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The combined effects of ocean warming and acidification on shallow-water meiofaunal assemblages

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ABSTRACT

Climate change due to increased anthropogenic CO_2 in the atmosphere is causing an increase in seawater temperatures referred to as ocean warming and a decrease in seawater pH, referred to as ocean acidification. The meiofauna play an important role in the ecology of marine ecosystems and the functions they provide. Using microcosms, meiofaunal assemblages were exposed to two temperatures (15 and 19 °C) and two pHs (pCO_2 of 400 and 1000 ppm), both individually and in combination, for a period of 90 days. The hypothesis that increased temperature will increase meiofaunal abundance was not supported. The hypothesis that a reduced pH will reduce meiofaunal abundance and species richness was supported. The combination of future conditions of temperature and pH (19 °C and pCO_2 of 1000 ppm) did not affect overall abundance but the structure of the nematode assemblage changed becoming dominated by a few opportunistic species.

1. Introduction

Climate change due to increased anthropogenic CO_2 in the atmosphere is causing an increase in seawater temperatures, ocean warming (OW) and a decrease in seawater pH, ocean acidification (OA) (IPCC, 2014; Gattuso et al., 2015). In intertidal and shallow subtidal environments, organisms experience significant variations in temperature and pH over tidal and diurnal cycles, which may in some cases already push species to the limits of their tolerances (Stillman and Somero, 1996; Wootton et al., 2008). Under future scenarios of climate change as these extremes increase, ecosystems may well experience significant changes in diversity and community structure (Gaylord et al., 2015; Boyd et al., 2016). Species will either acclimatise or adapt to the new conditions, or migrate to areas where the conditions remain within their tolerance limits. Those that can neither acclimatise, adapt nor migrate will eventually perish (Boyd et al., 2016). These changes in the ocean environment will likely drive changes in the ecology of the oceans (Gaylord et al., 2015).

The sedimentary environment is habitat for a wide diversity of organisms including prokaryotes (Corinaldesi, 2015), protozoans (Caron et al., 2012), algae (Norton et al., 1996) and invertebrates (Levin et al., 2001). In Chilean territorial waters over 5000 free-living benthic invertebrate species have so far been described (Lee et al., 2008). This enormous diversity may be under threat from climate change (Poloczanska et al., 2016), which may lead not only to reductions in diversity and biomass, but also to significant changes in the way that benthic ecosystems function and the services that they provide (Hewitt et al., 2016). The impact of OA on the biogeochemistry of marine sediments is complex (Gazeau et al., 2014). Sediment dwelling organisms are exposed to a highly variable environment and thus may already be experiencing temperature and pH extremes close to the means predicted under future scenarios for OW and OA. Boyd et al. (2016) suggest that it may well be these extremes of variation that will determine the impact of OW and OA rather than the means. The prevailing conditions within different oceanic basins may also have a significant effect on the responses of benthic fauna (Sperling et al., 2016), implying that the responses to OW and OA observed in the fauna may differ from basin to basin. Furthermore, OW and OA are not acting in isolation as other anthropogenically induced changes to the ocean environment will also add to the complexity of the ecosystem

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responses. Factors such as hypoxia (Mostofa et al., 2016; Sperling et al., 2016), changes in fresh water discharges from river systems (Philippart et al., 2011), eutrophication (Mostofa et al., 2016), metals (Wang et al., 2015), over exploitation of commercial species (Christensen et al., 2015), invasive species (Lord and Whitlatch, 2015), and the increased frequency of toxic algal blooms (Mostofa et al., 2016) will all affect the responses of organisms to OW and OA.

Meiofauna play an important role in the ecology of marine ecosystems and the functions they provide (Reiss et al., 2010). For example they are an essential component in the remineralisation of organic matter within the marine ecosystem (Evrard et al., 2010). Ocean acidification research has increased dramatically over the last decade (Browman, 2016), but publications concerning the impact of OA on sedimentary meiofauna remain few and far between (Widdicombe et al., 2009; Sarmento et al., 2015; Meadows et al., 2015; Álvarez-Castillo et al., 2015). The meiofauna represent a diverse assemblage of both prokaryotes (ciliates and foraminiferans) and eukaryotes, with a wide variety of physiological requirements and constraints (Giere, 2009). This high biological and physiological diversity along with other aspects of their ecology make them an ideal group for studying environmental change as they provide multiple responses to changes in their environment (Coull and Chandler, 1992), i.e. different species or groups of species will respond differently to the environmental changes resulting from OW and OA. Meiofauna also have logistical advantages for manipulative experiments: they are small (<1 mm), generally abundant and ubiquitous, they have short life cycles (a short as a few weeks in some cases), and direct benthic development; all of which makes them ideal for microcosm experiments (Lee and Correa, 2007; Fleeger and Carman, 2011). Much of the research into OA has focused on the responses of calcifying organisms (such as corals, molluscs and echinoderms) where reduced pH is thought to increase the energetic costs of maintaining calcified structures (Cyronak et al., 2016). However, for both calcifying and non-calcifying organisms changes in pH may affect a wide number of other metabolic processes such as acid-base regulation and osmoregulation (Cyronak et al., 2016; Freitas et al., 2016) which may impose increased energetic costs and reduce fitness.

Thus the objective of this research was to determine the responses of meiofaunal assemblages to OW and OA, both individually and in combination, at both the assemblage level and within the nematodes, typically the most abundant meiofaunal group. We propose the general hypotheses that: (i) OW will increase meiofaunal abundances, nematodes cultivated at increased temperatures typically exhibit shorter life-cycles (Stroustrup et al., 2016), thus increasing the number of generations per year and possibly population sizes. That (ii) OA will decrease meiofaunal abundances and species richness, as reduced pH will impose increased metabolic costs on the meiofauna, reducing fitness and survival rates. Finally, (iii) that if the first two hypotheses are supported then the combined effects of OW and OA will be antagonistic leading to a neutral effect on the meiofauna assemblages.

