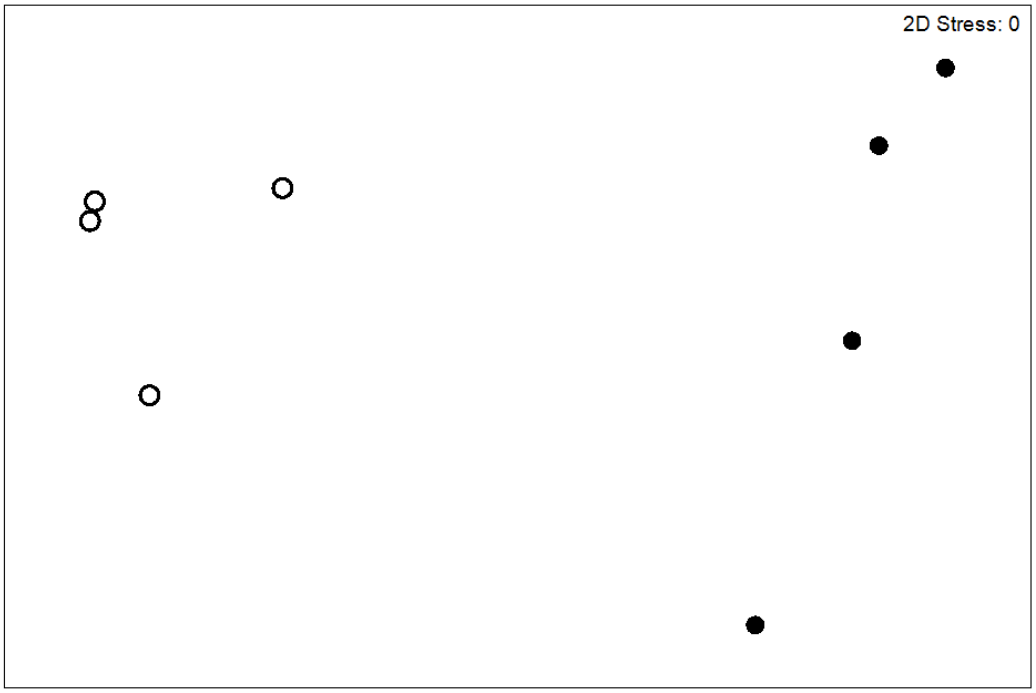

Fig. 4 Non-metric multi-dimensional scaling ordination plots for the Bray–Curtis similarity for the copepods community structure from ○ (open circles) Field and ● (closed circles) pH 8.0, 12 ºC samples

SIMPER analyses showed that the dissimilarities among field and control samples were due to the shift in the dominance of *Harpacticus obscurus* in field samples to *Tisbe* sp in control samples kept in laboratory (Table 2).

Table 2 Average abundance (Av. Abund.) and percent contribution (Contrib. %) of Harpacticoida species to average dissimilarity among field and control samples (pH 8, Temperature 12 ºC) (Cut off for low contributions: 90%)

Average dissimilarity = 87.83			
	Field	**pH 8, 12 ºC**	
Species	Av. Abund.	Av. Abund.	Contrib.%
Harpacticus obscurus	63.4	0	36.09
Tisbe sp	5.27	62.82	32.76
Dactylopusia vulgaris dissimilis	14.13	2.67	6.52
Ectinosoma sp2	0.96	8.01	4.16
Paradactylopodia sp	4.77	2.96	2.72
Amphiascoides golikovi	0	4.1	2.34
Ameiropsis mixta	3.52	1.18	2.01
Ectinosoma sp1	1.14	3.44	1.68
Amphiascoides sp1	0	2.52	1.43
Pseudobradya sp2	0.96	1.75	1.15

3.2 Copepod community

A total of 2160 copepod individuals was analyzed, 60.09% of which were identified as harpacticoids at the species level, 0.79% were adult cyclopoids, 38.7% were copepodites and 0.42% were broken animals which could not be determined to species. Among the harpacticoids, 12 families, 33 genera and 51 species were recorded (Table 3).

Table 3 List of Copepoda Harpacticoida species from phytal environment of the rocky shore at Mount Batten, Plymouth, UK

Order Harpacticoida Sars, 1903
Suborder Oligoarthra Lang, 1944

Family Laophontidae T. Scott, 1905
Laophonte cornuta Philippi, 1840
Laophonte setosa Boeck, 1865
Laophonte sima Gurney, 1927
Laophonte sp
Laophontinae sp1
Laophontinae sp2
Paralaophonte brevirostris (Claus, 1863)

Family Miraciidae Dana, 1846
Amonardia normani (Brady, 1872)
Amphiascoides golikovi Chislenko, 1977
Amphiascoides sp1
Amphiascopsis sp
Amphiascus minutus (Claus, 1863)
Amphiascus longarticulatus Marcus, 1974
Amphiascus (varians) sp
Amphiascus parvus Sars, 1906
Amphiascus angustipes Gurney, 1927
Bulbamphiascus sp
Delavalia sp
Haloshizopera lima Becker, 1974
Paramphiascella sp
Robertgurneya sp
Robersonia sp

Family Ameiridae Boeck, 1865
Ameira sp
Ameiropsis mixta Sars, 1907
Nitocra sp
Proameira hiddensoensis (Schäfer, 1936)
Proameira thetiensis Pallares, 1982
Psyllocamptus (L) *triarticulatus* Lang, 1965

Family Canthocamptidae Brady, 1880
Mesochra pygmaea (Claus, 1863)
Nannomesochra arupinensis (Brian, 1925)

Family Dactylopusiidae Lang, 1936
Dactylopusia vulgaris dissimilis Brian, 1921
Diarthrodes sp
Paradactylopodia sp

Family Ectinosomatidae Sars, 1903
Ectinosomatidae sp 1
Ectinosoma sp1
Ectinosoma sp2
Halectinosoma sp1
Halectinosoma sp2
Halectinosoma sp3
Pseudobradya sp1
Pseudobradya sp2
Pseudobradya sp3
Sigmatidium sp