2. Materials and methods

2.1. Mesocosms

All the experiments were conducted within mesocosms, designed to generate four treatment conditions: 15 °C/400 ppm CO_2 ("control"), 15 °C/1000 ppm CO_2, 19 °C/400 ppm CO_2, and 19 °C/1000 ppm CO_2. The treatments were selected to represent current conditions and one possible future conditions of OW and OA. 15 °C/400 ppm CO_2 represent current conditions in the seawaters adjacent to the laboratory (Bahía Herradura, Coquimbo, Chile: 29.4661 °S, 071.3547 °W), and 19 °C and 1000 ppm CO_2 represent possible future scenarios of OW and OA (Meinshausen et al., 2011). Constant seawater temperatures were maintained in the mesocosms using a recirculating system with temperature maintenance provided by two thermostat controlled heat exchanger units. Seawater was pumped from the bay adjacent to the laboratory and added to the circulating system for temperature conditioning. The temperature controlled water was then circulated through seawater tables (insulated fibre-glass, approximately 15 cm deep). The seawater pH levels were maintained by steady aeration of the microcosm units using premixed gases to generate the desired pCO_2 levels (Fig. S1). Dry oil free compressed air was mixed with compressed CO_2 (research grade, supplied by Indura S.A.) using mass flow controllers (Aalborg). The entire mesocosm unit was located on an outside concrete platform adjacent to the water-front and the mesocosms were shaded from direct sunlight by an opaque fabric tent (Fig. S2–D).

2.2. Microcosms

The microcosm units consisted of 156 × 156 × 86 mm plastic boxes (Ziploc™). The lid of the box was pierced with two connectors. The first connected to a plastic drip feed unit (Cosmoplas) used to maintain a constant flow (1 L^{-h}) of 1 μm filtered, temperature and pH treated seawater to the microcosm. The second connected to a short length of tubing, a right angle connector, and a micro-pipette tip to supply either dry air or pre-mixed air-CO_2 at a constant rate (Fig. S2–A). Bubbling the gasses directly through the water in each microcosm ensured that the desired pCO_2 was maintained throughout the experimental run. A series of small outflow holes were made on one side of the box, 35 mm from the base. Sediment was added to each microcosm to a depth of 15 mm and the outflow holes maintained a water depth over the sediment of 20 mm. The replicate microcosms were maintained in the temperature controlled seawater tables of the mesocosm system described above (Fig. S2–B,C). In order to keep the outflow holes just above the water level in the seawater tables the microcosm units were supported on glass platforms.

2.3. Seawater chemical analyses

Chemical analyses of the seawater in the microcosms was conducted twice a week in order to monitor the actual seawater conditions in each of the treatments, the following chemical variables were measured: pH, total alkalinity (A_T), temperature, salinity, pCO_2, pH in situ, pCO_2 in situ, [CO_3^{-2}], and the saturation state of Ω aragonite and calcite (Table 1). The pH measurements were made in a closed 25 ml cell, thermostatically controlled at 25.0 °C, with a Metrohm 713 pH meter (input resistance $>10^{13}$ Ohm, 0.1 mV sensitivity and nominal resolution 0.001 pH units) and a glass fixed ground-joint diaphragm electrode with Pt1000 (Metrohm 6.0257.000, Aquatrode plus) calibrated with 8.089 Tris buffer at 25.0 °C (DOE, 1994); pH values are therefore reported on the total hydrogen ion scale (DOE, 1994). Total Alkalinity (A_T) was determined by potentiometric titration in an open cell, according to Haraldsson et al. (1997). The accuracy was controlled against a certified reference material supplied by Andrew Dickson (Scripps Institution of Oceanography). The correction factor was approximately 1.002, corresponding to a difference of < 5 μmol kg^{-1}. Each sample was analysed using 2 or 3 replicates. Temperature and salinity were measured using a CTD (Ocean Seven 305) placed in the mixing tanks. The pH, A_T and hydrographic data were used to calculate the rest of the carbonate system parameters (pCO_2, pH in situ, pCO_2 in situ and [CO_3^{-2}]) and the saturation state of Ω aragonite and calcite, using CO_2 SYS software (Lewis and Wallace, 1998) set with Mehrbach solubility constants (Mehrbach et al., 1973) refitted by Dickson and Millero (1987).

Table 1
Average (±SE) conditions of the seawater used to maintain the microcosm units over a period of 90 days, under contrasting temperature and pCO_2 levels. Current and future pCO_2 levels are based on the rate of change in pH predicted by the most extreme scenario (RCP8.5 scenario) of atmospheric CO_2 for the beginning of the next century. See Meinshausen et al. (2011) for further details. A_T total alkalinity, pCO_2 partial pressure of carbon dioxide in seawater.

pCO_2 level (°C)	pH at 25 °C (pH units)	Temperature (°C)	A_T (μmol kg^{-1})	pCO_2 in situ (μatm)	$[CO_3^{2-}]$ in situ (μmol kg^{-1}SW)	Salinity	Ω calcite	Ω aragonite
Source seawater	7.892 ± 0.019	16.075 ± 0.168	2283.617 ± 5.313	434.744 ± 22.521	155.443 ± 5.521	34.096 ± 0.089	3.730 ± 0.132	2.398 ± 0.086
Current (15 °C)	7.906 ± 0.014	15.935 ± 0.183	2252.326 ± 2.360	405.210 ± 16.645	157.457 ± 4.061	34.394 ± 0.022	3.771 ± 0.097	2.424 ± 0.063
Future (15 °C)	7.566 ± 0.013	15.865 ± 0.185	2267.577 ± 2.105	1003.976 ± 36.109	78.564 ± 2.175	34.551 ± 0.029	1.879 ± 0.052	1.209 ± 0.034
Current (19 °C)	7.987 ± 0.009	19.385 ± 0.102	2266.659 ± 3.279	372.037 ± 9.263	185.755 ± 3.258	34.427 ± 0.031	4.459 ± 0.078	2.891 ± 0.051
Future (19 °C)	7.622 ± 0.014	19.408 ± 0.094	2257.027 ± 2.715	995.934 ± 39.590	89.540 ± 2.506	34.438 ± 0.017	2.149 ± 0.060	1.394 ± 0.039