Family Longipediidae Boeck, 1865
Longipedia sp

Family Normanellidae Lang, 1944
Normanella sp

Family Harpacticidae Dana, 1846
Harpacticus obscurus T. Scott, 1895

Family Pseudotachidiidae Lang, 1936
Idomene purpurocincta (Norman & T. Scott, 1905)

Family Peltidiidae Claus, 1860
Alteutha depressa (Baird, 1837)
Eupelte sp

Family Tisbidae Stebbing, 1910
Tisbe sp

Harpacticoida sp

Tisbe sp (37.42%), *Harpacticus obscurus* (11.91%), *Ectinosoma* sp2 (5.45%), *Ectinosoma* sp1 (4.8%), *Amphiascoides* sp1 (4.55%), *Paradactylopodia* sp (4.49%), *Dactylopusia vulgaris dissimilis* (4.26%), *Ameiropsis mixta* (3.94%), *Amphiascus longarticulatus* (3.25%), *Amphiascoides golikovi* (3.06%) and *Ameira* sp (1.67%), accounted for ~85% of total.

MDS ordination analyses indicated marked differences in the structure of copepod community among Field and treatment samples. Among treatments the most important difference was observed among samples maintained in pH 6.7 from the other pHs (Fig. 5).

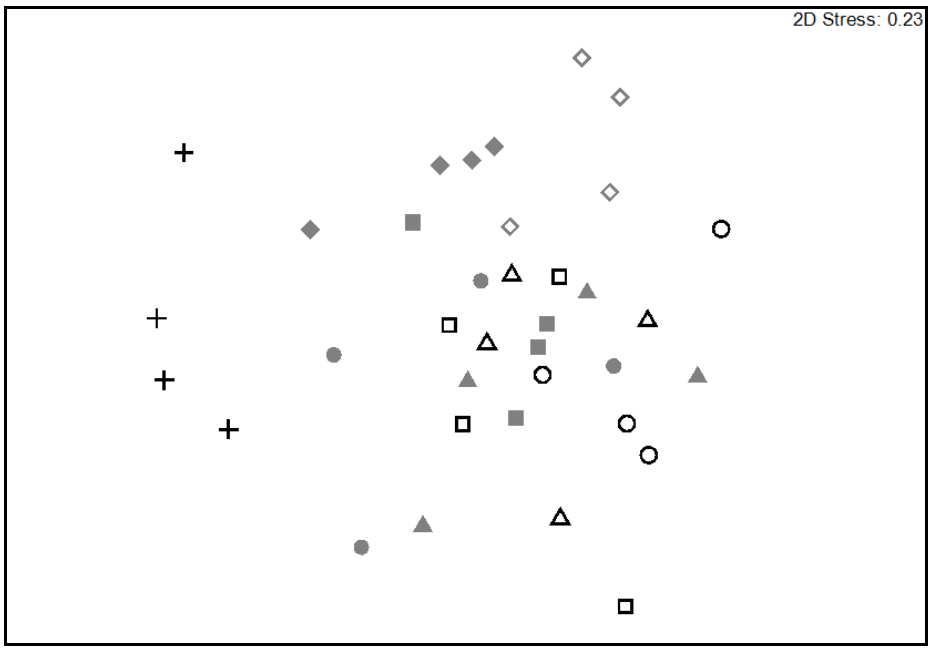

Fig. 5 MDS ordination plots for the Bray–Curtis similarity for Copepod community structure. + (cross) Field samples, ● (circle) 8.0, ▲ (triangle) 7.7, ■ (square) 7.3, ♦ (diamond) 6.7 (12 °C closed symbols, 16 °C open symbols)

The pattern illustrated in the MDS ordination was confirmed by PERMANOVA. Significant differences in the structure of the copepod community were

detected for the factor pH and Temperature, but not for the interaction between the two factors (Table 4).

Table 4 PERMANOVA results for the Copepod community exposed to different pH and temperatures. Significant values are highlighted in bold

Source	*df*	MS	*F*	*P*
Temperature (T)	1	3584.5	**2.52**	0.0044
pH	3	3147.1	**2.22**	0.0004
pH x T	3	1467.1	1.03	0.422
Residual	24	1420.3		

The response of copepod community structure to the different pH was due to differences among samples kept in pH 6.7 from the others, while no differences among control and the other treatments were detected (Table 5).

Table 5 Pair-wise *a posteriori* comparisons for pH. Significant values are highlighted in bold

pH Comparisons	*t*	*P*
8.0 x 7.7	0.821	0.759
7.7 x 7.3	1.000	0.466
7.7 x 6.7	**1.951**	0.0001
8.0 x 7.3	0.911	0.6551
8.0 x 6.7	**2.066**	0.0001
7.3 x 6.7	**1.839**	0.0002

SIMPER analyses showed that decreases in the density of *Ectinosoma* sp2 and *Tisbe* sp in samples kept in the pH 6.7 were important to these dissimilarities (Table 6).

Table 6 Percent contribution (Contrib. %) of Cyclopoida and Harpacticoida species to average dissimilarity (Diss.) among different pH (Cut off for low contributions: 70%)

8.0 *vs* 6.7		**7.7 *vs* 6.7**		**7.3 *vs* 6.7**	
Diss.= 63.88	Contrib. %	Diss.= 60.36	Contrib. %	Diss.= 58.61	Contrib. %
Amphiascus longarticulatus	5.84	*Ectinosoma* sp2	6.63	*Ectinosoma* sp2	6.18
Tisbe sp	5.80	*Tisbe* sp	6.45	*Tisbe* sp	6.02
Ectinosoma sp2	5.62	*Amphiascoides golikovi*	5.40	*Paradactylopodia* sp	5.51
Dactylopusia vulgaris dissimilis	5.04	*Amphiascoides* sp1	4.89	*Amphiascus longarticulatus*	5.31
Amphiascoides golikovi	4.81	*Delavalia* sp	4.85	*Amphiascoides* sp1	4.66
Laophonte cornuta	4.65	*Amphiascus longarticulatus*	4.82	*Laophonte cornuta*	4.44
Amphiascoides sp1	4.65	*Laophonte cornuta*	4.70	*Robertgurneya* sp	4.01
Paradactylopodia sp	4.54	*Ameiropsis mixta*	4.44	*Ameiropsis mixta*	3.68
Normanella sp	4.09	*Paradactylopodia* sp	4.32	*Pseudobradya* sp2	3.67
Ectinosoma sp1	4.07	Cyclopoida	4.23	*Ectinosoma* sp1	3.58
Ameira sp	4.01	*Dactylopusia vulgaris dissimilis*	4.14	*Dactylopusia vulgaris dissimilis*	3.54
Cyclopoida	3.76	*Ectinosoma* sp1	4.01	*Amphiascoides golikovi*	3.52
Ameiropsis mixta	3.22	*Normanella* sp	3.69	*Pseudobradya* sp1	3.25
Pseudobradya sp1	2.88	*Idomene purpurocincta*	3.39	*Idomene purpurocincta*	3.18
Pseudobradya sp2	2.86	*Pseudobradya* sp1	3.35	*Ameira* sp	3.17
Laophonte sima	2.84			*Normanella* sp	2.82
				Laophontinae sp2	2.53

SIMPER analyses showed that many species were important to dissimilarity between temperatures (Table 7).

Table 7 Percent contribution (Contrib. %) of Cyclopoida and Harpacticoida species to average dissimilarity (Diss.) between temperatures (Cut off for low contributions: 70%)

12 ºC *vs* 16 ºC	
Diss.= 57.39	Contrib.%
Amphiascoides sp1	5.18
Paradactylopodia sp	4.98
Amphiascus longarticulatus	4.81
Ectinosoma sp	4.68
Amphiascoides golikovi	4.38
Dactylopusia vulgaris dissimilis	4.25
Cyclopoida	4.11
Tisbe sp	4.11
Ameiropsis mixta	4.06
Ectinosoma sp1	3.83
Laophonte cornuta	3.81
Normanella sp	3.72
Ameira sp	3.68
Pseudobradya sp1	3.42
Delavalia sp	3.38
Robertgurneya sp	3.15
Idomene purpurocincta	2.93

ANOVA results for species richness, evenness and diversity showed no significant differences for the factors pH and temperature nor for interaction between the two factors ($p>0.1$ for all) (Fig. 6).

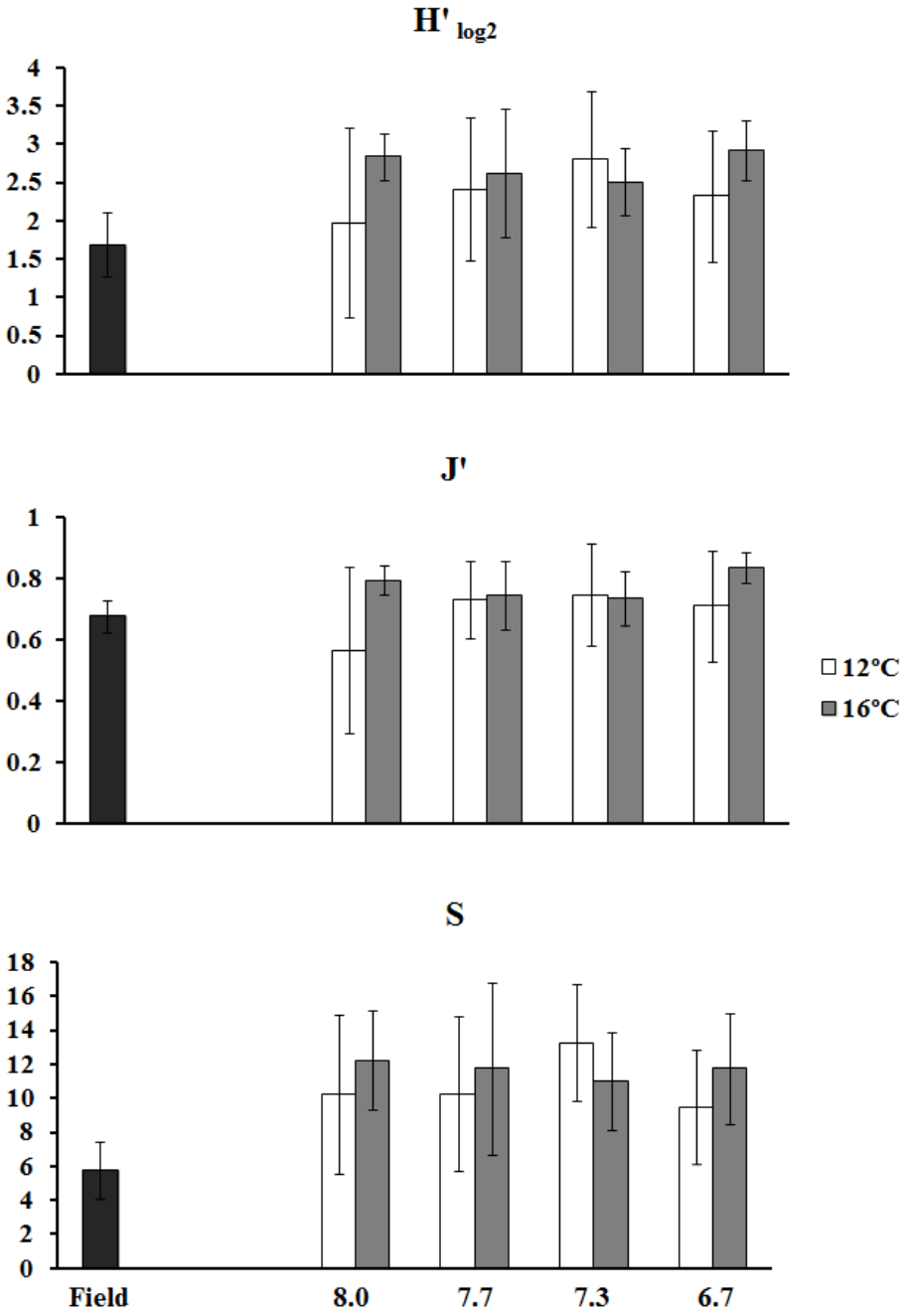

Fig. 6 Shannon diversity (H' log_2), Pielou's evenness (J') and Species richness (S) for copepod community at different pH and temperature. Values: mean ±95 % confidence intervals