2.4. Experimental design

The sediment used in the microcosms was collected from the southern end of the Bahía La Serena (29.9572 °S, 071.3133 °W), just to the north of Coquimbo. The sediment from Bahía La Serena is predominantly silicacious. Sediments were collected from the shallow sub-tidal (≈1 m) as it was not practical to generate tidal conditions within the microcosms. Approximately 20 L of sand was collected. Due to the known heterogeneous distribution of meiofauna, small amounts of sand were collected at random along an approximately 30 m stretch of shore. The sand for the microcosms was transported to the laboratory in two 10 L plastic buckets where its was mixed by hand in order to homogenise as far as possible the distribution of the meiofauna before the sand was added to the microcosms. Approximately 300 ml of sand was added to each microcosm and microcosm units were then randomly assigned to the four treatments. Microcosms were maintained under the treatment conditions for 90 days. Each microcosm was "fed" every 30 days with 1 g of rehydrated *Macrosystis* sp. flour, this organic material simulated the input of organic material to the beach ecosystem and serves as the base of the trophic chain, primarily as a substrate for bacterial and protozoan growth, which in turn feed the meiofauna. This was done in an attempt to avoid resource limitation causing effects on the meiofaunal assemblages within the microcosms. There were 5 replicate microcosms for each treatment, thus at the start of the experiment there were 20 microcosms.

2.5. Sampling

At the end of the experimental run the sediment was homogenised by stirring and a single 50 ml sediment sample was collected from each microcosm unit for quantification of the meiofaunal assemblage. This sample was fixed in 5% formalin made up with 45 μm filtered seawater. The remaining sediment was collected for analysis of photopigments, % organic material and granulometry; these samples were placed in a small plastic bags and frozen. Five replicate 50 ml quantitative meiofauna samples were also taken at the field site at the start of the experiment (T_0), and at the end of the experimental run (T_1), field samples were also collected for photopigment, % organic material and granulometry analysis. These samples provided information on the meiofaunal assemblage structure in the field at the start and end of the experimental run.

2.6. Sample analyses

The meiofauna from each quantitative sample were extracted using a two stage methodology. The fauna were removed from the substrate by the decantation method (Pfannkuche and Thiel, 1988), using 45 μm filtered fresh water. The extraction procedure was repeated five times. The material captured on the 45 μm sieve by the decantation method was then further processed using the Ludox flotation method (Burgess, 2001). This removes any remaining sediment from the sample. The samples were washed into 50 ml conical tubes with Ludox (a colloidal silica solution with a density of 1.15 g cm^{-3}). The samples were then centrifuged at 750 rpm for 15 min, after which the Ludox was poured slowly through a 45 μm sieve with care being taken not to re-suspend the sediment at the bottom of the tube.

The fauna extracted from the quantitative samples was then washed into embryo dishes with a glycerol solution (5% glycerol, 20% ethanol, 75% distilled water) and placed in a warm desiccator for a period of between 24 and 48 h. Once the water and alcohol had evaporated leaving the fauna in glycerol, the samples were mounted on large glass microscope slides (75 × 38 mm) within a wax ring. Where the fauna was present in very high densities or where there was a significant amount of organic material in a sample, the sample was divided between two or more slides to facilitate analysis. The meiofauna present in each sample were assessed using an Olympus BX43 compound microscope. Each slide was systematically examined at a magnification of ×100 and the abundance of each taxonomic group or species determined.

The frozen sediment for the photopigment, % organic material and granulometry analyses was first freeze-dried (Thermo Savant Modulyo D-230) for approximately 48 h. Analysis of the photopigments in the sediment samples was made using the standard methodology (Brito et al., 2009). Spectrophotomic measurements were then made using a Tecan Infinite200-PRO plate reader. Analysis of the organic material content of the sediment samples was made using the standard methodology (Eleftheriou, 2013) with samples ashed in a muffle-oven (Vulcan A-550) for 4 h at 450 °C. Analysis of the sediment granulometry was conducted using the standard mechanical sieving methodology (Eleftheriou, 2013) followed by the calculation of the standard sediment parameters (Folk, 1966), the graphic mean (Md), the inclusive graphic quartile deviation (QDI), and the inclusive graphic skewness (SkI).

2.7. Statistical analyses

All statistical analyses were conducted using R (R Core Team, 2016). Meiofauna data were analysed for treatment effects using a two-way ANOVA with subsequent post-hoc Tukey's tests. In addition PERMANOVA tests were also conducted, using the abundance data for all the groups of fauna and for the nematode species abundances, using the adonis function of the vegan package (Oksanen et al., 2016). Bray-Curtis dissimilarity matrices were used for the analysis. Equality of variances PERMDISP tests were also conducted using the betadisp function also in the package vegan. Data from field samples collected at the start of the experimental run (T_0) and the end of the experimental run (T_1) were compared with the data from the "control" microcosms (15 °C/400 ppm CO_2) using a one-way ANOVA to determine microcosm effects. The following diversity indices were calculated for the nematodes,

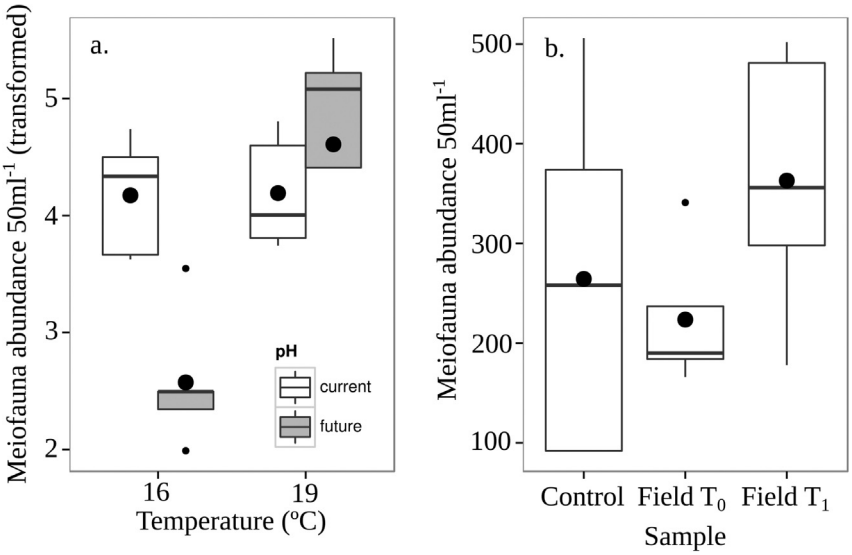

Fig. 1. (a.) Meiofauna abundance after 90 days of exposure in microcosms with different combinations of temperature (15 and 19 °C) and pH (pCO_2 of 400 and 1000 ppm). (b.) A comparison of meiofaunal abundance between field samples collected at the start (T_0) and end (T_1) of the 90 day experimental run with meiofaunal abundance in the control microcosms (15 °C and pCO_2 of 400 ppm) after 90 days. Total meiofauna abundances were Box-Cox power transformed ($\lambda = -0.092$).

Species richness (S), Simpson's index (1/D), and Simpson's evenness (E) using the vegan package (Oksanen et al., 2016). Shapiro test for normality and Bartlett's test for homogeneity were applied to the data. Where necessary the data were Box-Cox power transformed: total meiofauna abundances ($\lambda = -0.092$), turbellarian abundances ($\lambda = -0.001$), nematode abundances (treatment effects $\lambda = -0.339$, microcosm effects $\lambda = -0.131$); or Yeo-Johnson power transformed: foraminiferan abundances ($\lambda = 0.288$) and gastrotrichan abundances ($\lambda = -0.006$). Where normality could not be achieved with a power transformation, the data were rank transformed (Conover, 2012) prior to analysis: nematode diversity, chlorophyll-a and phaeopigments (treatment and microcosm effects).

Table 2
Two-way ANOVA examining the individual and interaction effects of temperature (15 and 19 °C) and pH (pCO_2 of 400 and 1000 ppm) on meiofauna exposed for 90 days in microcosms.

	d.f.	F.	p.		d.f.	F.	p.
Meiofauna (Abundance)				Nematode Richness (S)			
T °C	1	10.590	**0.005**	T °C	1	2.998	0.103
pH	1	3.510	0.079	pH	1	11.151	**0.004**
T °C x pH	1	10.210	**0.006**	T °C x pH	1	4.956	**0.041**
Residuals	16			Residuals	16		
Nematoda (Abundance)				Nematode Species Diversity (1/D)			
T °C	1	7.666	**0.014**	T °C	1	0.102	0.754
pH	1	2.756	0.116	pH	1	1.773	0.202
T °C x pH	1	11.602	**0.004**	T °C x pH	1	0.404	0.534
Residuals	16			Residuals	16		
Turbellaria (Abundance)				Nematode Species Evenness (E)			
T °C	1	10.397	**0.005**	T °C	1	2.034	0.173
pH	1	1.283	0.274	pH	1	0.079	0.782
T °C x pH	1	4.888	**0.042**	T °C x pH	1	5.316	**0.035**
Residuals	16			Residuals	16		
Foraminifera (Abundance)				Gastrotricha (Abundance)			
T °C	1	0.204	0.658	T °C	1	1.872	0.190
pH	1	6.523	**0.021**	pH	1	11.577	**0.004**
T °C x pH	1	2.218	0.156	T °C x pH	1	2.086	0.168
Residuals	16			Residuals	16		

Values in bold indicate statistically significant differences.

3. Results

The following meiofaunal groups were found in the sediments used in this study, the average proportional contribution of each group is in parenthesis: foraminiferans (6.5%), turbellarians (25.8%), nematodes (54.6%), harpacticoid copepods (6.7%), ciliates (2.9%), ostracods (0.3%), polychaetes (1.1%) and gastrotrichs (2.0%). In general abundances in the microcosms were more variable than in the field samples. The sediment used in the microcosms was fine sand (2.346 φ, 197 μm), moderately well sorted with a slight skew towards finer material. The results of the seawater chemistry analyses are shown in Table 1.