ANOVA results for copepod population parameters showed no differences for female/male ratio ($p>0.6$ for all comparisons) nor for the percentage of ovigerous

female (p>0.15 for all comparisons) for both pH and temperature or interaction between them. Copepodite ratio showed significant differences for factor pH ($F_{(3,24)}$= 3.12; p=0.045) and for factor Temperature ($F_{(1,24)}$= 5.41; p=0.029), but not for interaction between factors (p>0.18). The *a posteriori* Fisher test indicated that copepodite ratio at pH 6.7 was lower than at pH 7.7 (p=0.007) and that copepodite ratio was higher at 16 ºC. Malformed animals ratio showed significant differences for factor pH ($F_{(3,24)}$= 3.24; p=0.039). Fisher test indicated that the ratio of malformed animals in pH 6.7 was significantly higher when compared to pH 8.0 (p=0.014), 7.7 (p=0.013) and 7.3 (p=0.041) (Fig.7).

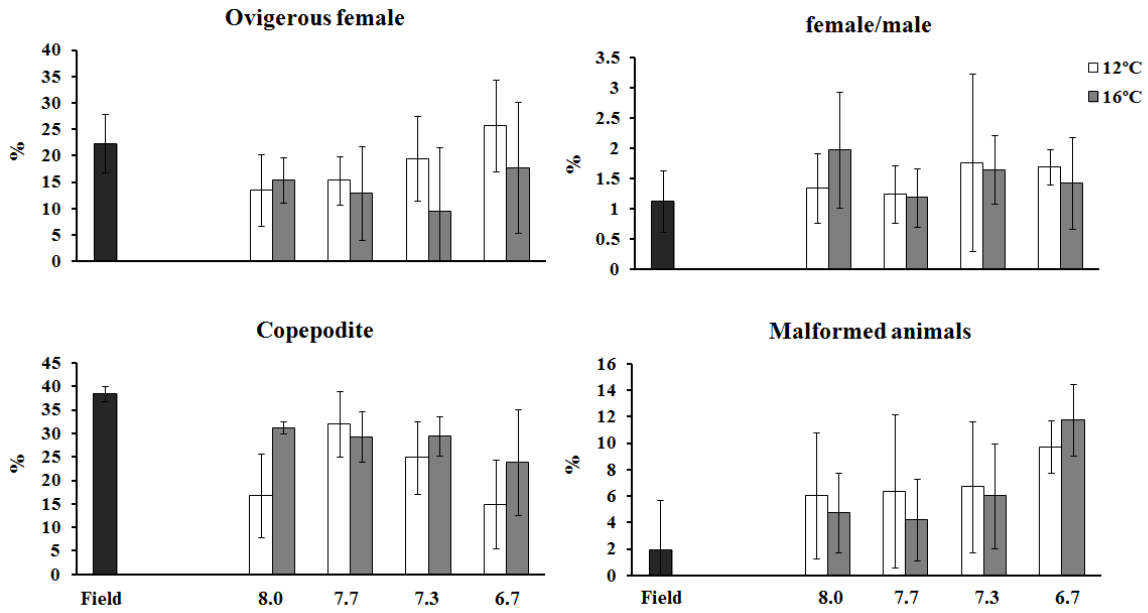

Fig. 7 Mean (±95 % confidence intervals) of ovigerous female, female/male ratios, copepodite and malformed animals ratios at different pH and temperatures

Only few species composing copepod community showed significant differences among treatments, and the majority were sensitive only at pH 6.7 (Fig. 8). The two-way ANOVA indicated that *Tisbe* sp showed significant interaction between the factors pH and Temperature ($F_{(3,24)}$= 5.22, p<0.01). Results for significant interactions between factors indicated that *Tisbe* sp showed higher densities at 12 ºC than at 16 ºC (p<0.001) in the pH 6.7. Moreover, density of *Tisbe* sp was the lowest in the pH 6.7 at 16 ºC than in the all other treatments (p<0.001). *Ectinosoma* sp2 was sensitive for the factor pH ($F_{(3,24)}$= 6.99, p<0.01) and Temperature ($F_{(1,24)}$= 5.33, p=0.03), but not for the interaction ($F_{(3,24)}$= 0.44, p=0.72). The a posteriori Fisher test showed that the density of *Ectinosoma* sp2 was lower in pH 6.7 when compared to all other pHs (p<0.01). Also, the density of *Ectinosoma* sp2 was higher at 16 ºC than at 12 ºC (p=0.03). For the species *Amphiascoides* sp1 significant differences were found only for the factor Temperature ($F_{(1,24)}$= 5.11, p=0.033) where higher densities were found at 16 ºC.

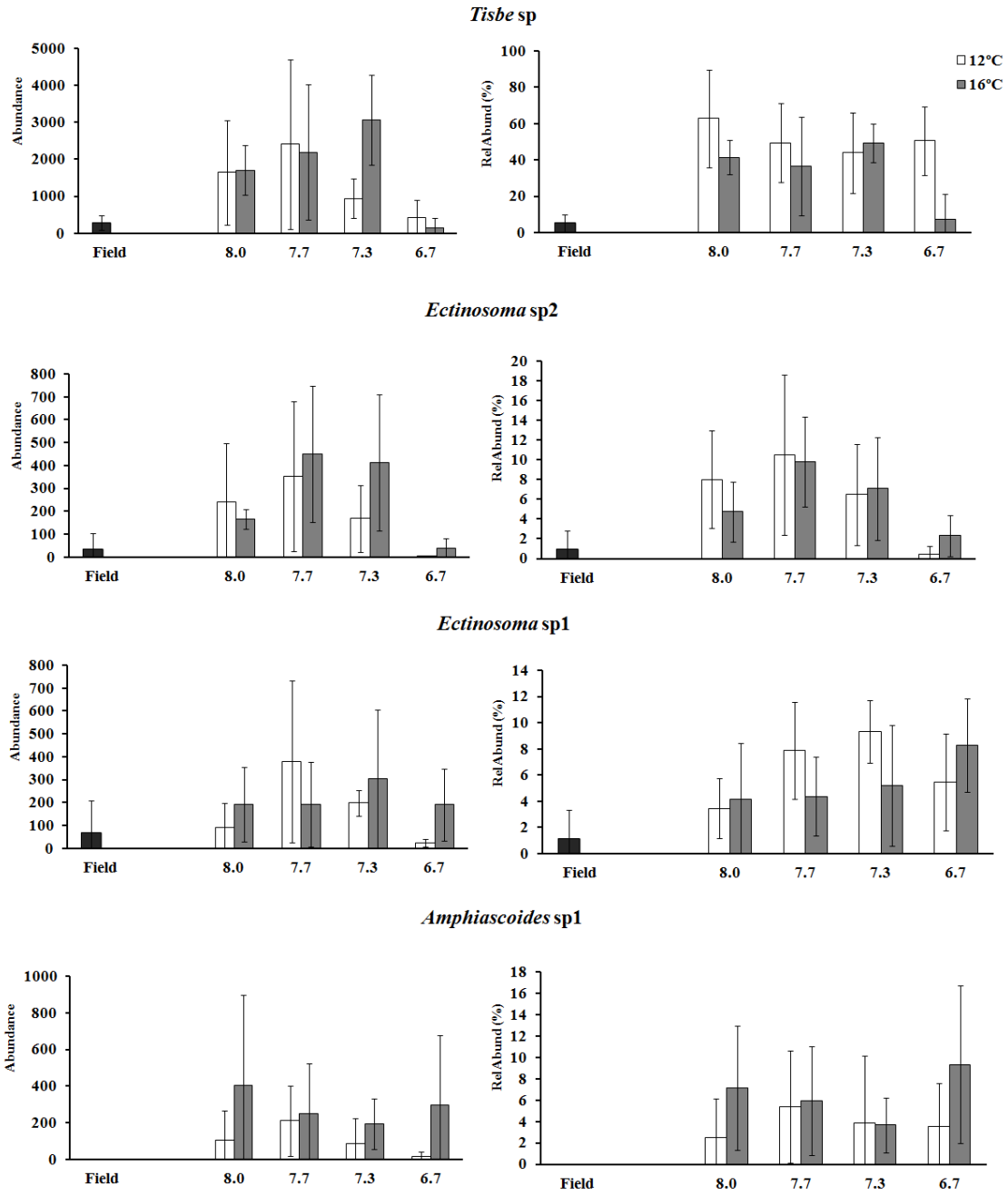

Fig. 8 The effects of pH and temperature on the mean abundance and relative abundance (±95 % confidence intervals) of the main harpacticoids species