Total meiofauna abundances (Fig. 1a) analysed using a two-way ANOVA (Table 2) indicated a significant effect of temperature (p. = 0.005) and the interaction between temperature and pCO_2 (p. = 0.006), pCO_2 alone was close to significance (p. = 0.079). Post-

Table 3
One-way ANOVA examining the differences between quantitative samples collected in the field at the start (T0) and end (T1) of the 90 day experimental run with quantitative samples collected from the control microcosms (15 °C and pCO_2 of 400 ppm) after 90 days.

	d.f.	F.	p.		d.f	F.	p.
Meiofauna (Abundance)				Nematode Species Richness (S)			
Sample	2	1.391	0.286	Sample	2	1.102	0.364
Residuals	12			Residuals	12		
Nematoda (Abundance)				Nematode Diversity (1/D)			
Sample	2	0.842	0.455	Sample	2	0.064	0.938
Residuals	12			Residuals	12		
				Nematode Species Evenness (E)			
				Sample	2	0.198	0.823
				Residuals	12		

hoc Tukey's tests indicated significantly lower total meiofaunal abundances at pCO_2 of 1000 ppm at 15 °C (p. = 0.012) but not at 19 °C. At pCO_2 of 1000 ppm total meiofauna abundances were higher at 19 °C compared to 15 °C (p. = 0.002), but no significant differences were observed between the temperature treatments at pCO_2 of 400 ppm. One-way ANOVA (Table 3) comparing meiofauna abundances (untransformed data) indicated that there were no significant differences between the T_0 and T_1 field samples and the "control" treatment (Fig. 1b). The PERMANOVA analysis on the multivariate meiofauna data (Table 4) indicated a significant effect of temperature (p. = 0.025), pCO_2 (p. = 0.022), and the interaction between temperature and pCO_2 (p. = 0.039). The PERMDISP tests were not significant for temperature (p. = 0.792) nor pCO_2 (p. = 0.153).

Table 4
Two-way PERMANOVA examining the individual and interaction effects of temperature (15 and 19 °C) and pH (pCO_2 of 400 and 1000 ppm) on the multivariate data for all meiofauna and nematode species exposed for 90 days in microcosms.

	Meiofauna			Nematodes		
	d.f.	F.	p.	d.f.	F.	p.
T °C	1	3.289	**0.025**	1	1.902	0.062
pH	1	3.814	**0.022**	1	2.463	**0.025**
T °C x pH	1	2.629	**0.039**	1	2.098	**0.041**
Residuals	16			16		

Values in bold indicate statistically significant differences.

Nematode abundance (Fig. 2a) analysed using a two-way ANOVA (Table 2) indicated a significant effect of temperature (p. = 0.014) and the interaction between temperature and pCO_2 (p. = 0.004). Post-hoc Tukey's tests indicate significantly lower nematode abundances at pCO_2 of 1000 ppm at 15 °C (p. = 0.012) but not at 19 °C. At pCO_2 of 1000 ppm nematode abundances were higher at 19 °C compared to 15 °C (p. = 0.002), but no differences were observed between the temperature treatments at pCO_2 of 400 ppm. A total of 39 nematode species (Table 5) were identified in the samples (field and microcosm). Nematode species richness (S, untransformed) (Fig. 2b) analysed using a two-way ANOVA (Table 2) indicated a significant effect of pCO_2 (p. = 0.004) and the interaction between temperature and pCO_2 (p. = 0.041). Post-hoc

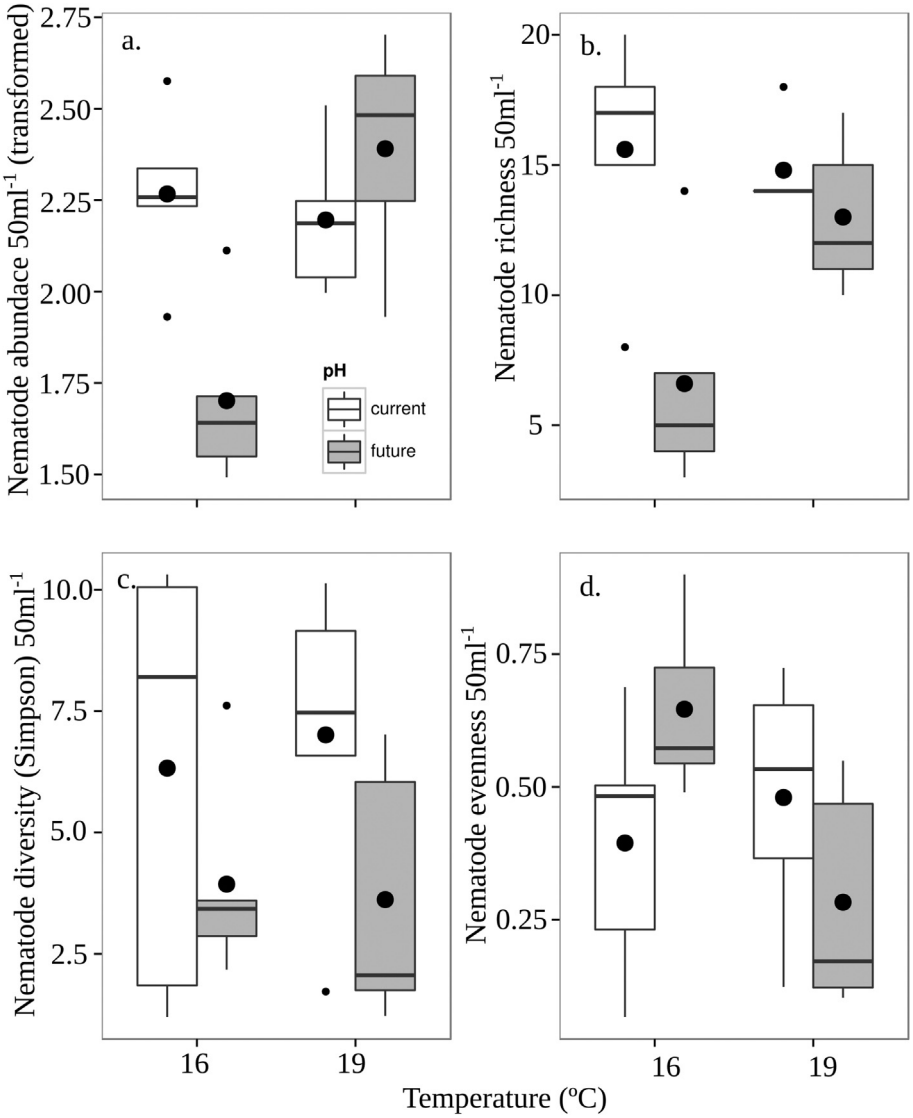

Fig. 2. A comparison of (a.) nematode abundance, (b.) nematode species richness, (c.) nematode diversity (Simpson's index), and (d.) nematode evenness (Simpson's evenness); after 90 days of exposure in microcosms with different combinations of temperature (15 and 19 °C) and pH (pCO_2 of 400 and 1000 ppm). Nematode abundances were Box-Cox power transformed ($\lambda = -0.339$) and nematode diversity (Simpson's index 1/D) data were rank transformed.