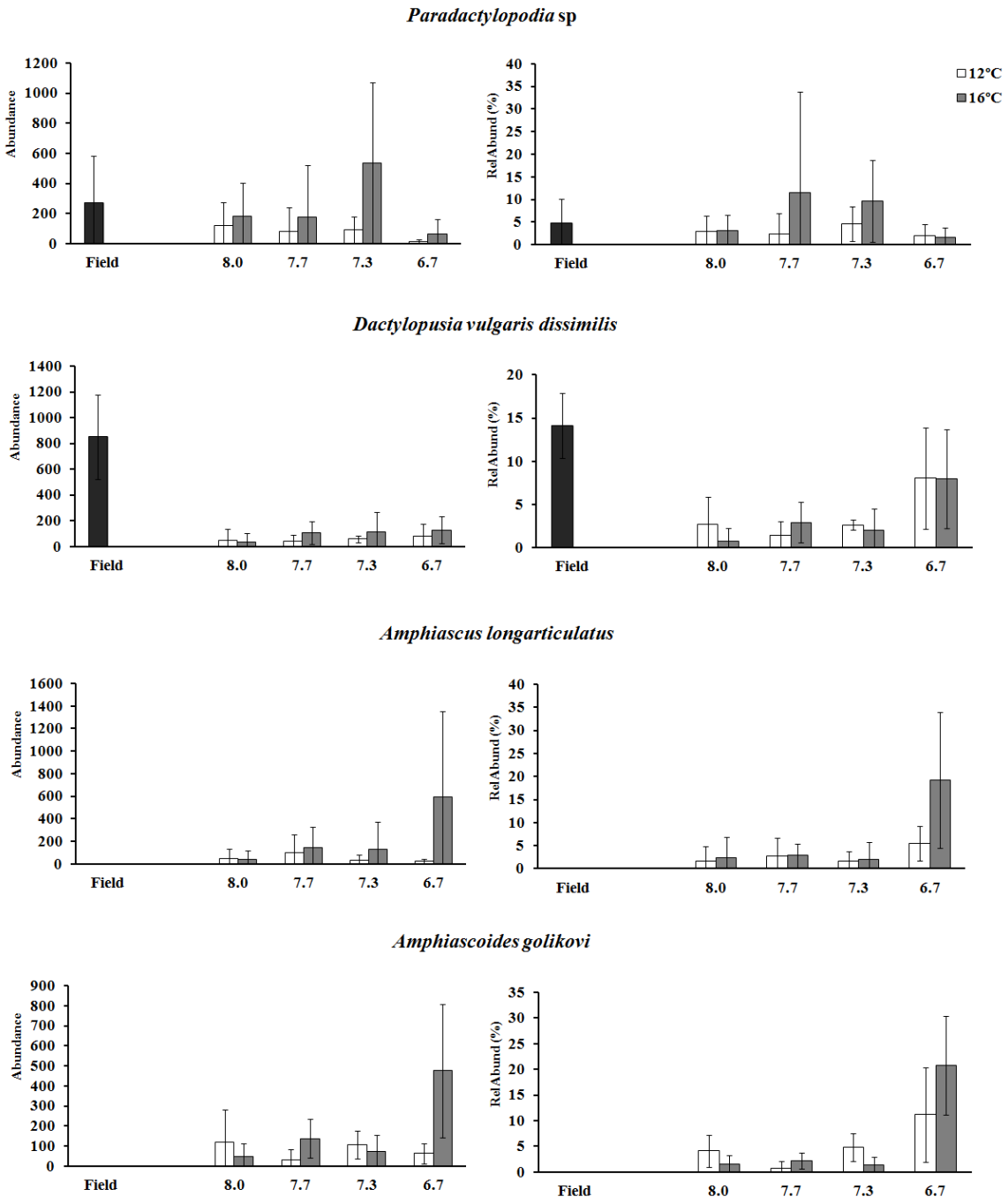

Fig. 8 *(Continued)*

4. Discussion

The fauna of harpacticoid copepods that colonized the artificial substrate units in the intertidal environment of the rocky shore at Mount Batten was similar in terms of genera to other phytal fauna reported by other authors in different parts of the world, such as the southeast of Brazil (Jakobi 1953; Curvêlo 1998), Ria Deseado, Argentina (Pallares 1968), Ashtamudi in India (Arunachalam and Nair 1988), Cook Strait (Hicks 1977), Wellington (Hicks 1986) and Island Bay (Coull and Wells, 1983) in New Zealand, Robin Hood's Bay and St Abbs in England (Hicks 1980), South Carolina (Coull et al. 1983) and Florida (Walters and Bell 1994) in the USA, British Columbia in Canada (Webb and Parsons 1992) and Port Phillip Bay in Australia (Jenkins et al. 2002). Moreover, this fauna presented high diversity value (H'= 3.65), which is in agreement with values reported for harpacticoid communities in phytal environment (Sarmento et al. 2012).

The concept of isocommunity or parallel ecological communities suggests that similar substrates, although geographically separated, are inhabited or colonized by the same set of dominant genera, although the species composition may vary between sites Por (1964). This hypothesis has been applicable to Copepoda Harpacticoida communities in phytal environments (Hicks 1977, 1980; Sarmento et al. 2012) and is reinforced here. The presence of a large number of cosmopolitan species (and genera) in this study should be stressed. Many species reported for Mount Batten were first described in early 20th century or even late 19th and have been cited as having considerable morphological variation or likely to belong to a complex of species with as yet unresolved taxonomy [see Wells (2007) for comments on the taxonomy of these species].

The results presented here give an indication on the potential impacts to intertidal copepods that are likely to occur across a range of predicted pH and temperature levels (Caldeira and Wickett 2003; IPCC 2014). They demonstrate that the predicted changes due to ocean acidification and warming could potentially alter copepod community structure.

Since ocean acidification and warming are both caused by increased atmospheric CO_2, the meiofauna organisms are being exposed to the two stressors simultaneously (Byrne 2011; Hale et al. 2011; Melatunan et al. 2013). In the present study the most significant separation of assemblages was observed among samples exposed to pH 6.7 from those subjected to other pH treatments, while no differences were observed among pH 8.0 and 7.7 nor 8.0 and 7.3. The same pattern of response was observed by meiofauna and nematodes from the same experiment. Meadows et al. (2015) found that meiofauna and nematodes community structure were both significantly affected by pH and temperature only, but no interaction was observed.

The response of total density of copepods was assessed by Meadows et al. (2015) and showed that copepod abundance was significantly affected by pH and temperature separately, but the interaction of the two stressors did not evoke a significant effect. Copepod abundance at pH 6.7 was significantly lower from abundance at other pH levels, for both the 12 and 16 °C treatments. At pH 7.3, abundance increases at 16 ºC was noticeable, but on average copepod abundances were greater at 16 °C (Meadows et al. 2015).

The response of a multispecies intertidal community to ocean warming and acidification is strongly influenced by direct effects on taxa and indirect effects through ecological interactions (Hale et al. 2011; Melatunan et al. 2013). Species interactions may attenuate or amplify the direct effects on individual species (Kroeker et al. 2012).

Moreover, the resistance of individual species to a single environmental stressor may be reduced in face of multiple stressors (Hale et al. 2011; Melatunan et al. 2013).

In general, harpacticoid species responded mainly to changes in pH 6.7. Differences between pH 8.0 and 6.7 samples were due to reductions in *Tisbe* sp and *Ectinosoma* sp2 densities at pH 6.7. However, at this pH, the densities of *A. longarticulatus* and *A. golikovi* increased. The same pattern was observed for differences between pH 7.7 and 6.7 samples, reductions in *Tisbe* sp and *Ectinosoma* sp2 densities at pH 6.7 and increases in the densities of *A. golikovi* were observed. For differences between pH 7.3 and 6.7 samples, it was observed reductions in *Tisbe* sp and *Ectinosoma* sp2 densities at pH 6.7 and increases in the densities of *A. longarticulatus*. These changes were also accompanied by differential response to temperature at this pH. Increases in the density of *A. longarticulatus* at pH 6.7 was higher at 16 ºC. At this pH, densities of *Tisbe* sp was higher at 12 ºC. In general, density of *Amphiascoides* sp1 was higher at 16 ºC.

Most of the previous studies investigating effects of ocean acidification on copepods (calanoids) have found that acidification (pH between 7.78 to 7.2) due to a wide range of CO_2 concentrations predicted for this century cause no significant effects on mortality, development, metabolism or reproductive parameters (Kurihara and Ishimatsu 2008; Mayor et al. 2012; McConville et al. 2013; Vehmaa et al. 2013; Hildebrant et al. 2014; Isari et al. 2015; Li et al. 2015). On the other hand, when the harpacticoid species *Tisbe battagliai* was exposed over three generations at four pH conditions (pH 7.67, 7.82, 7.95, and 8.06) some complex responses were observed. Naupliar production increased significantly at pH 7.95 compared with pH 8.06 followed by a decline at pH 7.82. Naupliar production at pH 7.67 was higher than pH 7.82. But

the multi-generational model predicted a gradual decline in naupliar production (Fitzer et al. 2012).

Despite the apparent tolerance of copepods to the pCO_2 predicted for a future ocean, when they are exposed in combination with increasing temperatures they could be sensitive to ocean acidification. Mayor et al. (2012) exposed *Calanus finmarchicus* to 1000 ppm CO_2-acidified seawater (pH~7.7), but found no significant effect on offspring viability. However, an interactive positive effect with temperature, i.e. higher viability with elevated temperature, was observed. Zervoudaki et al. (2014) showed that acidification (pH 7.83) does not have a direct effect on the vital rates of the copepod *Acartia clause*. Egg production rate and hatching success of *Acartia clause* however decreased at future pH in combination with rise temperature. Also, acidification and warming resulted in an increase of the excretion rate and the increase was higher than that observed by warming only. Incubations at 3000 µatm pCO_2 (pH 7.2) caused no changes in respiration rates, body mass and mortality in *Calanus glacialis* and *Calanus hyperboreus*. However, when *C. hyperboreus* were kept at different pCO_2 and temperatures, some sublethal stress was observed (Hildebrant et al. 2014). Vehmaa et al. (2013) found no significant effect of acidification on reproductive parameters in a pH (7.6) scenario projected for the year 2100. But a significant acidification- dependent effect in interaction with temperature reducing *Acartia bifilosa* antioxidant capacity was observed. Also, higher temperature also decreased egg viability, nauplii development, and oxidative status.

Some studies have applied CO_2 concentrations far beyond those expected in the next 100 years by the IPCC, and thus are relevant as they emulate potential Carbon Capture and Storage (CCS) conditions (e.g. Kurihara et al. 2004; Mayor et al. 2007; McConville et al. 2013). At these levels, copepods showed to be negatively affected in

reproduction but still not in mortality. Mayor et al. (2007) observed a reduction in hatching success of *Calanus finmarchicus* in response to ocean acidification (8000 ppm CO_2, pH 6.95), but not in growth (egg production and biomass loss). Kurihara et al. (2004) found that when exposed under conditions of +10,000 ppm CO_2 in seawater (pH 6.8), the egg production rates of *Acartia steueri* decreased significantly. But the survival rates of adult copepods were not affected when reared under increased CO_2 for 8 days, however longer exposure times could have revealed toxic effects of elevated CO_2 concentrations. Kurihara et al. (2004b) found that the hatching and nauplius mortality rate of *Acartia steueri* and *Acartia erythraea* tended to be negatively affected by increased CO_2 concentration, though, significant only above +5000 ppm CO_2 (pH 7.02). When exposed to a very high CO_2 treatment, (9830 ppm, pH 6.7), reductions of egg production rate and hatching success were observed for *Centropages typicus*, but not for *Temora longicornis* (McConville et al. 2013). These results indicate that CO_2 concentrations higher than ca. 2000 ppm potentially have negative impacts on reproduction of copepods, while lower concentrations appear not to have significant impact.

Contrarily to what was observed for single-species experiments, in the present study, copepod community was negatively affected at pCO_2 and pH that simulate carbon capture and storage site continuous point source leakage. Under this condition, the pattern of response showed to be complex. Despite the sensibility of the dominant *Tisbe* sp, other species had their densities increased. There is an increasing consensus in literature that experiments on community level can be more informative than single species ones, revealing complex changes in ecological and biological interactions. Though, a precautionary approach may be required when interpreting predictions from single species studies, since that many of the most striking consequences of climate

change will arise through altered species interactions (e.g. Fabricius et al. 2011; Kroeker et al. 2013; Gaylord et al. 2015). Therefore, the present results highlight the importance of studies conducted at community level.

When a tropical copepod community was exposed to a range of future scenarios of increasing warming and acidification, negative impacts were observed even at mild conditions demonstrating high sensibility to climate change (Sarmento et al. 2016c). Comparably, the results in the present study demonstrate that copepod communities from temperate areas would be more tolerant to changes in pH and temperature associated with climate change, since communities in these regions naturally experience high variability of abiotic factors. In fact, it should be stressed that all the studies founding no effects of ocean acidification (pH between 7.78 to 7.2), even at the worst forecast of CO_2 concentrations, were conducted with copepods from Arctic to temperate environments and/or from laboratory cultures (Kurihara and Ishimatsu 2008; Mayor et al. 2012; McConville et al. 2013; Vehmaa et al. 2013; Hildebrant et al. 2014; Isari et al. 2015; Li et al. 2015). Moreover, it is expected that the fauna from habitats characterized by strong abiotic variability (areas where volcanic emissions occur in the sea and where excessive respiration occurs in confined areas filled with plant and animal life, like in rockpools of the intertidal zone, but also in marine sediments or hypoxic bottom waters) would also exhibit higher tolerance to climate change expected for this century (Pörtner 2004). Pascal et al. (2010) suggested that difference in sensibility to ocean acidity of two harpacticoid species could be associated to the fact that the two copepod species are associated with different environments and thought copepods living in environments more prone to hypercapnia, such as mudflats where *Shizopera knabeni* lives, may be less sensitive to future acidification than *Amphiascoides stopus* found on large grains beaches. Li et al. (2015) found that

combined heat shock and ocean acidification (pCO_2 1000 μatm, pH~7.7) did not affect the mortality of *Tigriopus japonicus*, a copepod which inhabits a highly variable intertidal environment. However, species from the same locality, though with similar life histories, could present different tolerance to ocean acidification. Adult female of *Centropages typicus* and *Temora longicornis* collected from the western English Channel were exposed to very high CO_2 treatment and reduction of egg production rate and hatching success were observed for *C. typicus*, but not for *T. longicornis* (McConville et al. 2013).