Table 5
A list of the Families, Genera and Species of nematodes found in the samples used in this study.

Family	Genera	Species
Anoplastomatidae	*Chaetonema*	sp.
Anticomidae	*Anticoma*	*columba*
Axonolaimidae	*Odontophora*	*peritricha*
Ceramonematidae	*Dasynemoides*	sp.
	Psilonema	sp.
Chromadoridae	*Chromadorina*	sp.
	Dichromadora	sp.
	Hypodontolaimus	sp.
	Prochromadorella	sp.
	Rhips	sp.
	Spilopherella	*paradoxa*
Desmodoridae	*Desmodora*	sp.1
	Desmodora	sp.2
	Desmodora	sp.3
Desmoscolecidae	*Desmoscolex*	sp.
	Quadralinioides	sp.
	Tricoma	sp.
Enchelididae	*Calyptronema*	sp.
Epsilonematidae	*Epsilonema*	sp.
Leptolaimidae	*Leptolaimus*	sp.
Linhomoeidae	*Terschellingia*	*longicaudata*
Microlaimidae	*Microlaimus*	*gerlachi*
Oncholaimidae	*Curvolaimus*	*decipiens*
	Viscosia	sp.
Oxystominidae	*Halalaimus*	sp.
	Thalassolaimus	sp.
Rhabditidae	*Rhabditis*	sp.
Selachinematidae	*Gammanema*	sp.
Thoracostomatidae	*Enoplolaimus*	sp.
Tripyloididae	*Gairleanema*	sp.
Xyalidae	*Daptonema*	sp.1
	Daptonema	sp.2
	Omicronema	*litorium*
	Prorhynchonema?	sp.
	Rhynchonema	sp.
	Theristus	*problematicus*
	Theristus	sp.1
	Theristus	sp.2
	Trichotheristus	sp.

Tukey's tests indicated significantly lower species richness at pCO_2 of 1000 ppm at 15 °C (p. = 0.006) but not at 19 °C. At pCO_2 of 1000 ppm nematode abundances were higher at 19 °C compared to 15 °C (marginal significance, p. = 0.056), but no significant differences were observed between the temperature treatments at pCO_2 of 400 ppm. The PERMANOVA analysis on the multivariate nematode species data (Table 4) indicated a significant effect of pCO_2 (p. = 0.025), and the interaction between temperature and pCO_2 (p. = 0.041); the effect of temperature alone was marginally significant (p. = 0.062). The PERMDISP tests were not significant for temperature (p. = 0.911) nor pCO_2 (p. = 0.402). Nematode diversity (Simpson's index 1/D, Fig. 2c) analysed using a two-way ANOVA (Table 2) indicated no significant differences in diversity between the treatments, however, visually diversities at pCO_2 of 1000 ppm generally appear to be lower. Nematode evenness (Simpson's E, Fig. 2d) analysed using a two-way ANOVA (Table 2) indicated a significant interaction between temperature and pCO_2 (p. = 0.035). Post-hoc Tukey's tests indicated no significant pairwise differences, however the lower evenness observed at higher temperatures at pCO_2 of 1000 ppm was marginally significant (p. = 0.076).

Turbellarian abundances analysed using a two-way ANOVA (Table 2) indicated a significant effect of temperature (p. = 0.005) and the interaction between temperature and pCO_2 (p. = 0.042). Post-hoc Tukey's tests indicate at pCO_2 of 1000 ppm turbellarian abundances were higher at 19 °C compared to 15 °C (p. = 0.007), but no significant differences between temperature treatments were observed at pCO_2 of 400 ppm. No species level analysis of the turbellarians was possible as the fixation with formalin renders most diagnostic features unrecognisable. Foraminifera and Gastrotricha abundances analysed using a two-way ANOVA (Table 2) indicated significant negative effects of pCO_2 only (Foraminifera p. = 0.021; Gastrotricha p. = 0.004).

One-way ANOVA (Table 3) comparing data from the field samples collected at the start of the experimental run (T_0) and the end of the experimental run (T_1) with the data from the "control" microcososms (15 °C/400 ppm CO_2) indicated that there were no significant differences in nematode abundances (Fig. 3a), nematode species richness (Fig. 3b), nematode diversity (Fig. 3c), and nematode species evenness (Fig. 3d).

Both Chlorophyll-a and Phaeopigments data (Table 6) were rank transformed prior to analysis. A two-way ANOVA for both photopigment variables indicated no significant differences between treatments. One-way ANOVA comparing Chlorophyll *a* concentrations indicated that there were significant differences between the T_0 and T_1 samples and the "control" treatment (d.f. = 2, F = 6.529, p. = 0.012). Post-hoc Tukey's tests indicate that Chlorophyll *a* concentrations in the "control" treatment were significantly lower than in the T_0 samples (p. = 0.010), but not the T_1 samples. One-way ANOVA comparing Phaeopigment concentrations indicated that there were significant differences between the T_0 and T_1 samples and the "control" treatment (d.f. = 2, F = 3.923, p. = 0.049), but none of the post-hoc pairwise comparisons were significant. Sediment organic material (% organic matter) data (Table 6) anaysed using a two-way ANOVA indicated a significant effect of temperature (d.f. = 1, F = 8.563, p. = 0.010) and the interaction between temperature and pCO_2 (d.f. = 1, F = 6.705, p. = 0.020). Post-hoc Tukey's tests indicate at pCO_2 of 1000 ppm % organic matter was lower at 19 °C compared to 15 °C (p. = 0.006), but no significant differences were observed between the temperature treatments at pCO_2 of 400 ppm. One-way ANOVA comparing % organic material indicated that there were significant differences between the T_0 and T_1 samples and the "control" treatment (d.f. = 2, F = 9.742, p. = 0.003). Post-hoc Tukey's tests indicated that % organic material in the T_1 (p. = 0.002) and "control" treatment samples (p. = 0.026) were significantly higher than in the T_0 samples.