Tisbe sp was the dominant species in all treatment samples and the overall observed pattern of response to ocean acidification and warming was influenced by *Tisbe* sp. This species was not abundant in field samples but became dominant at laboratory conditions. This species was resistant to pH 7.7 and 7.3, with decreases in its density being observed only at pH 6.7. Species of the genera *Tisbe* are characterized as having high fecundity and short generation time, have very high levels of essential fatty acids, a wide range of body sizes, tolerate a wide range of environmental changes and have the ability to grow on different food sources and reach high population densities. *Tisbe* species are easily reared in the laboratory and have been extensively cultured for tests as live food for fish and crustacean larvae and for ecotoxicological bioassays as well (Gaudy et al. 1982; Williams and Jones 1999; Pinto et al. 2001; Souza-Santos et al. 2006; Diz et al. 2009; Souza-Santos et al. 2015).

Despite the no significant effects on *Tisbe* sp mortality observed at pH 7.7 and 7.3 in the present study, it is possible that sublethal impact on growth, size or copepods biomass could occur at these pH levels. Due to significant changes in growth, cuticle composition and naupliar production in *Tisbe battagliai* over three generations at four pH conditions (pH 7.67, 7.82, 7.95, and 8.06), Fitzer et al. (2012) suggested that

copepods subjected to ocean acidification induced stress preferentially reallocate resources towards maintaining reproductive output at the expense of somatic growth and cuticle composition. These responses may drive shifts in life history strategies that favour smaller brood sizes and females (Fitzer et al. 2012).

In agreement to Sarmento et al. (2016c) a positive increase of malformed adult animals with the increase level of warming and ocean acidification was observed in the present study. The analysis of this parameter suggest that, species that apparently can couple with the stress associated to ocean acidification and warming and entering the adult stage are still showing the consequences of its impacts (Sarmento et al. 2016c).

The results presented in this study demonstrated that the combination of elevated levels of CO_2 and ocean warming may have substantial effects on copepod communities from intertidal environment. Moreover, results stresses that ecological interactions may lead to complex community responses to pH and temperature changes that can not be encompassed by single species and/or single stressor experiments.

CONCLUSÕES

Modificações nas características físico-químicas da água do mar que podem ser determinadas pelas mudanças climáticas globais influenciaram a comunidade de meiofauna e associação de espécies de Copepoda Harpacticoida. Modificações na estrutura da comunidade de meiofauna foram observadas tanto em resposta à diminuição do pH, bem como em resposta ao aumento simultâneo da temperatura e acidificação da água do mar.

Quando expostos a diferentes níveis de redução do pH da água do mar, os grandes grupos taxonômicos da meiofauna demonstraram ser, no geral, tolerantes às condições de acidificação. Entretanto, quando expostos ao aumento simultâneo de temperatura e acidificação os grandes grupos da meiofauna foram negativamente afetados.

As modificações na estrutura da comunidade de meiofauna foram o resultado dos diferentes padrões de respostas de seus principais grupos taxonômicos. De forma geral, observa-se que, enquanto os Copepoda Harpacticoida são bastante sensíveis às mudanças no pH e temperatura, Nematoda apresentam grande resistência e comportamento oportunista tendo suas densidades aumentadas mesmo nos tratamentos mais severos.

Avaliando o impacto do aumento da temperatura e acidificação na estrutura populacional dos harpacticóides observa-se que, estágios de larva e juvenil apresentam maior sensibilidade quando comparados ao estágio adulto. Porém, mesmo aqueles animais que atingem o estágio adulto podem apresentar respostas subletais como malformação de suas estruturas morfológicas.

Os padrões de resposta antagônicos observado entre as diferentes espécies de Copepoda Harpacticoida sugerem que algumas espécies podem apresentar resistência ao

aquecimento e acidificação da água do mar. Tais características, somadas à flexibilidade alimentar bem como à diminuição das pressões ecológicas (e.g., predação e competição) podem ter favorecido o aumento dessas espécies em condições de elevada temperatura e acidificação da água do mar.

Os resultados apresentados ao nível de grandes grupos bem como ao nível de espécies de harpacticóides reforçam a importância da realização de experimentos no contexto de comunidades.

Diferentemente das comunidades de ambiente tropical, a comunidade de Copepoda Harpacticoida de região temperada, foi fortemente afetada apenas nos tratamentos mais severos. Esta aparente maior tolerância estaria associada ao fato de que animais de zonas entremáres de ambiente temperado experimentam grande variação dos fatores abióticos em seu ambiente natural, possuindo adaptações fisiológicas para sobreviver em ambientes tão variáveis e rigorosos.

Considerando a importância dos organismos da meiofauna para a cadeia alimentar e, em especial, a importância dos copépodes da Ordem Harpacticoida como item alimentar de muitos peixes, conclui-se que, as previsões de aumento de temperatura e acidificação da água do mar, que podem ser desencadeadas pelas mudanças climáticas globais, terão um efeito negativo na base da cadeia trófica dos ambientes bentônicos que podem desencadear mudanças nos níveis tróficos superiores comprometendo o funcionamento trófico desses ambientes.

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