4. Discussion

The results presented here suggest that an increase in seawater temperature from 15 to 19 °C alone will have no discernible impact on meiofaunal abundance, nematode abundance, nematode species richness, nematode diversity nor nematode evenness. Thus the hypothesis that increased temperature will increase meiofaunal abundance was not supported. With respect to seawater pH, the results indicate that a reduction in seawater pH from 7.9 (pCO_2 400 ppm) to 7.6 (pCO_2 1000 ppm) will have a negative impact on meiofaunal abundance, nematode abundance and nematode species richness. Thus the hypothesis that a reduced pH will reduce meiofaunal abundance and species richness was supported. The changes observed in the meiofaunal assemblages at the higher temperature, lower pH (higher pCO_2), or combination of the two, are taken as evidence of the potential effects of future OW and OA on meiofaunal assemblages.

Both meiofaunal and nematode abundances were significantly higher under the combined future OW and OA conditions, but species richness, diversity and evenness were all lower. Thus the combined effects of increased temperature and pCO_2 were not neutral and the third hypothesis postulated was rejected. The lowest nematode assemblage evenness values were associated with high temperature and low pH suggesting in that in these microcosms the assemblage was dominated by a few species, and

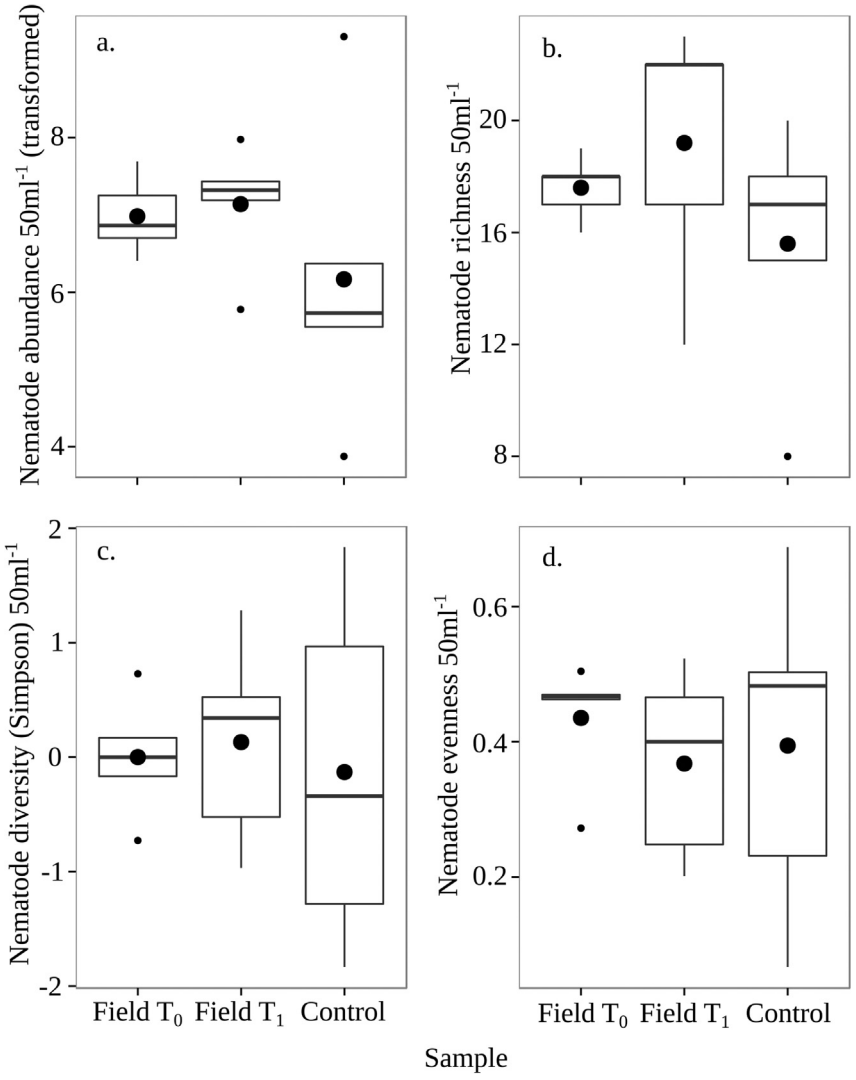

Fig. 3. A comparison of (a.) nematode abundance, (b.) nematode species richness, (c.) nematode diversity (Simpson's index), and (d.) nematode evenness (Simpson's evenness); between field samples collected at the start (T_0) and end (T_1) of the 90 day experimental run with meiofaunal abundance in the control microcosms (15 °C and pCO_2 of 400 ppm) after 90 days. Nematode abundances were Box-Cox power transformed ($\lambda = -0.131$) and nematode diversity (Simpson's index 1/D) data were rank transformed.

Table 6
Mean values (±standard deviation) of the variables: % organic material and the photopigments Chlorophyll-a and phaeopigments.

	% organic material		Chlorophyll-a		Phaeopigments	
	mean	s.d.	mean	s.d.	mean	s.d.
Field T_0	0.882	±0.043	0.314	±0.152	0.754	±0.662
Field T_1	1.044	±0.058	0.110	±0.044	0.556	±0.213
15°C/400 ppm	0.997	±0.075	0.033	±0.048	0.298	±0.118
15°C/1000 ppm	1.215	±0.166	0.232	±0.270	0.527	±0.722
19°C/400 ppm	0.977	±0.088	0.161	±0.180	0.500	±0.697
19°C/1000 ppm	0.900	±0.155	0.041	±0.057	0.615	±0.548

that was what was observed in the samples under the microscope. Indeed in the 19 °C/1000 ppm CO_2 microcosms just two species accounted for 72% of the fauna, those same two species accounted for only 34% of nematode abundance in the "control" microcosms. Both of these species display the characteristics of opportunistic or "r-selected" species, one species was from the family Rhabditidae and the second from the family Xyalidae. Why they dominated the "impacted" microcosms is open to speculation. The first possible cause is simple stochastic effects resulting from slight differences in the assemblage structure at the start of the experimental run and not due to the differential effects of the variables under examination (Gerwing et al., 2016). This "null" hypothesis could be tested in the future by increasing the number of replicates used or by repetition of the experiment. Assuming that the differences in the assemblage structure observed after 90 days are due to the combined effects of temperature and pH there are a number of potential causes. First, the different tolerances of different species to increases in temperature and decreases in pH could change the fitness of individual species in the assemblage which could in turn alter the relative competitiveness of species within the assemblage (Stachowicz et al., 2007). There is also the possibility that the species that increased significantly in abundance were released from predation pressure if their predators, typically other nematode species (Moens et al., 1999) and turbellarians from the family Otoplanidae, were less tolerant to the change in conditions. However, the abundance of the main predatory nematode species (*Gammanema* sp. and *Enoplolaimus* sp.) were low in all treatments and field samples; and turbellarian abundances were generally as high as nematode abundances. There may also have been a change in the availability of food resources that the opportunistic species

were able to take advantage of. However, Baragi and Anil (2016) reported that the synergistic effect of increased temperature and reduced pH on microbial communities was a reduction in abundances. On the other hand, they also reported that the situation was reversed when increased nutrients were available, something that could occur as photosynthesis may be enhanced under future climate change scenarios (Gao et al., 2012; Moreau et al., 2015).

Widdicombe et al. (2009) demonstrated using microcosm studies, that seawater acidification significantly altered nematode assemblages, though this was after exposure to lower pHs than we used. Indeed their Fig. 4 suggests that at pH 7.5 (our lower value was 7.6) nematode abundance, diversity and evenness could be interpreted as being unchanged or even slightly higher. This does not agree with the results presented here where both nematode abundance and species richness were significantly lower at pH 7.6. Sarmento et al. (2015) also found that seawater acidification significantly changed meiobenthic assemblages with different groups displaying different responses. For example, polychaetes and adult harpacticoid copepods were unaffected by lower pH (7.5) but copepod naupli were significantly more sensitive; while the nematodes, ostracods, turbellaria and tardigrades exhibited higher abundances at low pH. Again our results do not clearly agree as we found that both general meiofaunal abundance and nematode abundance were significantly lower at the lower pH. Finally, Meadows et al. (2015) demonstrated that the combination of ocean acidification and warming had substantial effects on the structural and functional characteristics of meiofaunal and nematode assemblages. They found, as did we, that nematode abundances increased with increased temperature and lower pH. This was also supported by the findings of Sarmento et al. (2017) comparing different climate change scenarios.

The results presented here show how meiofaunal assemblages from exposed shallow sub-tidal sediments change under experimental conditions. However the next question that needs to be asked is will the same results be observed under real world conditions? Natural fluctuations may already expose meiofauna to extremes close to the values we used in the microcosms (Boyd et al., 2016). The increase in temperature and reduction in pH of the coastal waters will be a gradual process occurring over decades (Smith et al., 2015), a period that will encompass hundreds to thousands of meiofaunal life-cycles. Thus not only will phenotypic plasticity (Levis and Pfennig, 2016) be important in the short term, but over the long-term there is a high probability that acclimation and adaptation will occur (Kelly and Hofmann, 2013). Though, undoubtedly, the different ranges of plasticity, and the acclimation and adaptation capacities of different species will likely result in changes to meiofaunal assemblage structure and dynamics.

Future research needs to incorporate additional variables in order to study the effects of climate change, such as hypoxia, and or contaminants (e.g. metals). As was stated in the introduction, environmental change is not restricted to changes in single variables, but occurs to a suite of variables at the same time (Mostofa et al., 2016). Many meiofaunal species are already exposed to anoxic, hypoxic and microxic conditions and thus have developed the physiological adaptations necessary to survive low oxygen conditions (Braeckman et al., 2013). This is especially true of those assemblages found in finer sediments, protected low energy sites, deeper water assemblages exposed to oxygen minimum zones (OMZ's), and areas with high concentrations of organic material. Changes in both temperature and pH of seawaters and sediments will also change the bioavailability of metals, increasing the concentrations of M^{2+} ions in the interstitial water and thus toxicity (Zeng et al., 2015; Stockdale et al., 2016).

In conclusion, we can expect that ocean warming and ocean acidification in combination will change the structure of meiofaunal assemblages. Nematode assemblages may become dominated by opportunistic species with the capacity to tolerate the physiological demands of increased temperature and low pH. Future research into ocean warming and ocean acidification needs to address three issues, 1. the changes in the benthic community structure and ecological functions, 2. the changes induced by additional stressors (e.g. metals, eutrophication, hypoxia) in the environment, and 3. whether phenotypic plasticity and evolutionary change will allow meiofauna to adapt to the changing environment without significant negative effects.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.marenvres.2017.09.002.

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